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Utilization of lipids by *Dentex dentex* L. (Osteichthyes, Sparidae) larvae during lecitotrophia and subsequent starvation

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Abstract

Total lipids, lipid classes and their associated fatty acids were measured in developing eggs, yolksac larvae and starving larvae (from fertilized egg to day 9 after hatch) of the common dentex *Dentex dentex* (L., 1758). The larvae of common dentex during lecitotrophia and subsequent starvation consumed 1.6 μg of total lipid per larvae per day. The overall decrease was mainly due to utilization of the major neutral lipids, TAG and SE (0.5 and 0.6 μg larvae^{-1} day^{-1}, respectively) which was 3.4-fold greater than that of the the major phosphoglycerides (primarily PC by 0.2 μg larvae^{-1} day^{-1}). There was net synthesis/conservation of PE during the first half of the study period before it decreased rapidly (0.2 μg larvae^{-1} day^{-1}) during the second half. PUFAs were principally catabolized (468.6 ng larvae^{-1} day^{-1}), primarily 22:6(n-3), 20:5(n-3) and 20:4(n-6) (221.8, 58.5 and 12.1 ng larvae^{-1} day^{-1}, respectively). Saturated and monounsaturated fatty acids were also utilized (227.2 and 256.7 ng larvae^{-1} day^{-1}, respectively), principally 16:0 and 18:1(n-9) that were both consumed at 149.8 and 156.7 ng larvae^{-1} day^{-1}, respectively. The rank order of utilization of fatty acids (ng larvae^{-1} day^{-1}) by *D. dentex* larvae from total lipids, PC and TAG coincided with the order of abundance of the different fatty acids in the respective lipid fractions. However, in PE, the most abundant fatty acid, DHA, was relatively conserved and 16:0, the second most abundant fatty acid, was catabolized to the greatest extent. *D. dentex* showed a pattern of lipid metabolism during early development similar to that of marine larval fish from temperate waters whose eggs contain high levels of total lipids, including an oil globule, and which preferentially utilize neutral lipids as the primary energy source.

Abbreviations: AA, all-*cis*-5,8,11,14-eicosatetraenoic acid (arachidonic acid, 20:4(n-6)); C, free cholesterol; DHA, all-*cis*-4,7,10,13,16,19-docosahexaenoic acid (22:6(n-3)); DMA, dimethyl acetal; DPA, all-*cis*-7,10,13,16,19-docosapentaenoic acid (22:5(n-3)); EPA, all-*cis*-5,8,11,14,17-eicosapentaenoic acid (20:5(n-3)); FFA, free fatty acid; HUFA, highly unsaturated fatty acids ($\geq C_{20}$ and with $\geq$ 3 double bonds); LA, all-*cis*-9,12-octadecadienoic acid (linoleic acid, 18:2(n-6)); LNA, all-cis-9,12,15-octadecatrienoic acid (α-linolenic acid, 18:3(n-3)); PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid(s); SE, steryl ester. TAG, triacylglycerol.
Note: PC and PE represent diradyl (diacyl + alkenilacyl + alkylacyl) glycerophosphocholine and glycerophosphoethanolamine respectively.

Introduction

Throughout embryogenesis and early larval development, fish larvae gain nutrients from the endogenous reserves of the yolk. In principle, fish eggs contain all the nutrients that the larvae utilize during the lecithotrophic phase to maintain homeostasis and development. The nutrient composition of fish eggs is species-specific, and the precise sequence of nutrient composition varies both qualitatively and quantitatively. Recent studies investigating the sequence of catabolic substrate oxidation in developing embryos and lecitotrophic larvae of several marine fish species indicate that lipids play a major role (Finn 1994; Rønnestad et al. 1994; Finn et al. 1996a,b). The lipid content and composition of fish eggs also varies between species and may change during the different stages of development depending on ambient conditions, physiological events and energy demands (Rainuzzo 1993; Sargent 1995; Wiegand 1996; Rainuzzo et al. 1997). Moreover, lipid reserves of fish eggs are used both as substrates for energy metabolism and structural components for membrane biogenesis in the developing larvae (Rainuzzo 1993; Finn 1994; Sargent 1995, Wiegand 1996; Rainuzzo et al. 1997). Neutral lipids are generally considered the most important energy reserve in marine fish eggs and larvae, whereas phosphoglycerides with their high concentration of essential (n-3) HUFA are utilized, not only for cell division and organogenesis, but also for energy in some species (Tocher et al. 1985a; Fraser et al. 1988; Rainuzzo 1993; Sargent 1995; Wiegand 1996; Rainuzzo et al. 1997). Thus, utilization of polar or neutral lipids as energy sources during early development in fish is also species-specific and different patterns of lipid metabolism are apparent (Tocher et al. 1985a; Koven et al. 1989; Tandler et al. 1989; Rainuzzo et al. 1992; Daniel et al. 1993; Ronnestad et al. 1994; Vázquez et al. 1994; Sargent 1995; Wiegand 1996; Rainuzzo et al. 1997).

Larvae of marine fish require (n-3) HUFA such as EPA (20:5(n-3); and DHA (22:6(n-3); acids (Watanabe and Kiron 1994; Sargent 1995; Wiegand 1996; Rainuzzo et al. 1997). These fatty acids, and particularly DHA, are specifically accumulated (Mourente et al. 1991; Mourente and Tocher 1992a,b; 1993a) and are required for the biosynthesis of cell membranes vital for normal development and functioning of the visual and neural systems of developing fish larvae (Sargent et al. 1990; Sargent et al. 1993; Sargent 1995; Mourente 1996; Wiegand 1996). As a result, failure to provide adequate quantities of the appropiate dietary fatty acids is a major factor in the unsuccessful rearing of marine fish larvae (Watanabe and Kiron 1994; Rainuzzo et al. 1997), due mainly to visual impairment and abnormal predatory behaviour (Navarro and Sargent 1992; Sargent et al. 1994; Sargent 1995; Wiegand 1996; Rainuzzo et al. 1997). Therefore, a major challenge in marine aquaculture is defining the nutritional requirements of marine larval fish. Determining the utilization of endogenous (yolk-sac) nutrients during embryonic and early larval development is one approach to define the nutritional requirements of larvae (Tocher et al. 1985a,b; Fraser et al. 1988; Tandler et al. 1989; Ostrowski and Divakaran 1991; Rainuzzo 1993; Finn 1994; Vázquez et al. 1994; Sargent 1995; Wiegand 1996; Rainuzzo et al. 1997). Thus, much knowledge of lipid metabolism in fish eggs and larvae derives from studies investigating whole eggs and larvae (Tocher et al., 1985a,b; Lie 1993; Rainuzzo 1993; Finn 1994; Watanabe and Kiron 1994; Vázquez et al. 1994; Sargent 1995; Wiegand 1996; Rainuzzo et al. 1997) or separated yolksac and larval bodies (Rønnestad et al. 1995).

The common dentex (*Dentex dentex* L.) is a member of the Sparidae family which is widely distributed along the Meditarranean and the Eastern Atlantic and it is considered a promising species for mariculture in the Mediterranean area (Glamuzina et al. 1989; Sweetman 1992; Cataudella et al. 1995; Pastor et al. 1995). In the present study, we investigated the changes occurring in lipid class and fatty acid contents of lipids from common dentex eggs and newly hatched larvae during lecitotrophia and subsequent starvation in order to elucidate lipid metabolism and utilization of yolk lipids as a first step to assessing the requirements of first feeding larvae.

Materials and methods

Broodstock management, egg and larval production

Broodstock maintenance and reproduction as well as egg and larval production were performed according to the methodology developed by Pastor et al. (1995) at the Estación de Acuicultura, Port d'Andraxt, Mallorca, Spain. Eggs were hatched in 400 l cylindroconical tanks, in an open circulation system with sea water at 18 °C (1–3 water exchange/day), at a density of 100 eggs per l. Newly hatched larvae developed at the expenses of yolksac and oil drop reserves and subsequent starvation until day 9 after hatch. Biometric

data of eggs and larvae are shown in Table 1. To enable comparison with other studies made at different temperatures, time was also expressed as physiological day-degrees to allow for the influence of temperature on development time (Hempel 1979), according to the equation:

$$\text{days-degrees} = [\text{temperature} \times (\text{hours}/24)] \,/\, q$$

where temperature (°C); q, Winberg coefficient to convert the rate of physiological processes at a standard temperature of 20 °C.

Sample collection

Eggs from the same batch, approximately 10–12 h after spawning were collected in an appropiate size mesh screen, rinsed in distilled water and blotted in filter paper before being frozen in liquid nitrogen and stored in the freezer at −80 °C until analysis. The same treatment was used for larvae from 1 to 9 days after hatching. Yolk sac and oil drop absortion had primarily occurred in larvae sampled by day 5 and by day 6 after hatching, respectively (Table 1).

Dry weight, biometric values, biochemical composition and energy content determinations

Replicates of preweighed samples (approximately 500 mg wet weight) were maintained at 110 °C for 24 h. The dry weights were determined after cooling *in vacuo* for at least 1 h. Individual dry weight was determined by weighting counted individual eggs or larvae (n = 15–30) on previously tared glass slides (triplicates) after maintaining the samples in the oven at the conditions described above. Biometric determinations were done by micrometric analysis for light microscopy. Protein content was determinned by the Folin-phenol reagent method, according to Lowry et al. (1951). Total lipid contents were determined gravimetrically after extraction as described below. Carbohydrate contents were determined by a colorimetric method using the phenol-sulphuric acid reagent (Dubois et al. 1956). Ash contents were measured gravimetrically after total combustion in a furnace at 550 °C. The gross energy content was calculated from the biochemical composition using values of 5.65, 9.45 and 4.20 kcal g^{-1} for protein, lipid and carbohydrates respectively (Henken et al. 1986).

Total lipid extraction, lipid class separation and quantification

Total lipid was extracted in triplicate samples of about 200 mg of wet weight egg or larvae after homogenization in chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant, basically according to Folch et al. (1957). Lipid classes were separated by high-performance thin-layer chromatography (HPTLC) on silica gel 60 plates, 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-9001PC dual-wavelength flying spot scanner (Olsen and Henderson 1989).

Total lipid and lipid classes fatty acid analyses

Lipid fractions, PC, PE and TAG were separated in 20 × 20 cm TLC by using the single-dimension double-development method indicated above (Olsen and Henderson 1989). The separated lipid classes were visualized by developing the plates with 1% 2′, 7′-dichlorofluoresceine in 98% methanol and viewing under UV light. Fatty acid methyl esters (FAME) from total lipids and lipid classes, scraped from the plates, were prepared by acid-catalyzed transmethylation for 16 h at 50 °C, using tricosanoic acid (23:0) as internal standard (Christie 1989). FAME were extracted and purified as described previously (Tocher and Harvie 1988) and were separated in a Hewlett-Packard 5890A Series II gas chromatograph equipped with a chemically bonded (PEG) Supelcowax-10 fused silica wall coated capillary column (30 m × 0.32 mm id, Supelco Inc., Bellefonte, USA), 'on column' injection system and flame ionization detection. Hydrogen was used as the carrier gas with an oven thermal gradient from an initial 50 °C to 180 °C at 25 °C per min and then to a final temperature of 235 °C at 3 °C per min. The final temperature was maintained for 10 min. Individual FAME were identified by comparison with known standards and quantified by means of a direct-linked PC and Hewlett-Packard ChemStation software.

Statistical analysis

Results are presented as means ± SD (n = 3 or 4). The data were checked for homogeneity of the variances by the Bartlett test and, where necessary, the

Table 1. The effect of the duration of food deprivation on biometric data, gross composition (dry weight percentage) and energy content (kcal•g^{-1}) of eggs and newly hatched lecitotrophic larvae of *Dentex dentex* during development reared at 18.1 °C

	Duration of food deprivation					
	Egg	Day 0	Day 1	Day 2	Day 3	Day 4
Days degree	−15.2	0.0	15.2	43.1	64.6	86.1
Total length (μm)	nd	2582 ± 22^{a}	3240 ± 49^{b}	3465 ± 49^{c}	3560 ± 73^{cde}	3545 ± 96^{cd}
Individual dry weight (ng)	650 ± 210^{a}	560 ± 110^{a}	530 ± 200^{a}	490 ± 130^{ab}	460 ± 230^{ab}	500 ± 30^{ab}
Yolk sac vol. (μm^3)	nd	227.7 ± 41.1^{a}	110.2 ± 19.0^{b}	68.9 ± 19.9^{bc}	17.6 ± 1.8^{cd}	7.8 ± 1.8^{d}
Oil drop vol. (μm^3)	nd	5.5 ± 0.8^{a}	4.2 ± 0.1^{b}	4.3 ± 0.1^{b}	2.0 ± 0.4^{c}	0.9 ± 0.0^{d}
dry weight (%)	7.8 ± 0.2^{a}	12.3 ± 0.3^{g}	12.2 ± 0.2^{g}	11.9 ± 0.1^{f}	11.1 ± 0.1^{c}	10.8 ± 0.1^{d}
Protein	60.5 ± 2.8^{de}	54.9 ± 2.4^{a}	57.3 ± 0.2^{abc}	58.1 ± 1.1^{bcd}	55.2 ± 0.6^{ab}	58.2 ± 2.7^{cd}
Lipid	24.4 ± 2.0^{cd}	31.9 ± 1.6^{a}	29.5 ± 0.6^{b}	26.1 ± 0.7^{c}	25.7 ± 0.6^{c}	23.1 ± 1.4^{de}
Carbohydrate	5.4 ± 0.1^{bc}	4.0 ± 0.2^{ef}	3.8 ± 0.1^{f}	4.2 ± 0.2^{de}	5.2 ± 0.3^{b}	4.6 ± 0.1^{b}
Ash	9.4 ± 1.0^{a}	9.0 ± 0.4^{a}	9.4 ± 0.1^{a}	11.3 ± 1.9^{a}	13.2 ± 0.4^{c}	13.3 ± 0.9^{c}
Energy from protein	3.4 ± 0.1^{cd}	3.1 ± 0.1^{a}	3.2 ± 0.1^{ab}	3.3 ± 0.1^{bc}	3.1 ± 0.0^{a}	3.3 ± 0.0^{bc}
Energy from lipid	2.3 ± 0.2^{de}	3.0 ± 0.1^{a}	2.8 ± 0.1^{b}	2.5 ± 0.1^{c}	2.4 ± 0.1^{cd}	2.2 ± 0.0^{ef}
Energy from carbohydrate	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Total Energy	5.9 ± 0.2^{b}	6.3 ± 0.1^{a}	6.2 ± 0.1^{a}	6.0 ± 0.1^{b}	5.7 ± 0.1^{c}	5.7 ± 0.1^{c}
	Day 5	Day 6	Day 7	Day 8	Day 9	
Days degree	107.7	129.2	150.8	172.3	193.8	
Total length (μm)	3635 ± 40^{cde}	3715 ± 89de	3739 ± 65^{c}	nd	nd	
Individual dry weight (ng)	420 ± 50^{ab}	450 ± 80^{ab}	350 ± 50^{ab}	290 ± 70^{ab}	180 ± 40^{b}	
Yolk sac vol. (μm^3)	nd	nd	nd	nd	nd	
Oil drop vol. (μm^3)	0.2 ± 0.0^{d}	nd	nd	nd	nd	
dry weight (%)	10.4 ± 0.1^{c}	11.1 ± 0.1^{e}	10.9 ± 0.3^{de}	9.6 ± 0.1^{b}	9.4 ± 0.1^{b}	
Protein	60.8 ± 2.0^{de}	62.4 ± 2.2^{ef}	64.0 ± 0.5^{f}	64.6 ± 0.8^{f}	64.8 ± 0.5^{f}	
Lipid	22.1 ± 1.2^{e}	16.5 ± 0.9^{f}	14.3 ± 0.6^{g}	14.4 ± 0.4^{g}	14.7 ± 0.4^{fg}	
Carbohydrate	4.4 ± 0.2^{cd}	5.6 ± 0.0^{a}	5.3 ± 0.4^{ab}	5.1 ± 0.1^{b}	5.3 ± 0.2^{ab}	
Ash	12.5 ± 0.2^{c}	15.0 ± 0.2^{d}	13.4 ± 0.0^{c}	15.5 ± 0.4^{d}	15.4 ± 0.6^{d}	
Energy from protein	3.4 ± 0.1^{cd}	3.5 ± 0.1^{de}	3.6 ± 0.1^{ef}	3.6 ± 0.0^{ef}	3.7 ± 0.0^{f}	
Energy from lipid	2.1 ± 0.1^{f}	1.5 ± 0.1^{g}	1.3 ± 0.1^{h}	1.3 ± 0.0^{h}	1.3 ± 0.0^{h}	
Energy from carbohydrate	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	
Total Energy	5.7 ± 0.1^{c}	5.2 ± 0.1^{d}	5.1 ± 0.0^{d}	5.1 ± 0.0^{d}	5.1 ± 0.0^{d}	

Results represent the mean ± SD ($n = 3$). SD = 0.0 implies an SD of <0.05. Mean values within horizontal rows with different superscript letters are significantly different ($p < 0.05$). If no superscript appears, values are not different. nd = not determined.

data were arc-sin transformed before further statistical analysis. Differences between mean values 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 $p < 0.05$. Changes of the variables during the experimental period were studyed by linear and non linear regression analysis (Zar 1984).

Materials

Potassium bicarbonate, potassium chloride, BHT and tricosanoic acid (> 99% pure) were from Sigma Chemical Co. (Alcobendas, Madrid, Spain). HPTLC (10 × 10 cm × 0.15 mm) and TLC (20 × 20 cm × 0.25 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.(Alcobendas, Madrid, Spain).

Results

Changes in gross composition and energy content

Total length showed a linear increase by aprox. 130.5 μm day^{-1}. Individual dry weight showed a significant downward trend that implied a linear loss of aprox. 3.6 μg of individual dry weight per day. Yolksac volume (μm^3) decreased according to an exponential model ($y = 241.3e^{-0.6t} - 15$; $R^2 = 0.94$) and oil drop volume decreased linearly by 1.1 μm^3 per day. Total lipid content also decreased linearly at a rate of 1.6 μg larvae^{-1} day^{-1}. Total energy consumption and energy consumption derived from lipids were linear and represented rates of 0.15 kcal g dry weight^{-1} day^{-1} and 0.21 kcal g dry weight^{-1} day^{-1}, respectively (Table 1).

Changes in lipid class composition

The percentage of total polar lipids increased significantly by 42.6% (mainly due to significant increases in PC (13.5%), PE (58.2%), PS (87.3%), PI (30%), PA (66.7%) and SM (43.5%)) with a concomitant significant decrease in the percentage of total neutral lipids (particularly TAG (82.4%) and SE (72.6%)) whereas the proportions of free cholesterol and free fatty acids increased by 64.4% and 58.8%, respectively, during the study period (Table 2). Calculating these data as μg lipid class per larvae (Figure 1), PC showed a linear decrease by 0.2 μg larva^{-1} day^{-1} during the whole study period. PE presented a different pattern, with the amount of PE per larvae being constant from egg to day 5 after hatching, whereas from day 5 to day 9 after hatching it showed a significant linear decrease amounting to 0.2 μg larva^{-1} day^{-1}. Similarly, C showed a slight increase from hatching to day 5 after hatch of 12.3 ng larva^{-1} day^{-1} and then a linear decrease from day 5 to day 9 after hatch of 0.2 μg larva^{-1} day^{-1}. TAG content decreased linearly by 0.5 μg larva^{-1} day^{-1} and SE also decreased linearly by 0.6 μg larva^{-1} day^{-1} during the whole study period. Both PC/PE and TAG/C ratios showed downward trends during the starvation period (Table 2).

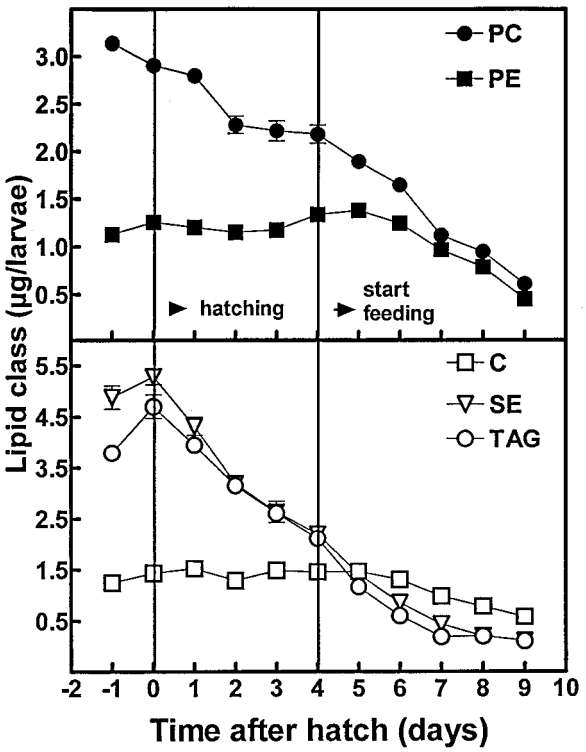

Figure 1. The effect of food deprivation on the content of major polar and neutral lipid classes of *Dentex dentex* larvae during early development.

Changes in total lipid fatty acid content

Total saturated fatty acids decreased due to significant decreases (2.2 μg mg total lipid^{-1} day^{-1}) in the content of the most abundant saturated fatty acid, 16:0, whereas the content of 18:0 increased significantly by 2.5 μg mg total lipid^{-1} day^{-1} during the same period (Table 3). However, when presented as ng of fatty acid larva^{-1} day^{-1}, 16:0 showed a linear decrease of 149.8 ng larva^{-1} day^{-1}, 18:0 showed a linear decrease of 32.5 ng larva^{-1} day^{-1} and total saturated fatty acids decreased by 227.2 ng larva^{-1} day^{-1}. Total monoene content showed significant linear decrease of 12.3 μg mg total lipid^{-1} day^{-1} mainly due to losses of 18:1(n-9) which decreased linearly by 7.4 μg mg total lipid^{-1} day^{-1} and 16:1(n-7) which decreased linearly 3.8 μg mg total lipid^{-1} day^{-1}. In absolute terms, total monoenes decreased linearly by 256.7 ng larva^{-1} day^{-1} with 18:1(n-9) and 16:1(n-7) decreasing linearly by 156.7 ng larva^{-1}day^{-1} and 63.3 ng larva^{-1} day^{-1}, respectively. Total PUFA content decreased linearly by 17.7 μg mg total lipid^{-1} day^{-1} due to decreased levels of 16:4(n-3), 18:2(n-

Table 2. The effect of inanition on lipid class composition (percentage of total lipid), triacylglycerol / cholesterol ratio and phosphatidylcholine/phosphatidylethanolamine ratio of eggs and newly hatched lecithotrophic larvae of *Dentex dentex* during early development

	Duration of food deprivation					
	Egg	Day 0	Day 1	Day 2	Day 3	Day 4
Total polar	34.4 ± 1.2^{ab}	31.5 ± 1.2^{a}	33.6 ± 0.8^{ab}	36.5 ± 1.4^{bc}	38.7 ± 0.7^{c}	43.5 ± 1.2^{d}
Phosphatidylcholine	19.8 ± 0.5^{ab}	16.3 ± 0.4^{d}	17.9 ± 0.3^{bcd}	17.8 ± 0.7^{cd}	18.8 ± 0.9^{abc}	18.9 ± 0.8^{abc}
Phosphatidylethanolamine	7.1 ± 0.3^{a}	7.0 ± 0.1^{a}	7.7 ± 0.1^{a}	9.0 ± 0.1^{b}	9.0 ± 0.3^{c}	11.5 ± 0.2^{d}
Phosphatidylserine	1.1 ± 0.5^{a}	1.4 ± 0.1^{a}	1.8 ± 0.1^{ab}	2.5 ± 0.2^{bc}	3.2 ± 0.2^{cd}	4.1 ± 0.4^{d}
Phosphatidylinositol	2.1 ± 0.3^{a}	2.2 ± 0.0^{ab}	2.1 ± 0.1^{a}	2.4 ± 0.2^{ab}	2.8 ± 0.1^{bc}	3.2 ± 0.2^{cd}
Phosphatidic acid	1.1 ± 0.2^{a}	1.4 ± 0.2^{a}	1.2 ± 0.1^{a}	1.6 ± 0.1^{ab}	1.6 ± 0.3^{ab}	2.1 ± 0.2^{bc}
Glycolipid	1.2 ± 0.1^{ab}	1.4 ± 0.6^{ab}	0.7 ± 0.4^{a}	1.4 ± 0.1^{ab}	1.1 ± 0.3^{ab}	1.8 ± 0.1^{b}
Sphingomyelin	1.3 ± 0.1^{abc}	1.2 ± 0.1^{ab}	1.5 ± 0.1^{abcd}	1.6 ± 0.1^{bcd}	1.1 ± 0.2^{a}	1.6 ± 0.1^{bcd}
Lyso-phosphatidylcholine	0.6 ± 0.1^{ab}	0.6 ± 0.1^{a}	0.7 ± 0.1^{a}	0.2 ± 0.0^{c}	0.2 ± 0.1^{c}	0.2 ± 0.1^{c}
Total neutral	62.5 ± 1.7^{ab}	65.7 ± 0.9^{a}	64.0 ± 0.6^{ab}	61.0 ± 1.1^{bc}	58.4 ± 1.0^{c}	53.5 ± 1.2^{d}
Cholesterol	7.8 ± 0.4^{a}	8.0 ± 0.9^{ab}	9.7 ± 0.6^{ab}	10.1 ± 0.4^{b}	12.5 ± 0.9^{c}	12.6 ± 0.6^{c}
Free fatty acid	trace^{a}	1.8 ± 0.6^{bcd}	1.4 ± 0.3^{b}	1.3 ± 0.3^{b}	1.5 ± 0.1^{bc}	3.6 ± 1.2^{de}
Triacylglycerol	23.9 ± 0.1^{ab}	26.3 ± 1.3^{b}	25.2 ± 0.8^{b}	24.6 ± 0.7^{ab}	22.1 ± 0.1^{a}	18.3 ± 0.2^{c}
Sterol ester	30.7 ± 1.4^{a}	29.5 ± 0.9^{a}	27.6 ± 1.1^{ab}	24.9 ± 0.8^{bc}	22.3 ± 1.8^{c}	19.1 ± 0.4^{d}
Triacylglycerol/cholesterol	3.0 ± 0.1^{a}	3.3 ± 0.2^{b}	2.6 ± 0.1^{c}	2.4 ± 0.0^{c}	1.8 ± 0.1^{d}	1.4 ± 0.0^{e}
Phosphatidylcholine/ Phosphatidylethanolamine	2.8 ± 0.0^{a}	2.3 ± 0.0^{b}	2.3 ± 0.0^{b}	2.1 ± 0.2^{b}	1.9 ± 0.0^{d}	1.6 ± 0.0^{e}
	Day 5	Day 6	Day 7	Day 8	Day 9	
Total polar	50.1 ± 1.0^{e}	55.9 ± 1.4^{f}	61.8 ± 0.5^{g}	61.9 ± 1.1^{g}	59.9 ± 0.7^{g}	
Phosphatidylcholine	20.4 ± 0.3^{ac}	22.1 ± 0.3^{ef}	22.3 ± 0.6^{ef}	22.7 ± 0.7^{f}	22.9 ± 0.0^{f}	
Phosphatidylethanolamine	14.8 ± 0.1^{e}	16.8 ± 0.6^{f}	19.4 ± 0.3^{g}	18.8 ± 0.2^{g}	17.0 ± 0.3^{f}	
Phosphatidylserine	5.5 ± 0.1^{e}	6.8 ± 0.1^{e}	8.9 ± 0.3^{f}	8.9 ± 0.3^{f}	8.7 ± 0.3^{f}	
Phosphatidylinositol	3.2 ± 0.1^{cd}	3.2 ± 0.2^{cd}	3.6 ± 0.2^{d}	3.2 ± 0.1^{cd}	3.0 ± 0.1^{cd}	
Phosphatidic acid	2.4 ± 0.3^{cd}	3.1 ± 0.1^{de}	3.8 ± 0.1^{e}	3.7 ± 0.2^{e}	3.3 ± 0.1^{e}	
Glycolipid	1.8 ± 0.1^{b}	1.8 ± 0.0^{b}	1.6 ± 0.4^{ab}	1.9 ± 0.3^{b}	2.1 ± 0.4^{b}	
Sphingomyelin	1.6 ± 0.1^{abcd}	1.9 ± 0.2^{de}	1.8 ± 0.0^{cde}	2.4 ± 0.1^{e}	2.3 ± 0.1^{e}	
Lyso-phosphatidylcholine	0.3 ± 0.1^{bc}	0.2 ± 0.0^{c}	0.3 ± 0.1^{bc}	0.3 ± 0.1^{bc}	0.4 ± 0.0^{abc}	
Total neutral	46.7 ± 1.1^{e}	41.3 ± 1.3^{f}	35.9 ± 0.6^{fg}	36.2 ± 0.9^{g}	37.9 ± 0.7^{g}	
Cholesterol	15.8 ± 0.9^{d}	17.6 ± 0.6^{de}	19.7 ± 0.6^{ef}	18.9 ± 0.9^{ef}	21.9 ± 0.8^{f}	
Free fatty acid	3.1 ± 0.1^{cde}	3.9 ± 0.9^{e}	3.5 ± 0.3^{de}	3.7 ± 0.7^{e}	3.4 ± 0.2^{de}	
Triacylglycerol	12.5 ± 0.1^{d}	8.2 ± 0.2^{e}	3.9 ± 0.2^{f}	4.9 ± 0.9^{f}	4.2 ± 0.3^{f}	
Sterol ester	15.2 ± 0.3^{e}	11.6 ± 0.4^{f}	8.8 ± 0.3^{g}	8.7 ± 0.6^{g}	8.4 ± 0.1^{g}	
Triacylglycerol/cholesterol	0.8 ± 0.0^{f}	0.5 ± 0.0^{g}	0.2 ± 0.0^{h}	0.2 ± 0.0^{h}	0.2 ± 0.0^{h}	
Phosphatidylcholine/ Phosphatidylethanolamine	1.4 ± 0.0^{f}	1.3 ± 0.0^{fg}	1.1 ± 0.0^{fg}	1.2 ± 0.0^{gh}	1.3 ± 0.0^{h}	

Results represent the Mean $\pm$ SD ($n = 3$). SD = 0.0 implies and SD of <0.05. Mean values within horizontal rows with different superscript letters are significantly different ($p < 0.05$). if no superscript appears values are not different.

6), 18:3(n-3), 18:4(n-3), 20:5(n-3) and 22:5(n-3). AA (20:4(n-6) showed a linear increase of 0.4 μg mg total lipid^{-1} day^{-1} and DHA also increased by 2.5 μg mg total lipid^{-1} day^{-1}. In contrast, when data were presented in absolute terms, total PUFA declined linearly by 468.6 ng larva^{-1} day^{-1}, with AA decreasing by 12.1 ng larva^{-1} day^{-1}, EPA decreasing by 58.5 ng larva^{-1} day^{-1} and DHA decreasing by 221.8 ng larva^{-1} day^{-1}, respectively. The rank order of utilization of fatty acids was 22:6(n-3) > 18:1(n-9) > 16:0 > 16:1(n-7) > 20:5(n-3) > 18:0 > 18:1(n-7) > 20:4(n-6).

Changes in fatty acid compositions in major lipid classes (PC, PE and TAG)

The effects of food deprivation on the fatty acid composition (% of total fatty acid within the lipid class) of the major polar lipid classes, PC and PE, from fertilized eggs to 9 days posthatch larvae are shown in Figures 2 and 3. For PC, when data are presented in absolute terms (ng fatty acid larvae^{-1}), total saturated fatty acids decreased linearly by 76.6 ng larva^{-1} day^{-1}, mainly due to a linear decrease in 16:0 of 55.9 ng larva^{-1} day^{-1}. Total monounsaturated fatty acids from PC decreased linearly by 25.5 ng larva^{-1} day^{-1} mainly due to a linear decrease in 18:1(n-9) of 14.6 ng larva^{-1} day^{-1}. Major PUFA in PC, AA, EPA and DHA, decreased linearly by 4.9, 20.7 and 71.0 ng larvae^{-1} day^{-1}, respectively, whereas total PUFA decreased by 118.8 ng larvae^{-1} day^{-1} . The rank order of utilization of the major fatty acids in PC was 22:6(n-3) > 16:0 > 18:1(n-9) > 20:5(n-3) > 18:0 > 16:1(n-7) > 20:4(n-6) > 18:1(n-7).

For PE, total saturated showed a linear downward trend of 8.8 ng larva^{-1} day^{-1} and linear decreases were also observed for total monoene and total DMA amounting to 3.8 and 1.3 ng larva^{-1} day^{-1}, respectively. PE total PUFA, mostly DHA, remained constant until day 5 after hatch and then decreased linearly by 67.6 ng larvae^{-1} day^{-1} and DHA by 57.4 ng larvae^{-1} day. The rank order of utilization of fatty acids in PE was 16:0 > 22:6(n-3) > 20:5(n-3) > 18:1(n-9) > total DMA > 18:0 > 18:1(n-7) > 20:4(n-6) > 16:1(n-7).

Changes in the proportions of TAG major fatty acids are shown in Figure 4. Major quantitative decreases were presented by total monoenes that decreased linearly by 177.0 ng larva^{-1} day^{-1}, mainly due to a linear decrease of 18:1(n-9) by 98.3 ng larva^{-1} day^{-1}. This was followed by total saturated fatty acids which decreased linearly by 167.4 ng larvae^{-1} day^{-1}, mainly due to significant linear decreases in 16:0 of 117.7 ng larva^{-1} day^{-1}. PUFAs also decreased linearly by 101.9 ng larva^{-1} day^{-1}, mainly due to a decrease in 22:6(n-3) of 64.1 ng larvae^{-1} day^{-1} . The rank order of utilization for fatty acids in TAG was 16:0 > 18:1(n-9) > 22:6(n-3) > 16:1(n-7) > 18:0 > 18:1(n-7) > 20:5(n-3) > 20:4(n-6).

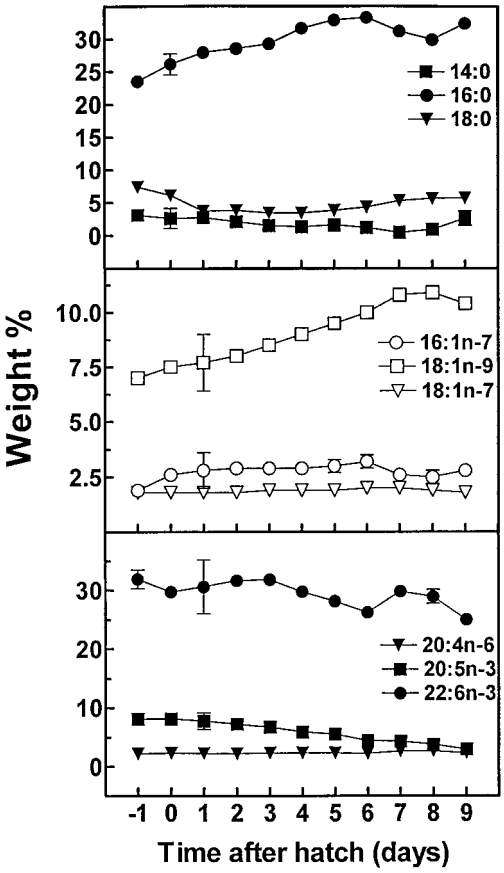

Figure 2. The effect of food deprivation on phosphatidylcholine major fatty acids of *Dentex dentex* larvae during early development.

Discussion

Marine fish larvae show a reduction in dry weight during starvation, primarily due to a decrease of total lipids, particularly after yolk sac absorption. This has been demonstrated in several species including plaice, *Pleuronectes platessa* (Rainuzzo et al. 1992; Rainuzzo 1993), Atlantic herring, *Clupea harengus* (Tocher et al. 1985b), cod, *Gadus morhua* (Fraser et al. 1988; Rainuzzo et al. 1992; Rainuzzo 1993; Van der Meeren

Table 3. The effect of inanition on the total lipid fatty acid content (μg fatty acid mg^{-1} total lipid) of eggs and newly hatched lecithotrophic larvae of *Dentex dentex* during early development

	Duration of food deprivation					
	Egg	Day 0	Day 1	Day 2	Day 3	Day 4
14:0	20.2 ± 4.4^{a}	12.9 ± 0.3^{abc}	13.4 ± 0.9^{abc}	12.6 ± 0.9^{abc}	21.5 ± 7.9^{a}	12.7 ± 3.1^{abc}
16:0DMA	4.6 ± 0.4^{a}	3.8 ± 1.0^{ab}	4.7 ± 0.1^{a}	4.0 ± 0.1^{a}	3.2 ± 0.1^{bc}	2.2 ± 0.1^{cd}
16:0	120.5 ± 4.3^{a}	101.5 ± 1.2^{cd}	108.8 ± 2.7^{abc}	108.0 ± 3.1^{abc}	118.3 ± 4.8^{ab}	106.9 ± 3.0^{abc}
16:1(n-7)	40.2 ± 0.5^{a}	35.9 ± 0.5^{bc}	37.6 ± 0.7^{b}	35.0 ± 1.2^{c}	31.1 ± 0.0^{d}	24.6 ± 0.0^{e}
18:0DMA	0.9 ± 0.4^{a}	0.9 ± 0.2^{a}	1.1 ± 0.1^{ab}	1.1 ± 0.2^{ab}	1.4 ± 0.3^{abc}	1.5 ± 0.1^{abc}
18:1DMA	3.8 ± 0.6^{ab}	4.0 ± 0.1^{a}	4.5 ± 0.2^{a}	3.9 ± 0.3^{ab}	3.6 ± 0.0^{ab}	2.9 ± 0.1^{bc}
18:0	33.6 ± 1.6^{bc}	29.4 ± 0.1^{a}	31.8 ± 0.9^{ab}	30.8 ± 0.4^{ab}	36.1 ± 2.1^{c}	35.9 ± 1.1^{c}
18:1(n-9)	104.4 ± 4.4^{a}	95.1 ± 1.0^{bc}	98.2 ± 2.1^{b}	93.2 ± 2.9^{c}	88.7 ± 1.7^{d}	73.7 ± 0.7^{e}
18:1(n-7)	20.6 ± 1.0^{a}	17.3 ± 0.1^{b}	18.2 ± 0.5^{b}	17.5 ± 0.4^{b}	17.0 ± 0.1^{b}	14.8 ± 0.1^{c}
18:2(n-6)	9.8 ± 0.5^{a}	9.9 ± 0.7^{a}	8.7 ± 0.1^{ab}	8.2 ± 0.3^{ab}	8.7 ± 0.8^{ab}	7.0 ± 0.3^{bc}
18:3(n-3)	3.6 ± 0.4^{a}	3.1 ± 0.0^{b}	3.0 ± 0.1^{bc}	2.7 ± 0.0^{c}	2.3 ± 0.1^{d}	1.8 ± 0.0^{e}
20:4(n-6)	10.3 ± 0.2^{ab}	9.9 ± 0.1^{a}	10.7 ± 0.2^{ab}	10.8 ± 0.1^{ab}	11.3 ± 0.1^{bc}	11.9 ± 0.1^{cd}
20:5(n-3)	36.8 ± 1.1^{a}	35.3 ± 0.1^{a}	36.2 ± 0.8^{a}	33.3 ± 0.7^{b}	31.3 ± 0.3^{c}	27.5 ± 0.6^{d}
22:5(n-6)	4.5 ± 0.1^{ab}	4.1 ± 0.1^{bc}	4.4 ± 0.0^{abc}	4.3 ± 0.0^{abc}	4.4 ± 0.0^{abc}	4.3 ± 0.0^{abc}
22:5(n-3)	7.7 ± 0.2^{a}	7.2 ± 0.0^{bc}	7.6 ± 0.1^{ab}	7.2 ± 0.1^{bc}	6.9 ± 0.0^{c}	6.1 ± 0.1^{d}
22:6(n-3)	177.4 ± 4.4^{ab}	167.2 ± 1.9^{a}	179.2 ± 4.3^{ab}	178.4 ± 4.0^{ab}	178.1 ± 2.2^{ab}	176.0 ± 2.7^{ab}
Total saturated	188.9 ± 13.2^{ab}	160.3 ± 0.7^{bc}	170.4 ± 3.7^{abc}	167.6 ± 3.3^{abc}	192.6 ± 15.4^{a}	172.4 ± 6.8^{abc}
Total monoenes	171.1 ± 4.3^{a}	154.3 ± 1.7^{b}	159.6 ± 3.0^{b}	151.3 ± 4.8^{b}	142.3 ± 1.5^{c}	117.7 ± 0.8^{d}
Total polyenes	330.1 ± 18.8^{a}	317.4 ± 3.3^{ab}	333.1 ± 6.3^{a}	319.9 ± 7.6^{ab}	316.0 ± 1.7^{ab}	291.2 ± 6.6^{bc}
Total DMA	9.4 ± 1.4^{a}	8.7 ± 0.9^{ab}	10.4 ± 0.5^{a}	9.1 ± 0.6^{ab}	8.2 ± 0.1^{ab}	6.6 ± 0.1^{bc}
Total(n-6)	33.1 ± 1.3^{ab}	31.6 ± 1.0^{abc}	31.9 ± 0.4^{abc}	31.0 ± 0.6^{bcd}	32.0 ± 0.4^{abc}	30.4 ± 0.5^{bcd}
Total(n-3)	297.0 ± 17.5^{a}	285.8 ± 2.2^{abc}	301.2 ± 5.9^{a}	289.0 ± 6.9^{ab}	284.0 ± 1.2^{abc}	260.8 ± 6.2^{bcd}
HUFA(n-6)	16.3 ± 0.2^{ab}	15.6 ± 0.1^{a}	16.3 ± 0.2^{ab}	16.4 ± 0.3^{ab}	17.1 ± 0.0^{abc}	17.3 ± 0.1^{abc}
HUFA(n-3)	231.3 ± 6.0^{ab}	218.6 ± 2.3^{abc}	231.2 ± 4.7^{ab}	225.8 ± 4.8^{ab}	224.8 ± 2.3^{abc}	216.7 ± 3.5^{abc}
		Day 5	Day 6	Day 7	Day 8	Day 9
14:0		13.9 ± 1.9^{ab}	5.9 ± 1.0^{bc}	7.2 ± 2.9^{bc}	3.7 ± 0.4^{a}	4.9 ± 3.9^{bc}
16:0DMA		1.3 ± 0.3^{de}	0.9 ± 0.0^{e}	0.9 ± 0.1^{e}	0.9 ± 0.1^{bc}	0.7 ± 0.0^{e}
16:0		112.2 ± 2.3^{abc}	104.1 ± 2.1^{bcd}	100.9 ± 10.5^{cd}	92.2 ± 3.3^{ab}	81.8 ± 8.2^{e}
16:1(n-7)		19.7 ± 0.2^{f}	13.4 ± 0.4^{g}	10.8 ± 1.1^{h}	9.9 ± 0.5^{d}	7.9 ± 0.3^{i}
18:0DMA		1.8 ± 0.5^{abc}	2.1 ± 0.1^{abc}	1.9 ± 1.0^{abc}	2.6 ± 0.1^{abc}	2.3 ± 0.6^{bc}
18:1DMA		2.2 ± 0.5^{cd}	2.3 ± 0.1^{cd}	1.7 ± 0.8^{d}	2.0 ± 0.2^{ab}	1.9 ± 0.0^{bc}
18:0		41.7 ± 0.8^{d}	43.8 ± 0.1^{d}	50.5 ± 4.2^{e}	48.6 ± 1.2^{c}	48.6 ± 2.4^{e}
18:1(n-9)		66.3 ± 1.7^{f}	50.0 ± 0.7^{g}	47.3 ± 4.2^{gh}	43.5 ± 1.2^{d}	41.4 ± 2.5^{i}
18:1(n-7)		13.7 ± 0.1^{c}	12.3 ± 0.1^{d}	12.1 ± 0.8^{d}	11.3 ± 0.5^{b}	10.1 ± 0.1^{e}
18:2(n-6)		9.6 ± 2.4^{a}	4.6 ± 0.0^{cd}	4.2 ± 0.4^{d}	4.1 ± 0.5^{ab}	3.3 ± 0.4^{d}
18:3(n-3)		1.5 ± 0.0^{e}	0.7 ± 0.0^{f}	0.5 ± 0.0^{f}	0.5 ± 0.0^{d}	0.4 ± 0.0^{f}
20:4(n-6)		12.5 ± 0.0^{de}	13.1 ± 0.1^{ef}	14.5 ± 1.1^{g}	13.7 ± 0.3^{bc}	13.2 ± 0.0^{ef}
20:5(n-3)		24.1 ± 0.1^{e}	19.4 ± 0.3^{f}	17.1 ± 1.2^{g}	14.2 ± 0.3^{c}	12.9 ± 0.1^{h}
22:5(n-6)		4.3 ± 0.0^{abc}	4.2 ± 0.0^{abc}	4.6 ± 0.5^{a}	4.3 ± 0.0^{abc}	4.0 ± 0.0^{c}
22:5(n-3)		5.3 ± 0.0^{e}	4.7 ± 0.0^{f}	4.6 ± 0.4^{f}	4.1 ± 0.0^{c}	3.7 ± 0.0^{g}
22:6(n-3)		178.5 ± 0.4^{ab}	186.2 ± 0.9^{ab}	210.3 ± 22.6^{c}	192.5 ± 5.7^{ab}	181.3 ± 2.7^{ab}

Table 3. Continued.

	Day 5	Day 6	Day 7	Day 8	Day 9
Total saturated	186.5 ± 5.3^{abc}	171.6 ± 2.9^{abc}	181.9 ± 22.0^{abc}	166.5 ± 7.3^{a}	155.1 ± 15.7^{c}
Total monoenes	104.8 ± 2.1^{e}	80.5 ± 1.1^{f}	75.2 ± 4.7^{fg}	68.5 ± 2.4^{c}	62.9 ± 3.1^{h}
Total polyenes	289.1 ± 7.6^{bc}	263.9 ± 0.1^{cd}	286.5 ± 29.6^{bc}	263.8 ± 3.3^{ab}	242.6 ± 0.9^{d}
Total DMA	5.3 ± 1.3^{c}	5.4 ± 0.1^{c}	5.0 ± 1.8^{c}	5.5 ± 0.3^{c}	4.9 ± 0.6^{c}
Total(n-6)	36.3 ± 4.5^{a}	27.5 ± 0.1^{cd}	29.3 ± 1.9^{cd}	29.3 ± 0.8^{bcd}	26.5 ± 0.8^{d}
Total(n-3)	252.8 ± 3.1^{d}	236.5 ± 0.2^{de}	257.1 ± 1.6^{e}	234.4 ± 4.1^{de}	216.2 ± 1.7^{e}
HUFA(n-6)	20.3 ± 1.5^{de}	18.0 ± 0.2^{bc}	20.9 ± 25.4^{c}	20.5 ± 0.6^{de}	18.5 ± 0.2^{cd}
HUFA(n-3)	215.4 ± 0.8^{bc}	217.0 ± 0.7^{abc}	240.2 ± 4.8^{ab}	220.4 ± 3.8^{abc}	204.8 ± 2.8^{a}

Results are means $\pm$ SD ($n = 3$). SD = 0.0 implies SD<0.05. Values bearing different superscript letters are significantly different ($p < 0.05$). If no superscript appears values are not different. DMA, dimethylacetal. HUFA, highly unsaturated fatty acid. Totals include minor components not shown.

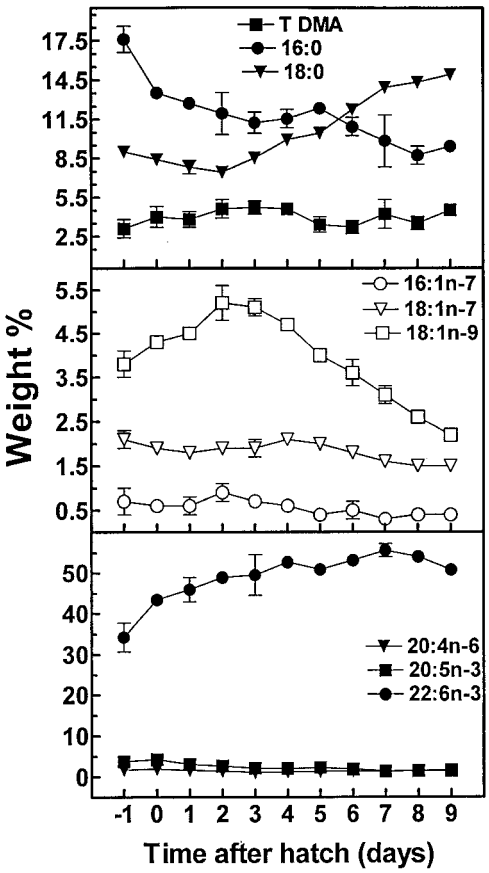

Figure 3. The effect of food deprivation on phosphatidylethanolamine major fatty acids of *Dentex dentex* larvae during early development.

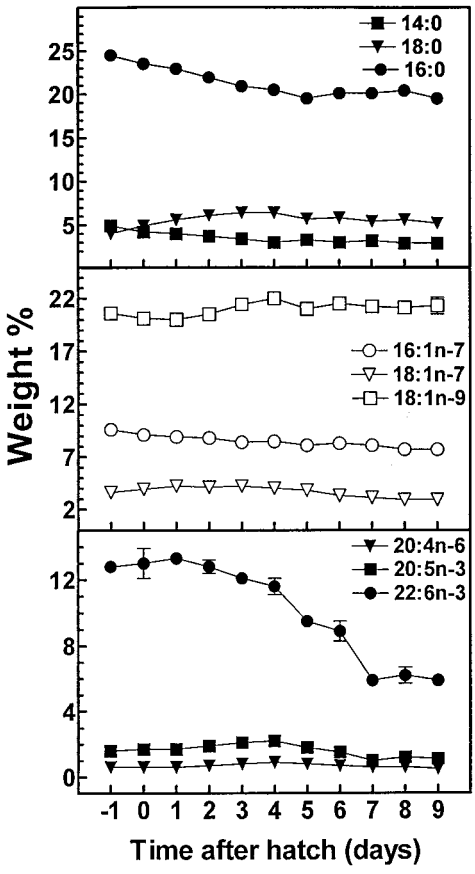

Figure 4. The effect of food deprivation on triacylglycerol major fatty acids of *Dentex dentex* larvae during early development.

et al. 1993), Atlantic halibut, *Hippoglossus hippoglossus* (Rainuzzo et al. 1992; Rainuzzo 1993; Whyte et al. 1993; Rønnestad et al. 1995), turbot, *Scophthalmus maximus* (Rainuzzo et al. 1992; Rainuzzo 1993), red

drum, *Sciaenops ocellata* (Vetter et al. 1983), dolphin fish, *Coryphaena hippurus* (Ostrowski and Divakaran 1991), red sea bream, *Pagrus major* (Tandler et al. 1989), gilthead sea bream, *Sparus aurata* (Koven et al. 1989; Mourente and Odriozola 1990; Rønnestad et al. 1994) and Senegal sole, *Solea senegalensis* (Mourente and Vázquez 1996). The common dentex larvae, which start feeding at day 4 after hatching when the mouth is open (Pastor et al. 1995), showed a dry weight decrease during starvation by 3.2 μg larva^{-1} day^{-1}, two-fold greater than that presented by gilthead sea bream (1.6 μg larvae^{-1} day^{-1}) and 1.5-fold than the Senegal sole (2.1 μg larva^{-1} day^{-1}) for the same rearing temperature (Parra 1998). This indicates a higher metabolic rate for the common dentex during yolk-sac stage and subsequent starvation.

The eggs of *D. dentex* are characterized by the presence of a single oil droplet and can be classified as high lipid content ($\approx$ 25% of egg dry mass) (Finn 1994). However, the sequence of catabolic substrate oxidation during the embriogenesis of *D. dentex* was carbohydrate (possibly glycogen), then protein (possibly including free amino acids). After hatching though, the order for catabolism throughout the whole period was lipid (82% depleted), then protein (62% reduction), then carbohydrate (56% depletion). This is in agreement with recent studies that have demonstrated that the principal source of energy during embryogenesis in several marine fish larvae is a pool of free amino acid in the lipoprotein yolk and that the oil droplet, if present, is consumed after hatch (Rønnestad et al. 1992, 1993, 1994; Wiegand 1996; Rainuzzo et al. 1997). The increase in the rate of oil globule absortion that occurred after hatching suggested that lipids derived from the oil globule are the dominant fuel for the common dentex larvae during this stage of development. The finding that oil globule absortion occurred after yolk absortion is consistent with studies on other species such as turbot (Rønnestad et al. 1992), Asian sea bass (*Lates calcarifer*) and rabbitfish (*Signatus guttatus*) (Bagarino 1986; Avila and Juario 1987), spot (*Leiostomus xanthurus* (Govoni 1980), red sea bream (Kitajima et al. 1993) and gilthead sea bream (Rønnestad et al. 1994). In the present study, the yolk-sac decreased exponentially whereas the oil globule decreased linearly.

Lipid metabolism during the early life of fish may differ in several ways among species, including the content and composition of lipids in the yolk, the time and rate of lipid combustion, the lipid class used for either combustion or tissue synthesis and the role of the different fatty acids (Verreth et al. 1994; Sargent 1995; Tocher 1995; Wiegand 1996; Rainuzzo et al. 1997). In the present study, neutral lipids were consumed at a higher average rate (1.1 μg neutral lipid larva^{-1} day^{-1}) than polar lipids (0.3 μg polar lipid larva^{-1} day^{-1}) and the overall decrease in the neutral lipids was 3.4 times greater than that of the polar lipids, as observed for Senegal sole (Mourente and Vázquez 1996) and gilthead sea bream (Rønnestad et al. 1994). Among the neutral lipid of common dentex, TAG and SE were the classes predominantly utilized as energy reserves. Free C significantly increased from hatching to day 5 after hatching, probably due to hydrolysis of SE, and then it also decreased. Similarly, PE was constant until day 5 after hatch then declined rapidly, whereas PC was catabolized during the whole study period. This pattern of lipid class utilization is contrary to that presented by cold water species with eggs with low lipid contents, lacking oil globule(s) and long periods of development such as Atlantic herring (Tocher et al. 1985b), cod (Fraser et al. 1988; Rainuzzo et al. 1992; Rainuzzo 1993), plaice (Rainuzzo et al. 1992; Rainuzzo 1993) or Atlantic halibut (Rainuzzo et al. 1992; Rainuzzo 1993; Rønnestad et al. 1995) but similar to that of temperate water species with high lipid contents, oil globule(s) and short periods of development such as red drum (Vetter et al. 1983), red sea bream (Tandler et al. 1989), turbot (Rainuzzo et al. 1992; Rainuzzo 1993), gilthead sea bream (Rønnestad et al. 1994) or Senegal sole (Mourente and Vázquez 1996). However, the decrease in total lipids from egg to 1 day old common dentex larvae by 58% presented by Tulli and Tibaldi (1997) for this species is 30 fold higher than the decrease in total lipids observed in the present study for the same period, even when the rearing conditions were very similar.

The catabolism of PC and conservation of PE in starved common dentex larvae can be achieved by *de novo* CDP-base: diacylglycerol phosphotransferase routes, Ca^{2+}-dependent base exchange reactions and deacylation / reacylation reactions (Gurr and Harwood 1991; Sargent et al. 1993a) and may be related to the important role of PE in biomembranes of neural and visual tissues during development (Sargent et al. 1993b; Mourente 1996). The PE synthesis and/or conservation has also been observed in other marine fish larvae (Rainuzzo et al. 1992; Rainuzzo 1993; Rønnestad et al. 1995; Mourente and Vázquez 1996). The products of PC catabolism may serve as a source of FFA, free inorganic phosphate for high energy nucleotides and choline for methyl metabolism and

neurotransmission (Tocher et al. 1985a; Fraser et al. 1988; Sargent 1995; Rainuzzo et al. 1997). It is also noteworthy that the synthesis and/or conservation of PE is linked to the retention of DHA, since PE synthesis utilizes preferentially substrates containing DHA (Tinoco 1982; Vance 1985; Sargent et al. 1993a). This has also been shown to occur in several marine fish larvae during early development (Rainuzzo et al. 1992; Rainuzzo 1993; Koven et al. 1993; Lie 1993; Van der Meeren et al. 1993; Rønnestad et al. 1995; Mourente and Vázquez 1996) and maybe related to the appearance of di-docosahexaenoyl molecular species (di-DHA) of phospholipids, particularly PE and PS, during retinogenesis (Bell and Dick 1993). Furthermore, the synthesis and accumulation of ethanolamine-plasmalogens in *D. dentex* larvae (data not shown), evidenced by the significant increase of DMAs in PE, is indicative of maturation of the neural system (Sastry 1985; Mourente and Tocher 1992a; Sargent et al. 1993a; Mourente 1996). The liberation of C from SE could also serve for myelination in the central nervous system (Sastry 1985) as well as precursor for bile salts (Gurr and Harwood 1991; Verreth et al. 1994; Rønnestad et al. 1995).

The nutritional status indicated by the TAG/C (Fraser 1989) and PC/PE ratios decreased significantly throughout the whole study period, denoting the effect of starvation and the necessity of providing food (at least green water) at this time to satisfy the energetic and nutritional demand of the larvae, since feeding starts in this species at day 4 after hatching, when the mouth is open (Pastor et al. 1995).

The total lipid fatty acid content of eggs and yolksac larvae of *D. dentex* are similar to results from previous studies with Senegal sole (Vazquez et al. 1994; Mourente and Vázquez 1996) but about twofold to those reported by Tulli and Tibaldi (1997) for the same species, including DHA/EPA ratios. Studies of fatty acid depletion in developing embryos and larvae of marine fish have found preferential utilization of fatty acids during yolksac stage and subsequent starvation (Koven et al. 1989; Wiegand et al. 1991; Wiegand 1996; Mourente and Vázquez 1996; Rainuzzo et al. 1997). The preferential catabolism of monounsaturated or saturated fatty acids along with the preferential retention of PUFA, particularly DHA during this period was the general pattern detected. However, it is noteworthy to observe that the rank order of utilization of fatty acids (ng fatty acid larva^{-1} day^{-1}) by common dentex larvae in total lipids, PC and TAG corresponded with the order of abundance of the different fatty acids in the respective fractions. The only exception was PE where DHA, the most abundant fatty acid, was conserved and catabolized after the 16:0 the second most abundant fatty acid in this fraction. It is also noteworthy that DHA/EPA ratios increased by 2.9, 2.2 and 4.4 fold for total lipid, PC and PE, respectively, denoting a preferential retention of DHA in these fractions. In contrast, DHA/EPA ratio decreased by 1.5 fold in TAG fraction. The conservation may also suggests that structures containing DHA are metabolically less accessible than those primarily containing EPA or DPA, or that there is a greater conversion of EPA to DHA (Finn 1994). It is important to recall the importance of DHA in the synaptogenesis and retinogenesis during early development of fish (Bell and Dick 1991; Mourente et al. 1991; Tocher et al. 1992; Sargent et al. 1993a,b; Sargent 1995; Mourente 1996) and also the important role of AA in biomembranes of salt secreting tissues and precursor of eicosanoids (Sargent 1995) . In any case, total DHA released from PC or TAG was primarily retained in the larval bodies to be used in deacylation / reacylation processes to synthesize other phosphoglycerides such as PE, that presented the highest relative retention of DHA, PS and PA or remain in the FFA pool to be used (data not shown). This seems to be in contrast to earlier studies in other marine fish species, where PC DHA was catabolized as energy substrate in the growing larvae (Tocher et al. 1985a; Fraser et al. 1988; Rainuzzo et al. 1992; Rainuzzo 1993; Rønnestad et al. 1995) but more in agreement to others that showed that the (n-3) PUFAs and particularly DHA were selectively mobilised for tissue synthesis (Tocher et al. 1992; Koven et al. 1993; Lie 1993; Rønnestad et al. 1994). Therefore, TAG appeared as the main energy substrate, which indicates a high efficiency of lipases degrading TAG together with bile salts-like molecules to allow the interaction TAG-lipase during early development of *D. dentex* (Gurr and Harwood 1991).

Regarding bioconversion of fatty acids and the low capacity of marine fish to elongate/desaturate C18 to C20 and C22 PUFA (Mourente and Tocher 1993b,c; 1994; Sargent et al. 1993a; Sargent 1995) common dentex, during larval development and starvation conditions, tends more to conserve important essential fatty acids than to biosynthesize *de novo* long chain PUFA.

In conclusion, common dentex larvae during early development and starvation consumes about 2.3% of its dry weight biomass per day (3.2 μg larva^{-1} day^{-1})

indicating a higher metabolic rate during lecitotrophia and subsequent starvation than other marine larval fish from temperate waters such as the gilthead sea bream and the Senegal sole. This dry weight consumption was mainly due to lipid catabolism (approximately a net consumption of 1.6 μg total lipid larva^{-1} day^{-1}) and net energy utilization of 0.15 kcal g dry weight^{-1} day^{-1}. Neutral lipids were consumed at a higher average rate (1.1 μg neutral lipid larva^{-1} day^{-1}) than polar lipids (0.3 μg polar lipid larva^{-1} day^{-1}) and the overall decrease in the neutral lipids (mainly TAG and SE by 0.5 and 0.6 μg larva^{-1} day^{-1}, respectively) was 3.4 faster than that of the polar lipids (primarily PC by 0.2 μg larva^{-1} day^{-1}). A net synthesis of PE was observed during the first half of the study period and then a fast declining (0.2 μg larva^{-1} day^{-1}) during the second half. PUFA were principally catabolized (468.6 ng larva^{-1} day^{-1}), primarily 22:6(n-3), followed by 20:5(n-3) and 20:4(n-6) (221.8, 58.5 and 12.1 ng larva^{-1} day^{-1}, respectively). Saturated and monounsaturated fatty acids were both utilized (227.2 and 256.7 ng larva^{-1} day^{-1}, respectively), principally 16:0 and 18:1(n-9) that were consumed at 149.8 and 156.7 ng larva^{-1} day^{-1}, respectively. The rank order of utilization of fatty acids (ng fatty acid larva^{-1} day^{-1}) by common dentex larvae in total lipids, PC and TAG corresponded with the order of abundance of the different fatty acids in the respective fractions, whereas in PE, where DHA, the most abundant fatty acid so far, it was conserved and catabolized after the 16:0 the second most abundant fatty acid in this fraction. Common dentex showed a pattern of lipid metabolism during early development similar to that of marine larval fish from temperate waters whose eggs contain high levels of total lipids, including an oil globule, and which preferentially utilize neutral lipids as the primary energy source.

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References

Avila, E. M. and Juario, J. V. 1987. Yolk and oil globule utilization and developmental morphology of the digestive tract epithelium in larval rabbitfish *Signatus guttatus* (Bloch). Aquaculture 65: 319–331.

Bagarino, T. 1986. Yolk resorption, onset of feeding and survival potential of larvae of three tropical marine fish species reared in the hatchery. Mar. Biol. 91: 449–459.

Bell, M. V. and Dick, J. R. 1991. Molecular species composition of the major diacyl glycerophospholipids from muscle, liver, retina and brain of cod (*Gadus morhua*). Lipids 26: 565–573.

Bell, M. V. and Dick, J. R. 1993. The appearance of rods in the eyes of herring and increased di- docosahexaenoyl molecular species of phospholipids. J. Mar. Biol. Ass. U. K. 73: 679–688.

Cataudella, S., Crosetti, D. and Marino, G. 1995. The sea breams. *In* Production of Aquatic Animals: Fishes. pp. 289–303. Edited by C. E. Nash and A. J. Novotny. Elsevier, Amsterdam.

Christie, W. W. 1989. Gas Chromatography and Lipids: A Practical Guide, 1st edition. The OilyPress, Ayr.

Daniel, E. S., Parrish, C. C., Somerton, D. C. and Brown, J. A. 1993. Lipids in eggs from first-time and repeat spawning Atlantic halibut,*Hippoglossus hippoglossus* (L.). Aquacult. Fish. Managem. 24: 187–191.

Dubois, M., Gilles, G. A., Hamilton, J. K., Rebels, P. A. and Smith, F. 1956. Colorimetric methods for determination of sugars and related substances. Anal. Chem. 3: 350–356.

Fewster, M. E., Burns, B. J. and Mead, J. F. 1969. Quantitative densitometric thin layer chromatography of lipids using cupric acetate reagent. J. Chromatogr. 43: 120–126.

Finn, R. N. 1994. Physiological Energetics of Developing Marine Fish Embryos and Larvae. Dr. Scient. Thesis, University of Bergen, Bergen.

Finn, R. N., Henderson, R. J. and Fyhn, H. J. 1996a. Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua* L.) II. Lipid metabolism and enthalpy balance. Mar. Biol. 124: 371–379.

Finn, R. N., Fyhn, H. J., Henderson, R. J. and Evjen, M. S. 1996b. The sequence of catabolic substrate oxidation and enthalpy balance of developing embryos and yolk-sac larvae of turbot (*Scophthalmus maximus* L.). Comp. Biochem. Physiol. 115A: 133–151.

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

Fraser, A. J. 1989. Triacylglycerol content as a condition index for fish, bivalve and crustacean larvae. Can. J. Fish. Aquat. Sci. 46: 1868–1873.

Fraser, A. J., Gamble, J. C. and Sargent, J. R. 1988. Changes in lipid content, lipid class and fattyacid composition of developing eggs and unfed larvae of cod (*Gadus morhua*). Mar. Biol. 99: 307–313.

Glamuzina, B., Jug-Dujakovic, J. and Katavich, I. 1989. Preliminary studies on reproduction and larval rearing of common dentex, *Dentex dentex* (Linnaeus 1758). Aquaculture 77: 75–84.

Govoni, J. J. 1980. Morphological, histological, and functional aspects of alimentary canal and associated organ development in larval *Leiostomus xanthurus*. Rev. Can. Biol. 39: 69–80.

Gurr, M. I. and Harwood, J. L. 1991. Lipid Biochemistry: An Introduction (4th edition). Chapman & Hall, London.

Hempel, G. 1979. Early Life History of Marine Fishes: The Egg Stage. University of Washington Press, Seattle.

Henken, A. M., Lucas, H., Tijssen, P. A. T. and Machiels, M. A. M. 1986. A comparison between methods used to determine the

energy content of feed, fish and faeces samples. Aquaculture 58: 195–201.

Kamler, E. 1992. Early Life History of Fish: An Energetic Approach. Chapman & Hall, London.

Kitajima, C., Yamane, Y., Matsui, S., Kihara, Y. and Furuichi, M. 1993. Ontogenic change in buoyancy in the early stage of red sea bream. Nippon Suisan Gakkaishi 59: 209–216.

Koven, W. M., Kissil, G. W. and Tandler, A. 1989. Lipid and n-3 requirement of *Sparus aurata* larvae during starvation and feeding. Aquaculture 79: 185–191.

Koven, W. M., Tandler, A., Sklan, D. and Kissil, G. W. 1993. The association of eicosapentaenoic and docosahexaenoic acids in the main phospholipids of different age *Sparus aurata* larvae with growth. Aquaculture 116: 71–82.

Lie, Ø. 1993. Changes in fatty acid composition of neutral lipids and glycerophospholipids in developing cod eggs. *In*: Physiology and Biochemistry of Marine Fish Larval Development. pp. 330–337. Edited by B. T. Walther and H. J. Fyhn. University of Bergen, Bergen.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with Folin-phenol reagent. J. Biol. Chem. 193: 265–275.

Mourente, G., 1996. Lipid and polyunsaturated fatty acid (PUFA) nutrition and neural system development in early stages of farmed marine fish. *In*: Improvement of the Commercial Production of Marine Aquaculture Species. pp. 23–29. Edited by G. Gajardo and P. Coutteau. Proc. Workshop on Fish and Mollusc Larviculture. Impresora Creces, Santiago de Chile.

Mourente, G. and Odriozola, J. M. 1990. Effect of broodstock diet on total lipids and fatty acid composition of larvae of gilthead sea bream (*Sparus aurata* L.) during yolksac stage. Fish Physiol. Biochem. 8: 103–110.

Mourente, G. and Tocher, D. R. 1992a. Lipid class and fatty acid composition of brain lipidsfrom Atlantic herring (*Clupea harengus*) at different stages of development. Mar. Biol. 112: 553–558.

Mourente, G. and Tocher, D. R. 1992b. Effect of weaning onto a pelleted diet on docosahexaenoic acid (22:6n-3) levels in brain of developing turbot (*Scophthalmus maximus* L.). Aquaculture 105: 363–377.

Mourente, G. and Tocher, D. R. 1993a. The effects of weaning on to a dry pellet diet on brainlipid and fatty acid compositions in postlarval gilthead sea bream (*Sparus aurata* L.). Comp. Biochem. Physiol. 104A: 605–611.

Mourente, G. and Tocher, D. R. 1993b. Incorporation and metabolism of ^{14}C-labelled polyunsaturated fatty acids in juvenile gilthead sea bream *Sparus aurata* L. *in vivo*. Fish Physiol. Biochem. 10: 443–453.

Mourente, G. and Tocher, D. R. 1993c. Incorporation and metabolism of ^{14}C-labelled polyunsaturated fatty acids in wild-caught juveniles of golden grey mullet, *Liza aurata*, *in vivo*. Fish Physiol. Biochem. 12: 119–130.

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

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

Mourente, G. and Vázquez, R. 1996. Changes in the content of total lipid, lipid classes and their fatty acids of developing eggs and unfed larvae of the Senegal sole, *Solea senegalensis* Kaup. Fish Physiol. Biochem. 15: 221–235.

Navarro, J. C. and Sargent, J. R. 1992. Behavioural differences in starving herring *Clupea harengus* L. larvae correlate with body levels of essential fatty acids. J. Fish Biol. 41: 509–513.

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

Ostrowski, A. C. and Divakaran, S. 1991. Energy substrates for eggs and prefeeding larvae ofthe dolphin *Coryphaena hippurus*). Mar. Biol. 109: 149–155.

Parra, M. G. 1998. Fisiología y Balance Energético Durante el Desarrollo Larvario de Peces Marinos (*Solea senegalensis* Kaup and *Sparus autara* Linneo) en Cultivo. Tesis Doctoral, Facultad de Ciencias del Mar, Universidad de Cádiz.

Pastor, E., Riera, F., Pou, S., Grau A. M. and Grau, A. 1995. Summary of investigations on reproduction and larval rearing of common dentex (*Dentex dentex* L.). ICES Mar. Sci. Symp. 201: 148–152.

Rainuzzo, J. R. 1993. Lipids in Early Stages of Marine Fish. Dr. Scient. Thesis, University ofTrondheim, Trondheim.

Rainuzzo, J. R., Reitan, K. I. and Jorgersen, L. 1992. Comparative study on the fatty acid andlipid composition of four marine fish larvae. Comp. Biochem. Physiol. 103B: 21–26.

Rainuzzo, J. R., Reitan, K. I. and Olsen, Y. 1997. The significance of lipids at early stages ofmarine fish: a review. Aquaculture 155: 103–115.

Rønnestad, I., Fyhn, H. J. and Gravningen, K. 1992. The importance of free amino acids to theenergy metabolism of eggs and larvae of turbot (*Scophthalmus maximus*). Mar. Biol. 114: 517–525.

Rønnestad, I., Groot, E. P. and Fyhn, H. J. 1993. Compartmental distribution of free amino acids and protein in developing yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus*). Mar. Biol. 116: 349–354.

Rønnestad, I., Koven, W. M., Tandler, A., Harel, M. and Fyhn, H. J. 1994. Energy metabolismduring development of eggs and larvae of gilthead sea bream (*Sparus aurata*). Mar.Biol. 120: 187–196.

Rønnestad, I., Finn, R. N., Lein, I. and Lie, Ø. 1995. Compartmental changes in the contents of total lipid, lipid classes and their associated fatty acid in developing yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus* L.). Aquacult. Nutr. 1: 119–130.

Sargent, J. R. 1995. Origin and functions of egg lipids: nutritional implications. *In*: Broodstock Management and Egg and Larval Quality. pp. 353–372. Edited by N. R. Bromage and R. J. Roberts.Blackwell Science, London.

Sargent, J. R., Batty, R. S., Bell, M. V., Fretwell, K. and Navarro, J. C. 1994. Docosahexaenoicacid, 22:6(n-3), and development of the retina in marine fish larvae. *In*: Proc. 3rd Int. Marine Biotechnology Conference. p. 95. Tromsoe, Norway.

Sargent, J. R., Bell, M. V., Henderson, R. J. and Tocher, D. R. 1990. Polyunsaturated fatty acids in marine and terrestrial food webs. *In*: Animal Nutrition and Transport Processes. 1. Nutrition in Wild and Domestic Animals. pp. 11–23. Edited by J. Mellinger. Karger, Basel.

Sargent, J. R., Bell, J. G., Bell, M. V., Henderson, R. J. and Tocher, D. R. 1993a. Themetabolism of phospholipids and polyunsaturated fatty acids in fish. *In*: Aquaculture: Fundamental and Applied Research. pp. 103–124. Edited by B. Lahlou and P. Vitiello. Coastal and Estuarine Studies 43, American Geophysical Union, Washington, DC.

Sargent, J. R., Bell, M. V. and D. R. Tocher. 1993b. Docosahexaenoic acid and the developmentof brain and retina in marine fish. *In*: Omega-3 Fatty Acids: Metabolism and Biological Ef-

fects. pp. 139–149. Edited by C. A. Devon, J. Baksaas and H. E. Krokan. Birkhauser Verlag, Basel.

Sastry, P. S. 1985. Lipids of nervous tissue: composition and metabolism. Progr. Lipid Res. 24: 69–176.

Sweetman, J. W. 1992. Larviculture of Mediterranean marine fish species: current status andfuture trends. J. World Aquacult. Soc. 23: 330–337.

Tandler, A., Watanabe, T., Satoh, S. and Fukusho, K. 1989. The effect of food deprivation onthe fatty acid profile of red sea bream (*Pagrus major*) larvae. Brit. J. Nutr. 62: 349–361.

Tinoco, J. 1982. Dietary requirements and functions of α-linolenic acid in animals. Progr. LipidRes. 21: 1–45.

Tocher, D. R. 1995. Glycerophospholipid metabolism. *In* Biochemistry and Molecular Biologyof Fishes. Vol. 4, pp. 119–157. Edited by P. W. Hochachka and T. P. Mommsen. Elsevier, Amsterdam.

Tocher, D. R., Fraser, A. J., Sargent, J. R. and Gamble, J. C. 1985a. Fatty acid composition of phospholipids and neutral lipids during embryonic and early larval development in Atlantic herring (*Cuplea harengus* L.). Lipids 20: 69–74.

Tocher, D. R., Fraser, A. J., Sargent, J. R. and Gamble, J. C. 1985b. Lipid class compositionduring embryonic and early larval development in Atlantic herring (*Cuplea harengus* L.). Lipids 20: 84–89.

Tocher, D. R. and Harvie, D. G. 1988. Fatty acid composition of the major phosphoglycerides from fish neural tissues; (n-3) and (n-6) polyunsaturated fatty acids in rainbow trout(*Salmo gairdneri*) and cod (*Gadus morhua*) brains and retinas. Fish Physiol. Biochem. 5: 229–239.

Tocher, D. R., Mourente, G. and Sargent, J. R. 1992. Metabolism of [1-^{14}C] Docosahexaenoate (22:6n-3), [1-^{14}C] Eicosapentaenoate (20:5n-3) and [1-^{14}C] Linolenate (18:3n-3) in braincells from juvenile turbot *Scophthalmus maximus*. Lipids 27: 494–499.

Tulli, F. and Tibaldi, E. 1997. Changes in amino acids and essential fatty acids during early larval rearing of dentex. Aquacult. Int. 5: 229–236.

Vance, D. E. 1985. Phospholipid metabolism in eucaryotes. *In*: Biochemistry of Lipids and Membranes. pp. 242–270. Edited by D. E. Vance and J. E. Vance. Benjamin/Cummings, Menlo Park.

Van der Meeren, T., Klungsøyr, J., Wilhelmsen, S. and Kvenseth, P. G. 1993. Fatty acid composition of unfed cod larvae *Gadus morhua* L. and cod larvae feeding on natural plankton in large enclosures. J. World Aquacult. Soc. 24: 167–185.

Vázquez, R., González, S., Rodríguez, A. and Mourente, G. 1994. Biochemical composition and fatty acid content of fertilized eggs, yolk sac stage larvae and first feeding larvae of theSenegal sole (*Solea senegalensis* Kaup). Aquaculture 119: 273–286.

Verreth, J., Custers, G. and Melger, W. 1994. The metabolism of neutral and polar lipids in eleuthero-embryos and starving larvae of the African catfish *Clarias gariepinus*. J. Fish Biol. 45: 961–971.

Vetter, R. D., Hodson, R. E. and Arnold, C. 1983. Energy metabolism in a rapidly developing marine fish egg, the red drum (*Sciaenops ocellata*). Can. J. Fish. Aquat. Sci. 40: 627–634.

Vitiello, F. and Zanetta, J. P. 1978. Thin layer chromatography of phospholipids. J. Chromatogr.166: 637–640.

Watanabe, T. and Kiron, V. 1994. Prospects in larval fish dietetics. Aquaculture 124: 223–251.

Whyte, J. N. C., Clarke, W. C., Ginther, N. G. and Jensen, J. O. T. 1993. Biochemical changes during embryogenesis of the Pacific halibut, *Hippoglossus stenolepsis* (Schmidt). Aquacult. Fish. Managem. 24: 193–201.

Wiegand, M. D. 1996. Composition, accumulation and utilization of yolk lipids in teleost fish.Rev. Fish Biol. Fish. 6: 259–286.

Wiegand, M. D., Kitchen, C. L. And Hataley, J. M. 1991. Incorporation of yolk fatty acids into body lipids of goldfish (*Carassius auratus* L.) larvae raised at two different temperatures. Fish Physiol. Biochem. 9: 199–213.

Zar, J. H. 1984. Biostatistical Analysis. 2nd edition. Prentice-Hall, Englewood Cliffs.