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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

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Abstract

Total lipids, lipid classes and their associated fatty acids were quantified in developing eggs, yolk-sac larvae and starving larvae (from day 1 to day 5 after hatching) of the Senegal sole, *Solea senegalensis* Kaup. Larvae during early development and starvation consumed about 0.6% of its dry weight per day, mainly due to lipid catabolism. There was a net consumption of approximately 1.7% total lipid per day, and a net energy utilization of 1.3 kcal g^{-1} dry weight biomass day^{-1}, mostly derived from lipid depletion. The overall decrease of total neutral lipids (mainly triacylglycerols and sterol esters) was 3.4 faster than that of total polar lipids (primarily phosphatidylcholine), with rates of 29.2 and 8.7 μg mg^{-1} dry weight biomass day^{-1}, respectively. There was a concomitant increase in PE, PS and phosphatidic acid during the period under study. Total saturated and total monounsaturated fatty acids were catabolized (primarily 16:0 and 16:1(n-7)) as energy substrates at rates of 7.4 and 10.9 μg mg^{-1} total lipid day^{-1}, whereas total PUFAs were conserved. DHA was specifically retained in PE, whereas EPA and DHA were catabolized in PC and triacylglycerol. Total DMA and AA contents in total lipid increased during early development and starvation. The data denote a pattern of lipid metabolism during early development of Senegal sole similar to that of other marine larval fish, with eggs containing high amounts of total lipids (presence of oil globule/s), from temperate waters and with short developmental periods; the pattern contrasts with fish larvae from eggs of cold water fish species that contain low levels of total lipids (lack of oil globule/s) and have long developmental periods.

Introduction

The metabolic capacity of the developing larvae of marine fish is a critical factor in relation to the animal's nutritional requirements up to the point of first feeding. Throughout embryogenesis and early larval development fish larvae obtain nutrients from the endogenous reserves of the yolk. In principle, fish eggs contain all the nutrients that the larvae utilize during the lecithotrophic phase, prior to

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; EPA, all-*cis*-5,8,11,14,17-eicosapentaenoic acid (20:5(n-3)); HUFA, highly unsaturated fatty acids (≥ C_{20} with ≥ 3 double bonds); PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine, PUFA, polyunsaturated fatty acid(s); SE, sterol ester; TAG, triacylglycerol
Note: PC and PE represent diradyl (diacyl + alkenilacyl + alkylacyl) glycerophosphocholine and glycerophospho-ethanolamine respectively.

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exogenous feeding, to support both homeostasis and development. The nutrient composition of fish eggs is species-specific, and the precise sequence of consumption varies both qualitatively and quantitatively. After protein, lipids are the most abundant constituent of most marine fish eggs, and protein, carbohydrates, free amino acids and lipids are all implicated as fuel in energy metabolism of eggs and larvae (Cetta and Capuzzo 1982; Kimata 1982, 1983a; Hemming and Buddington 1988; Rainuzzo 1993; Finn 1994).

The lipid content and composition of fish eggs also varies between species and may change during the different stages of development according to ambient conditions, physiological events and energy demands (Rainuzzo 1993; Sargent 1995). Fish eggs can be classified into different energetic categories according to their lipid content. The presence or lack of an oil globule(s) correspond to eggs with a high lipid (> 15% of egg dry mass) or low lipid (< 15% of egg dry mass) content. The former type are characterized by high amounts of neutral lipids, mainly TAG and SE/wax esters, and low amounts of polar lipids, whereas the second category presents high amounts of polar lipids, particularly PC, and low amounts of neutral lipids (Rainuzzo 1993; Finn 1994). The lipid reserves of fish eggs are used by the developing larvae both as substrates for energy metabolism and as structural components in membrane biogenesis (Hemming and Buddington 1988; Rainuzzo 1993; Finn 1994; Sargent 1995). Neutral lipids are generally considered the most important energy reserve in marine fish eggs and larvae (Vetter *et al.* 1983; Blaxter 1988), but phosphoglycerides with their high concentration of essential (n-3) HUFAs are utilized not only for cell division and organogenesis but also for energy by some species (Tocher *et al.* 1985a; Fraser *et al.* 1988; Falk-Petersen *et al.* 1986, 1989; Rainuzzo 1993; Sargent 1995). Thus, the preferential 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 (Kimata 1983a; Vetter *et al.* 1983; Cowey *et al.* 1985; Tocher *et al.* 1985a; Rainuzzo *et al.* 1992; Daniel *et al.* 1993; Fraser *et al.* 1987; Tandler *et al.* 1989; Koven *et al.* 1989; Mourente 1989; Vázquez *et al.* 1994; Rønnestad *et al.* 1994; Sargent 1995).

Larvae of many marine fishes require (n-3) HUFA such as EPA and DHA (Sargent *et al.* 1989; Watanabe and Kiron 1994; Sargent 1995). These fatty acids and particularly DHA are specifically accumulated for the formation of cell membranes vital for normal development and functioning of the visual and neural systems of fish larvae (Sargent *et al.* 1990; Mourente *et al.* 1991; Mourente and Tocher 1992b, 1993a; Sargent *et al.* 1993a,b; Sargent 1995). In consequence, failure to provide adequate quantities of the appropriate dietary fatty acids is a primary cause of the unsuccessful rearing of marine fish larvae (Watanabe and Kiron 1994), mainly due to visual impairment and abnormal predatory behaviour (Navarro and Sargent 1992; Sargent *et al.* 1994; Bell *et al.* 1995).

One of the most important challenges in marine aquaculture is larval nutrition, since a major difficulty in rearing marine fish larvae is the limited knowledge of their nutritional requirements. In consequence, an estimation of the utilization of endogenous nutrients from the yolk-sac that occurs during embryonic and early larval development can be a useful approach to the study of the nutritional requirements of fish larvae (Kimata 1983a,b; Tocher *et al.* 1985a,b; Heming and Buddington 1988; Fraser *et al.* 1988; Tandler *et al.* 1989; Ostrowski and Divakaran 1991; Rainuzzo 1993; Finn 1994; Vázquez *et al.* 1994; Sargent 1995). Thus, much of our understanding of lipid metabolism in fish eggs and larvae derives from studies investigating the changes that occur in lipid classes and their associated fatty acids throughout embryogenesis and early larval stages in whole eggs and larvae (Cetta and Capuzzo 1982; Vetter *et al.* 1983; Quantz 1985; Tocher and Sargent 1984; Tocher *et al.* 1985a,b; Rainuzzo 1993; Lie 1993; Finn 1994; Vázquez *et al.* 1994; Watanabe and Kiron 1994; Sargent 1995) or separated yolk-sac and larval bodies (Tocher *et al.* 1985a; Rønnestad *et al.* 1995).

The Senegal sole, *Solea senegalensis* Kaup, is a potential new species for mariculture in the temperate waters of the Mediterranean. Hence, techniques for its culture are being developed in southern European countries such as Portugal and Spain (Rodríguez 1984; Dinis 1992; Vázquez *et al.* 1994) and they may complement the methods used for gilthead sea bream (*Sparus aurata* L.) or sea bass (*Dicentrarchus labrax* L.) in duo-culture systems. In consequence, the objective of the present study

was to determine the changes occurring in lipid class and fatty acid contents from lipids of newly hatched Senegal sole larvae during food deprivation in order to elucidate the lipid metabolism and requirements of first feeding larvae.

Materials and methods

Broodstock

Wild broodstock of *S. senegalensis* from the salt marshes in the Bay of Cádiz (SW Spain) were caught in October 1992 and maintained in captivity for 2 years. Broodstock were stocked in 25 m^3, 1 m deep circular outdoor tanks at a density of 0.8–1.5 kg m^{-2} and a female to male ratio of 1:1. Fish were kept under natural photoperiod, with temperatures during the spawning season of 20 ± 1°C, salinity 37‰ and dissolved oxygen 5.8–9.3 ppm. A diet of fresh food comprising of whole minced cuttlefish (*Illex* spp.) five days a week; minced mussel meat (*Mytilus edulis*) was given once a week. The ration was given once per day at an average of 1–3% of broodstock biomass.

Egg production and larval rearing

Broodstock spawned naturally from March to May 1994 and all the eggs used in the present study belonged to a single batch spawned on the 11th of April 1994. The eggs had a diameter of 962.8 ± 51.9 µm and contained multiple oil droplets. Buoyant fertilized eggs were collected in an adequate mesh screen (500 µm) from the spawning tanks and were transferred to 500 l cylindrical tanks at a density of 60 individuals per l where they were maintained unfed during the study period. Eggs hatched approximately 24–26h after spawning and fertilization at a hatching and rearing temperature of 20 ± 1°C and yolk-sac larvae remained for a further 48–72h after hatching.

Sample collection

Eggs from the same batch, approximately 10–12h after spawning were collected in an adequate size mesh screen, rinsed in distilled water and blotted on filter paper before being frozen in liquid nitrogen and stored at –80°C until analysis. The same procedure was used for larvae from 1 to 5 days after hatching. Yolk-sac absorption had primarily occurred in larvae sampled by day 2 after hatching and no yolk-sac was observed in larvae sampled after day 3.

Dry weight, biochemical composition and energy content determinations

Three replicates of preweighed samples (approximately 500 mg wet weight of eggs and unfed larvae from 1 to 5 days after hatching were maintained 110°C for 24h. The dry weights were determined after cooling *in vacuo* for at least 1h. Protein content was determined 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 was measured gravimetrically after total combustion in a furnace at 550°C for 6h. 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 after homogenization in chloroform/methanol (2:1, v/v) containing 0.01% of butylated hydroxytoluene (BHT) as an antioxidant, basically according to the method of 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 classes including PC, PE and TAG were separated by the above procedure. The separated lipid classes were visualized by developing the plates with 1% 2',7'-dichlorofluorescein in 98% methanol and viewing under UV light. Fatty acid methyl esters from total lipids and bands of adsorbent containing lipid classes scraped from the plates, were prepared by acid-catalyzed transmethylation for 16h 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 determined quantitatively in a Hewlett-Packard 5890A Series II gas chromatograph equipped with a chemically bonded (PEG) Omegawax-320 fused silica wall-coated capillary column (30 m × 0.32 mm i.d., Supelco Inc., Bellefonte, USA), an on-column injection system and FID. Hydrogen was used as the carrier gas with an oven thermal gradient from an initial 50 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. Separated individual fatty acid methyl esters were identified by comparison with known standards and quantified using Hewlett-Packard 3365 ChemStation software in a computer linked to the gas chromatograph.

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 data were arc-sin transformed before further statistical analysis. Differences between mean values were analyzesd 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, U.K.). 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, U.K.).

Results

Gross composition and energy content

Dry weight, gross composition and energy content of eggs and early larvae of *S. senegalensis,* from day 1 to day 5 post-hatch, are presented in Table 1. The dry weight percentage showed a significant increase from fertilized egg to larvae 1 day after hatch possibly due to the loss of the perivitelline fluids but afterwards showed a downward trend due to starvation that resulted in a 2.4% decrease by day 5 at an average rate of 0.6% of the dry weight per day. The protein content presented a significant increase from fertilized eggs to one day old larvae but during the larval stages was relatively stable. Total lipid content together with total carbohydrates also showed a significant increase from egg to one day old larvae due to the increase in dry weight, but while total lipid showed a significant marked decline thereafter, total carbohydrates significantly increased during the same larval period under starvation conditions. Total lipids showed the greatest significant decrease, particularly during the larval period, amounting to 6.8% of the dry weight percentage for the whole period under study, at an average of 1.7% of the dry weight percentage per day. The largest decrease was between days 3 and 4 after hatching (3.2% of the dry weight). Total carbohydrates increased by 1.2% of the dry weight from fertilized eggs to 1 day old larvae and by 1.3% from day 1 to day 5 after hatch. Total energy content of eggs and larvae decreased by 5.4 kcal g^{-1} of dry weight in the whole period, mainly due to the utilization of energy derived from lipids (at an average of 1.6 kcal g^{-1} of dry weight biomass day^{-1}).

Lipid class composition

Lipid class compositions from fertilized *S. senegalensis* eggs to larvae 5 days after hatching

Table 1. The effect of the duration of food deprivation on gross composition (dry weight percentage) and energy content (kcal g^{-1}) of eggs and newly hatched larvae of *Solea senegalensis* during development

	Duration of food deprivation					
	Egg	Day 1	Day 2	Day 3	Day 4	Day 5
Dry weight (%)	9.2 ± 0.5^{a}	10.4 ± 0.1^{b}	9.0 ± 0.0^{a}	8.7 ± 0.1^{ac}	8.2 ± 0.1^{cd}	8.0 ± 0.2^{d}
Protein	64.4 ± 1.7^{a}	69.1 ± 0.6^{bc}	71.9 ± 1.0^{c}	68.4 ± 2.4^{abc}	66.6 ± 2.1^{ab}	69.7 ± 1.4^{bc}
Lipid	16.3 ± 1.5^{ab}	20.3 ± 0.8^{c}	19.5 ± 1.3^{c}	18.1 ± 0.6^{bc}	15.3 ± 1.1^{ab}	13.5 ± 0.4^{a}
Carbohydrate	1.7 ± 0.2^{a}	3.0 ± 0.1^{b}	3.4 ± 0.1^{c}	3.9 ± 0.0^{d}	4.0 ± 0.1^{dc}	4.3 ± 0.1^{c}
Ash	17.6 ± 1.1^{a}	7.6 ± 0.9^{bc}	5.2 ± 0.6^{c}	9.6 ± 1.6^{bc}	14.1 ± 0.4^{d}	12.5 ± 1.4^{d}
Energy from protein	36.4 ± 0.9^{a}	39.0 ± 0.3^{bc}	40.6 ± 0.5^{c}	38.6 ± 1.3^{abc}	37.6 ± 1.2^{ab}	39.4 ± 0.8^{bc}
Energy from lipid	15.4 ± 1.4^{ab}	19.2 ± 0.7^{b}	18.4 ± 1.2^{ab}	17.1 ± 0.5^{ab}	14.4 ± 1.0^{a}	12.7 ± 0.4^{a}
Energy from carbohydrate	0.7 ± 0.1^{a}	1.3 ± 0.0^{b}	1.4 ± 0.0^{c}	1.6 ± 0.0^{d}	1.7 ± 0.0^{c}	1.8 ± 0.0^{f}
Total Energy	52.5 ± 2.3^{a}	59.5 ± 1.8^{b}	60.4 ± 1.4^{b}	57.3 ± 1.3^{ab}	53.7 ± 1.6^{a}	53.9 ± 1.2^{a}

Results represent the mean ± 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 not superscript appears, values are not different.

Table 2. The effect of the duration of food deprivation on lipid class composition (percentage of total lipid), triacylglycerol/cholesterol ratio and phosphatidylcholine/phosphatidylethanolamine ratio of eggs and newly hatched larvae of *Solea senegalensis* during development

	Duration of food deprivation					
	Egg	Day 1	Day 2	Day 3	Day 4	Day 5
Total polar	37.9 ± 1.0^{a}	42.9 ± 2.4^{b}	43.5 ± 0.3^{b}	46.5 ± 0.2^{c}	51.9 ± 2.8^{d}	53.4 ± 0.7^{d}
Phosphatidylcholine	19.6 ± 0.8	21.5 ± 1.4	20.9 ± 0.5	20.6 ± 0.1	20.1 ± 0.7	19.7 ± 0.2
Phosphatidylethanolamine	8.0 ± 0.2^{a}	9.4 ± 0.6^{b}	9.7 ± 0.1^{b}	11.5 ± 0.1^{c}	14.4 ± 0.9^{d}	14.7 ± 0.4^{d}
Phosphatidylserine	1.3 ± 0.0^{a}	2.2 ± 0.0^{b}	2.8 ± 0.0^{b}	4.3 ± 0.8^{c}	5.9 ± 0.5^{d}	6.8 ± 0.2^{c}
Phosphatidylinositol	3.3 ± 0.1^{a}	3.9 ± 0.4^{ab}	4.3 ± 0.2^{bc}	4.7 ± 0.3^{bc}	4.8 ± 0.5^{c}	4.5 ± 0.3^{bc}
Phosphatidic acid	1.2 ± 0.0^{a}	1.4 ± 0.1^{ab}	1.7 ± 0.1^{bc}	2.0 ± 0.0^{c}	2.7 ± 0.3^{d}	2.9 ± 0.0^{d}
Glycolipid	2.6 ± 0.2^{ab}	2.5 ± 0.1^{ab}	2.2 ± 0.3^{ac}	2.0 ± 0.0^{c}	2.5 ± 0.1^{ab}	2.9 ± 0.2^{b}
Sphingomyelin	1.1 ± 0.1^{ab}	1.3 ± 0.2^{bc}	1.4 ± 0.0^{c}	0.8 ± 0.1^{d}	0.9 ± 0.1^{ad}	1.1 ± 0.0^{ab}
Lyso-phosphatidylcholine	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}	0.3 ± 0.0^{b}	0.4 ± 0.1^{ab}	0.5 ± 0.1^{a}	0.6 ± 0.1^{a}
Total neutral	61.8 ± 1.0^{a}	56.9 ± 2.3^{b}	56.1 ± 0.3^{b}	53.2 ± 0.3^{c}	47.7 ± 2.9^{d}	46.2 ± 0.7^{d}
Cholesterol	9.8 ± 0.2^{a}	9.3 ± 2.1^{a}	14.8 ± 0.4^{b}	18.5 ± 0.3^{c}	20.3 ± 1.1^{c}	25.2 ± 1.3^{d}
Free fatty acid	0.7 ± 0.1^{a}	1.5 ± 0.1^{b}	2.1 ± 0.1^{c}	4.6 ± 0.0^{d}	6.6 ± 0.3^{c}	7.7 ± 0.2^{f}
Triacylglycerol	28.3 ± 0.5^{a}	26.1 ± 0.3^{b}	22.7 ± 0.1^{c}	18.0 ± 0.3^{d}	10.6 ± 1.1^{c}	4.8 ± 0.4^{f}
Sterol ester	22.9 ± 0.9^{a}	20.0 ± 0.1^{ab}	16.5 ± 0.3^{b}	11.9 ± 0.4^{c}	10.1 ± 2.7^{c}	8.5 ± 1.7^{c}
Triacylglycerol/cholesterol	12.9 ± 0.4^{a}	2.8 ± 0.9^{a}	1.5 ± 0.3^{b}	0.9 ± 0.2^{bc}	0.5 ± 0.1^{bc}	0.2 ± 0.0^{c}
Phosphatidylcholine/ Phosphatidylethanolamine	2.4 ± 0.2^{a}	2.3 ± 0.4^{a}	1.5 ± 0.3^{b}	0.9 ± 0.2^{bc}	0.5 ± 0.1^{bc}	0.2 ± 0.0^{c}

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.

during fasting are shown in Table 2. The percentage of total polar lipids increased significantly by 29% (mainly due to significant increases in PE, PS, PI and phosphatidic acid with conservation of PC (the major polar lipid) with a concomitant significant decrease in the percentage of total neutral lipids (particularly TAG and SE) whereas the proportions of C and free fatty acids increased by factors of 61.1% and 90.1%, respectively, during the study period. When considering these data as μg

Table 3. The effect of the duration of food deprivation on the total lipid fatty acid content (µg fatty acid mg^{-1} total lipid) of eggs and newly hatched larvae of *Solea senegalensis* during development

	Duration of food deprivation					
Fatty acid	Egg	Day 1	Day 2	Day 3	Day 4	Day 5
14:0	14.6 ± 3.6	15.3 ± 6.2	8.1 ± 1.3	5.9 ± 0.6	7.2 ± 3.4	8.9 ± 2.8
15:0	11.4 ± 2.7	12.0 ± 2.4	10.4 ± 0.1	9.2 ± 1.1	15.3 ± 1.8	15.9 ± 5.5
16:0	128.3 ± 9.5^{a}	125.0 ± 6.1^{a}	106.8 ± 4.1^{b}	98.4 ± 1.4^{b}	91.4 ± 5.4^{bc}	77.5 ± 5.4^{c}
16:0DMA	2.7 ± 0.2^{a}	2.5 ± 0.1^{a}	2.9 ± 0.4^{a}	4.2 ± 0.1^{b}	4.0 ± 0.4^{b}	4.6 ± 0.2^{b}
16:1(n-7)	45.2 ± 2.4^{a}	46.9 ± 4.7^{a}	32.2 ± 1.0^{b}	24.5 ± 0.8^{c}	16.8 ± 0.4^{d}	12.6 ± 2.1^{d}
16:2	7.5 ± 1.1	7.9 ± 1.2	5.8 ± 0.3	5.4 ± 0.2	5.9 ± 1.4	6.2 ± 2.2
16:3	8.1 ± 0.9	8.3 ± 1.5	6.8 ± 0.3	6.8 ± 0.2	6.6 ± 0.8	7.3 ± 1.8
16:4	5.3 ± 1.0^{a}	5.1 ± 1.1^{ab}	3.6 ± 0.3^{ab}	3.2 ± 0.2^{b}	3.5 ± 0.1^{ab}	5.3 ± 0.9^{d}
18:0DMA	1.1 ± 0.1^{a}	1.1 ± 0.1^{a}	1.4 ± 0.1^{a}	1.9 ± 0.0^{b}	2.4 ± 0.1^{c}	2.9 ± 0.2^{d}
18:1(n-9)DMA	0.9 ± 0.2^{a}	0.3 ± 0.0^{b}	1.1 ± 0.1^{a}	1.4 ± 0.0^{c}	1.4 ± 0.1^{c}	1.5 ± 0.0^{c}
18:1(n-7)DMA	0.5 ± 0.1^{a}	0.5 ± 0.0^{a}	0.5 ± 0.1^{a}	0.8 ± 0.0^{b}	0.7 ± 0.1^{b}	0.7 ± 0.0^{b}
18:0	31.4 ± 0.2^{a}	32.8 ± 1.9^{a}	33.5 ± 1.5^{a}	37.9 ± 0.4^{b}	41.8 ± 0.4^{c}	44.6 ± 1.7^{c}
18:1(n-9)	55.5 ± 3.1^{a}	57.0 ± 1.2^{a}	47.8 ± 2.1^{b}	43.8 ± 0.4^{bc}	39.2 ± 1.3^{c}	44.2 ± 1.9^{bc}
18:1(n-7)	17.9 ± 0.8^{a}	16.9 ± 1.1^{ab}	15.5 ± 0.6^{bc}	14.6 ± 0.2^{c}	12.5 ± 0.1^{d}	11.6 ± 0.5^{d}
18:2(n-6)	4.5 ± 1.0	4.2 ± 0.8	3.5 ± 0.5	3.2 ± 0.2	4.2 ± 1.2	3.3 ± 1.1
18:3(n-3)	3.4 ± 0.2	3.2 ± 0.5	2.9 ± 0.1	2.9 ± 0.1	2.9 ± 0.2	2.8 ± 0.2
18:4(n-3)	3.0 ± 0.2^{ab}	3.2 ± 0.3^{abc}	3.4 ± 0.1^{bc}	2.4 ± 0.7^{a}	4.0 ± 0.1^{c}	3.6 ± 0.1^{bc}
20:4(n-6)	11.3 ± 0.9^{a}	12.2 ± 0.1^{a}	12.5 ± 0.6^{ab}	14.2 ± 0.3^{bc}	15.0 ± 0.1^{c}	14.7 ± 1.2^{c}
20:3(n-3)	2.3 ± 0.3^{a}	1.0 ± 0.1^{b}	0.8 ± 0.1^{b}	0.7 ± 0.1^{b}	0.7 ± 0.1^{b}	0.7 ± 0.2^{b}
20:4(n-3)	2.4 ± 0.1^{a}	2.5 ± 0.2^{a}	3.1 ± 0.3^{a}	3.9 ± 0.2^{b}	4.4 ± 0.3^{b}	5.8 ± 0.3^{c}
20:5(n-3)	31.9 ± 0.2^{a}	31.4 ± 1.9^{a}	28.9 ± 1.5^{ab}	26.7 ± 0.5^{b}	20.4 ± 0.7^{c}	15.5 ± 0.9^{d}
22:5(n-6)	2.4 ± 0.1^{a}	2.4 ± 0.1^{a}	2.4 ± 0.1^{a}	2.3 ± 0.6^{a}	2.5 ± 0.1^{a}	3.9 ± 0.7^{b}
22:5(n-3)	26.7 ± 1.3^{a}	26.5 ± 0.9^{a}	24.5 ± 0.7^{ab}	20.6 ± 0.2^{b}	14.9 ± 2.6^{c}	28.3 ± 1.8^{a}
22:6(n-3)	188.7 ± 13.7	188.5 ± 7.8	188.7 ± 10.4	182.9 ± 1.8	182.6 ± 0.6	183.9 ± 12.5
Total saturated	193.2 ± 2.7^{a}	191.9 ± 17.4^{ab}	167.8 ± 7.6^{abc}	158.6 ± 4.1^{bc}	163.9 ± 10.6^{abc}	156.1 ± 19.8^{c}
Total monoenes	130.6 ± 6.3^{a}	131.9 ± 6.0^{a}	106.7 ± 3.5^{b}	92.8 ± 1.5^{b}	75.4 ± 1.6^{c}	75.9 ± 11.5^{c}
Total polyenes	308.5 ± 15.7	305.3 ± 14.1	296.0 ± 12.7	284.3 ± 1.8	275.9 ± 1.1	289.9 ± 35.6
Total DMA	5.2 ± 0.5^{ab}	4.4 ± 0.4^{a}	5.8 ± 0.6^{b}	8.3 ± 0.2^{c}	8.6 ± 0.7^{cd}	9.8 ± 0.4^{d}
Total (n-9)	64.8 ± 3.3^{a}	65.6 ± 0.9^{a}	55.9 ± 2.5^{ab}	50.9 ± 0.6^{bc}	44.1 ± 1.1^{c}	48.6 ± 8.8^{bc}
Total (n-7)	65.3 ± 3.0^{a}	65.8 ± 5.8^{a}	49.7 ± 1.7^{b}	40.9 ± 0.8^{c}	30.6 ± 0.5^{d}	25.9 ± 2.7^{d}
Total (n-6)	33.2 ± 1.2	33.3 ± 1.4	30.7 ± 1.1	31.7 ± 0.8	33.4 ± 3.1	34.8 ± 3.5
Total (n-3)	275.3 ± 16.0	272.0 ± 13.2	265.3 ± 12.9	252.6 ± 1.3	242.5 ± 2.0	255.1 ± 32.5
HUFA (n-6)	19.6 ± 2.2	19.4 ± 0.9	19.8 ± 0.9	21.5 ± 0.4	21.8 ± 1.0	23.8 ± 2.9
HUFA (n-3)	255.4 ± 18.1	252.2 ± 10.7	248.5 ± 12.9	237.1 ± 2.7	225.4 ± 2.7	236.0 ± 30.0

Results represent the means ± SD (n = 3). SD = 0.0 implies an SD < 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. Totals include some minor components not shown. DMA, dimethylacetal. HUFA, highly unsaturated fatty acid (> C20 and with at least 3 double bonds); tr, trace.

lipid class per mg dry weight (data not shown) total polar lipids remained constant during most of the period, decreasing at day 5 due to significant decreases in PC from day 3 after hatching onwards. The other polar lipids (such as PE, PS, PI and phospatidic acid) increased significantly throughout the study period. Total neutral lipid content decreased significantly mainly due to significant decreases in the absolute contents of the major neutral lipid classes, TAG and SE. Both the PC/PE and TAG/cholesterol ratios showed a downward trend during the starvation period.

Total lipid fatty acid content

Total lipid fatty acid content of eggs and newly hatched larvae of *S. senegalensis,* from fertilization to day 5 after hatching are presented in Table 3. Total saturated fatty acids decreased signifi-

Table 4. The effect of the duration of food deprivation on the fatty acid content (μg fatty acid mg^{-1} total lipid) from the phosphatidylcholine fraction of eggs and newly hatched larvae of *Solea senegalensis* during development

	Duration of food deprivation					
Fatty acid	Egg	Day 1	Day 2	Day 3	Day 4	Day 5
14:0	3.2 ± 0.2^{a}	2.2 ± 0.4^{ab}	2.3 ± 0.6^{ab}	2.0 ± 0.1^{b}	1.6 ± 0.1^{b}	1.7 ± 0.4^{b}
15:0	1.0 ± 0.1^{a}	1.2 ± 0.2^{a}	1.0 ± 0.2^{a}	1.1 ± 0.1^{a}	3.8 ± 0.3^{b}	3.2 ± 0.2^{c}
16:0	49.9 ± 3.1	58.8 ± 4.9	55.7 ± 6.2	60.3 ± 5.7	61.1 ± 2.6	55.2 ± 5.3
16:1(n-7)	6.4 ± 0.7	7.3 ± 1.6	4.9 ± 2.3	6.9 ± 1.2	7.0 ± 0.3	6.1 ± 0.6
16:2	0.8 ± 0.2	0.9 ± 0.3	1.1 ± 0.3	1.0 ± 0.3	0.9 ± 0.1	0.9 ± 0.1
16:3	0.9 ± 0.1	1.1 ± 0.3	0.9 ± 0.2	1.0 ± 0.2	1.1 ± 0.0	1.0 ± 0.1
16:4	1.2 ± 0.1^{a}	1.3 ± 0.2^{a}	1.3 ± 0.2^{a}	1.6 ± 0.2^{ab}	2.0 ± 0.0^{b}	1.9 ± 0.1^{b}
18:0	6.3 ± 0.2^{a}	6.8 ± 0.4^{a}	6.6 ± 0.4^{a}	7.5 ± 0.4^{ab}	8.2 ± 0.5^{bc}	9.1 ± 0.7^{c}
18:1(n-9)	12.9 ± 0.4^{a}	13.8 ± 0.6^{a}	13.1 ± 0.9^{a}	16.9 ± 1.1^{b}	21.4 ± 0.9^{c}	22.5 ± 1.8^{c}
18:1(n-7)	3.6 ± 0.4^{a}	4.1 ± 0.6^{ab}	3.6 ± 0.7^{a}	4.3 ± 0.6^{ab}	5.5 ± 0.1^{b}	5.1 ± 0.7^{ab}
18:2(n-6)	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	0.9 ± 0.1
18:3(n-3)	0.3 ± 0.0	0.2 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
18:4(n-3)	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.0	0.4 ± 0.0
20:4(n-6)	2.9 ± 0.1^{a}	3.4 ± 0.1^{b}	3.5 ± 0.1^{b}	4.3 ± 0.2^{c}	4.3 ± 0.1^{c}	3.9 ± 0.2^{d}
20:3(n-3)	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
20:4(n-3)	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
20:5(n-3)	8.3 ± 0.4^{ab}	9.5 ± 0.0^{c}	9.4 ± 0.5^{c}	9.2 ± 0.2^{bc}	7.8 ± 0.2^{a}	5.7 ± 0.4^{d}
22:5(n-6)	1.1 ± 0.1^{a}	1.2 ± 0.1^{a}	1.0 ± 0.1^{a}	1.0 ± 0.2^{a}	0.6 ± 0.0^{b}	0.6 ± 0.1^{b}
22:5(n-3)	5.7 ± 0.7^{a}	5.6 ± 0.6^{a}	4.9 ± 0.5^{ab}	4.0 ± 0.6^{bc}	2.7 ± 0.1^{cd}	1.9 ± 0.1^{d}
22:6(n-3)	52.3 ± 6.1^{a}	50.1 ± 3.9^{a}	45.4 ± 3.8^{ab}	38.1 ± 4.7^{bc}	29.2 ± 1.7^{cd}	23.5 ± 1.4^{d}
Total saturated	62.4 ± 3.6	71.1 ± 5.9	67.3 ± 7.6	73.2 ± 6.4	77.8 ± 3.5	72.3 ± 6.8
Total monenes	27.8 ± 1.2^{a}	30.4 ± 3.0^{ab}	25.8 ± 4.3^{a}	32.5 ± 3.5^{ab}	38.2 ± 1.4^{b}	37.3 ± 3.4^{b}
Total polyenes	76.6 ± 7.1^{a}	77.2 ± 3.6^{a}	71.4 ± 4.2^{ab}	64.0 ± 4.5^{bc}	52.5 ± 1.9^{cd}	42.4 ± 2.6^{d}
Total (n-9)	17.5 ± 0.6^{ab}	18.5 ± 0.9^{ab}	16.9 ± 1.4^{a}	20.9 ± 1.6^{b}	25.4 ± 0.9^{c}	25.9 ± 2.1^{c}
Total (n-7)	10.2 ± 1.1	11.7 ± 2.2	8.9 ± 2.8	11.5 ± 1.9	12.8 ± 0.5	11.4 ± 1.3
Total (n-6)	6.7 ± 0.2^{a}	7.9 ± 0.5^{bc}	7.8 ± 0.5^{bc}	8.5 ± 0.5^{c}	8.3 ± 0.1^{c}	7.2 ± 0.4^{ab}
Total (n-3)	69.8 ± 7.0^{a}	69.2 ± 4.1^{a}	63.5 ± 4.2^{ab}	55.5 ± 4.8^{bc}	44.2 ± 2.1^{cd}	35.2 ± 2.2^{d}
HUFA (n-6)	4.7 ± 0.4^{a}	5.6 ± 0.3^{bc}	5.6 ± 0.2^{bc}	6.1 ± 0.1^{c}	6.1 ± 0.2^{c}	5.1 ± 0.3^{ab}
HUFA (n-3)	67.1 ± 7.3^{a}	66.2 ± 4.5^{a}	60.8 ± 4.6^{ab}	52.4 ± 5.3^{bc}	40.4 ± 2.0^{cd}	31.5 ± 1.9^{d}

Legend as in Table 3.

cantly primarily due to significant decreases in the content of 16:0 (the most abundant saturated) whereas the content of 18:0 (the second major saturated) increased significantly during the same period. Other minor saturated fatty acids such as 14:0 and 15:0 did not change throughout the study period. Total monoene content showed a significant decrease greater than that of total saturated fatty acids. This decrease was due to losses of major monoenes such as 18:1(n-9) and 18:1(n-7) and, particularly, to the decrease in 16:1(n-7). In any case, the decreases in the fatty acids of the (n-7) series were more important than those presented by the (n-9) monoenes. Total PUFA content did not show significant variations mainly due to conservation of the levels of 18:2(n-6), 18:3(n-3) and DHA (the major PUFA component). AA showed an upward trend and, in contrast, EPA and 22:5(n-3) declined significantly. Neither total (n-6) and (n-3) PUFA or HUFA showed significant variations. Total DMA content presented a significant increase due to significant increases of all its components.

Fatty acid contents of phosphatidylcholine and phosphatidylethanolamine

Tables 4 and 5 show the effects of food deprivation on the fatty acid content of the major polar lipid classes, PC and PE, from fertilized eggs to 5 days posthatch larvae of *S. senegalensis*. In PC, total

Table 5. The effect of the duration of food deprivation on the fatty acid content (μg fatty acid mg^{-1} total lipid) from the phosphatidylethanolamine fraction of eggs and newly hatched larvae of *Solea senegalensis* during development

	Duration of food deprivation					
Fatty acid	Egg	Day 1	Day 2	Day 3	Day 4	Day 5
14:0	0.7 ± 0.3^{a}	0.4 ± 0.1^{a}	1.5 ± 0.4^{b}	1.7 ± 0.1^{b}	0.5 ± 0.2^{a}	0.6 ± 0.1^{a}
15:0	1.0 ± 0.2^{a}	0.9 ± 0.1^{a}	1.2 ± 0.3^{a}	1.7 ± 0.1^{ab}	3.0 ± 1.0^{c}	2.8 ± 0.4^{bc}
16:0DMA	2.7 ± 0.2^{a}	2.5 ± 0.1^{a}	2.9 ± 0.4^{a}	4.2 ± 0.1^{b}	4.0 ± 0.4^{b}	4.6 ± 0.2^{b}
16:0	7.4 ± 0.6^{ab}	6.7 ± 0.2^{a}	8.0 ± 0.5^{bc}	11.1 ± 0.4^{d}	9.6 ± 0.5^{e}	9.0 ± 0.1^{e}
16:1(n-7)	1.3 ± 0.4^{a}	1.1 ± 0.3^{a}	1.4 ± 0.3^{ab}	2.1 ± 0.2^{b}	1.5 ± 0.1^{ab}	1.2 ± 0.2^{a}
16:2	0.5 ± 0.1^{ab}	0.3 ± 0.0^{a}	0.5 ± 0.1^{ab}	0.8 ± 0.0^{b}	0.7 ± 0.2^{b}	0.8 ± 0.2^{b}
16:3	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	1.0 ± 0.0	0.9 ± 0.1	0.8 ± 0.0
16:4	0.6 ± 0.1^{a}	0.6 ± 0.0^{a}	0.6 ± 0.1^{a}	0.8 ± 0.0^{b}	0.9 ± 0.1^{b}	0.9 ± 0.0^{b}
18:0DMA	1.1 ± 0.1^{a}	1.1 ± 0.1^{a}	1.4 ± 0.1^{a}	1.9 ± 0.0^{b}	2.4 ± 0.1^{c}	2.9 ± 0.2^{d}
18:1(n-9)DMA	0.9 ± 0.2^{a}	0.3 ± 0.0^{b}	1.1 ± 0.1^{a}	1.4 ± 0.0^{c}	1.4 ± 0.1^{c}	1.5 ± 0.0^{c}
18:1(n-7)DMA	0.5 ± 0.1^{a}	0.5 ± 0.0^{a}	0.5 ± 0.1^{a}	0.8 ± 0.0^{b}	0.7 ± 0.1^{b}	0.7 ± 0.0^{b}
18:0	3.9 ± 0.3^{a}	3.8 ± 0.4^{a}	4.7 ± 0.0^{b}	6.8 ± 0.0^{c}	8.9 ± 0.4^{d}	10.2 ± 0.1^{e}
18:1(n-9)	2.2 ± 0.1^{ab}	2.0 ± 0.1^{ab}	1.9 ± 0.1^{a}	2.6 ± 0.1^{c}	2.6 ± 0.1^{c}	2.3 ± 0.2^{bc}
18:1(n-7)	1.4 ± 0.2^{ab}	1.0 ± 0.0^{c}	1.3 ± 0.1^{bc}	2.1 ± 0.1^{d}	1.8 ± 0.1^{de}	1.7 ± 0.1^{ae}
18:2(n-6)	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.5 ± 0.0^{b}	0.3 ± 0.0^{c}	0.3 ± 0.0^{c}
18:3(n-3)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
18:4(n-3)	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}
20:4(n-6)	0.9 ± 0.0^{a}	0.9 ± 0.1^{a}	0.9 ± 0.0^{a}	1.2 ± 0.0^{b}	1.5 ± 0.1^{c}	1.7 ± 0.0^{d}
20:3(n-3)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:4(n-3)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:5(n-3)	1.2 ± 0.2^{a}	1.4 ± 0.2^{ab}	1.4 ± 0.0^{ab}	1.5 ± 0.1^{ab}	1.8 ± 0.2^{b}	1.8 ± 0.1^{b}
22:5(n-6)	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.0
22:5(n-3)	1.4 ± 0.4	1.4 ± 0.2	1.4 ± 0.2	1.1 ± 0.0	1.4 ± 0.4	1.0 ± 0.0
22:6(n-3)	13.9 ± 4.2^{a}	14.8 ± 2.7^{a}	16.1 ± 2.1^{ab}	16.0 ± 0.6^{ab}	24.7 ± 6.5^{b}	21.7 ± 2.0^{b}
Total saturated	14.5 ± 0.8^{a}	13.5 ± 0.8^{a}	17.6 ± 1.5^{b}	23.3 ± 0.3^{c}	23.7 ± 1.9^{c}	24.6 ± 0.4^{c}
Total monoenes	5.5 ± 0.2^{a}	4.5 ± 0.1^{b}	5.2 ± 0.2^{ab}	7.3 ± 0.4^{c}	7.6 ± 0.3^{c}	7.0 ± 0.5^{c}
Total polyenes	21.4 ± 4.3^{a}	21.9 ± 3.2^{a}	23.8 ± 2.2^{ab}	24.5 ± 0.8^{ab}	34.2 ± 7.4^{b}	30.9 ± 2.0^{b}
Total DMA	5.2 ± 0.5^{ab}	4.4 ± 0.4^{a}	5.8 ± 0.6^{b}	8.3 ± 0.2^{c}	8.6 ± 0.7^{cd}	9.8 ± 0.4^{d}
Total (n-9)	2.6 ± 0.2^{ab}	2.3 ± 0.2^{a}	2.4 ± 0.1^{a}	3.0 ± 0.1^{b}	4.1 ± 0.2^{c}	3.8 ± 0.2^{c}
Total (n-7)	2.8 ± 0.4^{ab}	2.2 ± 0.3^{a}	2.8 ± 0.3^{ab}	4.3 ± 0.3^{c}	3.5 ± 0.1^{bc}	3.2 ± 0.3^{b}
Total (n-6)	2.9 ± 0.2^{ab}	2.4 ± 0.1^{a}	2.9 ± 0.3^{ab}	3.1 ± 0.0^{b}	3.8 ± 0.3^{c}	3.9 ± 0.1^{c}
Total (n-3)	18.5 ± 4.6^{a}	19.4 ± 3.3^{a}	20.9 ± 2.2^{ab}	21.4 ± 0.9^{ab}	30.5 ± 7.2^{b}	27.0 ± 2.1^{b}
HUFA (n-6)	1.7 ± 0.0^{a}	1.8 ± 0.1^{a}	1.8 ± 0.1^{a}	1.5 ± 0.0^{a}	2.4 ± 0.3^{b}	2.5 ± 0.1^{b}
HUFA (n-3)	16.9 ± 4.8^{a}	17.9 ± 3.1^{ab}	19.3 ± 2.2^{ab}	19.0 ± 0.8^{ab}	28.3 ± 7.3^{b}	24.9 ± 2.1^{b}

Legend as in Table 3.

saturated fatty acids did not vary significantly throughout the starvation period, due to conservation of 16:0, the major component. Other saturated fatty acids such as 18:0 (second major saturated) and 15:0 significantly increased, whereas 14:0 decreased significantly from egg to 5 day posthatch larvae. Total monounsaturated fatty acids from PC increased by 25.5%. The increments, quantitatively more important after yolk-sac absorption, were principally due to the contributions of its predominant component 18:1(n-9), whereas other monoenes such as 16:1(n-7) and 18:1(n-7) did not contribute significantly to the total increase. Total PUFA content of PC decreased by 44.6% during the period under study mainly due to losses after the yolk-sac had been reabsorbed. The individual PUFAs that contributed to the decreased PUFA were, in order of importance, DHA, 22:5(n-3) and EPA, which showed losses of 28.8, 3.8 and 2.6 μg mg^{-1} total lipid, respectively. In contrast, AA, the most important n-6 PUFA, significantly increased. In consequence, total n-3 HUFA and n-6 HUFA contents decreased and increased, respectively. The C18 PUFAs, either of the n-3 or n-6 series, did not present any significant variations throughout the period considered in this study.

Table 6. The effect of the duration of food deprivation on the fatty acid content (µg fatty acid mg^{-1} total lipid) from the triacylglycerol fraction of eggs and newly hatched larvae of *Solea senegalensis* during development

	Duration of food deprivation					
Fatty acid	Egg	Day 1	Day 2	Day 3	Day 4	Day 5
14:0	14.4 ± 0.9^{a}	10.9 ± 0.4^{b}	5.8 ± 0.8^{c}	3.4 ± 0.6^{d}	1.6 ± 0.1^{e}	0.8 ± 0.1^{e}
15:0	4.8 ± 0.7	3.7 ± 1.1	3.4 ± 1.1	2.9 ± 0.9	3.1 ± 0.2	3.5 ± 0.4
16:0	82.3 ± 6.7^{a}	66.2 ± 4.0^{b}	38.5 ± 2.8^{c}	24.0 ± 0.7^{d}	11.3 ± 0.5^{e}	5.7 ± 0.3^{e}
16:1(n-7)	33.8 ± 2.8^{a}	28.4 ± 0.6^{b}	18.9 ± 1.0^{c}	12.7 ± 0.6^{d}	6.1 ± 0.5^{e}	3.3 ± 0.6^{e}
16:2	1.3 ± 0.1^{a}	1.1 ± 0.2^{ab}	0.8 ± 0.1^{bc}	0.8 ± 0.1^{bc}	0.8 ± 0.0^{bc}	0.6 ± 0.2^{c}
16:3	2.5 ± 0.2^{a}	2.0 ± 0.1^{b}	1.3 ± 0.1^{c}	0.9 ± 0.0^{d}	0.6 ± 0.1^{de}	0.3 ± 0.1^{e}
16:4	3.3 ± 0.2^{a}	2.9 ± 0.1^{a}	2.0 ± 0.1^{b}	1.5 ± 0.0^{c}	1.1 ± 0.0^{d}	0.7 ± 0.3^{d}
18:0	13.0 ± 0.9^{a}	11.4 ± 0.6^{b}	7.6 ± 0.4^{c}	5.7 ± 0.1^{d}	2.9 ± 0.1^{e}	1.8 ± 0.1^{e}
18:1(n-9)	30.7 ± 2.1^{a}	28.8 ± 1.7^{a}	19.4 ± 1.1^{b}	12.6 ± 0.2^{c}	5.3 ± 0.4^{d}	2.5 ± 0.1^{d}
18:1(n-7)	10.6 ± 0.9^{a}	9.7 ± 0.9^{a}	6.6 ± 0.6^{b}	4.2 ± 0.1^{c}	1.8 ± 0.1^{d}	0.9 ± 0.0^{d}
18:2(n-6)	1.7 ± 0.1^{a}	1.5 ± 0.1^{a}	1.0 ± 0.1^{b}	0.7 ± 0.0^{c}	0.3 ± 0.1^{d}	0.3 ± 0.0^{d}
18:3(n-3)	1.0 ± 0.1^{a}	0.9 ± 0.1^{a}	0.6 ± 0.0^{b}	0.4 ± 0.1^{c}	0.2 ± 0.0^{d}	0.1 ± 0.0^{d}
18:4(n-3)	1.1 ± 0.1^{a}	0.9 ± 0.1^{b}	0.6 ± 0.1^{c}	0.4 ± 0.0^{d}	0.2 ± 0.0^{de}	0.3 ± 0.0^{e}
20:4(n-6)	1.9 ± 0.2^{ab}	2.1 ± 0.1^{a}	1.7 ± 0.0^{b}	1.8 ± 0.1^{b}	0.9 ± 0.1^{c}	0.4 ± 0.0^{d}
20:3(n-3)	0.4 ± 0.1^{a}	0.4 ± 0.0^{a}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.1 ± 0.0^{c}	0.1 ± 0.0^{c}
20:4(n-3)	0.5 ± 0.1^{a}	0.4 ± 0.1^{a}	0.2 ± 0.0^{b}	0.2 ± 0.1^{b}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}
20:5(n-3)	10.9 ± 1.1^{a}	10.6 ± 0.4^{a}	7.0 ± 0.1^{b}	5.1 ± 0.3^{c}	2.0 ± 0.2^{d}	0.7 ± 0.1^{d}
22:5(n-6)	0.5 ± 0.0^{a}	0.6 ± 0.1^{a}	0.3 ± 0.0^{b}	0.2 ± 0.0^{bc}	0.1 ± 0.0^{c}	0.1 ± 0.0^{c}
22:5(n-3)	7.4 ± 0.8^{a}	7.1 ± 0.6^{a}	4.6 ± 0.3^{b}	3.2 ± 0.1^{c}	1.4 ± 0.1^{d}	0.7 ± 0.0^{d}
22:6(n-3)	44.6 ± 4.8^{a}	39.1 ± 2.4^{a}	25.3 ± 1.3^{b}	18.5 ± 0.7^{c}	7.5 ± 0.8^{d}	2.9 ± 0.1^{d}
Total saturated	119.6 ± 8.5^{a}	97.2 ± 4.3^{b}	59.1 ± 5.2^{c}	39.0 ± 2.3^{d}	20.8 ± 0.9^{e}	12.9 ± 0.2^{e}
Total monoenes	87.1 ± 6.4^{a}	78.2 ± 3.7^{a}	51.9 ± 3.3^{b}	33.7 ± 0.6^{c}	15.3 ± 0.9^{d}	8.3 ± 0.4^{d}
Total polyenes	80.3 ± 8.1^{a}	72.3 ± 3.9^{a}	47.7 ± 1.8^{b}	35.7 ± 1.3^{c}	18.3 ± 2.3^{d}	8.8 ± 0.3^{d}
Total (n-9)	41.6 ± 2.7^{a}	38.9 ± 2.1^{a}	25.4 ± 2.7^{b}	16.1 ± 1.1^{c}	7.2 ± 0.5^{d}	3.9 ± 0.4^{d}
Total (n-7)	45.1 ± 3.9^{a}	39.0 ± 1.5^{b}	26.2 ± 1.2^{c}	17.4 ± 0.5^{d}	8.0 ± 0.5^{e}	4.3 ± 0.6^{e}
Total (n-6)	8.0 ± 0.8^{a}	7.5 ± 0.2^{ab}	5.4 ± 0.3^{bc}	5.0 ± 0.2^{c}	4.9 ± 1.8^{c}	2.6 ± 0.1^{d}
Total (n-3)	72.2 ± 7.4^{a}	64.7 ± 3.7^{a}	42.3 ± 1.7^{b}	30.7 ± 1.2^{c}	13.4 ± 1.0^{d}	6.1 ± 0.3^{d}
HUFA (n-6)	4.1 ± 0.2^{a}	4.3 ± 0.1^{a}	3.1 ± 0.0^{b}	2.8 ± 0.1^{b}	1.5 ± 0.1^{c}	0.8 ± 0.1^{d}
HUFA (n-3)	64.3 ± 6.9^{a}	57.9 ± 3.4^{a}	37.7 ± 1.7^{b}	27.5 ± 1.1^{c}	11.2 ± 0.9^{d}	4.5 ± 0.2^{d}

Legend as in Table 3.

In PE, most of the constituent fatty acids tended to increase from fertilized eggs to 5 day post-hatch larvae, especially saturated fatty acids and PUFA (Table 5). Total saturated fatty acids increased by 42.0%, mainly due to significantly increased 18:0, whereas 16:0 (the major saturated fatty acid in PE) and minor saturated fatty acids such as 14:0 and 15:0 did not vary significantly. As with total saturated fatty acids, total PUFA in PE increased largely after yolk-sac reabsorption. Major contributions to the increase in total PUFA were made by DHA, EPA and AA, at 7.8, 0.6 and 0.8 µg mg^{-1} total lipid, respectively, whereas C18 PUFAs and 22:5(n-3) did not vary significantly. Similar trends were shown by both n-3 and n-6 PUFA and HUFA. Total monoenes, which were low in PE, and total DMA contents both showed an increasing trend.

Fatty acid content of triacylglycerol

The fatty acid content of TAG from fertilized eggs to 5 day old larvae of *S. senegalensis* are presented in Table 6. A general significant decreasing trend was observed in all fatty acids in TAG throughout the study period, but particularly the larval phase. Major quantitative decreases were observed in total saturated fatty acids (by 89,2% and net losses of 106.7 µg mg^{-1} total lipid), followed by total monoenes and PUFA (90.5% and 89.1% and net losses of 78.8 and 71.5 µg mg^{-1} total lipid, respectively). The most abundant fatty acids in TAG were, in decreasing order, 16:0, DHA and 18:1(n-9), which decreased by 76.6, 41.7 and 28.2 µg mg^{-1} total lipid, respectively in the whole period.

Discussion

The eggs of the Senegal sole are characterized by the presence of multiple oil droplets and classified as high lipid content (> 15% of egg dry mass) (Finn 1994), and in the present study, total lipid accounted for 16.3% of the total dry weight of the fertilized eggs. As a result of starvation, marine fish larvae show a reduction in the dry weight, mostly due to a rapid decrease of total lipids during early development and particularly after yolk-sac absorption. This has been demonstrated in species such as plaice, *Pleuronectes platessa* (Ehlrich 1974a; Rainuzzo *et al.* 1992; Rainuzzo 1993), herring, *Clupea harengus* (Ehlrich 1974b; Tocher *et al.* 1985b), cod, *Gadus morhua* (Fraser *et al.* 1988; Rainuzzo *et al.* 1992; Van der Meeren *et al.* 1993; Rainuzzo 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* (Kimata 1983a; Tandler *et al.* 1989) and gilthead sea bream, *Sparus aurata* (Mourente 1989; Koven *et al.* 1989; Mourente and Odriozola 1990; Rønnestad *et al.* 1994). In the present study, *S. senegalensis*, a marine flatfish species with a rapid rate of development, showed a significant decrease by 2.4% in dry weight from newly hatched larvae to 5 day old starved larvae accompanied by the concomitant significant decrease of total lipids by 6.8% (on a dry weight basis). Most of the decrease in dry weight was due to lipid consumption, since total protein content was relatively stable and did not contribute to catabolism throughout the whole period. Protein contents, on a dry weight basis, of eggs and larvae of *S. senegalensis* are similar to the mean value of 66.3% reviewed by Kamler (1992) for fish eggs, but below the values estimated for several marine fish eggs by Finn (1994). A similar pattern was shown by gilthead sea bream (Rønnestad *et al.* 1994), although it is possible that free amino acids could also be a significant energy substrate during the egg stage of *S. senegalensis*. Nevertheless, *S. senegalensis* larvae during early development and starvation conditions, used lipids in part to be oxidized as fuel for energy (80%) and in part to be converted to carbohydrates (20%) *via* glycerol (Gurr and Harwood 1991). Thus, in terms of the whole energy metabolism and in contrast to other flatfish species such as turbot or lemon sole, *Microstomus kitt* (Rønnestad 1992), lipids provide most of the energy required by Senegal sole larvae after hatching and supply most of the catabolic demand. The energetic balance during the period under study showed that energy from lipids was consumed at a daily rate of 1.6 kcal per gram of dry biomass, 0.3 kcal g^{-1} day^{-1} were used for carbohydrate synthesis and 1.3 kcal g^{-1} day^{-1} were catabolized for maintenance and basic vital processes.

Lipid metabolism during the early life of fish may differ greatly among species, mainly with regard to the amount and composition of lipids in the yolk, the time and level of lipid combustion, the class of lipid used for either combustion or tissue synthesis and the role of the different fatty acids (Verreth *et al.* 1994; Sargent 1995). In the present study, neutral lipids were consumed at a higher average rate (29.2 µg neutral lipid mg^{-1} dry weight day^{-1}) than polar lipids (8.7 µg neutral lipid mg^{-1} dry weight day^{-1}) and the overall decrease in the neutral lipids was 3.4-fold faster than that of the polar lipids. A similar pattern of lipid catabolism was presented by *S. aurata* (Rønnestad *et al.* 1994). Lipids in the early life history of fish seem to be related to different needs, depending upon the temperature at which egg and larvae develop and the length of the developmental period (Vetter *et al.* 1983; Rainuzzo 1993; Sargent 1995). Among the neutral lipid classes of *S. senegalensis,* TAG and SE were mainly used as energy reserves and decreased at rates of 25.2 and 15.9 µg mg^{-1} dry weight day^{-1}, respectively, whereas free fatty acids and C produced by hydrolysis of the above increased at daily rates of 3.9 and 8.8 µg mg^{-1} dry weight. PC was the most catabolized polar lipid class (average rate 9.6 µg mg^{-1} dry weight day^{-1}) together with PI, glycolipid and sphingomyelin (rates not exceeding 1 µg mg^{-1} dry weight day^{-1}) but in contrast PE, PS and phosphatidic acid were synthesized at rates of 1.0, 2.4 and 0.6 µg mg^{-1} dry weight day^{-1}, respectively. This pattern of lipid class utilization is contrary to that presented by species with eggs lacking oil globule(s), with low lipid contents, living in cold water habitats and having long periods of development such as Atlantic salmon, *Salmo salar* (Cowey *et al.* 1985), herring (Tocher *et al.* 1985b), cod (Fraser *et al.* 1988;

Rainuzzo *et al.* 1992; Rainuzzo 1993), plaice (Rainuzzo *et al.* 1992; Rainuzzo 1993), African catfish, *Clarias gariepinus* (Verreth *et al.* 1994) and Atlantic halibut (Falk-Petersen *et al.* 1986, 1989; Rainuzzo *et al.* 1992; Rainuzzo 1993; Rønnestad *et al.* 1995), but similar to that of species with oil globule(s), high lipid contents, living in temperate waters habitats and having 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) and gilthead sea bream (Mourente 1989; Rønnestad *et al.* 1994).

The catabolism of PC and the synthesis and conservation of PE and PS in starved *S. senegalensis* larvae can be achieved by *de novo* CDP-base route, Ca^{2+}-dependent base exchange reactions and deacylation/reacylation processes of lyso forms for the incorporation of specific fatty acids (Gurr and Harwood 1991; Sargent *et al.* 1993a). The hydrolysis products of PC may serve as a source of free fatty acids, free inorganic phosphate for high energy nucleotides and choline for methyl metabolism and neurotransmission (Tocher *et al.* 1985a; Fraser *et al.* 1988; Sargent 1995). PE synthesis and conservation as observed here in starved larvae of *S. senegalensis* has also been reported in other marine fish larvae (Rainuzzo *et al.* 1992; Rainuzzo 1993; Rønnestad *et al.* 1995) and may be related to the important role of this lipid in membrane structure of neural and visul tissues (Sargent *et al.* 1993b). It is also noteworthy that the synthesis and conservation of PE is linked to the retention or conservation of DHA, since PE synthesis utilizes preferentially substrates containing DHA (Tinoco 1982; Vance 1985; Sargent *et al.* 1993a). Retention of DHA 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) and may be 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 PE-plasmalogens in *S. senegalensis* larvae, demonstrated by the significant increase of DMAs in PE, is indicative of the maturation of the neural system (Sastry 1985; Mourente and Tocher 1992a; Sargent *et al.* 1993a). The liberation of C from SE could also be involved in myelination of the central nervous system (Sastry 1985) and serve as a precursor for bile salts (Gurr and Harwood 1991; Verreth *et al.* 1994; Rønnestad *et al.* 1995). However, the presence of high levels of free fatty acid may indicate that the rate of release of fatty acids from the different lipid classes by lipases may exceed rates at which they are catabolized (Cowey *et al.* 1985). The nutritional status indicated by the TAG/cholesterol ratio (Fraser 1989) decreased significantly from day 1 after hatching onwards (Table 2), suggesting that the depletion of the yolk-sac already on day 2 point to the imminent need of exogenous feeding at this time to satisfy the energetic and nutritional demand of the larvae.

The total lipid fatty acid content of eggs and yolk-sac larvae of *S. senegalensis* was 7–18% lower than results from a previous study but it was richer in total (n-3) PUFA (Vazquez *et al.* 1994). This could be due to different broodstock diets, different time of spawning season or the fact that the animals were second year spawners (McEvoy *et al.* 1993; Daniel *et al.* 1993; Sargent 1995). Total lipid fatty acid catabolism showed a very specific pattern. The total monoenes showed the greatest decrease (54.7 $\mu g\ mg^{-1}$ total lipid) due to contributions, in decreasing order, from 16:1(n-7), 18:1(n-9) and 18:1(n-7) (at rates of 6.5, 2.3 and 1.3 $\mu g\ mg^{-1}$ total lipid day^{-1}, respectively). Total saturates decreased (by 37.1 $\mu g\ mg^{-1}$ total lipid) mainly due to 16:0 that showed quantitatively the greatest and fastest decrease (average rate 10.2 –g mg^{-1} total lipid day^{-1}), similar to data showed for gilthead sea bream by Rønnestad *et al.* (1994), but in contrast, 18:0 increased during the whole period. Surprisingly, total PUFA did not show a significant decrease as a consequence of the specific conservation of DHA, which predominated and its content remained unaltered, and the quantitatively small significant increase of AA. EPA and 22:5(n-3) were catabolized at similar rates, but since fatty acid bioconversion rates are not known in *S. senegalensis* larvae, it can only be speculated whether EPA and 22:5(n-3) were converted to DHA or selectively catabolized. The conservation of DHA may also suggest that structures containing DHA are metabolically less accessible than those primarily containing EPA (Finn 1994). It is important to note the importance of DHA in synaptogenesis and retinogenesis during early de-

velopment of fish (Bell and Dick 1991; Mourente *et al.*; Tocher *et al.* 1992; Sargent *et al.* 1993a,b; Sargent 1995) and also the important role of AA in biomembrane of salt secreting tissues (Bell *et al.* 1983; Cowey *et al.* 1985) and as a precursor of eicosanoids (Sargent 1995).

(n-3) PUFAs, primarily DHA, were released upon PC hydrolysis whereas major saturates such as 16:0 and 18:0 and monoenes such as 18:1(n-9) and 18:1(n-7) increased in this phospholipid during development and starvation of *S. senegalensis* larvae. DHA was the fatty acid that presented the most rapid decline in PC, decreasing during the period under study by 28.8 $\mu g\ mg^{-1}$ total lipid at an average rate of 5.8 $\mu g\ mg^{-1}$ total lipid day^{-1}. From data on the total lipid fatty acid content it appears that DHA is not used as an energy substrate by *S. senegalensis*, due to its persistent conservation throughout development. This is in contradiction with previous results, where a significant decrease of DHA content was observed between egg and yolk-sac larvae (Vázquez *et al.* 1994), but in agreement with the results of Koven *et al.* (1989) who reported conservation of DHA compared to EPA in starving but not in feeding larvae of gilthead sea bream. Possible explanations of this fact are the potentially higher metabolic rate of the non-starved *S. senegalensis* larvae in the previous study, due to its predatory behaviour, since green water and rotifers were added to the tanks from day 2 after hatch onwards, and the different fatty acid content of the earlier batch of eggs and larvae. In any case, DHA released from PC or TAG (total decrease 41.7 $\mu g\ mg^{-1}$ total lipid and an average rate 8.3 $\mu g\ mg^{-1}$ total lipid day^{-1}) was primarily retained in the larval bodies to be used in deacylation/reacylation processes to synthesize other phosphoglycerides such as PE, that presented a relative retention of DHA by 35.9% (total incorporation 7.8 $\mu g\ mg^{-1}$ total lipid), PS and phosphatidic acid or remain in the free fatty acid pool (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 (Fraser *et al.* 1988; Falk-Petersen *et al.* 1989; Rainuzzo *et al.* 1992; Rainuzzo 1993; Rønnestad *et al.* 1995) but in agreement with other studies that showed that the (n-3) PUFAs and particularly DHA were selectively mobilized for tissue synthesis (Tocher *et al.* 1985a, 1992; Lie 1993; Koven *et al.* 1993; Rønnestad *et al.* 1994). However, *S. senegalensis*, during starvation conditions, seem to present the highest rate of DHA retention with regard to previous studies. Therefore, TAG appeared as the main energy substrate, presenting large depletions of total saturated and total monounsaturated fatty acids (primarily 16:0, 16:1(n-7) and 18:1(n-9)) at rates of 21.3 and 15.8 $\mu g\ mg^{-1}$ total lipid day^{-1}, respectively. This fact indicates a high efficiency of lipases degrading TAG during early development of *S. senegalensis* (Gurr and Harwood 1991).

Regarding bioconversion of fatty acids, 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) and the results presented above, it seems that *S. senegalensis*, during larval development and starvation conditions, the conservation of essential C20 and C22 PUFA in eggs and larval tissues, and particularly DHA, predominated over the contribution of these fatty acids coming from biosynthesis of their precursors. However, a small net increase in AA was evident.

In conclusion, *S. senegalensis* larvae during early development and starvation consume about 0.6% of the dry weight biomass per day mainly due to lipid catabolism (net consumption of approximately 1.7% total lipid per day) and net energy utilization of 1.3 kcal g^{-1} dry weight biomass day^{-1}. The overall decrease of total neutral lipids (mainly TAG) was 3.4-fold faster than total polar lipids (primarily PC) and net synthesis of PE, PS and phosphatidic acid was observed. Saturated fatty acids and monoenes were primarily catabolized as energy fuels whereas essential PUFAs, mainly DHA and AA, were retained.

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