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Biochemical composition and fatty acid content of fertilized eggs, yolk sac stage larvae and first-feeding larvae of the Senegal sole (*Solea senegalensis* Kaup)

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ABSTRACT

Changes in biochemical composition and fatty acid content were investigated during the early development of the Senegal sole (*Solea senegalensis* Kaup). The pattern of lipid utilization in this rapidly developing marine flatfish species favored neutral lipids, particularly triacylglycerol and sterol ester fractions. Fertilized eggs and yolk sac larvae were richer in neutral lipids, which decreased during development. In contrast, a significant increase occurred to proportions of phospholipids, mainly due to significant increases in minor classes such as phosphatidylserine, phosphatidylinositol and phosphatidic acid/cardiolipin, whereas major phospholipid classes such as phosphatidylcholine and phosphatidylethanolamine remained constant during development. Saturated and monounsaturated fatty acids such as 16:0, 16:1*n*-7, 18:1*n*-9 and 18:1*n*-7 were utilized to a greater extent than polyunsaturated fatty acids as energy substrates. A requirement for long-chain polyunsaturated fatty acids such as eicosapentaenoic acid (20:5*n*-3) and docosahexaenoic acid (22:6*n*-3) is likely since no evidence of bioconversion from their precursors was found. A requirement for arachidonic acid (20:4*n*-6) is also suggested as it is specifically retained throughout development.

INTRODUCTION

Reliable rearing techniques and mass-production of commercially valuable common sole (*Solea solea* L.) juveniles has been successfully achieved in the United Kingdom and France during the last two decades (Shelbourne, 1975; Devauchelle et al., 1987; Baynes et al., 1993). In contrast, Senegal sole, a

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species well adapted to warm climates and commonly exploited in extensive aquacultural production of some southern Europe countries such as Spain or Portugal, has been little studied (Drake et al., 1984; Rodriguez, 1984; Dinis, 1992). At the present time, there is little information on the developmental biology of *S. senegalensis* which would help to assess the rearing potential of this species.

The ability to maintain good growth and high survival rate for marine fish larvae depends on an understanding of their nutritional requirements. Several studies have examined changes in protein, lipid and carbohydrate during early development of fish (Cetta and Capuzzo, 1982; Kimata, 1982, 1983; Vetter et al., 1983; Ostrowski and Divakaran, 1991; Whyte et al., 1993). Some authors (Fyhn and Serigstad, 1987; Fraser et al., 1988; Fyhn, 1989) have also suggested that the pattern of nutrient use by embryos and pre-feeding larvae may indicate the nutritional needs of the first-feeding fish larvae. This presumes that the biochemical system for the metabolism of endogenous nutrients would also be the same once the fish begins exogenous feeding (Ostrowski and Divakaran, 1991).

Lipids are one of the most important components of fish eggs, providing energy reserves and components of cell biomembranes (Sargent et al., 1989). In general, lipids (Tocher and Sargent, 1984; Sargent et al., 1989) and free amino acids (Fyhn and Serigstad, 1988; Fyhn, 1989) have been regarded as substrates for aerobic energy production in marine fish during early development. In fishes such as the winter flounder (*Pseudopleoronectes americanus*) and red sea bream (*Pagrus major*) lipid is used mainly after hatching, with protein and carbohydrate utilized before hatching (Cetta and Capuzzo, 1982; Kimata, 1983). The fatty acid requirements of some larval flatfish species have been much studied (Gatesoupe et al., 1977; Minkoff, 1987; Tzoumas, 1988; Bolla, 1989; Rigby, 1990; Dickey-Collas and Geffen, 1992). However, nutritionally important polyunsaturated fatty acids (mainly eicosapentaenoic acid (20:5*n*-3) and docosahexaenoic acid (22:6*n*-3)) may be lost as a consequence of utilizing phospholipids as an energy source (Tocher et al., 1985a,b; Fraser et al., 1988; Koven et al., 1989; Rainuzzo et al., 1992). In red sea breem, 22:6*n*-3 decreased in both neutral and polar lipids to a greater extent than major saturated and monounsaturated fatty acids (Kimata, 1983).

In the present paper we aimed to estimate the metabolic characteristics and nutritional requirements of Senegal sole first-feeding larvae by studying the changes in biochemical composition and fatty acid content that occur during the rapid development of the embryonic and yolk sac larval stages.

MATERIALS AND METHODS

Broodstock

Wild broodstock of Senegal sole (*Solea senegalensis*) from the salt marshes in the Bay of Cadiz (SW Spain) were caught in October 1992 and maintained

in captivity for 5 months before they spawned naturally for the first time. Broodstock were adequately treated to remove external parasites (5 ppm Furanace bath) and then placed in 10-m^3 rectangular tanks about 1 m in depth. Fish were kept under a natural photoperiod, at a temperature of 19 ± 1 °C and a constant salinity of 34‰ at a density of 2 fish/m^2. Three males were stocked for every female. A diet of fresh food comprising mussels (*Mytilus edulis*), cuttlefish (*Ilex* sp.) and polichaete (*Nereis* sp.) was given once per day to an average of 3% total broodstock wet weight.

Egg production and larval rearing

First spawning occurred during the period from the end of April to early June and buoyant fertilized eggs were collected in a mesh screen (500 μm) from the spawning tanks. Eggs from the same batch were transferred to 500-litre fibreglass cylindroconical tanks for hatching and larvae were stocked at a density of 60 individuals per litre. Eggs hatched at approximately 16–20 h after spawning and yolk sac larvae lasted for about 36 h after hatching. Larvae were offered rotifers (*Brachionus plicatilis*) at a density of 5 individuals/ml, cultured on marine Haptophyceae algae (*Isochrysis galbana*) from day 2 to day 5 post-hatch, and the same algae was used to produce "green water" at a density of 150 000–200 000 cells/ml.

Sample collection

Eggs from the same batch, approximately 10–12 h after spawning, were collected in a mesh screen, washed in distilled water and blotted on filter paper before being frozen in liquid nitrogen and stored in a freezer at −80 °C for analysis. The same treatment was used with yolk sac larvae (36 h after hatching) and first-feeding larvae fed rotifers (5 days after hatching).

Dry weight, elemental composition and biochemical composition determinations

Triplicates of preweighed samples (approx. 500 mg wet weight of rotifer, eggs, yolk sac larvae and first-feeding larvae) were dried at 110 °C for 24 h. The dry weights were determined after cooling in vacuo for at least 1 h. The elemental composition (C, N, H) analyses were performed in an elemental analyzer (Carlo Erba 1106), using cyclohexanone 2,4-dinitrophenylhydrazone as a standard. Triplicates of approximately 1 mg dry weight were accurately weighed and analyzed. Protein content was obtained by multiplying the nitrogen content by 6.25. Total lipid content was determined gravimetrically after extraction as described below. Carbohydrate content was 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.

Total lipid extraction, lipid class separation and quantification

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

Total lipid fatty acid analyses

Fatty acid methyl esters were prepared by acid-catalyzed transmethylation of the total lipids for 16 h at 50°C, using nonadecanoic acid (19:0) as the internal standard (Christie, 1989). Methyl esters were extracted and purified as described previously (Tocher and Harvie, 1988). The fatty acid methyl esters were analyzed in a Hewlett-Packard 5890A gas chromatograph equipped with a chemically bonded (PEG) Omegawax-320 fused silica wall coated capillary column (30 m×0.32 mm i.d., Supelco Inc., Bellefonte, USA) using hydrogen as carrier gas with a thermal gradient from 185°C to 235°C. Individual fatty acid methyl esters were identified by comparison with known standards and quantified using a Hewlett-Packard 3394 recording integrator.

Statistical analysis

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

RESULTS

Changes in the biochemical composition of fertilized eggs, yolk sac larvae and first-feeding larvae are shown in Table 1. The dry weight (% of wet weight) significantly increased from egg to yolk sac larvae, but no significant differences were observed between dry weights of yolk sac larvae and first-feeding larvae. Total protein and total lipid contents were not significantly different among different developmental stages, although the mean values showed a downward trend. No significant difference was also detected in total carbohydrate content between egg and yolk sac larvae, although a significant increase occurred between non-feeding yolk sac larvae and first-feeding larvae. However, ash content decreased while a significant increase in non-identified compounds ("others" in Table 1) was observed.

Total carbon content remained constant between egg and yolk sac larvae

TABLE 1

Dry weight, gross composition (protein, lipid, carbohydrate, ash, carbon, nitrogen and hydrogen), C/N ratios and lipid class compositions in fertilized eggs (A), yolk sac stage larvae (B) and first-feeding larvae of Senegal sole

	Developmental stage		
	(A)	(B)	(C)
Dry weight (%)	8.5 ± 0.2^{a}	10.9 ± 0.6^{b}	10.5 ± 0.7^{b}
Protein (%DW)	56.4 ± 2.5	55.5 ± 0.4	50.5 ± 1.2
Lipid (%DW)	11.7 ± 0.4	11.4 ± 0.7	11.0 ± 0.8
Carbohydrate (%DW)	2.9 ± 0.1^{a}	3.2 ± 0.4^{a}	4.9 ± 0.1^{b}
Ash (%DW)	21.7 ± 2.6^{a}	16.2 ± 0.9^{b}	17.8 ± 0.2^{ab}
Others (%DW)	7.3 ± 0.4^{a}	13.7 ± 0.9^{b}	15.8 ± 1.1^{b}
Carbon (%DW)	44.1 ± 0.5^{a}	44.1 ± 0.7^{a}	36.9 ± 1.1^{b}
Nitrogen (%DW)	8.6 ± 0.4^{ab}	8.8 ± 0.1^{a}	8.0 ± 0.2^{b}
Hydrogen (%DW)	7.0 ± 0.2^{a}	7.0 ± 0.2^{a}	5.9 ± 0.2^{b}
C/N ratio	5.1 ± 0.3^{a}	5.0 ± 0.2^{ab}	4.6 ± 0.2^{b}
Total polar lipids (%)	32.9 ± 4.8^{a}	43.1 ± 4.0^{ab}	51.1 ± 5.4^{b}
Sphingomyelin	0.9 ± 0.2	1.7 ± 0.7	0.9 ± 0.2
Phosphatidylcholine	21.8 ± 3.3	24.1 ± 2.6	23.1 ± 2.3
Phosphatidylethanolamine	7.4 ± 0.9	10.0 ± 1.0	10.2 ± 1.0
Phosphatidylserine	0.4 ± 0.1^{a}	2.1 ± 0.3^{b}	3.8 ± 0.4^{c}
Phosphatidylinositol	1.3 ± 0.2^{a}	2.6 ± 0.3^{b}	3.4 ± 0.3^{b}
Phosphatidic acid/cardiolipin	1.0 ± 0.1^{a}	1.6 ± 0.2^{a}	2.3 ± 0.2^{b}
Glycosylglycerides	0.9 ± 0.1^{a}	1.4 ± 0.3^{a}	4.0 ± 0.6^{b}
Pigments	tr	tr	2.5 ± 0.2
Total neutral lipids (%)	65.3 ± 5.0^{a}	56.9 ± 4.0^{ab}	48.6 ± 5.0^{b}
Cholesterol	9.1 ± 2.2^{a}	9.9 ± 0.4^{ab}	13.9 ± 1.4^{b}
Free fatty acids	0.9 ± 0.2^{a}	2.9 ± 0.4^{b}	9.5 ± 0.6^{c}
Triacylglycerol	31.0 ± 3.5^{a}	27.9 ± 2.9^{a}	17.3 ± 1.9^{b}
Sterol esters	23.3 ± 2.8^{a}	16.2 ± 1.5^{b}	7.7 ± 1.0^{c}

Data are means $\pm$s.d. ($n=3$). s.d. $=0.0$ implies an s.d. of <0.05. Values in each row bearing a different superscript letter are significantly different ($P<0.05$).

stages, but a significant decrease occurred between yolk sac larvae and first-feeding larvae. Nitrogen content remained constant and hydrogen content showed a significant decrease from yolk sac larvae to first-feeding larvae. The C/N ratios showed a downward trend throughout development.

The proportion of total neutral lipids was predominant in egg and yolk sac larvae stages, but in first-feeding larvae phospholipids accounted for 51.1% of the total lipids. Total polar lipids also increased during development, mainly due to a significant increase in phosphatidylserine (which increased by 81%

TABLE 2

Changes in total lipid fatty acid content (μg fatty acid/mg total lipid) in fertilized eggs (A), yolk sac stage larvae (B) and first-feeding larvae (C) of the Senegal sole

Fatty acid	Developmental stage		
	(A)	(B)	(C)
14:0	19.9±2.2^{a}	15.4±0.2^{a}	24.6±1.4^{b}
15:0	24.6±2.1^{ab}	25.0±0.4^{a}	20.8±1.0^{b}
16:0	194.7±11.5^{a}	164.2±3.3^{b}	108.7±2.3^{c}
17:0	17.5±0.9^{a}	14.6±0.3^{b}	11.3±0.2^{c}
18:0	30.8±1.3^{a}	32.1±0.6^{a}	38.4±0.7^{b}
20:0	3.9±0.6^{a}	2.6±0.2^{b}	2.5±0.1^{b}
22:0	0.3±0.0^{a}	0.4±0.1^{a}	1.2±0.3^{b}
Total saturated	291.9±18.6^{a}	254.4±4.4^{b}	207.5±2.4^{c}
16:1*n*-7	64.9±3.8^{a}	53.3±1.2^{b}	29.5±0.8^{c}
18:1*n*-9	110.2±4.8^{a}	97.0±4.9^{b}	62.4±1.1^{c}
18:1*n*-7	26.9±1.3^{a}	23.6±0.3^{b}	18.5±0.5^{c}
20:1*n*-9	7.2±0.9^{a}	5.2±0.1^{b}	6.7±0.2^{c}
20:1*n*-7	3.1±1.2^{a}	4.0±0.1^{ab}	5.9±0.2^{b}
22:1*n*-11	1.5±0.8^{ab}	3.0±1.0^{a}	0.6±0.3^{b}
22:1*n*-9	2.5±0.5	3.3±0.8	1.9±0.0
Total monounsaturated	216.2±11.0^{a}	189.5±4.7^{b}	125.5±3.1^{c}
Total *n*-9	119.8±6.2^{a}	105.5±4.3^{b}	71.1±1.3^{c}
Total *n*-7	94.9±3.9^{a}	81.6±1.2^{b}	54.4±1.5^{c}
16:2	5.0±1.5	5.4±0.3	4.1±0.7
16:3	7.4±0.3^{a}	5.9±0.1^{b}	4.1±0.1^{c}
16:4	9.0±1.1	9.2±0.3	7.3±0.2
18:2*n*-6	6.7±0.2^{a}	6.3±0.2^{a}	27.4±0.5^{b}
18:3*n*-3	3.4±1.0^{a}	2.6±0.3^{a}	11.3±0.2^{b}
18:4*n*-3	4.5±0.9^{a}	3.8±0.3^{a}	19.9±0.4^{b}
20:2*n*-6	4.1±0.2^{a}	3.7±0.1^{a}	1.9±0.1^{b}
20:3*n*-6	1.4±0.0	1.4±0.0	1.5±0.1
20:3*n*-3	1.9±0.1^{a}	0.5±0.2^{b}	1.2±0.3^{c}
20:4*n*-6	21.3±0.6	21.2±0.4	22.0±0.3
20:4*n*-3	3.8±0.2^{a}	2.8±0.1^{b}	11.5±0.2^{c}
20:5*n*-3	26.6±0.9^{a}	23.2±0.4^{b}	12.9±0.2^{c}
22:2*n*-6	1.5±0.1^{a}	1.8±0.1^{a}	0.5±0.1^{b}
22:3*n*-6	2.4±0.1	1.7±0.6	2.3±1.1
22:3*n*-3	9.0±0.3^{a}	8.4±0.2^{a}	4.7±0.1^{b}
22:4*n*-6	2.0±0.1	1.9±0.2	1.8±1.0
22:4*n*-3	1.1±0.1^{a}	0.5±0.2^{b}	0.1±0.0^{c}
22:5*n*-6	17.6±0.6^{a}	16.4±0.2^{a}	14.8±0.3^{b}
22:5*n*-3	37.4±1.3^{a}	31.3±0.7^{b}	14.2±0.3^{c}
22:6*n*-3	102.8±3.7^{a}	91.0±2.5^{b}	87.1±1.3^{b}
Total polyunsaturated	270.7±9.1^{a}	240.5±4.5^{b}	252.1±4.7^{ab}
Total *n*-6	63.5±3.3^{a}	60.7±1.2^{a}	77.3±1.8^{b}
Total *n*-3	207.1±5.8^{a}	179.3±3.5^{b}	174.3±2.8^{b}

Data are means ±s.d. ($n=3$). s.d.=0.0 implies an s.d. of <0.05. Values in each row bearing a different superscript letter are significantly different ($P<0.05$). Totals include some minor components not shown.

from egg to yolk sac larvae and by 45% from yolk sac larvae to first-feeding larvae), phosphatidylinositol, phosphatidic acid/cardiolipin and glycosylglyceride fractions. In contrast, total neutral lipids showed a declining trend due to losses in triacylglycerol and sterol ester fractions (Table 1).

Changes in total lipid fatty acid content, expressed as μg fatty acid per mg

of total lipid, throughout the different developmental stages, are shown in Table 2. The biochemical composition and fatty acid contents of the rotifer *B. plicatilis*, fed on marine Haptophycean microalgae (*I. galbana*), used to feed the larvae just after the yolk sac was reabsorbed, are shown in Table 3. Total fatty acid content showed a significant decrease throughout development. Total fatty acid content decreased by 12.1% from fertilized egg to yolk sac larvae and by 15.5% from yolk sac larvae to first-feeding larvae. Total saturated fatty acids significantly decreased during development, mainly due to losses of 16:0, 17:0 and 20:0. Total monounsaturated fatty acids also declined and either component of the *n*-9 (such as 18:1*n*-9 or 20:1*n*-9) or the *n*-7 series (such as 16:1*n*-7 or 18:1*n*-7) showed significant decreases. Total polyunsaturated fatty acids exhibited a significant decrease of approximately 11.1% from fertilized egg to yolk sac larvae stage and only a 4.2% decrease from yolk sac larvae to first-feeding larvae. C_{18} fatty acids such as 18:2*n*-6, 18:3*n*-3 and 18:4*n*-3 did not show any significant difference between egg and yolk sac larvae but then exhibited a significant increase from yolk sac larvae to first-feeding larvae. It is noteworthy that arachidonic acid (20:4*n*-6) content remained constant during development. In contrast, fatty acids containing five double bonds such as eicosapentaenoic acid (20:5*n*-3) and docosapentaenoic acid (22:5*n*-3) significantly decreased during the whole period. Docosahexaenoic acid (22:6*n*-3) significantly decreased (11.5%) from egg to yolk sac larvae, but no significant difference was found between the contents of yolk sac larvae and first-feeding larvae.

DISCUSSION

It has been suggested (Heming and Buddington, 1988) that changes in the chemical composition of the egg or yolk sac larvae during development may indicate nutritive needs during early feeding stages. The order and magnitude of macronutrient catabolism appears to vary with the physiological requirements of each fish species. In the present study, Senegal sole did not show significant decreases in total protein and total lipid during development; however, downward trends were shown by mean values, indicating a possible use of both protein and lipid during development. Therefore, the C/N ratio, which is considered an indicator of the lipid/protein ratio (Anger, 1988), decreased during development, suggesting a preferential utilization of lipid instead of protein as the energy source. This fact agrees with those found in the red drum (*Sciaenops ocellata*) (Vetter et al., 1983) and the dolphin (*Coryphaena hippurus*) (Ostrowski and Divakaran, 1991). The predominance in the utilization of lipid seems to be related to the metabolic needs of warm water marine fish that have rapid periods of embryonic and yolk sac development. Carbohydrates do not seem to have an important energetic role in

Senegal sole, since a significant increase was observed from yolk sac larvae to first-feeding larvae.

Triacylglycerol is usually the primary form of energy storage in egg and yolk sac larvae of many fish species (Fraser et al., 1987; Fraser, 1989). In the present study, triacylglycerol content decreased, as did triacylglycerol/cholesterol ratios. However, this should not be interpreted as an undernourishment condition of first-feeding larvae, but merely demonstrates a different pattern of energetic metabolism to that of the Atlantic herring (*Clupea harengus*) and cod (*Gadus morhua*) larvae (Tocher et al., 1985b; Fraser et al., 1987, 1988). In the rapid development of Senegal sole, triacylglycerol reserves apparently were used to obtain the necessary metabolic energy to enhance development. Therefore, phospholipid synthesis was taking place as the proportions of phosphatidylserine, phosphatidylinositol and phosphatidic acid/cardiolipin did increase significantly, while major phospholipid classes such as phosphatidylcholine and phosphatidylethanolamine remained constant during development (Table 1). The significant increase in free fatty acids in yolk sac larvae reflects a rapid lipid metabolism. Catabolism of triacylglycerol is required to utilize fatty acids as substrates for β-oxidation to obtain metabolic energy and also to shuttle unsaturated fatty acids for phospholipid and biomembrane synthesis. The greater increase in the free fatty acid fraction observed in the first-feeding larvae was due to the high level of this fraction contained in the rotifer diet, as can be seen from the data in Table 3. Similar observations have been reported by developing larvae of red drum (Vetter et al., 1983), red sea bream (Kimata, 1983), gilthead sea bream (*Sparus aurata*) (Mourente, 1989) and turbot (*Scophthalmus maximus*) (Rainuzzo et al., 1992). Other species such as the plaice (*Pleuronectes platessa*) have an intermediate pattern, using both neutral and polar lipids for energetic purposes (Rainuzzo et al., 1991, 1992). In contrast, most cold water fish, such as Atlantic herring, cod or the halibut (*Hippoglossus hippoglossus*), utilize phospholipids (mainly phosphatidylcholine) as the primary energy source (Tocher et al., 1985a,b; Fraser et al., 1987, 1988; Rainuzzo et al., 1991, 1992). Obviously, a substantial portion of phospholipid and cholesterol is retained in the developing larval body (Sargent et al., 1989).

It is evident that Senegal sole utilize saturated and monounsaturated fatty acids to a greater extent than polyunsaturated fatty acids during early development (Table 2). Major saturated and monounsaturated fatty acids such as 16:0, 16:1*n*-7, 18:1*n*-9 and 18:1*n*-7 were consumed from egg to first-feeding larvae for energetic purposes. Polyunsaturated fatty acids did present different patterns of utilization. C_{18} fatty acids such as 18:2*n*-6, 18:3*n*-3 and 18:4*n*-3 remained constant from egg to yolk sac larvae and then accumulated in lipids of first-feeding larvae (possibly in the triacylglycerol fraction) due to the high content of these fatty acids in the rotifer diet (Table 3). However, although no significant differences were shown in the content of 18:2*n*-6,

TABLE 3

Gross composition (dry weight percentage), lipid class composition (total lipid percentage) and total lipid fatty acid content (μg fatty acid/mg dry weight) in rotifer *Brachionus plicatilis* cultured with the marine algae *Isochrysis galbana* used to feed Senegal sole larvae

Dry weight	16.0±0.7	14:0	5.1±0.1
		15:0	5.0±0.1
Protein	36.2±1.4	16:0	8.6±0.2
Lipid	11.6±0.6	17:0	0.8±0.0
Carbohydrate	6.8±0.5	18:0	2.3±0.1
Ash	17.2±0.6	20:0	0.1±0.0
Others	28.2±1.1	22:0	0.4±0.1
		Total saturated	22.4±0.2
Carbon	37.8±1.1	16:1*n*-7	3.9±0.1
Nitrogen	5.8±0.2	18:1*n*-9	5.4±0.1
Hydrogen	5.8±0.2	18:1*n*-7	1.6±0.0
C/N ratio	6.5±0.3	20:1*n*-9	1.9±0.0
		20:1*n*-7	0.3±0.0
Total polar lipids	35.2±1.9	22:1*n*-11	0.3±0.1
		22:1*n*-9	0.7±0.3
Phosphatidylcholine	7.1±0.5	Total monoenes	14.2±0.3
Phosphatidylethanolamine	9.1±0.7	Total *n*-9	7.9±0.1
Phosphatidylserine	1.1±0.1	Total *n*-7	5.9±0.2
Phosphatdiylinositol	3.8±0.3	16:2	0.8±0.1
Phosphatidic acid/cardiolipin	2.3±0.2	16:3	0.5±0.0
Glycolipids	3.5±0.6	16:4	0.9±0.0
Sphyngomyelin	0.4±0.1	18:2*n*-6	10.3±0.0
Pigments	7.9±2.7	18:3*n*-3	7.0±0.1
		18:4*n*-3	3.3±0.1
Total neutral lipids	60.1±2.9	20:2*n*-6	0.4±0.0
		20:3*n*-6	0.4±0.0
Cholesterol	9.7±0.6	20:3*n*-3	0.6±0.0
Free fatty acid	12.0±0.4	20:4*n*-6	0.9±0.1
Triacylglycerol	32.6±2.4	20:4*n*-3	2.2±0.0
Sterol ester	5.7±0.6	20:5*n*-3	1.5±0.1
		22:2*n*-6	0.2±0.0
		22:3*n*-6	0.2±0.0
		22:4*n*-6	0.2±0.0
		22:5*n*-6	0.4±0.0
		22:3*n*-3	0.1±0.0
		22:4*n*-3	0.3±0.0
		22:5*n*-3	0.3±0.0
		22:6*n*-3	1.8±0.1
		Total polyunsaturated	32.9±0.3
		Total *n*-6	14.0±0.2
		Total *n*-3	18.8±0.1

Data are means ±s.d. ($n=3$). s.d.=0.0 implies an s.d. of <0.05. Totals include some minor components (<0.1%) not shown.

18:3*n*-3 and 18:4*n*-3 between egg and yolk sac larvae, mean values showed a downward trend. This may indicate that they were not being utilized to a great extent either for energy purposes or as precursors for desaturation or elongation to longer-chain more unsaturated fatty acids. When larvae start to feed, competition for Δ^6-desaturase may arise due to the high content of both 18:2*n*-6 and 18:3*n*-3 in the rotifer diet (Sargent et al., 1989). Major C_{20} polyunsaturated fatty acids showed two different patterns of utilization during development. First, *n*-3 C_{20} fatty acids, such as 20:5*n*-3, showed a significant decrease. This may indicate that this particular fatty acid was being used for either energetic purposes or as a precursor to be elongated to 22:5*n*-3 and then Δ^4-desaturated to docosahexaenoic acid, which is very important in fish development (Sargent et al., 1993). It is generally considered at the present time, that marine fish are incapable of converting C_{18} polyunsaturated fatty acids to their highly unsaturated C_{20} and C_{22} products due to a deficiency in the Δ^5 fatty acid desaturase (Sargent et al., 1990). Possibly, the whole process of polyunsaturated fatty acid bioconversion during Senegal sole development was compromised with very low activity due to inhibition of the Δ^6-desaturase by the end-product, 22:6*n*-3, contained in egg and yolk sac larvae lipid reserves (Sargent et al., 1989). Interestingly, arachidonic acid remained constant during development. Despite the great preponderance of *n*-3 polyunsaturated fatty acids in marine fish membrane lipids, prostaglandins in marine fish are known to be synthesized primarily from 20:4*n*-6 rather than 20:5*n*-3 (Sargent et al., 1990). The persistence in the retention of arachidonic acid during the early development of the Senegal sole may well indicate a specific requirement for this particular fatty acid by the first-feeding larvae of this species. This seems to be in agreement with the fact that starved larvae of cod (*G. morhua*) and dolphin (*C. hippurus*) preferentially retain 22:6*n*-3 and 20:4*n*-6 in their polar lipid fractions, which strongly suggests that these fatty acids are important to survival in these species (Fraser et al., 1988; Ostrowski and Divakaran, 1990).

Docosahexaenoic acid is essential for the development of brain and retina in marine fish (Bell and Dick, 1991; Mourente et al., 1991; Mourente and Tocher, 1992, 1993; Tocher et al., 1992; Sargent et al., 1993) and deficient fish may show behavioural abnormalities possibly due to visual and neural impairment (Navarro and Sargent, 1992). The significant decrease in 22:6*n*-3 content from egg to yolk sac larvae with concomitant parallel decreases in 20:5*n*-3 and 22:5*n*-3 indicates that little, if any, bioconversion or docosahexaenoic acid synthesis was occurring during non-feeding development. Thus, 22:6*n*-3 must be supplemented to the first-feeding larvae to achieve good growth and performance of the developing larvae, since a significant depletion of 20:5*n*-3, 22:5*n*-3 and 22:6*n*-3 was occurring during development to *S. senegalensis* larvae, as established in species such as *S. aurata* (Koven et al., 1989, 1990; Mourente et al., 1993) or *C. hippurus* (Ostrowski

and Divakaran, 1990). This is not in agreement with the results found in turbot larvae (Minkoff, 1987), common sole larvae (Tzoumas, 1988) and plaice larvae (Dickey-Collas and Geffen, 1992). The Haptophycea marine algae *I. galbana* contains high levels of 22:6*n*-3 (Mourente et al., 1990, 1993) and the rotifer, *B. plicatilis*, cultured on this algae appears to be a good live first food for Senegal sole larvae since the content of 22:6*n*-3 did not significantly decrease from yolk sac larvae to first-feeding 5 days after hatching. First-feeding larvae of several marine fishes, such as cod or halibut, are reported to ingest algal cells in considerable amounts (Van der Meeren, 1991; Naas et al., 1992). Thus the larvae of Senegal sole might have digested the *I. galbana* in the "green water", and also contributed to the nutrition of the first-feeding larvae. However, there is some controversy since some authors emphasise the role of the *n*-3 highly unsaturated fatty acids (such as 20:5*n*-3 and 22:6*n*-3) and others maintain that species such as the common sole (Minkoff, 1987; Tzoumas, 1988) and plaice (Dickey-Collas and Geffen, 1992) do not require 22:6*n*-3 in the diet if adequate 20:5*n*-3 is present. In any case, further research is needed to assess nutritional requirements and good performance of Senegal sole in culture. Studying the variation in fatty acid composition of the major phospholipid and neutral lipid classes could explain whether *n*-3 highly unsaturated fatty acids (20:5*n*-3, 22:5*n*-3 and 22:6*n*-3) occurring in neutral lipid fractions are preferentially utilized (mainly triacylglycerol) while being selectively retained, and possibly augmented in major phospholipid classes.

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