



Lipid enrichment for Senegalese sole (*Solea senegalensis*) larvae: effect on larval growth, survival and fatty acid profile

S. MORAIS^{1,3,*}, L. NARCISO¹, E. DORES² and P. POUSSÃO-FERREIRA²

¹Laboratório Marítimo da Guia, Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Estrada do Guincho, 2750-642 Cascais, Portugal; ²IPIMAR/CRIPSul Av. 5 de Outubro s/n, 8700-305 Olhão, Portugal; ³Current address: Sofia Morais, CCMAR, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal; *Author for correspondence (e-mail: smorais@ualg.pt; phone: +351-289 800 900; fax: +351-289 818 353)

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Abstract. Results from three larval Senegalese sole (*Solea senegalensis*) feeding trials using non-enriched *Artemia* and *Artemia* enriched with Super HUFA[®], Arasco[®], sunflower oil and microalgae are presented and the effects on larval survival, growth and fatty acid (FA) composition are reported. The FA profile of Senegalese sole eggs was analysed to gather information about the nutritional requirements of the early larval stages and a high DHA/EPA ratio (4.3) was found. However, there was no evidence of a high dietary demand for DHA or EPA, given that no relationship was found between dietary HUFA concentration and larval growth and survival. When larvae were fed non-enriched *Artemia* a significantly better growth and comparable survival were obtained than with *Artemia* enriched with Super HUFA[®] (containing the highest HUFA level and DHA/EPA ratio). The FA profiles of the larvae generally reflected those of their diets. DHA was an exception, as it was present in high proportions, even in larvae fed DHA-deficient prey. Total FAME concentration decreased during larval development, with SFA, MUFA and PUFA being equally consumed; HUFA appeared to be less used, with its relative concentration being either kept constant (particularly EPA and ARA) or increased (DHA). A specific requirement for ARA in the first larval stages could not be confirmed but it was always present in considerable amounts, even in larvae fed an ARA poor diet.

Abbreviations: ARA – arachidonic acid, 20:4n-6; DAH – days after hatching; DHA – docosahexaenoic acid, 22:6n-3; DPA – docosapentaenoic acid, 22:5n-3; EPA – eicosapentaenoic acid, 20:5n-3; FA – fatty acid; FAME – fatty acid methyl esters; HUFA – highly unsaturated fatty acids; LA – linoleic acid, 18:2n-6; LNA – linolenic acid, 18:3n-3; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; SFA – saturated fatty acids

Introduction

Southern Europe is an area of extensive fish production, but a rapid increase in the production of European seabass (*Dicentrarchus labrax* Linnaeus, 1758) and gilt-head seabream (*Sparus aurata* Linnaeus, 1758) has caused a decline in market prices for these species. The Senegalese sole (*Solea senegalensis* Kaup, 1858) is commonly raised in extensive polyculture (in earthen ponds) in the South of Portugal and Spain (Drake et al. 1984; Rodriguez 1984; Dinis 1986, 1992), where it has a higher growth rate than seabass (Drake et al. 1984; Dinis and Reis 1995).

In addition, a high price and market demand has stimulated producers to ongrow this species. Hatchery production of Senegalese sole for intensive or semi-intensive culture seems to be economically viable (Dinis 1992) but there is still insufficient knowledge regarding larval and juvenile nutritional requirements (Dinis and Reis 1995; Dinis et al. 1999).

Senegalese sole larvae are fed on live prey (*Brachionus plicatilis* O.F. Muller, 1786 and *Artemia* sp.) during the first 40 days after hatching (DAH), although earlier weaning on inert feeds has been attempted (Cañavate and Fernández-Díaz 1999; Dinis et al. 1999). The nutritional value of live prey is, therefore, a key factor in the success of larval rearing. Lipids are very important nutritionally, as a result of their double role as an energy source and as structural components of biological membranes (Sargent et al. 1993). Fish have a dietary requirement for polyunsaturated fatty acids (PUFA) of the (n-3) series, and the content of the (n-3) highly unsaturated fatty acids (HUFA) docosahexaenoic acid (DHA; 22:6n-3) and eicosapentaenoic acid (EPA; 20:5n-3), is considered a central factor in determining the nutritional value of a prey for rearing marine fish larvae (Watanabe et al. 1983; Sargent et al. 1993; Rainuzzo et al. 1997; Sargent et al. 1997, 1999).

In the present work, three feeding trials were conducted using non-enriched *Artemia* and *Artemia* enriched with different products – two commercial products (Super HUFA[®] and Arasco[®]), a sunflower oil emulsion and an algal mixture (50% *Tetraselmis chui* Butcher and 50% *Isochrysis galbana* Parke) – with the objective of studying the effect of the prey lipid profile on growth, survival and fatty acid (FA) composition of larval Senegalese sole. The FA profile of sole eggs was also analysed to gain insight into the FA requirements of the earliest larval stages.

Materials and methods

Larval culture

Three feeding experiments were carried out using larvae obtained from eggs derived from naturally spawning, wild caught, Senegalese sole broodstock kept at the IPIMAR/CRIPSul (Olhão, Portugal) hatchery. During the 24 h incubation period, eggs were placed in 200 l cylindrical-conical tanks, in a flow-through system (0.8–1 l min⁻¹), with filtered seawater (20 µm), gentle bottom aeration, a temperature of 19 ± 1 °C, a salinity of 36 ± 1 g l⁻¹ and an oxygen concentration of 8 mg l⁻¹. Larvae were stocked at 25 larvae l⁻¹ in 200 l cylindrical-conical fibreglass tanks, with a continuous flow (0.8–1 l min⁻¹, gradually increased to 2 l min⁻¹) of filtered (20 µm) and aerated seawater. Temperature was maintained at 20 ± 1 °C, salinity at 36 ± 1 g l⁻¹ and oxygen was kept close to saturation. Larvae were reared under a light:dark regime of 14:10 h. Each treatment had three replicate tanks that were stocked at the onset of exogenous feeding (2 DAH) and all groups were fed identically until 8 DAH. Larvae were fed *Brachionus plicatilis* from 2 to 5 DAH, *Artemia* 'type A.F.' (INVE nv, Belgium) from 3 to 10 DAH and enriched *Artemia* 'type E.G.' (enrichment grade) (INVE nv, Belgium) from 8 DAH until trials were

terminated. *Artemia* and rotifer densities were maintained at approximately 1 and 5 ml⁻¹, respectively, by adding prey throughout the day.

Live prey culture and experimental diets

Microalgae were batch-cultured (Coutteau 1996) and used at peak concentration. Intermediate volumes (2 and 10 l) were used to inoculate 80 l plastic (PVC) bags, where they were cultured at 20 ± 1 °C and 36 ± 1 g l⁻¹, in filtered and sterilised seawater, with F2 nutritive medium, continuous aeration and illumination. Production of *B. plicatilis* was carried out in 500 l cylindrical-conical tanks, at 26 ± 1 °C and a salinity of 20 g l⁻¹. The rotifers were cultured on baker's yeast and enriched with Protein Selco[®] (INVE nv, Belgium), according to manufacturer's recommendations. *Artemia franciscana* Kellogg 1906 cysts were decapsulated and incubated under standard conditions (Sorgeloos et al. 1986) and the newly hatched nauplii were washed and concentrated. In each feeding trial two treatments were tested:

Trial 1 – Non-enriched *Artemia* versus *Artemia* enriched with Super HUFA[®] (Salt Creek Inc., USA);

Trial 2 – *Artemia* enriched with either sunflower oil emulsion (1 ml of sunflower oil emulsified with 0.05 g of egg lecithin and 19 ml of seawater) or microalgae (50% *Tetraselmis chui* and 50% *Isochrysis galbana*, aff. 'Tahitian');

Trial 3 – *Artemia* enriched with either Arasco[®] emulsion (Martek Biosciences, USA) (1 ml of Arasco[®] emulsified with 0.05 g of egg lecithin and 19 ml of seawater) or microalgae (50% *Tetraselmis chui* and 50% *Isochrysis galbana* aff. 'Tahitian').

Artemia E.G. were enriched for 24 h, in vigorously aerated 20 l tanks, at a density of 200–300 nauplii ml⁻¹, 27 ± 1 °C and a salinity of 36 ± 1 g l⁻¹. The enrichment product was added at 0 and 12 h, with each dose consisting of 0.1 g l⁻¹ Super HUFA[®], 10 ml l⁻¹ sunflower oil emulsion and 0.2 g l⁻¹ Arasco[®] emulsion. Microalgal enrichment was conducted in concentrated cultures of 10 l of *Tetraselmis chui* and 10 l of *Isochrysis galbana* aff. 'Tahitian'. Enriched nauplii were harvested and washed with UV-filtered seawater. Non-enriched *Artemia* were treated similarly.

Sampling and biochemical analysis

Samples of 20 larvae were removed periodically from each replicate tank and total length and dry weight were recorded. Dry weight (DW) was measured using a high precision Sartorius Supermicro[®] balance (±0.2 µg), after freeze-drying in a Savant VP100[®]. Larval survival rates were estimated at the end of each experiment, by counting the total number of larvae remaining in the tanks. Survival was only corrected for the larvae sampled at the end of the trial.

Biochemical analyses were performed to determine the FA compositions of Senegalese sole eggs (approximately 12 h after spawning), enriched and non-enriched

Artemia and of larvae 20 and 26 DAH (in trials 1 and 2) and 26 and 36 DAH (in trial 3). Twenty larvae (pooled sample) were removed for analysis from each tank while, for eggs and *Artemia*, samples of 100 mg DW and 150 mg DW were analysed, respectively. Freeze-dried samples were ground in a Potter homogeniser with chloroform–methanol–water (2:2:1.8) (Bligh and Dyer 1959). After saponification and esterification of the lipid extracts (Metcalf and Schmitz 1961), the fatty acid methyl esters (FAME) were injected into a capillary column (30 m fused silica, 0.32 I.D.) installed in a Varian Star 3400CX gas–liquid chromatograph (GLC). Helium was used as carrier gas, at a flow rate of 1 ml min⁻¹; oven temperature was 180 °C for 7 min and then 200 °C (with a temperature gradient of 4 °C min⁻¹) over a period of 71 min. Both the injector and the FID detector were set at 250 °C. GLC data acquisition and handling was done using a Varian integrator 4290 connected to the GLC. Peak quantification was carried out with a Star Chromatography workstation and peak identification was carried out by reference to cod liver oil chromatograms. For each treatment samples were analysed from two of the tanks. An internal standard (19:0) was included in the samples, and FA composition is presented in terms of µg FA per mg sample DW.

Statistical analysis

To assess differences in growth (total length and dry weight) of larvae submitted to different dietary treatments in each trial, the results obtained at each sampling date were analysed using a one-way ANOVA. In some cases, the assumption of homogeneity of variances was not met and the transformation, e^x , was used. Survival data were analysed after arcsine transformation. Significance was accepted at $P < 0.05$ (Zar 1996).

Results

Larval growth and survival

In trial 1, statistically significant differences in both length and dry weight were observed at 21 and 24 DAH, with non-enriched *Artemia* inducing better growth than *Artemia* enriched with Super HUFA[®] (Figure 1A). In the second trial, no statistically significant differences between treatments were found in total length, although a significant difference in dry weight was found at 21 DAH (Figure 1B); however, later in the trial, there were no significant differences in dry weight. In the third trial the dietary treatments did not induce significant differences in both growth parameters measured.

Survival rates were highly variable, not only between trials, but also within treatments (Figure 2), and no statistically significant differences were found between treatments in any of the trials.

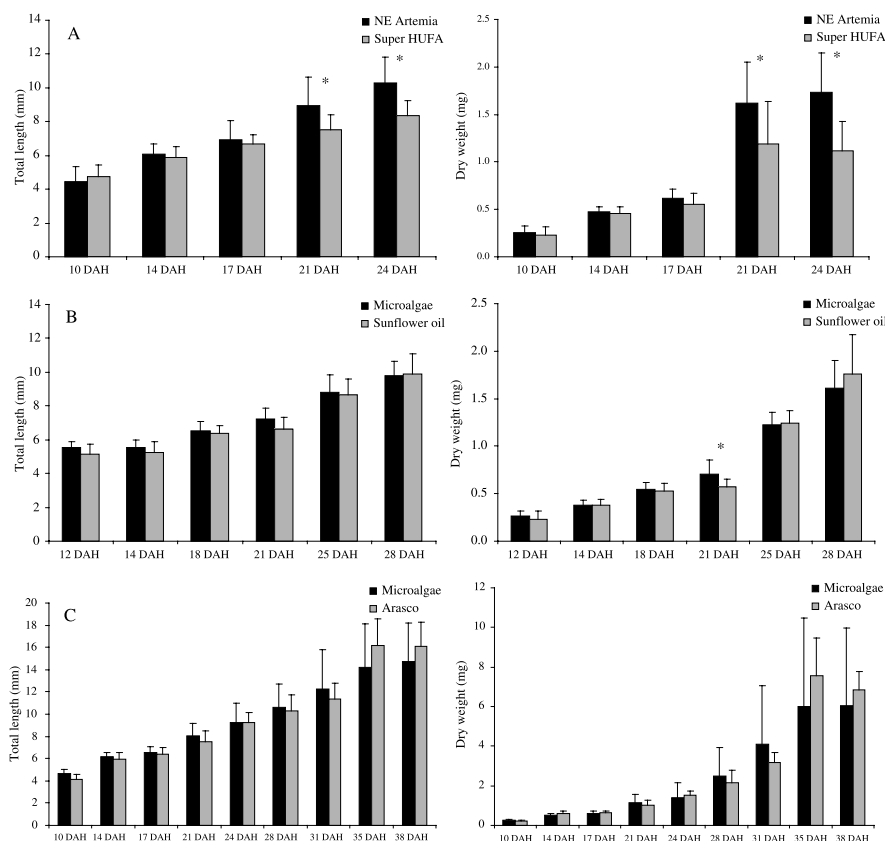


Figure 1. Larval growth – total length (mm) and dry weight (mg), measured at different sampling times during the trials. A – Trial 1: larvae fed non-enriched *Artemia* (NE *Artemia*) or *Artemia* enriched with Super HUFA[®]; B – Trial 2: larvae fed *Artemia* enriched with sunflower oil or with microalgae; C – Trial 3: larvae fed *Artemia* enriched with Arasco[®] or with microalgae. Data are means \pm S.D. of triplicate tanks ($n = 60$). Asterisks indicate statistically significant differences between treatments, at a specific sampling date.

S. senegalensis eggs and *Artemia* fatty acid profile

The FA composition of Senegalese sole eggs had a predominance of saturated fatty acids (SFA) mostly 16:0, followed by monounsaturated fatty acids (MUFA) (particularly 18:1n-9, 16:1n-7 and 18:1n-7) and finally PUFA, mainly EPA and DHA. The eggs were characterised by a relatively high DHA/EPA ratio (4.3), with absolute amounts of EPA and DHA of 2.0 and 8.4 $\mu\text{g mg DW}^{-1}$, respectively, and the (n-3)/(n-6) ratio was found to be 5.4 (Table 1).

The FA profiles of the non-enriched and enriched *Artemia* differed (Table 1). Total FAME and PUFA contents appeared highest in *Artemia* enriched with sunflower oil, followed by Arasco[®], microalgae and Super HUFA[®], whereas

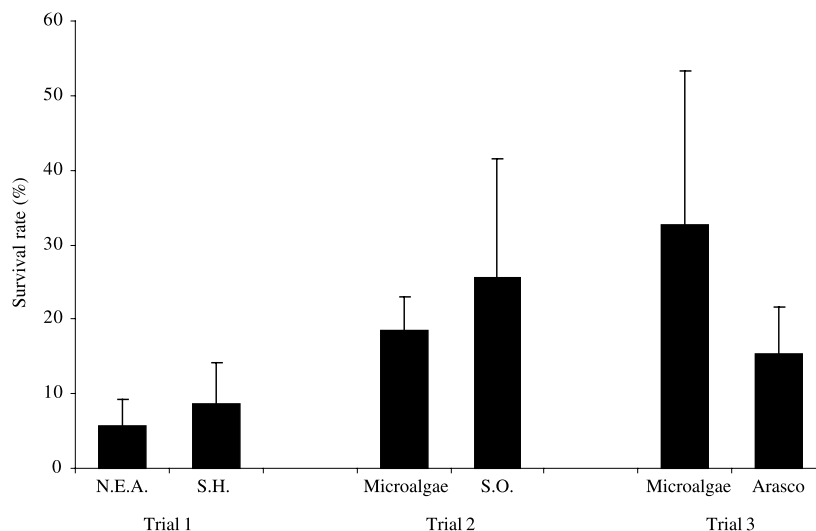


Figure 2. Survival rate (%) of larvae fed different diets, in the three trials. N.E.A. – non-enriched *Artemia*; S.H. – Super HUFA[®]; S.O. – sunflower oil. Data are means \pm S.D. of triplicate tanks.

non-enriched *Artemia* presented the lowest values. The high FAME content was mostly associated with high levels of MUFA in the sunflower oil, Arasco[®] and microalgae diets and SFA, particularly in the microalgae treatment, while the highest content of PUFA was caused mainly by high levels of linoleic (LA; 18:2n-6) and linolenic (LNA; 18:3n-3) acids in these diets. In terms of HUFA, contents appeared highest in *Artemia* enriched with Super HUFA[®], followed by Arasco[®], as a result of the high DHA and EPA concentration (the highest of all prey) in *Artemia* enriched with Super HUFA[®] and a high arachidonic acid (ARA; 20:4n-6) level found in *Artemia* enriched with Arasco[®]. EPA was present in the composition of all *Artemia* (more in the enriched prey), while the DHA content was very low and comparable to the non-enriched *Artemia* (with the exception of *Artemia* enriched with Super HUFA[®]). The ARA content was high in *Artemia* enriched with Arasco[®], comparable in the remaining enriched *Artemia* and lowest in non-enriched *Artemia*. Finally, the highest DHA/EPA and (n-3)/(n-6) ratios were achieved in *Artemia* enriched with Super HUFA[®], while the Arasco[®] and sunflower oil emulsions induced the lowest ratios.

Larval fatty acid profile

Comparing the FA concentration of the larvae submitted to different dietary treatments (Table 2), at the end of the first trial, 26 DAH larvae fed non-enriched *Artemia* presented a marginally higher total FAME concentration than larvae fed *Artemia* enriched with Super HUFA[®], as a result of its relatively higher SFA,

Table 1. Fatty acid composition of *S. senegalensis* eggs, of non-enriched (N.E.) *Artemia* and of *Artemia* enriched in the different tested products (values are means \pm S.D. of duplicate samples).

Fatty acids $\mu\text{g mg}^{-1}$ DW	Sole eggs	<i>Artemia</i>				
		N.E. <i>Artemia</i>	Microalgae	Sunflower oil	Super HUFA	Arasco
14:0	3.8 \pm 0.01	0.7 \pm 0.06	1.7 \pm 0.13	1.3 \pm 0.03	1.2 \pm 0.08	1.3 \pm 0.06
16:0	22.9 \pm 0.19	7.6 \pm 0.02	18.6 \pm 2.35	15.6 \pm 0.57	10.6 \pm 0.01	14.0 \pm 0.40
17:0	0.6 \pm 0.00	0.9 \pm 0.03	2.2 \pm 0.23	1.6 \pm 0.06	1.2 \pm 0.05	1.4 \pm 0.01
18:0	3.7 \pm 0.05	3.5 \pm 0.01	6.7 \pm 0.79	6.1 \pm 0.20	4.4 \pm 0.01	6.9 \pm 0.26
Σ SFA	32.3 \pm 0.25	13.7 \pm 3.15	31.0 \pm 3.70	25.8 \pm 0.83	18.6 \pm 0.42	25.5 \pm 0.83
16:1n-7	7.2 \pm 0.01	3.3 \pm 0.08	8.1 \pm 1.05	7.8 \pm 0.30	5.2 \pm 0.21	6.9 \pm 0.11
18:1n-9	8.0 \pm 0.03	11.6 \pm 0.22	23.9 \pm 3.22	25.8 \pm 0.93	16.4 \pm 0.19	27.1 \pm 0.71
18:1n-7	2.9 \pm 0.03	5.5 \pm 0.63	11.1 \pm 1.73	10.7 \pm 0.42	7.3 \pm 0.73	10.1 \pm 0.23
20:1n-9	1.5 \pm 0.01	0.7 \pm 0.02	0.6 \pm 0.11	0.6 \pm 0.02	1.4 \pm 0.15	0.7 \pm 0.13
Σ MUFA	21.7 \pm 0.05	22.0 \pm 0.10	45.7 \pm 5.88	46.3 \pm 1.68	32.5 \pm 0.18	46.1 \pm 1.36
Anteiso 15:0	0.1 \pm 0.00	0.5 \pm 0.14	1.4 \pm 0.15	1.2 \pm 0.04	0.6 \pm 0.18	1.0 \pm 0.01
Iso 16:0	0.2 \pm 0.04	0.4 \pm 0.01	1.1 \pm 0.04	0.9 \pm 0.01	0.5 \pm 0.02	0.8 \pm 0.01
Anteiso 17:0	0.1 \pm 0.00	0.8 \pm 0.04	1.7 \pm 0.23	1.7 \pm 0.06	0.9 \pm 0.01	1.4 \pm 0.04
Σ Branched	0.8 \pm 0.04	2.1 \pm 0.19	5.1 \pm 0.54	4.4 \pm 0.15	2.5 \pm 0.19	3.8 \pm 0.06
18:2n-6	0.7 \pm 0.01	3.1 \pm 0.02	5.1 \pm 0.57	13.1 \pm 0.40	3.6 \pm 0.05	6.1 \pm 0.12
18:3n-3	0.2 \pm 0.01	8.5 \pm 0.28	18.2 \pm 1.65	26.4 \pm 0.97	12.1 \pm 0.08	22.1 \pm 0.21
18:4n-3	0.1 \pm 0.04	1.4 \pm 0.04	3.1 \pm 0.13	4.4 \pm 0.12	2.1 \pm 0.02	3.5 \pm 0.03
20:4n-6	0.7 \pm 0.01	0.6 \pm 0.01	1.3 \pm 0.25	1.6 \pm 0.05	1.3 \pm 0.22	9.8 \pm 0.60
20:4n-3	0.9 \pm 0.17	0.3 \pm 0.01	0.8 \pm 0.34	0.8 \pm 0.01	1.2 \pm 0.40	0.5 \pm 0.03
20:5n-3	2.0 \pm 0.12	1.4 \pm 0.05	3.7 \pm 0.73	5.1 \pm 0.11	8.1 \pm 0.60	4.7 \pm 0.08
22:6n-3	8.4 \pm 0.72	0.1 \pm 0.02	0.4 \pm 0.12	0.1 \pm 0.00	4.9 \pm 0.31	0.1 \pm 0.01
Σ PUFA	14.9 \pm 0.90	16.1 \pm 0.51	34.5 \pm 3.59	52.7 \pm 1.67	36.0 \pm 1.86	48.3 \pm 1.10
Σ HUFA	14.2 \pm 0.94	3.0 \pm 0.22	7.9 \pm 1.30	8.7 \pm 0.18	18.2 \pm 1.97	16.5 \pm 0.74
Σ n-3	13.0 \pm 0.88	12.0 \pm 0.60	27.3 \pm 2.76	37.5 \pm 1.21	30.3 \pm 1.49	31.4 \pm 0.30
Σ n-6	2.4 \pm 0.02	4.0 \pm 0.09	7.3 \pm 0.83	15.2 \pm 0.46	5.7 \pm 0.37	16.8 \pm 0.81
n-3/n-6 ratio	5.4 \pm 0.32	3.0 \pm 0.23	3.8 \pm 0.05	2.5 \pm 0.01	5.3 \pm 0.09	1.9 \pm 0.07
DHA/EPA	4.32 \pm 0.64	0.07 \pm 0.01	0.10 \pm 0.05	0.02 \pm 0.00	0.61 \pm 0.01	0.02 \pm 0.00
Σ FAME	70.8 \pm 0.63	57.0 \pm 7.24	121.7 \pm 14.40	142.5 \pm 4.73	93.5 \pm 1.68	130.2 \pm 3.50

Only fatty acids present at $>1 \mu\text{g mg}^{-1}$ DW are included in the table but totals (Σ) include all identified fatty acids.

MUFA and PUFA (mostly LNA and ARA) composition. Larvae fed *Artemia* enriched with Super HUFA[®] had a considerably higher DHA and a comparable EPA concentration and, consequently, a higher DHA/EPA ratio. Similarly, the (n-3)/(n-6) ratio was higher in these larvae. In the second trial, 26 DAH larvae fed *Artemia* enriched with sunflower oil presented a substantially higher total FAME composition, resulting from a higher SFA, MUFA and PUFA (mainly LN and LNA) concentration. On the other hand, 26 DAH larvae fed *Artemia* enriched with microalgae presented a slightly higher HUFA concentration (particularly of DHA) and the DHA/EPA and (n-3)/(n-6) ratios were somewhat higher in these larvae. Finally, close to the end of the third trial, 36 DAH larvae fed *Artemia* enriched with Arasco[®] had a higher level of total FAME, SFA, MUFA and PUFA (particularly

Table 2. Absolute fatty acid composition (μg fatty acids mg^{-1} DW) of *S. senegalensis* larvae fed different experimental diets, at 20, 26 or 36 days after hatching (DAH), in the three different trials (values are means \pm S.D. of duplicate samples).

Fatty acids $\mu\text{g mg}^{-1}$ DW	Trial 1				Trial 2				Trial 3			
	N.E. <i>Artemia</i>		Super HUFA [®]		Sunflower oil		Microalgae		Arasco [®]		Microalgae	
	20 DAH	26 DAH	20 DAH	26 DAH	20 DAH	26 DAH	20 DAH	26 DAH	26 DAH	36 DAH	26 DAH	36 DAH
14:0	1.3 \pm 0.71	0.9 \pm 0.01	1.9 \pm 0.47	1.1 \pm 0.04	1.9 \pm 0.02	2.1 \pm 0.52	3.0 \pm 0.04	1.3 \pm 0.29	1.1 \pm 0.18	0.7 \pm 0.15	1.0 \pm 0.12	0.7 \pm 0.17
16:0	14.4 \pm 4.80	13.9 \pm 1.20	20.4 \pm 2.75	13.6 \pm 0.46	20.3 \pm 0.27	14.9 \pm 0.76	24.4 \pm 0.94	13.9 \pm 0.40	14.2 \pm 0.83	9.3 \pm 2.19	11.9 \pm 0.57	8.9 \pm 2.19
17:0	2.0 \pm 0.38	2.2 \pm 0.01	2.6 \pm 0.09	2.0 \pm 0.01	2.5 \pm 0.13	1.6 \pm 0.03	2.6 \pm 0.16	1.5 \pm 0.03	1.6 \pm 0.24	1.1 \pm 0.32	1.3 \pm 0.16	0.7 \pm 0.22
18:0	9.2 \pm 2.67	8.7 \pm 0.60	12.6 \pm 0.60	7.7 \pm 0.23	11.3 \pm 0.01	8.5 \pm 0.27	13.0 \pm 0.61	8.2 \pm 0.23	8.8 \pm 0.55	5.6 \pm 1.15	7.0 \pm 0.50	5.6 \pm 1.35
22:0	1.7 \pm 0.85	1.7 \pm 1.28	1.2 \pm 0.35	0.6 \pm 0.04	1.7 \pm 0.40	1.0 \pm 0.18	1.9 \pm 0.01	0.9 \pm 0.31	1.1 \pm 0.21	0.7 \pm 0.23	1.0 \pm 0.78	0.6 \pm 0.12
Σ SFA	29.8 \pm 9.97	28.6 \pm 0.37	40.6 \pm 3.03	26.0 \pm 1.12	39.6 \pm 0.61	30.1 \pm 0.39	47.2 \pm 1.43	26.8 \pm 0.16	27.7 \pm 1.61	18.0 \pm 3.64	23.2 \pm 2.39	17.2 \pm 3.93
16:1n-7	3.7 \pm 0.80	4.2 \pm 0.12	6.4 \pm 1.44	4.5 \pm 0.06	7.3 \pm 0.13	5.0 \pm 0.05	8.0 \pm 0.75	3.9 \pm 0.45	4.0 \pm 0.36	2.8 \pm 0.86	3.4 \pm 0.35	2.1 \pm 0.69
18:1n-9	16.0 \pm 3.68	16.7 \pm 1.03	24.9 \pm 2.87	16.3 \pm 0.87	25.1 \pm 0.94	18.6 \pm 1.64	26.2 \pm 4.07	15.2 \pm 1.84	20.7 \pm 2.86	12.3 \pm 4.40	14.2 \pm 0.04	8.6 \pm 2.69
18:1n-7	8.4 \pm 1.44	9.2 \pm 0.42	11.4 \pm 2.94	8.0 \pm 0.09	13.6 \pm 0.09	8.8 \pm 0.06	14.7 \pm 2.01	7.7 \pm 0.70	8.8 \pm 0.83	5.7 \pm 1.87	7.5 \pm 0.38	4.2 \pm 1.17
20:1n-9	1.3 \pm 0.95	1.2 \pm 0.31	1.9 \pm 0.44	1.2 \pm 0.01	1.2 \pm 0.04	0.8 \pm 0.05	1.3 \pm 0.34	0.9 \pm 0.07	1.0 \pm 0.11	0.6 \pm 0.10	0.9 \pm 0.28	0.4 \pm 0.08
Σ MUFA	32.2 \pm 8.55	33.7 \pm 0.04	47.0 \pm 8.37	31.8 \pm 0.96	49.3 \pm 0.93	35.9 \pm 2.75	52.7 \pm 7.39	29.6 \pm 2.74	36.3 \pm 3.75	22.5 \pm 7.06	27.9 \pm 0.63	16.1 \pm 4.65
Iso 16:0	0.6 \pm 0.09	0.6 \pm 0.01	0.9 \pm 0.25	0.7 \pm 0.02	1.3 \pm 0.09	1.0 \pm 0.51	1.8 \pm 0.10	0.9 \pm 0.49	0.7 \pm 0.18	0.5 \pm 0.11	0.6 \pm 0.10	0.4 \pm 0.04
Iso 17:0	0.7 \pm 0.17	0.8 \pm 0.03	0.7 \pm 0.51	0.8 \pm 0.04	1.2 \pm 0.00	0.8 \pm 0.09	1.4 \pm 0.09	0.8 \pm 0.04	0.7 \pm 0.04	0.5 \pm 0.12	0.7 \pm 0.04	0.4 \pm 0.15
Anteiso 17:0	0.9 \pm 0.10	1.0 \pm 0.01	1.2 \pm 0.21	0.8 \pm 0.00	1.8 \pm 0.18	1.0 \pm 0.09	1.7 \pm 0.23	0.8 \pm 0.13	1.0 \pm 0.18	0.7 \pm 0.23	1.0 \pm 0.01	0.5 \pm 0.11
Σ Branched	2.7 \pm 0.50	2.9 \pm 0.04	3.6 \pm 0.58	2.8 \pm 0.15	5.2 \pm 0.28	3.5 \pm 0.48	5.8 \pm 0.25	3.1 \pm 0.44	3.0 \pm 0.44	1.9 \pm 0.63	2.7 \pm 0.10	1.5 \pm 0.34
18:2n-6	5.3 \pm 0.67	4.7 \pm 0.47	12.4 \pm 7.79	4.6 \pm 0.79	14.6 \pm 0.38	10.8 \pm 0.20	8.8 \pm 1.86	5.4 \pm 0.75	5.6 \pm 0.27	3.3 \pm 0.90	5.3 \pm 0.20	2.8 \pm 0.79
18:3n-3	7.7 \pm 1.19	8.2 \pm 0.06	11.0 \pm 2.47	7.2 \pm 0.23	18.8 \pm 0.45	10.4 \pm 1.25	18.1 \pm 4.20	8.3 \pm 2.65	10.2 \pm 1.30	7.2 \pm 2.81	8.8 \pm 1.24	4.3 \pm 1.47
18:4n-3	1.6 \pm 0.45	1.6 \pm 0.22	2.5 \pm 0.18	1.5 \pm 0.08	2.6 \pm 0.11	1.5 \pm 0.05	2.9 \pm 0.57	1.2 \pm 0.20	1.8 \pm 0.58	0.9 \pm 0.25	1.5 \pm 0.20	0.6 \pm 0.13
20:4n-6	4.7 \pm 1.60	5.1 \pm 0.01	5.3 \pm 0.47	3.7 \pm 0.69	4.5 \pm 0.25	3.3 \pm 0.57	5.4 \pm 0.41	3.7 \pm 0.11	12.3 \pm 2.23	5.9 \pm 2.03	4.0 \pm 1.19	2.7 \pm 0.57
20:3n-3	1.4 \pm 0.10	1.8 \pm 0.67	2.8 \pm 1.17	0.6 \pm 0.07	2.1 \pm 0.01	2.0 \pm 0.44	3.2 \pm 0.33	1.8 \pm 0.25	1.4 \pm 0.47	1.1 \pm 0.19	1.6 \pm 0.30	1.2 \pm 0.09
20:4n-3	2.4 \pm 1.04	2.4 \pm 1.00	2.5 \pm 0.52	1.5 \pm 0.06	3.1 \pm 0.87	1.7 \pm 0.72	3.0 \pm 0.08	2.0 \pm 0.19	1.1 \pm 0.52	1.0 \pm 0.14	1.7 \pm 1.07	1.1 \pm 0.15
20:5n-3	5.9 \pm 3.25	6.0 \pm 0.84	10.1 \pm 2.76	7.0 \pm 0.98	6.0 \pm 0.21	3.7 \pm 0.77	6.4 \pm 0.86	3.9 \pm 0.31	3.1 \pm 0.20	2.5 \pm 0.04	3.7 \pm 0.97	2.4 \pm 0.63
22:5n-3	2.6 \pm 0.88	3.1 \pm 0.29	4.9 \pm 1.24	3.5 \pm 0.04	2.5 \pm 0.28	2.3 \pm 0.35	2.9 \pm 0.15	2.4 \pm 0.30	2.5 \pm 0.16	1.6 \pm 0.28	2.7 \pm 0.37	1.5 \pm 0.44
22:6n-3	5.2 \pm 2.82	6.1 \pm 1.05	13.7 \pm 3.24	9.6 \pm 0.74	3.7 \pm 0.18	3.3 \pm 0.28	4.2 \pm 0.29	4.0 \pm 0.31	3.0 \pm 0.32	2.6 \pm 0.43	3.5 \pm 0.02	2.6 \pm 0.83

Table 2. (continued)

Fatty acids $\mu\text{g mg}^{-1}$ DW	Trial 1				Trial 2				Trial 3			
	N.E. <i>Artemia</i>		Super HUFA [®]		Sunflower oil		Microalgae		Arasco [®]		Microalgae	
	20 DAH	26 DAH	20 DAH	26 DAH	20 DAH	26 DAH	20 DAH	26 DAH	26 DAH	36 DAH	26 DAH	36 DAH
Σ PUFA	39.0 ± 12.27	42.4 ± 3.38	67.9 ± 3.10	41.1 ± 1.01	60.5 ± 0.48	40.8 ± 1.28	57.3 ± 8.37	34.3 ± 4.16	43.5 ± 1.07	28.0 ± 6.15	35.4 ± 4.43	21.3 ± 4.36
Σ HUFA	24.2 ± 10.94	27.6 ± 2.76	41.7 ± 7.63	27.6 ± 0.79	24.3 ± 1.41	17.9 ± 2.88	27.3 ± 1.74	19.4 ± 0.50	25.6 ± 0.23	16.4 ± 2.18	19.5 ± 5.47	13.2 ± 1.68
Σ n-3	27.2 ± 9.00	30.0 ± 2.05	48.0 ± 9.42	31.3 ± 0.15	39.0 ± 0.43	25.1 ± 0.59	41.0 ± 6.23	23.6 ± 3.27	23.6 ± 1.22	17.4 ± 3.25	24.1 ± 1.73	14.2 ± 4.19
Σ n-6	11.8 ± 3.27	12.4 ± 1.33	19.9 ± 6.85	9.8 ± 0.46	21.5 ± 0.05	15.6 ± 0.69	16.3 ± 2.14	10.7 ± 0.89	19.9 ± 2.29	10.6 ± 2.91	11.3 ± 2.69	7.1 ± 0.17
n-3/n-6	2.3 ± 0.11	2.4 ± 0.09	2.7 ± 1.27	3.2 ± 0.11	1.8 ± 0.02	1.6 ± 0.03	2.5 ± 0.05	2.2 ± 0.12	1.2 ± 0.20	1.7 ± 0.15	2.2 ± 0.37	2.0 ± 0.54
DHA/EPA	0.89 ± 0.02	1.04 ± 0.32	1.37 ± 0.06	1.38 ± 0.02	0.62 ± 0.01	0.89 ± 0.28	0.66 ± 0.04	1.02 ± 0.16	0.97 ± 0.04	1.07 ± 0.16	0.97 ± 0.26	1.07 ± 0.06
Σ FAME	109.5 ± 32.19	112.9 ± 3.88	172.1 ± 8.01	106.7 ± 2.15	170.0 ± 0.43	121.6 ± 1.53	172.5 ± 19.30	99.6 ± 6.97	116.5 ± 7.06	74.0 ± 18.34	95.0 ± 8.00	59.3 ± 13.85

Only fatty acids present at $>1 \mu\text{g mg}^{-1}$ DW are included in the table but totals (Σ) include all identified fatty acids.

LNA and ARA), while a lower (n-3)/(n-6) ratio was found in these larvae, compared with those with a diet of *Artemia* enriched with microalgae. The EPA and DHA concentration and thus the DHA/EPA ratio were similar in both larvae. A comparative analysis of the dietary treatments showed a similar pattern at each sampling date in trials 2 and 3. However, in trial 1, as a result of the decrease with time in the concentration of most FA in the Super HUFA[®] treatment, in contrast with the increase observed in larvae fed non-enriched *Artemia*, a more homogenous larval FA profile was found between treatments at 26 DAH than at 20 DAH.

The larval FA profile was analysed twice during the experimental period in all trials, in order to examine changes during larval development (Table 2). A substantial decrease in total FAME, SFA, MUFA, PUFA, HUFA, ARA, EPA and DHA concentration was observed in all treatments, except for larvae fed non-enriched *Artemia*; in this case, a slight increase was observed from 20 to 26 DAH. Between sampling dates there was a decrease of 24–43% in SFA, 27–44% in MUFA and 33–40% in PUFA. Within the PUFA, HUFA were slightly less utilised, with a decline of 26–36% being noted. The reduction in total SFA, MUFA and PUFA was caused mainly by the decline in 16:0, 18:0, 16:1n-7, 18:1n-9, 18:1n-7, LA and LNA. The DHA concentration was generally only slightly reduced during larval development, except in larvae fed *Artemia* enriched with Super HUFA[®] and with microalgae (in the third trial) – 30% and 26% decrease, respectively (in contrast to 5–13% in the other treatments). The decrease in EPA during larval development was generally much more pronounced (19–39%) than DHA, which resulted in an increase in the DHA/EPA ratio; the exception was the Super HUFA[®] treatment, in which the DHA/EPA ratio remained stable. The larval utilisation of ARA during development was relatively high in larvae fed *Artemia* enriched with Arasco[®] (52%) and average (27–33%) in the remaining treatments.

The quantitatively most important FA were also expressed in terms of percentage of total FAME, to eliminate quantitative differences between larvae submitted to different dietary treatments and better analyze qualitative changes (Table 3). One result that stands out is the relative stability that is maintained in the proportion of the different FA classes, within each dietary treatment, during larval development. No particular pattern of change was noted for SFA, MUFA or PUFA, with its relative levels being stable, slightly increased or decreased depending on the trial and dietary treatment. However, in all trials and treatments, the proportion of HUFA was either increased or remained stable; the relative proportion of DHA increased in all cases, while the percentage of EPA and ARA in relation to total FAME remained rather stable, except for the percentage of ARA which decreased over development in larvae fed *Artemia* enriched with Arasco[®].

Discussion

In the present study, no correlation was found between the two analysed larval performance parameters, that is, growth and survival. Survival was lower than reported in other studies – 33.4 and 29–87% at 19 DAH (Dinis 1992; Dinis et al.

Table 3. Relative fatty acid composition (% of total FAME) of *S. senegalensis* larvae fed different experimental diets, at 20, 26 or 36 days after hatching (DAH), in the three different trials.

Fatty acids % total FAME	Trial 1				Trial 2				Trial 3			
	N.E. <i>Artemia</i>		Super HUFA [®]		Sunflower oil		Microalgae		Arasco [®]		Microalgae	
	20 DAH	26 DAH	20 DAH	26 DAH	20 DAH	26 DAH	20 DAH	26 DAH	26 DAH	36 DAH	26 DAH	36 DAH
14:0	1.2	0.8	1.1	1.0	1.1	1.7	1.8	1.3	0.9	0.9	1.1	1.2
16:0	13.2	12.3	11.9	12.8	11.9	12.3	14.2	13.9	12.1	12.6	12.5	15.0
17:0	1.8	1.9	1.5	1.9	1.5	1.3	1.5	1.5	1.4	1.5	1.4	1.2
18:0	8.4	7.7	7.3	7.2	6.7	7.0	7.5	8.2	7.5	7.6	7.4	9.5
22:0	1.5	1.5	0.7	0.5	1.0	0.8	1.1	0.9	0.9	0.9	1.1	1.1
Σ SFA	27.2	25.3	23.6	24.4	23.3	24.8	27.3	26.9	23.7	24.3	24.4	29.0
16:1n-7	3.4	3.7	3.7	4.2	4.3	4.1	4.6	3.9	3.5	3.7	3.6	3.4
18:1n-9	14.6	14.8	14.4	15.3	14.8	15.2	15.2	15.2	17.7	16.6	15.0	14.5
18:1n-7	7.7	8.1	6.6	7.5	8.0	7.2	8.5	7.7	7.6	7.7	7.9	7.1
20:1n-9	1.2	1.1	1.1	1.1	0.7	0.7	0.8	0.9	0.9	0.8	0.9	0.7
Σ MUFA	29.4	29.8	27.3	29.8	29.0	29.5	30.5	29.7	31.1	30.3	29.4	27.2
18:2n-6	4.8	4.1	7.2	4.3	8.6	8.9	5.1	5.4	4.8	4.5	5.6	4.8
18:3n-3	7.0	7.2	6.4	6.8	11.1	8.6	10.5	8.3	8.8	9.8	9.3	7.3
18:4n-3	1.5	1.4	1.5	1.4	1.5	1.2	1.7	1.2	1.5	1.3	1.6	1.1
20:4n-6	4.3	4.5	3.1	3.5	2.6	2.7	3.1	3.7	10.6	7.9	4.2	4.5
20:3n-3	1.3	1.6	1.6	0.6	1.3	1.7	1.9	1.8	1.2	1.5	1.7	2.0

Table 3. (continued)

Fatty acids % total FAME	Trial 1				Trial 2				Trial 3			
	N.E. <i>Artemia</i>		Super HUFA [®]		Sunflower oil		Microalgae		Arasco [®]		Microalgae	
	20 DAH	26 DAH	20 DAH	26 DAH	20 DAH	26 DAH	20 DAH	26 DAH	26 DAH	36 DAH	26 DAH	36 DAH
20:4n-3	2.2	2.1	1.4	1.4	1.8	1.4	1.7	2.0	1.0	1.4	1.8	1.8
20:5n-3	5.4	5.3	5.8	6.6	3.5	3.0	3.7	3.9	2.7	3.3	3.9	4.1
22:5n-3	2.4	2.7	2.9	3.3	1.5	1.9	1.7	2.4	2.2	2.2	2.9	2.5
22:6n-3	4.7	5.4	7.9	9.0	2.2	2.7	2.5	4.0	2.6	3.5	3.7	4.4
Σ PUFA	35.6	37.5	39.4	38.5	35.6	33.5	33.2	34.5	37.3	37.8	37.3	35.8
Σ HUFA	22.1	24.4	24.2	25.9	14.3	14.7	15.8	19.4	22.0	22.1	20.6	22.3
Σ n-3	24.8	26.6	27.9	29.3	22.9	20.7	23.8	23.7	20.2	23.5	25.4	23.9
Σ n-6	10.8	11.0	11.6	9.1	12.6	12.9	9.4	10.8	17.1	14.3	11.9	12.0

Only the quantitatively most important fatty acids are represented.

1999, respectively) or 71.6% at 23 DAH (Cañavate and Fernández-Díaz 1999). Apart from nutritional and broodstock effects, the use of cylindrical-conical tanks during the whole experimental period in this study was not the most adequate for sole, which becomes benthic around 17 DAH (depending on temperature).

A relationship between the dietary levels of (n-3) HUFA, particularly DHA and EPA, and larval survival has been observed in several marine species, such as seabream (Salhi et al. 1994) and red seabream, *Pagrus major* (Temminck and Schlegel) (Watanabe et al. 1989; Watanabe 1993). However, in other studies conducted with seabream (Koven et al. 1990, 1992; Pousão-Ferreira et al. 1999) and turbot, *Scophthalmus maximus* (Linnaeus, 1758) (Rainuzzo et al. 1994; Reitan et al. 1994; Estévez et al. 1999), no evidence was found relating higher levels of (n-3) HUFA with a higher larval survival. Similarly, the results from the present study appear to support the lack of correlation between the prey's HUFA concentration and larval survival in Senegalese sole. The most striking example was seen in trial 1, in which *Artemia* enriched with Super HUFA[®], containing higher PUFA, HUFA, DHA, EPA and ARA levels than non-enriched *Artemia*, did not result in a significantly improved survival.

In addition, the results presented in this study do not substantiate a correlation between dietary HUFA concentration and sole's growth performance. Again, the results obtained in trial 1 are the most evident in this respect, as larvae fed non-enriched *Artemia* showed a significantly better growth than larvae fed *Artemia* enriched with Super HUFA[®]. Growth in seabream and red seabream has been positively related to a high dietary HUFA content and a high DHA/EPA ratio, particularly at the onset of exogenous feeding (Watanabe et al. 1989; Mourente et al. 1993; Watanabe 1993). On the contrary, studies by Rainuzzo et al. (1994), Reitan et al. (1994) and Estévez et al. (1999) showed no correlation between the dietary (n-3) HUFA content and the growth rate of turbot larvae. Additionally, Howell and Tzoumas (1991) found that a higher DHA inclusion level did not improve *Solea solea*'s growth rate and high growth and survival rates could be achieved with diets almost deficient in DHA. These authors suggest that Dover sole is less demanding in terms of (n-3) HUFA dietary requirements, when compared with many other marine species, and that there is no need for enhancing the *Artemia* DHA concentration, as long as there is a sufficient provision of EPA.

It may be hypothesised that the experimental period might have been too short and that the dietary influence may be felt more strongly later in development. Although short in this species, the endogenous feeding period could affect the performance of the larvae during the early developmental stages and thus dietary effects may be more evident in later stages. The requirements and performance of the larvae may depend not only on their ontogenetic stage but also on the FA reserves in their yolk-sacs or biochemical composition at the start of feeding, which may be highly variable and dependent on the broodstock nutritional status (Lavens et al. 1995; Estévez et al. 1999). Additionally, Heath and Moore (1997) found that Dover sole, *Solea solea* (Linnaeus, 1758), larval diet had a significant impact in juvenile growth, while studies performed on turbot larvae (Støttrup and Attramadal 1992) revealed that the PUFA content of rotifers did not have a significant

immediate effect on growth and survival but it became later evident in larval survival, during the *Artemia* stage.

Eggs of Senegalese sole were analysed, in an attempt to indicate the nutritional requirements of the first larval stages. Quantifying larval nutritional requirements is not an easy task and therefore it is usually agreed that the best way is to simulate the lipid content of the eggs or yolk reserves of the species in culture (Mourente and Vásquez 1996; Narciso 1999; Pousão-Ferreira et al. 1999; Sargent et al. 1999). A diet with a lipid composition equivalent to that of the yolk of eggs or yolk sac larvae should be ideal for the larval development after the onset of exogenous feeding, since the yolk reserves have to meet all the nutritive requirements and metabolic needs of the larvae during the endogenous feeding stage (Watanabe and Kiron 1994; Rainuzzo et al. 1997). The FA profile of the Senegalese sole eggs indicates a requirement for high dietary DHA/EPA ratios. Analyses of other marine fish egg's (*Sparus aurata*, *Pagrus pagrus* (Linnaeus, 1758) and *Diplodus sargus* (Linnaeus, 1758)), at the same stage of development, have been conducted in our laboratory (unpublished data) and have revealed a DHA/EPA ratio of 2–3, in contrast with the ratio of 4.3 found for *S. senegalensis* eggs. Nonetheless, the absolute concentration of both DHA and EPA in the sole's eggs was not very elevated and was even substantially lower than in the other analysed species. The egg composition is most probably related to the benthic diet of the broodstock, based on squid supplemented with polychaetes during final maturation (Dinis et al. 1999), and reveals the need to include relatively high levels of DHA (in relation to the EPA content) to obtain high DHA/EPA ratios in the larval diet. This is probably the biggest challenge in the nutrition of the first larval stages, given the selective DHA catabolism by *Artemia* (Dhert et al. 1993; Danielsen et al. 1995; Triantaphyllidis et al. 1995) and the difficulty in increasing the DHA levels in marine oils without simultaneously raising the EPA levels (Sargent et al. 1999). The enrichment products tested in the present work were probably not the most suitable for the larval rearing of Senegalese sole. All products were below the required DHA/EPA ratio; the highest value was obtained in *Artemia* enriched with Super HUFA[®] (0.6), which is far from the value measured in the eggs. As for the (n-3)/(n-6) ratio, the results were also less than desirable – Super HUFA[®] was the only product that induced a (n-3)/(n-6) ratio similar to that of the eggs. Therefore, further work should be conducted using commercial products or marine oil emulsions with a composition closest to the egg's FA profile.

As found in other species (Rainuzzo et al. 1994; Reitan et al. 1994; Pousão-Ferreira et al. 1997; Estévez et al. 1999), the results from this study show that, in general, the larval FA profile reflected the composition of the diet. The main exception seems to be DHA – although its level is superior in larvae fed live prey with a higher DHA content (Super HUFA[®]), when it is present in small or trace amounts in the diet, the larvae still have this essential FA in considerable amounts. This is probably the result of a selective conservation of the DHA present in the egg yolk, although some degree of biosynthesis may also be hypothesised. When feeding larvae with non-enriched *Artemia* there were also signs of EPA and ARA conservation, as larvae fed this deficient diet still contain EPA and ARA in amounts comparable to the other treatments.

In all trials there was a decrease in total FAME concentration during larval development. This could be expected, given that fish larvae are characterised by fast metabolism and high nutritional demands, using lipids as a source of metabolic energy and essential FA. Mourente and Vásquez (1996) estimated that Senegalese sole larvae consume around 1.7% of total lipid per day during early development and starvation, while Vásquez et al. (1994) determined a 12.1% decrease in the total FA content from fertilised egg to yolk sac larvae and a further 15.5% decline from yolk sac larvae to first-feeding larvae. Additionally, Vásquez et al. (1994) and Mourente and Vásquez (1996) showed that total SFA and MUFA were catabolised as energy substrates, while PUFA either conserved or increased their concentration. Although PUFA can be catabolised for the production of energy, SFA or MUFA are typically more easily and preferentially catabolised in marine animals (Rainuzzo et al. 1997). In the present study, however, SFA, MUFA or PUFA (mostly 16:0, 18:0, 16:1n-7, 18:1n-9, 18:1n-7, LA and LNA) were equally consumed during larval development. Only HUFA appeared to be conserved, with its relative concentration being either kept constant or increased. It should however be noted that Vásquez et al. (1994) and Mourente and Vásquez (1996) looked at eggs and younger larval stages. Nonetheless, some of the major trends were still observed in the present work.

Within the HUFA, DHA was the most conserved FA, with its relative composition increasing in all dietary treatments. This clear conservation of DHA during larval development seems to support data from Mourente and Vásquez (1996) and contradict an earlier study by Vásquez et al. (1994), where a significant decrease was observed in the DHA content between egg and yolk-sac larvae. In the case of the Super HUFA[®] treatment, given that it induced higher DHA levels in the enriched preys and in the reared larvae, the larvae metabolised a considerable amount of DHA during development (30%). Even so, there was still an increase in relative terms from 20 to 26 DAH. EPA and ARA, also considered essential FA for marine fish species, were consumed (in absolute amounts) but their relative concentration remained stable (or increased very slightly) during larval development in most treatments. An exception was larvae fed *Artemia* enriched with Arasco[®] in which a decrease in the relative concentration of ARA was noted from 26 to 36 DAH. In larvae fed non-enriched *Artemia*, EPA and ARA were not consumed during development. A likely explanation is that EPA and ARA are below the minimum requirement in non-enriched *Artemia*, being therefore conserved by the larvae. On the contrary, in *Artemia* enriched with Arasco[®], ARA was most probably well above larval requirements.

At present, there is no indication whether EPA is metabolised for energetic purposes or used as a precursor to be elongated to DPA (Docosapentaenoic Acid; 22:5n-3) and further desaturated to DHA. Nevertheless, it is unlikely that Senegalese sole larvae have the ability to biosynthesise DHA from EPA and DPA, even if in limited amounts, as recorded in turbot larvae (Linares and Henderson 1991), given that in the non-enriched *Artemia* treatment EPA and DPA were conserved or even slightly increased while the DHA level still rose during larval development.

Vásquez et al. (1994) and Mourente and Vásquez (1996) indicate a specific requirement for ARA in the first larval stages. The same has been described for turbot larvae (Linares and Henderson 1991; Rainuzzo et al. 1994), where the level

of this FA was shown to be independent of diet and was selectively retained during starvation. A specific requirement for ARA could not be confirmed in the present study. Nonetheless, it is noteworthy that this FA was always present in considerable amounts, even in larvae fed non-enriched *Artemia*, where the ARA content was the lowest of the analysed preys.

Conclusions

1. Larval FA profile reflected the composition of their diet, with the exception of DHA which, although being in higher amounts in larvae fed prey with a higher DHA content, was always present in considerable amounts even with a DHA-poor diet.
2. A decrease in total FAME concentration (mostly 16:0, 18:0, 16:1n-7, 18:1n-9, 18:1n-7, LA and LNA) occurred during larval development, with SFA, MUFA and PUFA being equally consumed; only HUFA (particularly DHA) appeared to be relatively conserved.
3. Senegalese sole eggs have a DHA/EPA ratio (4.3) higher than that found in most marine species eggs (2–3), which may indicate a high DHA dietary requirement, relative to EPA.
4. No correlation was found between the prey's HUFA concentration or DHA/EPA ratio and Senegalese sole larval growth and survival. In fact, larval sole could be grown quite well on non enriched *Artemia*, a prey which is deficient in terms of essential FA, with a better growth being achieved than with *Artemia* enriched with Super HUFA[®], which was the prey with the highest HUFA level and DHA/EPA ratio.
5. Future studies should analyse the effect of a HUFA-deficient diet, as is the case of non-enriched *Artemia*, on the later performance of sole larvae; following the larvae until a more advanced stage of development may help clarify some of the questions raised by this study.

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