Full Length Research Paper

Effects of n-3 fatty acids on dentex larvae survival and growth

Ali Ait Ali [1*], Nadia Rharbi[2], Houda Akharbache[1] and Said Sedki[1]

[1]Centre Spécialisé en Aquaculture, BP. 31-INRH. M'diq. Morocco.
[2]Université Hassan II. Faculté des sciences Ain Chock, Casablanca, Morocco.

The rotifer *Brachionus plicatilis* (S-type) and *Artemia* were fed with three enrichment types (Nanno: *Nannochloropsis occulata*, CEP: Commercial Enrichment Products: Selco[®] from INVE Aquaculture NV and CLO: Cod Liver Oil emulsion). The polyunsaturated fatty acid composition in the regimes was tested to estimate survival and growth of common dentex larvae. During rotifers feeding phase, EPA (20:5n-3) contents were of 4.6 ± 1.46 mg g^{-1} DW for Nanno regime, 5.1 ± 0.22 for the CEP regime and 6.1 ± 0.87 mg g^{-1} DW for the CLO regime; for DHA (22:6n-3), CLO regime contains the highest level (7.2 ± 1.26 mg g^{-1} DW) than the two other regimes. During *Artemia* feeding phase, EPA contents were 2.4 ± 0.05, 3.2 ± 0.03 and 8.3 ± 0.41 mg g^{-1} DW and in DHA 0.4 ± 0.00, 4.4 ± 0.13 and 8.6 ± 0.69 mg g^{-1} DW respectively for Nanno, CEP and CLO enrichments. The weight growth of larvae showed a significant difference (p < 0.05) for the rotifers and *Artemia* feeding phases. The respective SGR for the three enrichments are 8.8, 8.9 and 9.2 and survival rates were of 3.15, 4.03 and 8.35% respectively for Nanno, CEP and CLO regimes.

Key words: *Dentex dentex*, live-preys, n-3 HUFA, larvae, growth, survival.

INTRODUCTION

The major problem for mass production of common dentex is the high mortalities during the larval phase. This mortality can be associated to environmental factors (temperature, salinity, water renewal rate, lightening intensity, and handling). The priority of investigations should be focused up on the nutritional aspects, especially the nutritional value of preys during the first stages of feeding. Their nutritional value depends on the nature of the food source, particularly contents in (n- 3) highly unsaturated fatty acids ((n-3) HUFA), such as docosahexaenoic acid (DHA, 22:6n3) and eicosapentaenoic acid (EPA, 20:5n3). The lipid sources in the enrichment diets differ in lipid class composition (McEvoy et al., 1996; Tocher et al., 1995), (n-3) HUFA content (Dhert et al., 1993; Evjemo et al., 1997) and DHA/EPA ratio (Naess et al., 1995; Evjemo et al., 1997). The low diets in fatty acids can be at the origin of massive mortality in larval phase for aquacultured marine fish species. The enrich-ment protocols improve the HUFA content rotifers and *Artemia* nauplii (Léger et al., 1987; Takeuchi et al., 1992; Watanabe, 1993) . Sorgeloos et al. (1988) reported a strong correlation between the EPA content and larvae survival and between DHA content and the growth of Asiatic sea bass larvae. Watanabe (1993) conclude that EPA and DHA increase survival and growth of larvae of several marine fishes. Koven et al. (1990) suggested that such acids function as essential component of biomembranes, and their level in the tissue phospholipid fraction are associated with larval growth.

The purpose of this study was to determine the effectiveness of live prey's enrichment with three different diets and their effects on survival and growth of common dentex larvae.

MATERIAL AND METHODS

Brood stock and origin of *Dentex*'s larvae

The brood stock was acquired in March 2001 and acclimatized to captivity conditions. Parents were maintained in a polyester rect-

---

*Corresponding author. E-mail: a.aitali2@caramail.com.

Table 1. Fatty acid composition (mg g^{-1} DW) of *Dentex*'s eggs and larvae. Data represent means ± SEM

	Egg	newly hatched larvae
Total lipids(%DW)	0.38±0.00[a]	1.97±0.01[b]
14.0	4.6±0.01	4.8±0.23
15.0	1.8±0.01	1.7±0.04
16.0	20.4±0.39[a]	16.5±0.25[b]
17.0	2.6±0.01	2.5±0.03
18.0	6.2±0.08[a]	4.5±0.03[b]
16:1	8.3±0.13[a]	9.1±0.14[b]
17:1	1.5±0.01[a]	1.2±0.01[b]
18:1n-9c	16.9±0.22[a]	18.0±0.08[b]
18:1n-7	4.0±0.06	3.7±0.00
20:1n-9	1.9±0.04	1.8±0.01
22:1n-9	1.2±0.00	1.2±0.02
24:1n-9	1.3±0.00	1.2±0.00
18:2n-6c	2.6±0.04	2.7±0.04
20:2n-6	5.3±0.01	5.3±0.01
18:3n-6	4.1±0.00	4.2±0.00
20:3n-6	nd	0.1±0.01
20:4n-6	1.2±0.02[a]	0.9±0.01[b]
18:3n-3	0.9±0.21	0.9±0.01
20:3n-3	nd	nd
20:5n-3	3.6±0.08[a]	4.7±0.04[b]
22:5n-3	1.0±0.01[a]	1.5±0.04[b]
22:6n-3	16.9±1.10[a]	22.1±0.69[b]
Saturated	35.3±0.50[a]	30.2±0.57[b]
Mono saturated	35.1±1.05	36.2±0.09
DHA/EPA	4.7±0.19	4.7±0.10
n-3 HUFA	21.5±1.39[a]	28.3±0.78[b]

n-3 HUFA = > 20:3n-3. nd= not detected
Means ± SEM having different letters indicate that treatments are significantly different (P<0.05) according to Kruscal Wallis test. (n = 2)

rectangular tank, of volume 10 m^3. The renewal sea water rate was maintained about 300 - 500%d^{-1} and oxygenation was provided by two air inflows. The temperature (14 - 22°C) and salinity (36 - 38 ppm) conditions were natural. The spawning was spontaneous, and floating eggs were collected out of broodstock tank through a mesh screen (500 µm). They were incubated in 100 liters polycarbonate cylindro-conical tanks. The temperature of incubation was kept at the same value of that in broodstock's tank where spawning was happen. Photoperiod and thermo-period were natural; light intensity during the incubation phase was included between 80 - 100 lx. The duration of hatching varied from 60 to 72 h according to the temperature of incubation during spawning season.

Larviculture and live food

To realize this experiment, six cylindro- conical polycarbonate tanks, covered with black plastic sheet, with volume of 200 liters (noted from B$_1$ to B$_6$), received each 6000 freshly hatched larvae of common dentex. The initial density was 30 larvae per liter. The parameters of larval rearing were the same for all tanks, except changes of prey's enrichment which were subject of this study.

Larval feeding

For all the tanks, larvae were fed on rotifers "S type", the density was of 10 to 20 individuals mL^{-1} from the opening mouth until 35-th day of larval rearing. Fresh *Artemia* (AF strain) from day 25 to day 30, and EG strain from day 30. For tanks 1 and 4 enrichment was in base of *Nannochloropsis*; tanks 2 and 5, it was in base of commercial enrichment products and for tanks 3 and 6 it was in base of the cod liver oil emulsion (Emulsion was prepared in base of 40% hen's egg yolk and 60% of cod liver oil using electric blender). Rotifers and Artemia were enriched in cylindroconical tanks of volume 30 L; water temperature varied around 20°C. The rotifers density was about 100.000 rot L^{-1}; That of Artemia was around 50.000 Ind L^{-1}. For Nanno enrichment, the used concentration of algae varied between 5 and 10 million cells mL^{-1}. The used doses for CEP were as described in Selco prospectus (Selco$^{®}$ INVE Aquaculture, Ghent, Belgium) and the used dose for CLO enrichment was 1 g of emulsion per million rotifers. Enrichment period was 12 h for all the tanks. Each enrichment was tested in replicate. The regimes affectation in various larval tanks was made at random in every row of tanks.

Survival, length and weight sampling

Dead larvae were collected by siphoning and recorded daily starting from day 12, survival was adjusted to be 100% at day 12 of the experiment, while at the end, the number of missing fish (never less than 5%) was calculated and distributed over the experiment as a proportion of actually observed mortality. Sampling of larvae was made to measure total length of 20 larvae per tank, while larvae were weighted at day 0 and 7 (2 x 50 Ind); at days 15, 27, 32, 50 and 70 twenty larvae per tank were weighted for every sampling.

Fatty acid analysis

Every three days, approximately two million of rotifers were filtered from the three regimes trough 64 µm mesh screens. For *Artemia*, every five days one million of *Artemia* nauplii were harvested in each enrichment regime. The rotifers and *Artemia* harvested were frozen and lyophilized.

Total lipids were extracted and measured gravimetrically according to Folch et al. (1957) using dichloromethane instead of chloroform.

Fatty acid methyl esters were prepared by acid-catalyzed transmethylation of total lipids using boron trifluoride methanol according to Santha and Ackman (1990) and were analysed in a Varian 3400 gas chromatograph. The chromatograph was equipped with a DB Wax fused silica capillary column (30 m x 0.25 mm i.d., film thickness: 0.25 µm, J and W Scientific, Folsom, CA). Helium was used as carrier gas (1.4 mLmn^{-1}) and the thermal gradient was 100 to 180°C at 8°/mn, 180 to 220°C at 4°/mn and a constant temperature of 220°C during 25 min. Injector (Split/ splitless with automatic passage) and flame ionisation detector temperatures were 260 and 250°C respectively. Fatty acid methyl esters were identified by comparison with known standard mixtures (Sigma ref 189-13) and quantified using a computer system. Data were collected and processed using the Star computer package (varian).

Statistical analysis

Total length and body weight were compared by one-way analysis of variance (ANNOVA) followed by Kruscal Wallis test. A significant level of 5% was used for both tests (Sokal et al., 1981).

Statistical study was done by the Statgraph plus 5.1 (ICLARM Software project, MC P. O. Box 2631, 0718 Makati, Manila, Philip-

Table 2. Fatty acid composition (mg g^{-1} DW) of rotifers fed on the three regimes. Data represent means ± SEM

Regimes	R(Nanno)[1]	R(CEP)[2]	R(CLO)[3]
Total lipids (%DW)	2.31±0.02[a]	1.41±0.01[b]	3.27±0.08
14.0	4.7±0.02[a]	1.8±0.04[b]	2.8±0.43[c]
15.0	5.6±0.00	5.4±0.01	5.5±0.04
16.0	17.0±0.32[a]	12.5±0.23[b]	12.1±0.83[b]
17.0	6.4±0.00	6.5±0.01	6.3±0.01
18.0	3.7±0.04[a]	5.3±0.06[b]	4.2±0.09[c]
16:1	19.5±0.08[a]	19.5±0.08[a]	6.8±0.48[c]
17:1	4.7±0.01[a]	4.2±0.01[b]	4.3±0.01[b]
18:1n-9c	5.5±0.13[a]	17.0±0.03[b]	20.1±1.10[c]
18:1n-7	5.4±0.09[a]	6.0±0.12[b]	3.6±0.08[b]
20:1n-9	2.0±0.08[a]	3.5±0.06[b]	5.1±0.04[c]
22:1n-9	3.6±0.01[a]	4.1±0.02[b]	4.1±0.08[b]
24:1n-9	2.2±0.00	2.2±0.01	2.1±0.00
18:2n-6c	4.0±0.11[a]	7.0±0.01[b]	5.2±0.10[c]
20:2n-6	nd	0.1±0.00	0.1±0.001
18:3n-6	1.4±0.02[a]	1.7±0.01[b]	1.7±0.04[b]
20:3n-6	0.4±0.01	0.6±0.18	0.3±0.01
20:4n-6	3.0±0.25[a]	1.5±0.04[b]	1.1±0.08[c]
18:3n-3	1.0±0.05	1.4±0.16	1.2±0.07
20:3n-3	0.3±0.00	0.4±0.01	0.3±0.01
20:5n-3	4.6±1.46[a]	5.1±0.22[b]	6.1±0.87[c]
22:5n-3	4.0±0.46[a]	3.7±0.16[b]	3.0±0.55[c]
22:6n-3	0.3±1.86[a]	3.1±0.17[b]	7.2±1.26[c]
Saturated	37.5±0.37[a]	31.6±0.32[b]	30.9±1.40[c]
Mono saturated	42.9±0.18[a]	49.4±0.38[b]	46.0±1.77[c]
DHA/EPA	0.2±0.03[a]	0.60±0.01[b]	1.20±0.04[c]
n-3 HUFA	8.9±0.42[a]	11.9±0.57[b]	16.3±1.05[b]

n-3 HUFA = > 20:3n-3. nd = not detected
Means ± SEM followed by different index are significantly different (P<0.05) according to Kruscal Wallis test. (n = 2) [1] R(Nanno) = Rotifers fed on Nannochloropsis regime [2] R(CEP) = Rotifers fed on commercial enrichment products regime [3] R(CLO) = Rotifers fed on cod liver oil regime.

Table 3. Fatty acid composition (mg g^{-1} DW) of *Artemia* fed on the three regimes. Data represent means±SEM.

Regimes	A(Nanno)[1]	A (CEP)[2]	A (CLO)[3]
Total lipids (%DW)	1.45±0.01[a]	1.65±0.02[a]	1.94±0.01[b]
14.0	2.7±0.07[a]	4.4±0.06[b]	2.9±0.10[a]
15.0	3.2±0.00	3.3±0.00	3.3±0.04
16.0	12.2±0.21[a]	5.6±0.25[b]	10.0±0.45[c]
17.0	4.8±0.01[a]	4.4±0.01[b]	4.6±0.00[c]
18.0	8.5±0.06[a]	5.8±0.10[b]	6.3±0.09[c]
16:1	4.3±0.06[a]	2.6±0.06[b]	3.6±0.20[c]
17:1	2.8±0.05[a]	2.1±0.00[b]	2.5±0.01[c]
18:1n-9c	22.0±0.32[a]	18.7±0.37[b]	20.4±0.32[c]
18:1n-7	11.0±0.25[a]	2.0±0.19[b]	7.0±0.12[c]
20:1n-9	3.8±0.06[a]	4.4±0.00[b]	4.7±0.07[c]
22:1n-9	0.1±0.00	0.2±0.08	0.2±0.01
24:1n-9	2.1±0.00	2.3±0.30	2.2±0.01
18:2n-6c	5.2±0.04[a]	10.6±1.03[b]	5.7±0.04[c]
20:2n-6	4.4±0.03[a]	4.1±0.03[b]	4.2±0.01[b]
18:3n-6	3.3±0.01	3.2±0.00	3.4±0.03
20:3n-6	0.7±0.00[a]	0.4±0.01[b]	1.8±0.11[c]
20:4n-6	nd	0.1±0.00	0.2±0.05
18:3n-3	17.5±0.19[a]	3.8±0.04[b]	12.3±0.05[c]
20:3n-3	0.5±0.01	0.2±0.08[b]	0.2±0.01[b]
20:5n-3	2.4±0.06[a]	3.2±0.03[a]	8.3±0.41[b]
22:5n-3	0.1±0.00[a]	0.5±0.01[b]	1.0±0.08[a]
22:6n-3	0.4±0.00[a]	4.4±0.13[b]	8.6±0.69[c]
Saturated	31.5±0.33[a]	33.6±0.42[b]	28.1±0.49[c]
Mono saturated	48.1±0.19[a]	34.3±0.13[b]	42.2±0.44[c]
DHA/EPA	0.17±0.03[a]	1.38±0.04[b]	1.04±0.02[c]
n-3 HUFA	2.9±0.95[a]	8.1±1.39[b]	17.9±1.39[c]

n-3 HUFA = > 20:3n-3. nd = not detected
Means ± SEM followed by different index are significantly different (P<0.05) according to Kruscal Wallis test. (n = 2)[1] A (Nanno) = Artemia fed on Nannochloropsis regime, [2] A (CEP) = Artemia fed on commercial enrichment products regime, [3]A (CLO) = Artemia fed on cod liver oil regime, Survival of dentex's larvae for the three enrichments.

pines) total lengths and weights of larvae were submitted to a logarithmic transformation while the survival rates were treated by an angular transformation.

RESULTS

Eggs and larvae analysis

Eggs and newly hatched larvae (Table 1) had the same content of 18:3n-3 (0.9 mg g^{-1} DW). n-3 HUFA content was higher in larvae (28.3 ± 0.78 mg g^{-1} DW), than in eggs (21.5 ± 1.39 mg g^{-1} DW). The DHA/EPA ratio was the same in both, while the contents of DHA and EPA were higher in newly hatched larvae than in eggs.

Rotifers analysis

Rotifers fed on the three regimes (Table 2) showed a same content of 18:3n-3 and 20:3n-3, but rotifers en-iched with Nanno regime showed a lower EPA content (4.6 ± 1.46 mg g^{-1} DW) and a lower DHA content (0.3 ± 1.86 mg g^{-1} DW). DHA/EPA is of 0.02 ± 0.03; 0.60 ± 0.01 and 1.20 ± 0.04 respectively for Nanno, CEP and CLO regimes. n- 3 HUFA contents were respectively 8.9 ± 0.46, 11.9 ± 0.57 and 16.3 ± 1.05 mg g^{-1} DW.

Artemia analysis

For *Artemia* (Table 3), CLO regime showed a higher content in 20:5n-3 and 22:6n-3, respective values are of
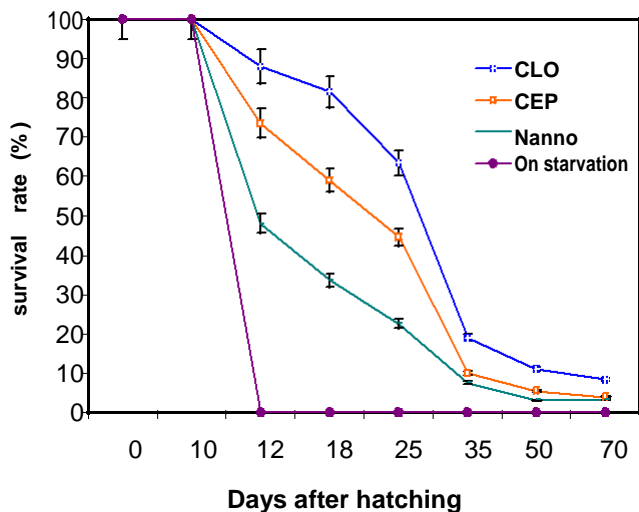
Figure 1. Survival rate (%) of dentex's larvae. Bars indicate ±SEMP < 0.05, there is significant difference according to Kruscal Wallis test. (n = 2)
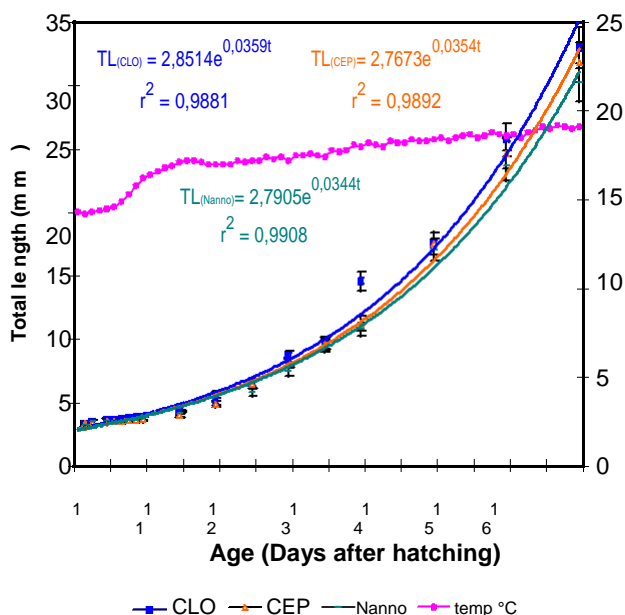
Figure 2. Linear growth curve of dentex's larvae fed on the three enrichment diets and temperatures during larval phase. Bars indicate ±SEM.

8.3 ± 0.41 and 8.6 ± 0.69 mg g^{-1} DW. The DHA/EPA ratios were of 0.17 ± 0.03, 1.38 ± 0.04 and 1.04 ± 0.02 respectively for Nanno, CEP and CLO enrichments. n-3 HUFA contents were respectively 2.9 ± 0.32, 8.1 ± 0.55 and 17.9 ± 1.05 mg g^{-1} DW.

All the obtained survival rates (Figure 1) were lower than 10% (ranged between 3 and 8% on day 70 of larval rearing). Collapses were observed from day 12 to day 35. Larvae fed on preys enriched with *Nannochloropsis*

Table 4. Linear growth performances of dentex's larvae during the entire period

Enrichment	Nanno	CEP	CLO
Initial length	2.62±0.07	2.62±0.07	2.62±0.07
Day 25	5.82±0.11[a]	6.48±0.09[b]	6.54±0.24[c]
Day 35	9.47±0.17[a]	9.66±0.23[b]	9.73±0.24[c]
Final length	30.22±0.38[a]	31.85±0.53[b]	32.98±0.26[c]
TL SGR %	3.50±0.04[a]	3.60±0.06[b]	3.61±0.01[c]

Means ± SEM affected with different letters by exposing on the same age are significantly different (P< 0.05) according to Kruscal Wallis test. (n = 20), SGR =specific growth rate = 100* (ln final length- ln initially length)/no days.

showed low survival rates particularly from second week, while there was an appearance of the swim bladder hyperinflation phenomenon. Towards D_{30}, cannibalism appeared and caused high mortalities. The hyperinflation phenomenon was observed when larvae were fed on preys enriched with *Nannochloropsis*, but decrease when larvae were fed on preys enriched with commercial enrichment products or with cod liver oil emulsion. Survival rates during the entire period were of 3.15% for Nanno enrichment, 4.03% for CEP enrichment and 8.35% for CLO enrichment. The highest regime in lipids content gave the highest survival rate compared with the lowest regime in lipids content.

Linear growth of larvae

Figure 2 showed a fast growth rate during larval phase. The study of the linear growth of larvae in this experiment gave results presented on Table 4. The linear growth showed a significant difference from day 15 (rotifers feeding phase: day 3 to day 25) until day 70 (*Artemia* feeding phase: day 25 to day 70). Day 25 correspond to the introducing *Artemia* in feeding sequence and day 35 corresponds to the end of rotifers feeding phase.

Weight growth of *Dentex*'s larvae

The results of weight growth of *Dentex*'s larvae in this experiment are presented in Figure 3 and Table 5. At the beginning of experiment, the average weight of larvae was the same (0.20 ± 0.00 mg), at D_{70} larvae on the three diets are going to grow differently during rotifers and *Artemia* feeding phases. CLO regime gave the highest growth (123.50 ± 1.50 mg) compared to the CEP regime (106.00 ± 1.00 mg) and Nanno regime (101.50 ± 1.50 mg). The SGR are of 8.8, 8.9 and 9.2 respectively for Nanno, CEP and CLO enrichments.

DISCUSSION

Data on the nutritional requirements of marine fish larvae
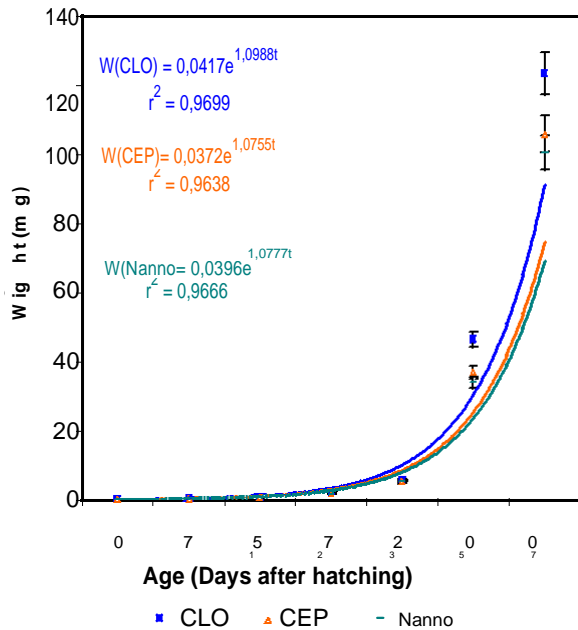
Figure 3. Weight growth curve (mg) of dentex's larvae fed on the three enrichments. Bars indicate ±SEM.

Table 5. Weight growth performances of dentex larvae during the entire period.

Enrichment	Nanno	CEP	CLO
Initial weight	0.20±0.00	0.20±0.00	0.20±0.00
Day 27	1.61±0.06[a]	1.88±0.04[b]	6.54±0.24[c]
Final weight	101.50±1.50[a]	106.00±1.00[b]	123.50±1.50[c]
SGR %	8.80±0.04[a]	8.9±0.09[b]	9.2±0.05[c]

Means ± SEM affected with different letters by exposing on the same age are significantly different (P<0.05) according to Kruscal Wallis test. (n = 20), SGR = specific growth rate = 100* (ln final weight- ln initially weight)/no days.

from mouth opening are scarce and largely concern fatty acids (Watanabe and Kiron, 1994) probably because other micronutrients are not easy to measure in the small sample available. Lavens and Sorgeloos (1996) showed that rotifers grow in a formulated food having a good composition in HUFA and containing 5.4, 4.4 and 15.6 mg g^{-1} of dry weight in DHA, EPA and (n-3) HUFA, respectively. In this study, only CLO regime meets this requirement (rotifers feeding phase: 7.2 ± 1.26, 6.1 ± 0.78 and 16.3 ±1.05 mg g^{-1} DW; Artemia feeding phase: 8.6 ± 0.69, 8.3 ± 0.41 and 17.9 ± 1.39 mg g^{-1} DW respectively in DHA, EPA and n-3 HUFA). Rotifers fed with the Nanno regime present a weak rate in DHA. Values cited by Rodriguez et al. (1998) concerning minimal content in EPA: 6 mg g^{-1} of dry weight; DHA: 8 mg g^{-1} of dry weight and in (n-3) HUFA: 15 mg g^{-1} of dry weight are equal to those EPA rotifers composition fed on CLO regime, that for the DHA is nearby; while (n-3) HUFA content was slightly superior. Koven et al. (1990) observed that the enrichment of rotifers with various oils increases the growth rate of gilt-head sea bream larvae (*Sparus aurata*), but it has no influence on the survival. In this experiment, rotifers feeding phase (until D$_{25}$) shows an increase of survival for CLO regime, in which DHA / EPA is 1.20 ± 0.04. Kraul (1993) noted that the first survivals (0 - 9 days) of Mahi mahi larvae are not affected by the fatty acids composition and suggests that it is due probably to high endogenous level in EPA and DHA contained in the yolk sac of larvae. Contents in DHA eggs (16.90 ± 1.10 mg g^{-1} of dry weight) and larvae (22.10 ± 0.69 mg g^{-1} of dry weight), obtained during this work were very high. These values begin to fall during the first days

of larval rearing (Watanabe, 1993). The DHA / EPA values for the three regimes: 0.02 ± 0.03, 0.60 ± 0.01 and 1.20 ± 0.04 respectively obtained for Nanno, CEP and CLO are lower than those reported by Rodriguez et al. (1998) and Ibeas et al. (1997) which meet for good growth of gilt-head sea bream. The necessity to introduce *Artemia* in food for the development of marine fish larviculture stimulated the research efforts to improve the nutritional value of *Artemia*'s nauplii (Watanabe et al., 1978; Léger et al., 1987). The success of the nauplii enrichment in DHA depends not only on the enrichment regime and conditions, but also on *Artemia*'s strain (Evjemo et al., 1997; Furuita et al., 1996; Han et al., 2000a,b) . The improvement of the nutritional quality of *Artemia*, notably by polyunsaturated fatty acids and vitamins contributes to the improvement of survival, growth and stress to larvae (Merchie et al., 1995) . The results obtained during this experiment for *Artemia* DHA content (between 0.40 ± 0.00 and 8.60 ± 0.69 mg g^{-1} of dry weight) were much lower than those reported by Triantaphylidis et al. (1995) and which are included between 13.7 and 25.1 mg g^{-1} of dry weight and those cited by Kyungmin et al. (2000) and which are included between 22.7 and 37 mg g^{-1} of dry weight. It's the same for values obtained by Evjemo et al. (1997) and Coutteau and Mourente (1997) with respective values of 36.0 and 21.0 mg g^{-1} of dry weight. In the present work the values of DHA/EPA ratio for *Artemia* phase (0.17 ± 0.03, 1.38 ± 0.04 and 1.04 ± 0.02 respectively for Nanno, CEP and CLO regimes) remain within the limits of values reported by Rodriguez et al. (1998) for gilt-head sea bream. They are lower than values reported by Sargent et al. (1999) where the DHA / EPA ratio must be equal to 2 by based on the wild zooplankton composition and confirmed by the experimental data of breeding. These results remain slightly superior to those we found for the three regimes. The food of larvae does not only have to contain high fatty acids content but also with suitable proportions in DHA/EPA (Sargent et al., 1999). Food imbalance in HUFA during the larval development can affect growth, survival and pigmentation of fish larvae (Reitan et al., 1994; Estévez et al., 1999; Sargent et al., 1999). The survival rates of common dentex are very low; the survi-

val rate obtained in hatchery phase was 1.5 - 2.8% (Pastor et al., 1995; Bibiloni et al., 1993a; Koumoundouros et al., 1998; Rueda et Martinez, 2001). During this experiment the survival rates obtained were 8.35% for preys enriched with the cod liver oil emulsion, 4.03% for the commercial enrichment and 3.15% for the enrichment with *N. occulata* at D_{70}. They are superior to previous cited results but very lower than those cited by Koumoundouros et al. (2004) with survival rate between 25 to 35% and a production cycle of 54 – 62 days in semi-intensive conditions. The period during which a massive mortality of larvae happed is situated between D_{12} and D_{35}. Referring to published works, it seems that the authors do not agree about the age where take place higher mortalities; Franicevic (1991) finds that the maximum of mortality is situated between D_6 and D_{15} and D_{25-30}; Pastor et al. (1995) note that mortality take place on D_{9-15} and after D_{25}. For Riera et al. (1993), mortalities take place on D_{6-15} and D_{25-30} while for Abellan (1997) mortalities are continuous and begin on D_{12} and from D_{22} to D_{45}. Concerning growth, there was a significant differrence for linear and weight growth in the end of the larval rearing (D_{70}).

Conclusion

The survival rates of larvae, although relatively weak, show that diets with high lipids content were highest than other regimes with a low content in lipids. Live-preys fatty acids composition is very important also for a harmonious larvae development. Highest content in fatty acids gives highest survival and best growth, but apparently it is the DHA / EPA factor turns out to be the most important to ensure a good survival and high growth.

ACKNOWLEDGEMENT

REFERENCES

Abellan E, Garcia-Alcazar A, Arizcun M, Delgado J, Martin P (1997). Experiencias preliminares sobre reproduction y cultivo del denton (*Dente dentex* L.) In : Actas VI Congresso Nac. Acuicult. De Costa J, Abellan E, Garcia B, Ortega A, Zamora S. (eds), Cartagena, 1997. Ministerio de Agricultura, Pesca y alimentation, Madrid. pp. 477-482.

Bibiloni G, Cladera A, Gomila MA, Massuti S, Zaratiegui I (1993ã). A small scale experiment on rearing of *Dentex dentex*. In: From discovery to commercialisation. European Aquaculture Society. Spec. Publ. N° 19. Carrillo M, Dahle L, Morales J, Sorgeloos P, Svenning N, Wyban J (eds). Oostende, Belgium. p. 317.

Coutteau P, Mourente G (1997). Lipids class and their content of n-3 highly unsaturated fatty acids (HUFA) in *Artemia* Franciscana after hatching, HUFA enrichment and subsequent starvation. Mar. Biol. 130: 81-91.

Dhert P, Sorgeloos P, Devresse B (1993). Contribution towards a specific DHA enrichment in the live food *Brachionus plicatilis* and *artemia sp.* In Reinersten H, Dahle LA, Jorgensen L, Tvinnereim K (Eds). Preceeding of the first International Conference of fish farming Technology. Trodheim, Norway. 9-12 August 1993, Balkema AA, Rotterdam. pp. 109-115.

Estévez A, Mc Evoy LA, Bell JG (1999). Growth, survival, lipid composition and pigmentation of turbot (*Scophtalmus maximus*) larvae fed live prey enriched in arachidonic and eicosapentanoic acids. Aquaculture. 80: 321-343.

Evjemo JO, Coutteau P, Olsen Y, Sorgeloos P (1997). The stability of DHA in two *Artemia* species following enrichment and subsequent starvation. Aquaculture. 155: 135-148.

Folch J, Lees M, Sloanne-Stanley GH (1957). A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226(2): 497-509.

Franicevic V (1991). Preliminary results of the larval rearing of common dentex, *Dentex dentex* (Linneaus, 1758). EAS. Special publication. 14: 106-107.

Furuita H, Takeushi T, Toyota M, Watanabe T (1996). EPA and DHA requirements in early juveniles red sea bream using HUFA enriched Artemia. Fish Sci. 62: 246-251.

Han K, Geurden I, Sorgeloos P (2000a). Enrichment strategies for Artemia using emulsions providing different le vels of n-3 highly unsaturated fatty acids. Aquaculture. 183: 335-347.

Han K, Geurden I, Sorgeloos P (2000b). Comparison of docosahexanoic acid (22:6n3) emulsions in various Artemia strains during enrichment and subsequent starvation. J. World Aquaculture Soc. 31: 469-475.

Ibeas C, Cejas JR, Fores R, Badia P, Gomez T, Hernandez AL (1997). Influence of eicosapentaenoic to docosahexaenoic acid ratio (EPADHA) of dietary lipids on growth and fatty acid composition of gilthead seabream (*Sparus aurata*) juveniles Aquaculture 150: 91-102.

Koumoundouros G, Carrilllo J, Divanach P, Kentouri M (2004). The rearing of common dentex *Dentex dentex* (L.) During the hatchery and on-growing phases. Aquaculture. 240: 165-173.

Koumoundouros G, Divanach P, Kentouri M (1998). Embryonic and yolk-sac larval development of *Dentex dentex* (Linnaeus 1758) (Osteichthyes, Sparidae). Mar. Life 6: 41-50.

Koven WM, Tandler A, Kissil GW, Skland D, Friezlander O, Harel M (1990). The effect of dietary (n-3) polyunsaturated fatty acids on growth, survival and swim bladder development in *Sparus aurata* larvae. Aquaculture. 91: 131-141.

Kraul S (1993). Larviculture of the Mahi mahi *Coryphaena hippurus* in Hawaii, USA. J.world Aquac. Society. 24: 410-421.

Kyungmin H, Geurden I, Sorgeloos P (2000). Comparison of docosahexanoic acid (22:6n3) levels in various strains during enrichment and subsequent starvation. J. World Aquac. Society 31(3): 469-475.

Lavens P, Sorgeloos P (1996). Manual of the production and use of live food for aquaculture. FAO Fisheries Technical Paper No. 361. FAO. Rome.

Léger Ph, Naessens E, Foucquaert L, Sorgeloos P (1987). International Study on Artemia. XXXV. Techniques to manipulate the fatty acid profile in *Artemia nauplii* and the effect on its nutritional effectiveness for the marine crustacean Mysidopsis bahia (M). pp. 411-424. In : *Artemia* Research and its Applications - Vol. 3-
SORGELOOS P, BENGSTON D.A, DECLEIR W, JASPERS E. (Eds)- Universa Press - Wetteren-Belgium. p. 556.

McEvoy LA, Navarro JC, Hontario F, Amat F, Sargent JR (1996). Two novel Artemia enrichment diets containing polar lipid. Aquaculture. 144 : 339-352.

Merchie G, Lavens P, Dhert P, Dehasque M, Nellis H, Deleenheer A, Sorgeloos P (1995). Variation in ascorbic acid content in different live food organisms. Aquac. Nutr. 7(3): 143-151.

Naess T, Germail-Henry M, Naess KE (1995). First feeding of Atlantic halibut (Hippoglossus hipoglossus) using different combination in the first stage of Artemia sp. Mar. Biol. 111: 461-465.

Pastor E, Riera F, Pou S, Grau AM, Grau A (1995). A summary of investigations on reproduction and larval rearing of common dentex, Dentex dentex (Linnaeus, 1758). ICES Mar. Sci. Symp. 201: 148-152.

Reitan KI, Rainuzzo JR, Olsen Y (1994). Influence of lipid composition

of live feed on growth, survival and pigmentation of turbot larvae. Aquac. Int. 2: 33-48.

Riera F, Pastor E, Grau AM, Grau A,Massuti E (1993). Experiencias en cultivos del denton. In : Actas IV Congresso Nac. Acuicult., Cervino, A., Landin A, de Coo A, Guerra A, Torre M (eds), Villanova de Aroussa, (1993). Centro de Investigaciones Marinas, Pontevedra. pp. 143-148.

Rodriguez C, Perez JA, Badia P, Izquierdo MS, Fernandez-Palacio H, Lorenzo Hernandez A (1998). The n-3 highly unsaturated fatty acid requirements of gilt-head sea bream (Sparus aurata) L.) Larvae when using an appropriate DHA/EPA ratio in the diet. Aquac. 169: 9-23.

Rueda FM, Martinez FJ (2001). A review of the biology and potential aquaculture of Dentex dentex. Rev. Fish Biol. Fish. 11: 57-70.

Santha NC, Ackman RG (1990). Nervonic acid versus tricosanoic acid as internal standards in quantitative gas chromatographic analyses of fish oil longer-chain n-3 polyunsaturated fatty acid methyl esters. J. Chromatogr. Biomed. Appl. 533: 1-10.

Sargent JR, McEvoy L, Estévez A, Bell G, Bell M, Henderson J, Tocher D (1999). Lipid nutrition of marine fish during early development: Current status and future directions. Aquaculture. 179: 217-229.

Sokal RR, Rohlf FG (1981). Biometry. The principles and practice of statistics in biological research. 2nd edn. WH Freeman, New york.

Sorgeloos P, Leger PH, Lavens P (1988). Improved larval rearing of European and Asian seabass, seabream, mahimahi, siganid and mikfish using enrichment diets for Brachionus and Artemia. World aquaculture. 19: 78-79.

Takeuchi T, Toyota M, Watanabe T (1992). Comparison of lipid and n-3 highly unsaturated fatty acids incorporation between Artemia enriched with various type of oil by direct method. Nippon Suisan Gakkaichi. 58: 277-281.

Tocher DR, Lou DC (1995). Improving the n-3 HUFA composition of Artemia using microcapsules containing marine oil. Aquaculture. 134: 91-99.

Triantaphylidis GV, Coutteau P, Sorgeloos P (1995). The stability of n-3 highly unsaturated fatty acids in various Artemia populations following enrichment and subsequent starvation. In: Lavens P, Jaspers E, Roelants I (Eds). Larvi 95 fish and shellfish symposium. European Aquac. Society. Special Publication . 24: 149-153.

Watanabe T (1993). Importance of docosahexanoic acid in marine larval fish. J. Aquac. Society. 24: 153-161.

Watanabe T, Oowa F, Kitajima C, Fujita (1978). Nutritional quality of brine shrimp, Artemia salina, as a living feed from the viewpoint of essential fatty acids for fish. Bulletin of the Japanese Society of Sciences Fisheries . 44(10): 1115-1121.

Watanabe T, Kiron V (1994). Prospect in larval fish dietetics. Aquaculture. 124: 223-251.