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Full Length Research Paper

Comparative study on growth and survival of larval and juvenile *Dicentrarchus labrax* rearing on rotifer and *Artemia* enriched with four different microalgae species

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In the present study, two experiments were carried out, the first one at age from 4th to 24th days post hatching (dph) which include *Dicentrarchus labrax* larvae rearing on rotifer and *Artemia* enriched with four types of algae as follows: *Chlorella salina, Dunaleilla salina, Nannochloropsis salina* and *Tetraselmis chuii* (ch_1, D_1, N_1 and T_1). At the end of the experiment, mean body length of 5.4, 11.9, 11.0 and 10.01 mm and a survival rate of 79.4, 73.8, 63.5 and 30.0% were achieved. Larvae fed with algae cultured in basal medium of ch_{cont}, D_{cont}, N_{cont} and T_{cont} reached 9.1, 9.5, 8.0 and 8.0 mm with survival rate of 31.7, 40.4, 30.5 and 22.4% by 25 (dph), respectively. In the second trial, juvenile *D. labrax* (25 - 60 dph) fed with *Artemia* metanauplii, enriched by ch_1 (the best result recommended from 1st experiment) increased their mean total length to 35.5±1.4 mm at age 60 dph. The total carbohydrate and total protein in the algae species used to enrich rotifer and *Artemia* significantly increased for ch_1, D_1, N_1 and T_1. Also total amino acid significantly increased at $P < 0.001$. The total fatty acid and total unsaturated fatty acid in the algae significantly increased ($P < 0.001$) for ch_1, D_1, N_1 and T_1 taking into consideration that the state of $C_{22:6}$ significantly increased. The ch_1 gave better growth and survival percentage followed by D_1 for enrich *Brachionus plicatilis* and newly hatched *Artemia*.

Key word. Larval and juvenile *Dicentrarchus labrax* – Enriched Rotifer and Artemia.

INTRODUCTION

Sea bass, *Dicentrarchus labrax* (L.), is one of the most important commercial fish in Egypt. In recent years, the yield of fry collected from natural sources for rearing on fish farm, as well as the yields of mature fish for market, has fluctuated. This has led to attempts to induce spawning in hatcheries with the aim of obtaining a large supply of fry. Larval culture of most marine fish species is dependent on live food, such as rotifers and Artemia to ensure successful larval development to metamorphoses. The early stages of marine fish larvae typically have incomplete formed digestive systems and most species cannot be reared on artificial diet at the first feeding stage (Cahu and Zambonino, 2001).

Marine fish fry, especially *D. labrax,* suffer high mortality during the early rearing period in hatcheries. This may be attributed to several factors, one of which is the availability, and nutritional adequacy of live food provided to the larvae at various stages of their development. Microalgae comprise the base of the food chain in the marine environment. Therefore, these organisms are indispensable in the commercial rearing of various species as a food source for all growth stages of most marine animals. Microalgae are also used to increase zooplankton mass (rotifers, copepods, and brine shrimp) which serve, in turn, as food for larvae and early juveniles of crustaceans and marine fish in marine hatcheries. During the rearing of marine fish larvae, microalgae ('green water') are added directly to larva rearing tanks, as they are believed to play a role in improving water quality, nutrition of larvae, and microbial control.

Microalgae species can vary significantly in their nutritional value which may also change under different culture conditions

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Abbreviations: TL, Total length; **TFA,** total fatty acid; **USFA,** unsaturated fatty acid; **DHA,** docosahexaenic acid; **Ch,** *Chlorella salina*; **D,** *Dunaleilla salina*; **N,** *Nannochloropsis salina*; **T,** *Tetraselmis chuii*; $_{cont,}$ control; $_1$, treatment group.

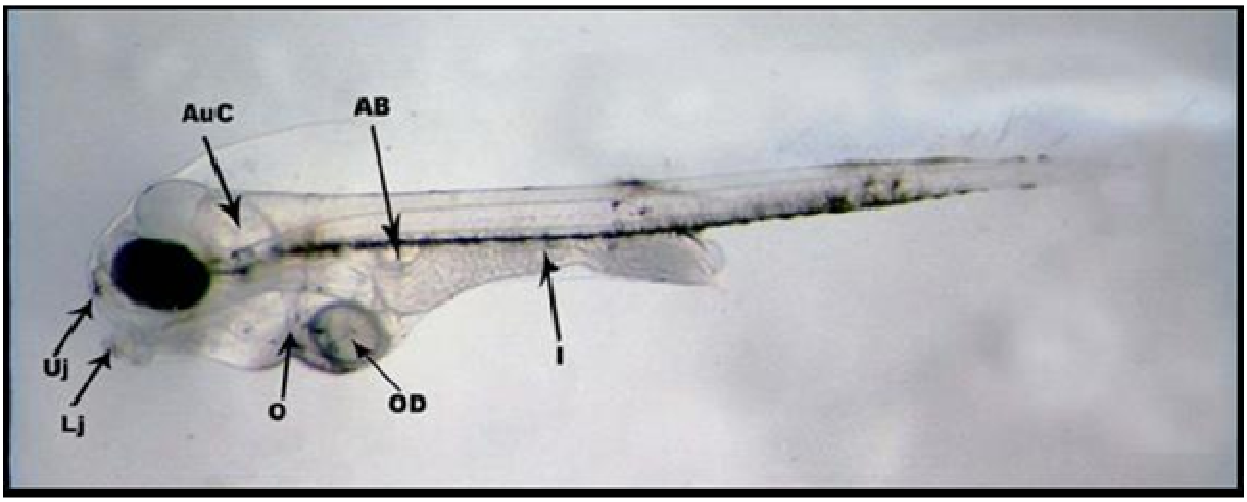

Figure 1a. Larva of *D. labrax* at the 4th day after hatching (bright field) [15 × 1.5].

(Enright et al., 1986 and Brown et al, 1997). Nevertheless, a carefully selected mixture of microalgae can offer excellent nutrition for larvae, either directly or indirectly (through enrichment of zooplankton). The nutrition adequacy of live food provided to the newly hatched larvae plays an important role in growth and increases their survival rate.

The nutritional adequacy of zooplankton used for feeding newly hatched larvae in marine hatcheries depends on the nutritional value of microalgae used to enrich it. Brine shrimp, *Artemia salina*, and the rotifer, *Brachionus plicatilis*, are the most suitable organisms for feeding newly hatched fish larvae. Increasing the nutritive value of these two organisms may in turn increase the survival and growth of the larvae to which they are fed.

Progress in commercial culture of many marine animals is currently hampered by an inconsistent supply of food. This is due in part, to the difficulty and expense associated with securing large, predictable quantities of high quality live feeds, especially microalgae and rotifers.

The present study was proposed to compare the growth and survival percentage of *D. labrax* larvae fed on *B. plicatilis* and *A. salina* enriched with *Chlorella salina, Dunaleilla salina, Nannochloropsis salina* and *Tetraselmis chuii* cultured in experimental medium(1) or in a basal medium as a control produced from algae laboratory and to evaluate the biochemical composition of each algal species to understand its nutritional value.

MATERIALS AND METHODS

Fish rearing and maintenance

Fertilized eggs of *D. labrax* were obtained from spawn of locally-maintained brood stock at the E1 kilo-21 hatchery in west Alexandria. The eggs were packed in plastic bags filled with oxygen, kept in darkness during transport to the National Institute of Oceanography Laboratory (NIOF). Eggs were acclimatized, and then incubated in triplicate 80 L glass aquaria at an initial density of 30 eggs L^{-1} in gently flowing sea water at constant temperature (21-23°C). Oxygen saturation was over 85%; salinity (39 ppt); and pH (7.5). Ammonia and nitrite concentrations were <0.012 mg L^{-1}; viable eggs were separated from dead (sinking) eggs by siphoning. The percentage of viable eggs was 60 - 70%.

The newly hatched larvae were stocked at a density of 7 individuals (ind) L^{-1} in twenty four glass aquaria (80 L capacity each). The aquaria were covered with black plastic sheeting to maintain dark conditions. Larva rearing was carried out in static aerated water up to 3 days post hatching (dph). The water was partially replaced (5 - 6% daily) from 4 to 12 dph. Out flowing water was strained through a 160 µg mesh. The water exchange rate was increased gradually with the age of the larvae. Debris and dead larvae were siphoned out each day before changing the water. The dead larvae in each aquarium were counted and recorded. At the beginning of 4 dph, larvae were fed with live *B. plicatilis* enriched with one of the eight microalga cultures: Ch_1, D_1, N_1 and T_1 or for controls Ch_{cont}, D_{cont}, N_{cont} and T_{cont}, respectively, received from algae laboratory. Each experiment was done in triplicate for statistical analyses.

First trial (4 - 24 dph)

The feeding regimes for larvae were started at age 4 dph (Figure 1a), when mouths were opened, the following sequence of feeding was conducted

1 - Rotifer *B. plicatilis* enriched by ch_1, D_1, N_1 and T_1, 2 - Control ch_{cont}, D_{cont}, N_{cont} and T_{cont}, respectively, at low density of 2 - 3 ind ml^{-1} on 4 dph; 3 - 5 ind ml^{-1} from 5 - 10 dph (Figures 1b and c); 5 - 10 ind ml^{-1} from 11 - 15 dph (1d ande).

At age 10 dph, the larvae were ready to accept nauplii of *A. salina*. These were fed at less than 0.2 ind ml^{-1} 10 - 12 dph, and increased to 0.5 - 2.0 ind ml^{-1} 13 - 24 dph (Figure 1f). 'Green water' of each algal species was added to each of the fish larva tanks feeding with both rotifers and *Artemia* to enhance its nutritional value.

Continual addition of Ch_1 or Ch_{cont}, D_1 or D_{cont}, N_1 or N_{cont} and T_1 or T_{cont} to each fish larva rearing tank was carried out to ensure enrichment for the live prey with the algae. The algae were added at a density of 150 000 - 200 000 cells ml^{-1} water daily to each aquarium; this was done in triplicate.

The first trial was continued until 24 dph when the fry were completely developed and their dorsal and anal fins were formed (Figure 1e). The

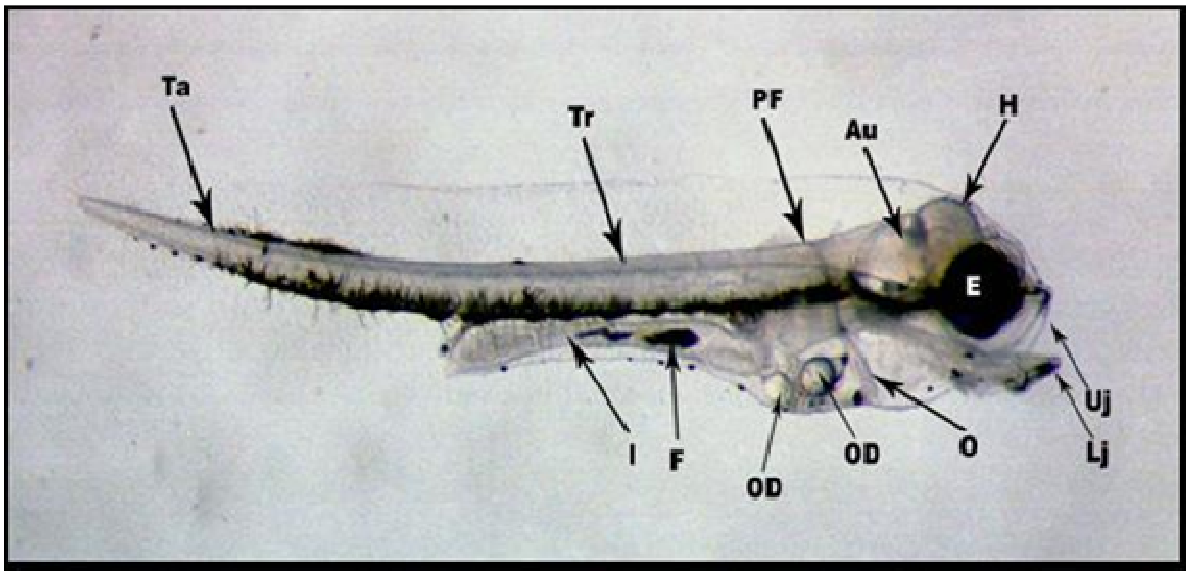

Figure 1b. Larva of *D. labrax* at the 7th day after hatching (bright field) [12 × 1.5].

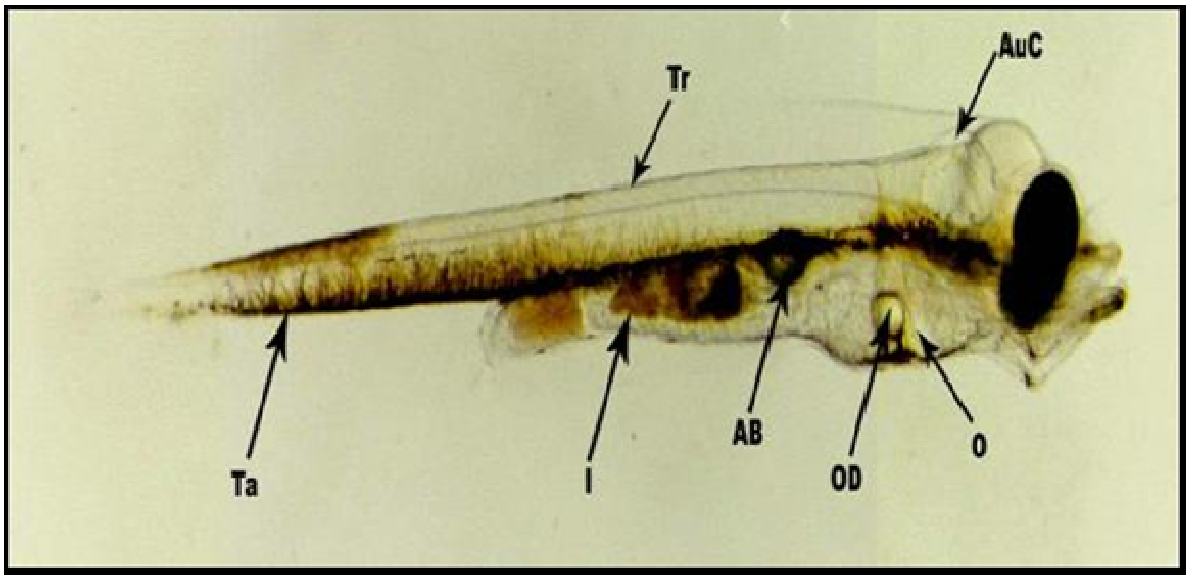

Figure 1c. Larva of *D. labrax* at the 9th day after hatching (bright field) [12 × 1.5].

growth, survival and mortality rate were recorded. Measurement of the newly hatched larvae and larval stages were made under a stereo-microscope (Wild, Heerbrugg). The measurements were taken, using an eyepiece micrometer calibrated with a standard decimal millimeter.

Second trial (25 - 60 dph)

At 25 dph, the remaining fry from the twenty four aquaria were transferred to a three aquarium (80 L capacity), filled with filtered sea water at a temperature range of 21 - 23°C. Acidity, ammonia, nitrite, dissolved oxygen and salinity were controlled daily. In the second trial the (Ch_1) media were used, which give high growth rate. Microalgae was added to increase the oxygen content of the water, and to reduce the concentration of ammonia, thus serving as a water conditioner. Rearing larvae were fed twice daily with *Artemia* metanauplii, increasing the density of feed daily according to total length (TL) of the fry. The TL increased from 10 to 35 mm; feeding rates from 0.5 to 2 ind ml^{-1}. The trial continued to 60 dph.

Biochemical composition of algae species

Total protein was extracted from the algal cells, according to the method of Rauch (1981). Protein content, both total and water-soluble, was determined following Hartree (1972). Individual amino acids, with the exception of tryptophan, were extracted and identified by the method of Speckman et al. (1958) using a Beckman 119-cl amino acid

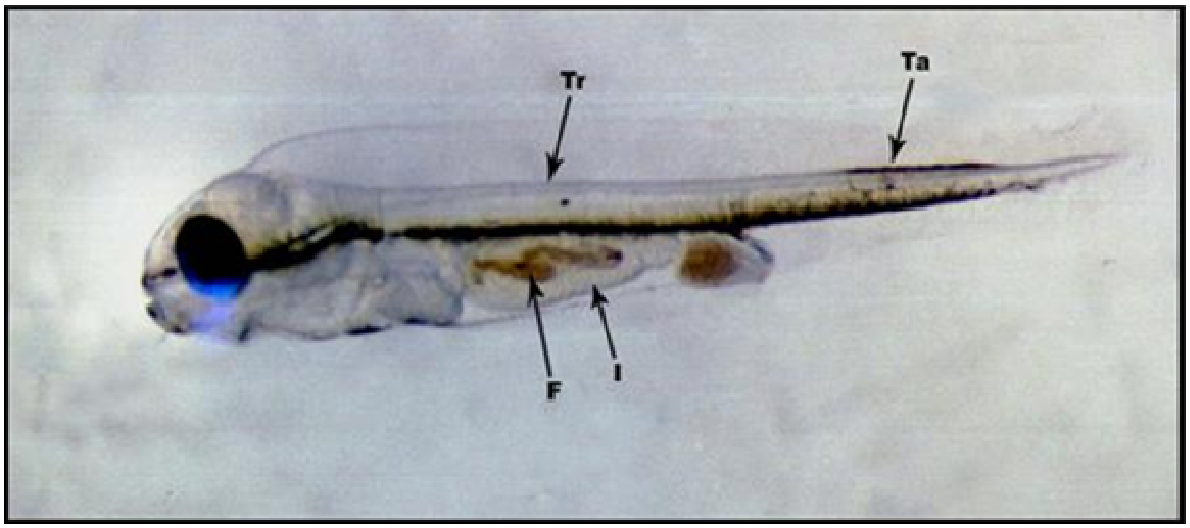

Figure 1d. Larva of *D. labrax* at the 12th day after hatching (bright field) [10 × 1.5].

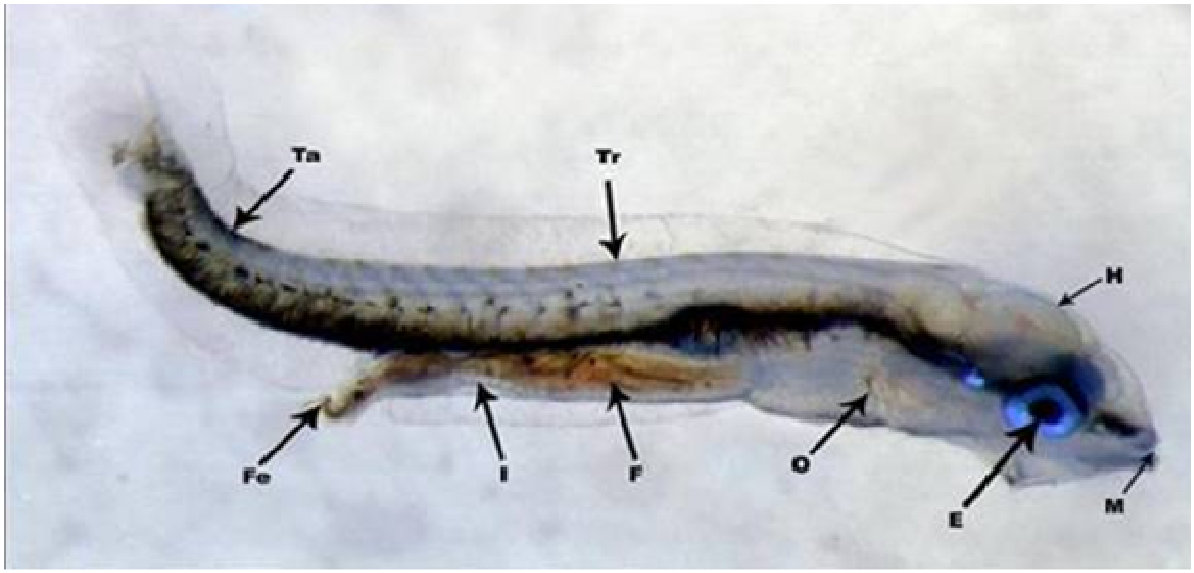

Figure 1e. Larva of *D. labrax* at the 15th day after hatching (bright field) [10 × 1.5].

analyzer. Carbohydrate content was estimated according to the methods of Dubois et al. (1959). The total lipid was extracted according to Bligh and Dyer (1959). Preparation of fatty acid methyl ester from total lipid was performed following Radwan (1978). All analyses for identification of fatty acid fractions were performed using gas chromatography (GC system Hp, Germany, serial No 6890 D 1530 A serial DE 00000348) equipped with a flame ionization detector; the packing column material was SP-2340.

Statistical analysis

Data were analyzed using statistical package for the social sciences (SPSS) version 11.5 and Excel. Analysis of data was done using student t-test to calculate the level of significance. The multiple range tests of mean differences was applied and was set at $P < 0.01$ level.

RESULTS

First trial 4 - 24 dph

Survival rate of D. labrax larvae

The mean survival rates for *D. labrax* larvae under the eight feeding regimes repeated in triplicate and the equation representing the relation between age and mean survival percentage during the period 5-25 dph are shown in Figures 2, 3, 4 and 5. At 10 dph, the mean survival rate of larvae fed *B. plicatilis* and *A. salina* enriched by *C. salina, D. salina, N. salina and T. chuii*, respectively, cultured in Ch_1, D_1, N_1 and T_1 were 95.3, 92.4, 87.2 and 79.0%, and 86.4, 84.1, 73.4

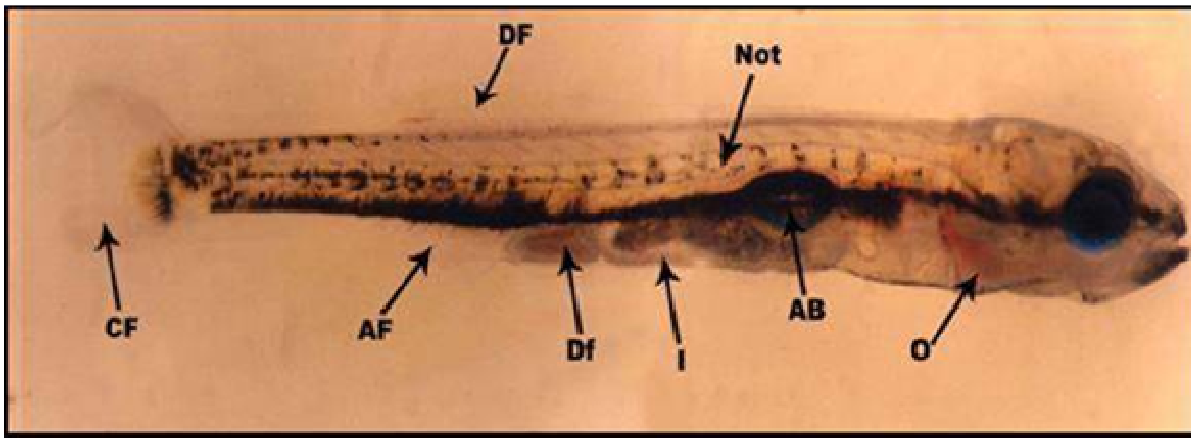

Figure 1f. Larva of *D. labrax* at the 19th day after hatching (bright field) [8 × 1.5].

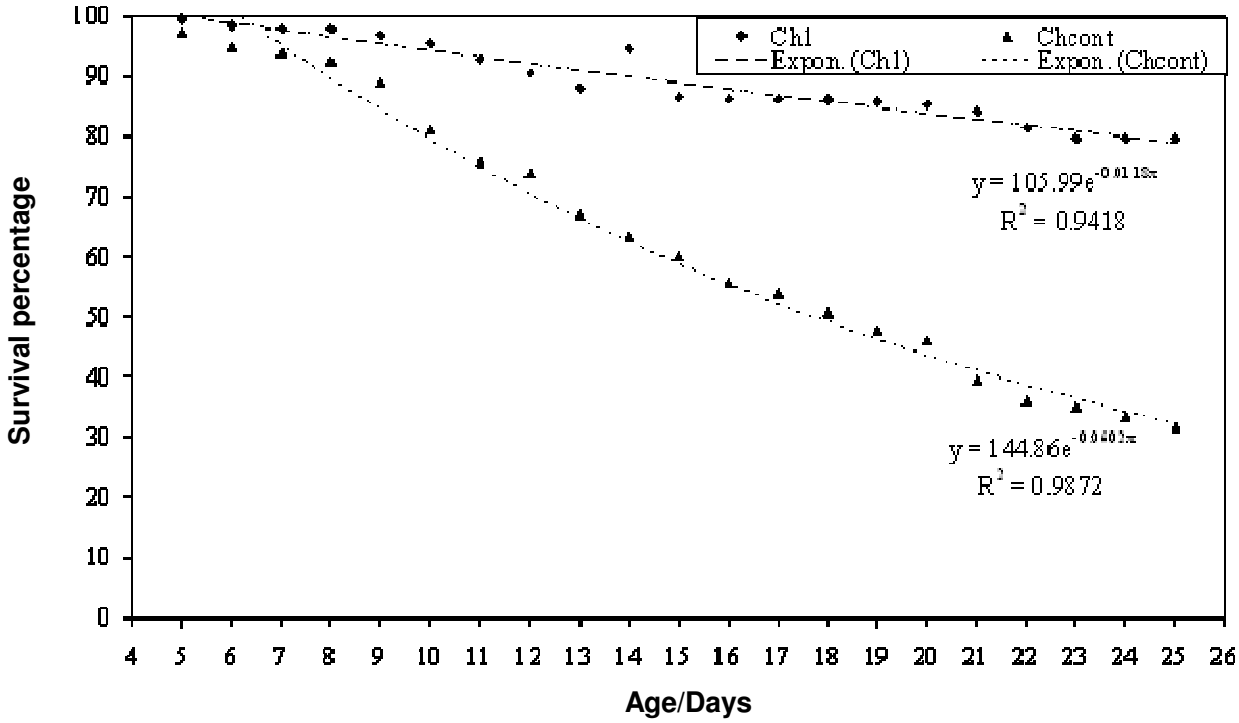

Figure 2. The relation between mean survival and age/days after hatching (dph) of *D.labrax* at feed regimes enriched by *Chlorella salina*.

and 56.3%, respectively, at 15 dph. The larvae fed on the algae grown in Ch_{cont}, D_{cont}, N_{cont} and T_{cont}, for the same time periods, showed mean survival rates of 80.9, 82.8, 87.2 and 74.4% and 60.0, 64.4, 50.1 and 53.1% respectively, at 15 dph. At the rearing period of 15 - 20 dph, the mean survival rate of larvae receiving the food enriched with Ch_1 and D_1 decreased slightly, from 86.4 and 84.1% to 85.2 and 79.1%, and then continue to decrease still it reached 79.5 and 73.8% at age 25 dph. Also, the survival rate of larvae receiving the food enriched with N_1 and T_1 progressively decreased in the rearing period 15 - 20 dph, from 73.4 and 56.3% to 69.4 and 43.2% and to 63.5 and 30.0% by 25 dph.

The mean survival rate of *D. labrax* fed with *B. plicatilis* and newly hatched *Artemia* enriched with Ch_{cont}, D_{cont}, N_{cont} and T_{cont} decreased from 60.0, 64.2, 50.1 and 53.1% to 46.0, 53.3, 41.3 and 42.3%, respectively, over the rearing period from 15 - 20 dph and these rate progressively decreased to 31.7, 40.1, 30.5 and 22.4% respectively, by 25 dph. At the conclusion of the first experiment, the mean survival rate of larvae fed on Ch_1, D_1, N_1 and T_1 enriched algae were 79.5, 73.8, 63.5 and 30.0%, respectively, where as Ch_{cont}, D_{cont}, N_{cont} and T_{cont} were 31.7, 40.4, 30.5 and

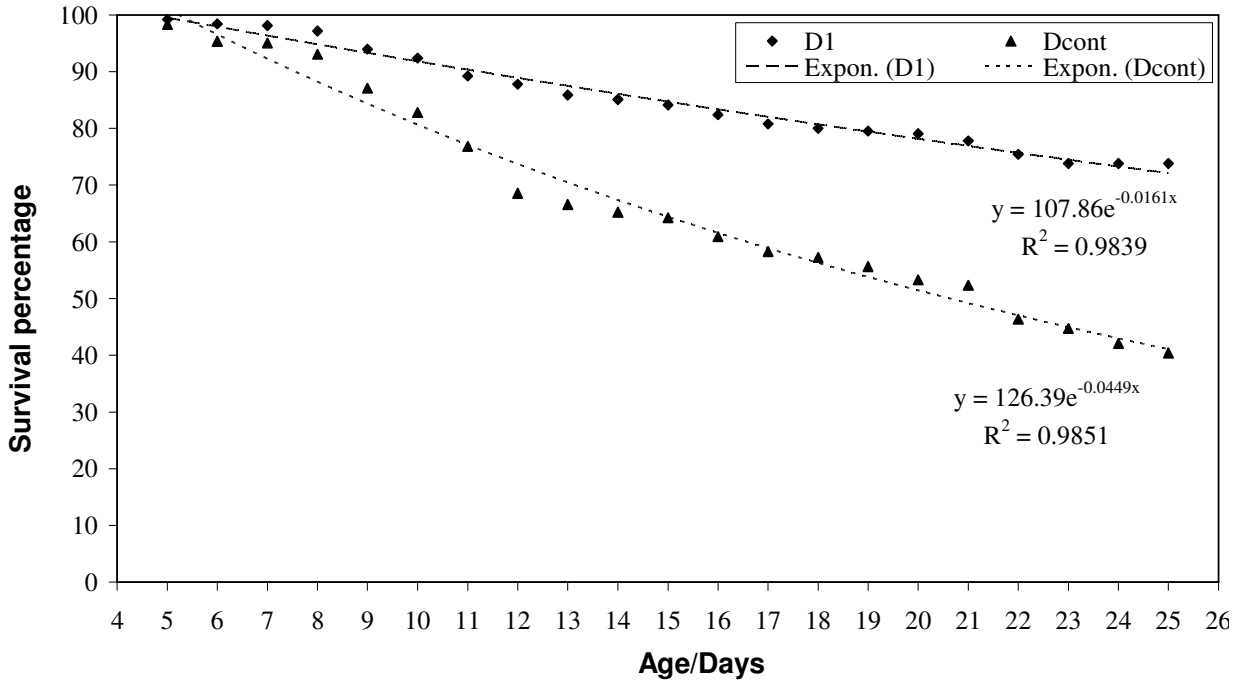

Figure 3. The relation between mean survival and age/days after hatching (dph) of *D.labrax* at feed regimes enriched by *Dunaleila salina*.

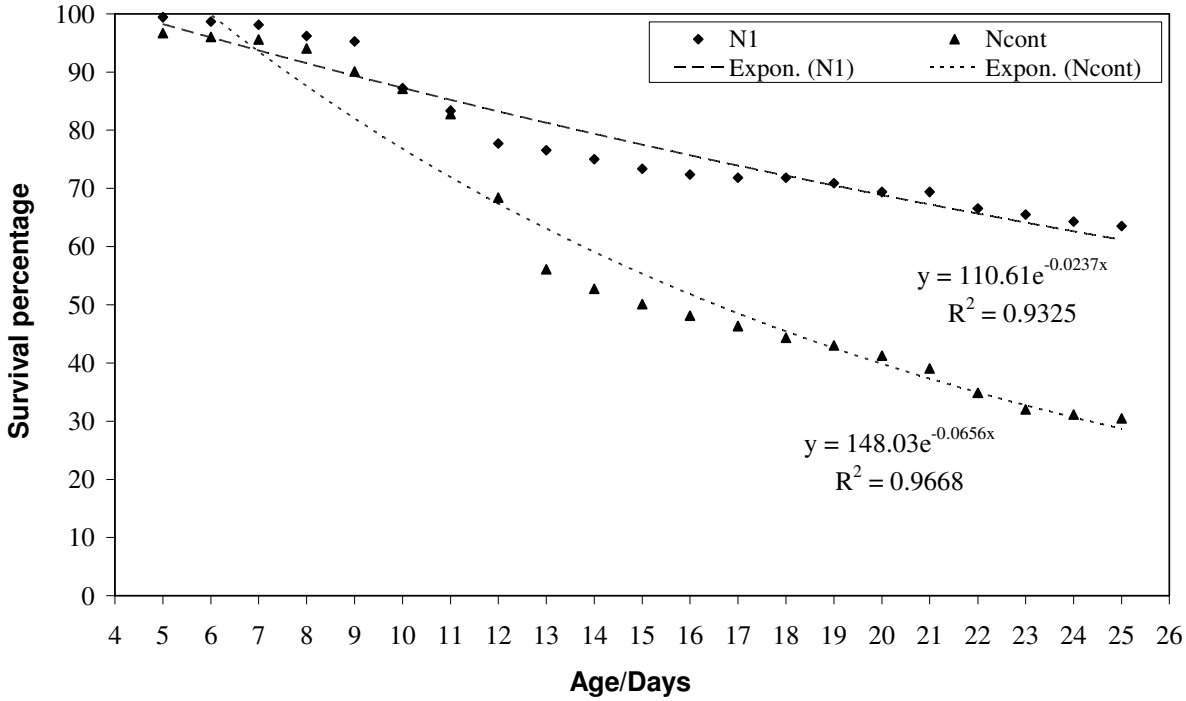

Figure 4. The relation between mean survival and age/days after hatching (dph) of *D.labrax* at feed regimes enriched by *Nannochloropsis salina*.

22.4%, respectively. It could be concluded that *Chlorella salina* culture in the modified experimental media was the best alga followed by *D. salina* which could be used to enrich *rotifers* and *Artemia* as food to marine fish larvae

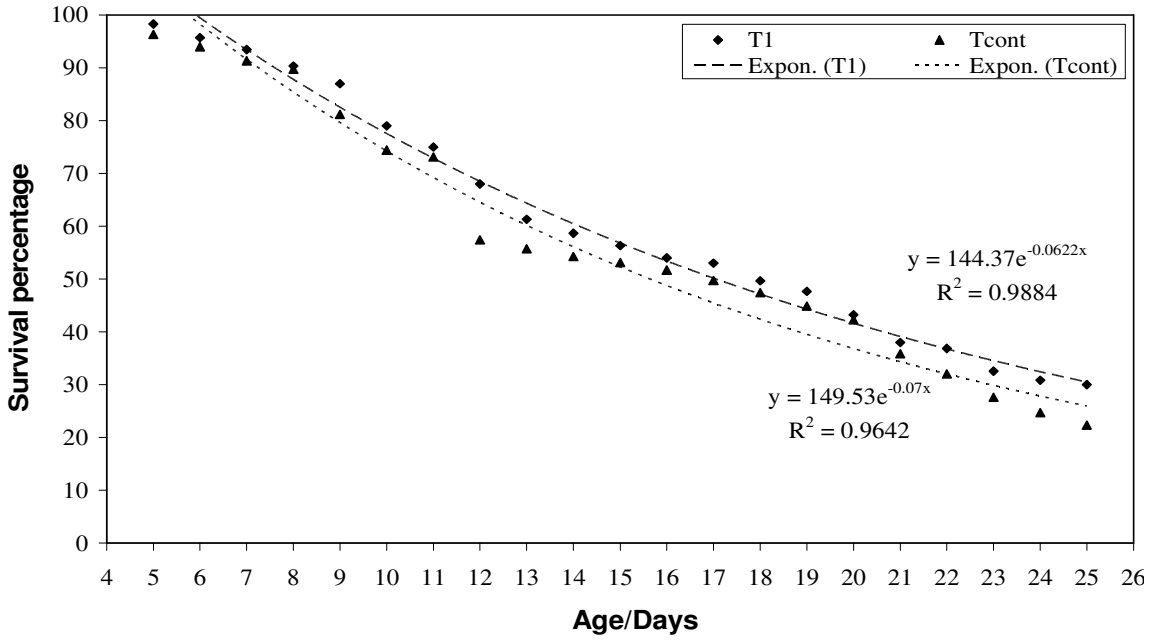

Figure 5. The relation between mean survival and age/days after hatching (dph) of *D.labrax* at feed regimes enriched by *Tetrasalmis chuii*.

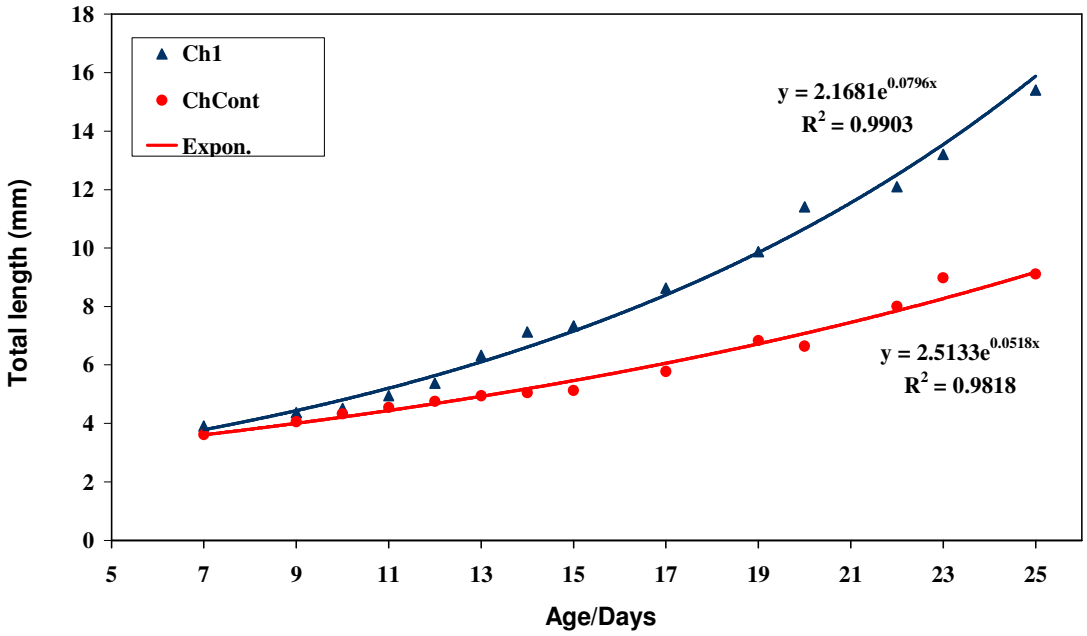

Figure 6. Growth in length of *D. labrax* from the 7th to 25th days after hatching feeding *Chlorella salina* culture in experimental and basal media.

especially *D. labrax* larvae.

Growth of D. labrax larvae

The relationship between mean growth in TL and eight feeding regime during the rearing period 5 - 25 dph is recorded in Figures 6,7,8 and 9 for C. *salina*, D. *salina*, N. *salina* and T. *chuii*, respectively. The mean TL of larvae increased from 3.9 and 3.6 mm at 7 dph to 5.4 and 4.8 mm at 12 dph with feed enriched with Ch_1 and Ch_{cont}, respectively. From 12-25 dph, the mean TL of larvae

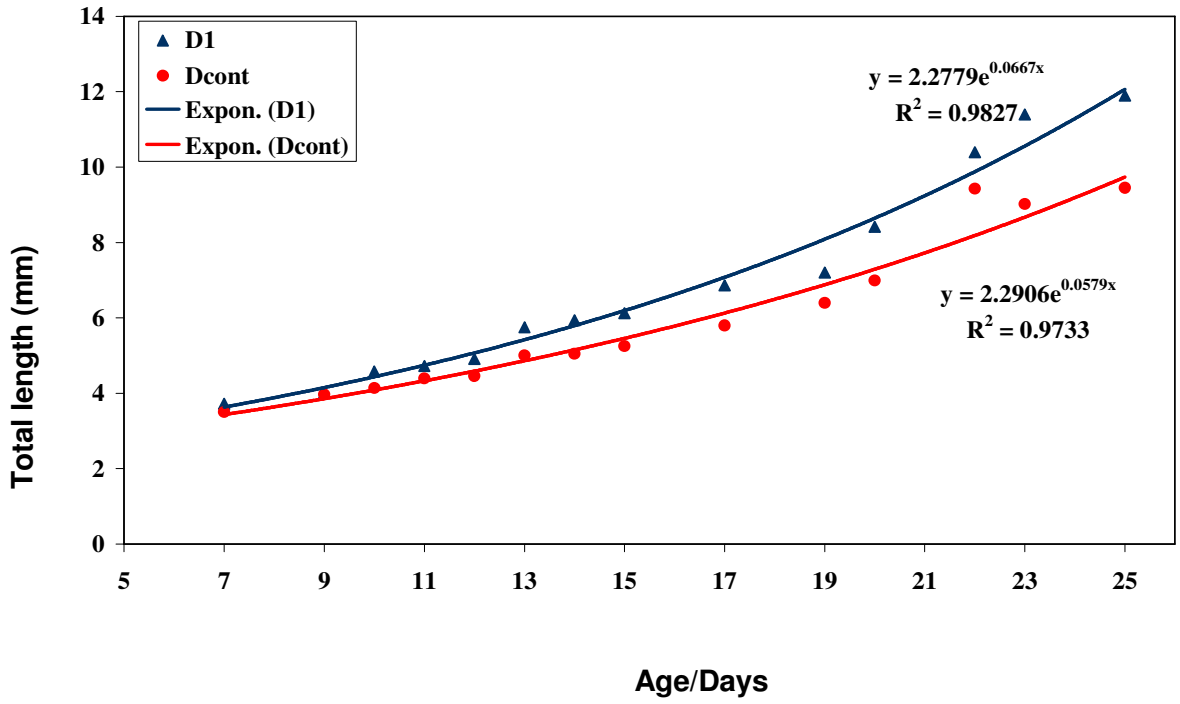

Figure 7. Growth in length of *D. labrax* from the 7th to 25th days after hatching feeding *Dunaleila salina* culture in experimental and basal media.

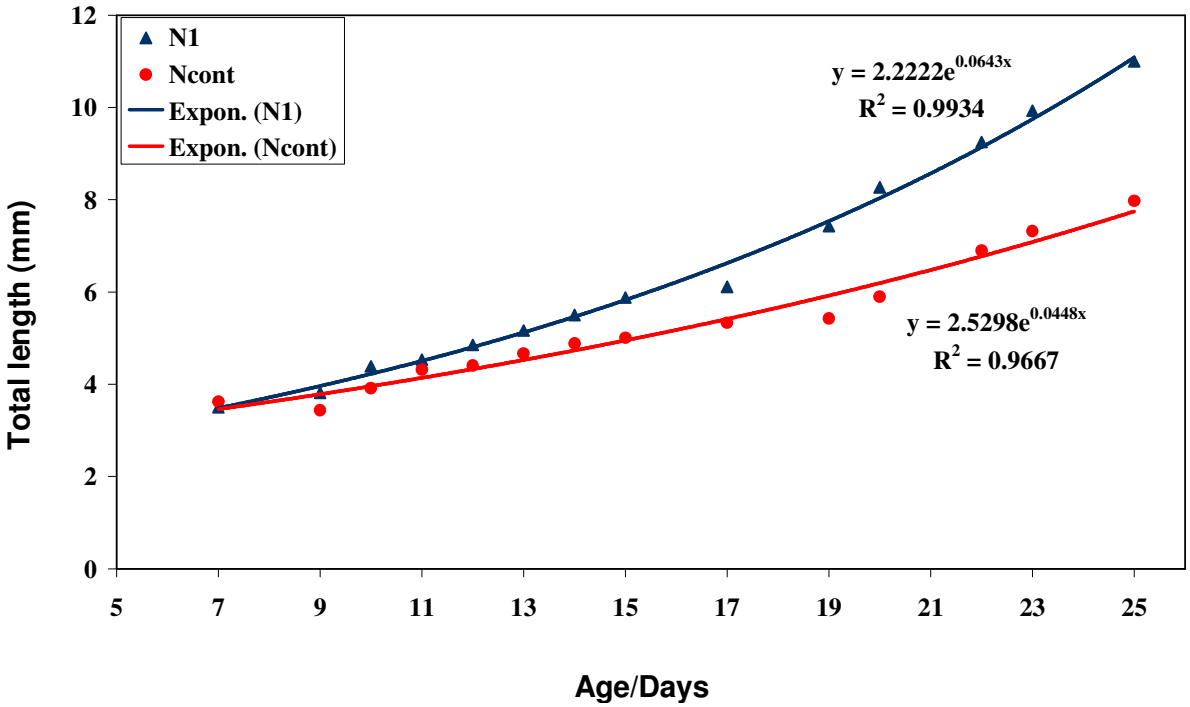

Figure 8. Growth in length of *D. labrax* from the 7th to 25th days after hatching feeding *Nannochloropsis salina* culture in experimental and basal media.

receiving food enriched with Ch1 increased by 10 mm, while the mean length of larvae fed on food enriched with Ch_{cont} increased by 4.4 mm. The mean TL of larvae increased from 3.7 and 3.5 mm at 7 dph to 4.9 and 4.5mm

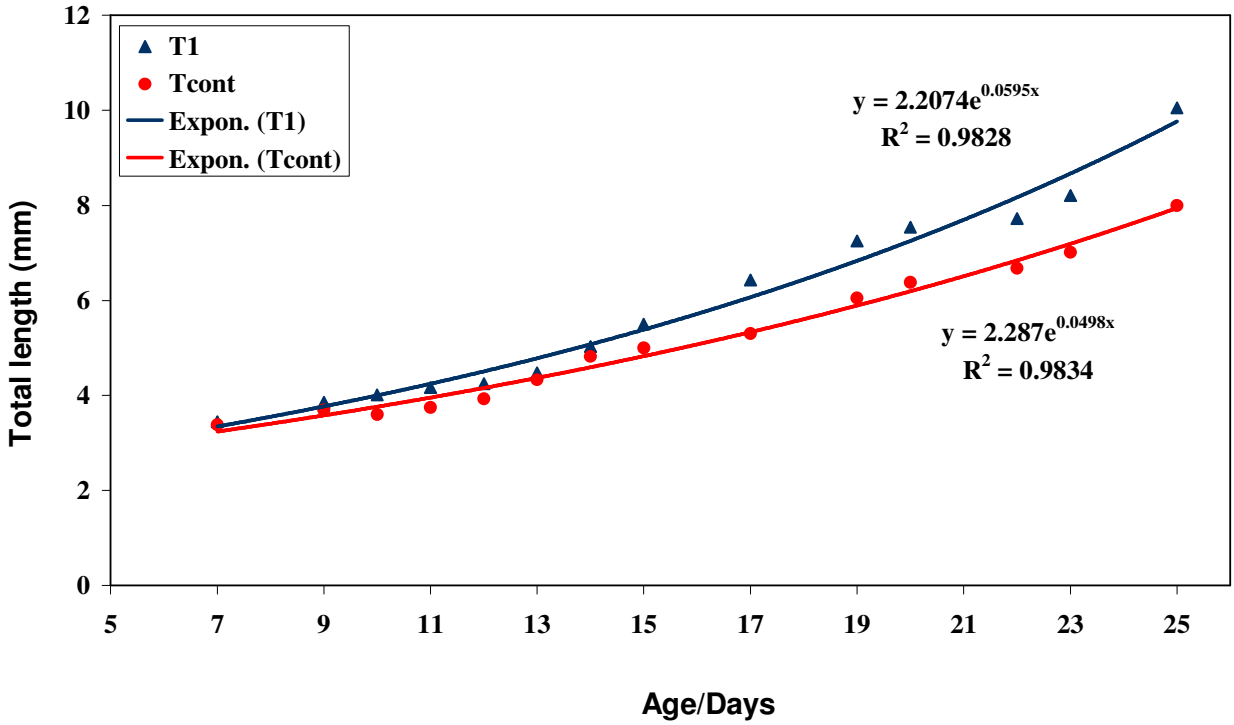

Figure 9. Growth in length of *D. labrax* from the 7th to 25th days after hatching feeding *Tetraselmis chuii* culture in experimental and basal media.

Table 1. Relation between increment/day in length and age of *D.larbax laravae* feeding different food regimes during the period of 7 to 25 days old after hatching.

Growth Age/day	Rate of increase in length/day mm							
	Ch_1	Ch_{cont}	D_1	D_{cont}	N_1	N_{cont}	T_1	T_{cont}
7 – 10	0.3	0.2	0.3	0.2	0.3	0.1	0.2	0.1
10 – 15	0.6	0.2	0.3	0.2	0.3	0.2	0.3	0.2
15 – 20	0.8	0.3	0.5	0.4	0.5	0.2	0.4	0.3
20 – 25	0.9	0.5	0.7	0.5	0.6	0.4	0.5	0.3

at 12 dph with feed enriched with D_1 and D_{cont} and from 3.5 and 3.6 mm to 4.9 and 4.4 mm with feed enriched with N_1 and N_{cont} while for T_1 and T_{cont} the mean TL of larvae increased from 3.5 and 3.4 mm to 4.3 and 3.9 mm for the same period.

From 12-25 dph, the mean TL of larvae receiving food enriched with D_1, N_1 and T_1 increased by 7.0, 6.1 and 5.8 mm, respectively, while the mean length of larvae fed on food enriched with D_{cont}, N_{cont} and T_{cont} increased by 5.0 mm, 3.6 mm and 4.1 mm, respectively.

At 25 dph (the end of the experiment), the mean TL of the larvae reached 15.4, 11.9, 11.0 and 10.01 mm when fed the Ch_1, D_1, N_1 and T_1 enriched food respectively, compared to 9.1, 9.5, 8.0 and 8.0 mm with Ch_{cont}, D_{cont}, N_{cont} and T_{cont}, respectively.

Increments of increase in length day^{-1} of *D. labrax* during the rearing period 7- 25 dph are recorded in Table 1. The rate of mean growth day^{-1} in TL increased significantly from 0.3, 0.3, 0.3 and 0.2 mm day^{-1} during the period from 7 -10 dph to 0.9, 0.7, 0.6 and 0.5 mm day^{-1} during the period from 20 - 25 dph in larvae fed on Ch_1, D_1, $N_1$and T_1, respectively enriched food, while, in the case of Ch_{cont}, D_{cont}, N_{cont} and T_{cont} respectively, they increased from 0.2, 0.2, 0.1 and 0.1 mm day^{-1} to 0.5, 0.5, 0.4 and 0.3 mm day^{-1} over the same period.

We can conclude that *C. salina* culture in the modified experimental medium (Ch_1) was considered as the best algae followed by *D. salina* that gave the best growth and survival percentage when compared with the other tested algae followed by *N. salina* and finally *T. chuii*.

Second trial 25 - 60 dph

In this trial the *D. labrax* larvae in triplicate were fed metanauplii enriched with *C. salina* cultured on the Ch_1 to

Table 2. Growth of *D. labrax* larvae 25 - 60 dph feeding on *Artemia* metanauplii enriched by *C. salina* cultured in experimental medium (Ch_1).

Age (days)	No. of samples	Min (mm)	Max (mm)	Mean total length (mm)	±SED
25	8	6.0	12.0	8.7	2.2
30	12	8.4	16.5	11.2	2.5
35	10	9.8	20.6	12.7	3.3
40	10	10.9	24.3	16.7	4.8
45	12	12.0	26.0	17.1	5.2
48	14	16.0	27.0	21.8	4.0
50	9	20.0	29.0	24.9	2.9
55	8	25.0	30.0	27.5	1.6
60	8	34.0	38.0	35.5	1.4

Table 3. The relationship between mean increase in total length and rate of increase in total length day^{-1} with age of *D. labrax* larvae during the period, 25 - 60 dph.

Age day^{-1} growth	25 - 35	35 - 45	45 - 55	55 - 60
Increase in total length (mm)	4.0	4.4	10.4	8.0
Rate of increase in total length mm day^{-1}	0.4	0.5	1.0	1.6

evaluate its effect on mean survival percentage and mean growth rate during the metamorphases period. The results revealed 98% of survival rate and the growth of *D. labrax* are presented in Table 2. Growth, represented by mean TL (mm), increased from 8.7±2.2 mm at the beginning of the trial to 35.5±1.4 mm at end of the trial (60 dph). The relationship between age and mean TL is represented by the following equation:

$$Y = 3.3242e^{0.039x} \qquad R^2 = 0.9848 \qquad r = 0.9924$$

The mean increase of TL was 4.0 mm for the period from 25-35 dph. It increased progressively to 4.4 and 10.4 mm during the period 35-45 dph and 45-55 dph, respectively. At the end of the trial, the mean growth increased to 8.0 mm for the period 55 - 60 dph (Table 3). Thus, the mean growth increment in length day^{-1} during the period 25 - 35 dph was 0.4 mm. It increased gradually to 0.5 and 1.0 mm for the period 35 - 45 and 45 - 55 dph, respectively, and reached 1.6 mm day^{-1} over the period from 55 - 60 dph (Table 3).

Biochemical composition of algae species

Data concerning total carbohydrate, protein, amino acids, essential amino acid, fatty acid, unsaturated fatty acid and docosahexaenic acid (DHA) C_{2216} content for Ch_{cont}; Ch_1; D_{cont}; D_1;N_{cont}, N_1 and T_{cont} and T_1 are recorded in Table 4. It is clear from the results that carbohydrate content significantly increased in Ch_1, D_1, N_1 and T_1 respectively. In correlation with the results obtained for total protein content of four tested algae significantly increased in Ch_{cont}, D_{cont}, N_{cont} and T_{cont} compared to Ch_1, D_1, N_1, and T_1.

The total amino acid and total essential amino acid significantly increased at $P < 0.001$ in Ch_{cont}, D_{cont}, N_{cont} and T_{cont} compared with Ch_1, D_1, N_1 and T_1.

The total fatty acid (TFA) and total unsaturated fatty acid (USFA) significantly increased from Ch_{cont}, D_{cont}, N_{cont} and T_{cont} to culture for Ch_1, D_1, N_1 and T_1 taking into consideration the state of DHA in the four tested algae which increased significantly. It is surprising to mention that Ch_1 gave the highest value followed by D_1, N_1 and T_1.

DISCUSSION

The results of this investigation showed that at the 4th dph, the eyes were completely formed, the mouth opened and body axis was straight which helped larvae to identify their food as internal-external feeding starts. This description agrees with Kvenseth et al. (1996) who stated that the development of a fundamental eye occurred at the time when the larval halibut was observed to capture prey and when the digestive systems appeared histologically functional.

Comparison of the eight feeding regimes against the mean TLs of *D. labrax* larvae with age day^{-1} during the rearing period 5 - 25 dph revealed that the larvae fed with rotifers and *Artemia* nauplii enriched with *C. salina* cultured in Ch_1 gave higher survival and TL followed by D_1, N_1 and T_1 than the control group. Dinis (1992) reported

Table 4. Total carbohydrate (µg ml^{-1} culture), protein (µg ml^{-1} culture), amino acid (µg g^{-1} fwt), essential amino acid (µg g^{-1} fwt), fatty acid (µg ml^{-1} culture), polyunsaturated fatty acid (µg ml^{-1}) culture and docosahexaenic acid (C226) µg ml^{-1} culture in alga species.

Nutrients	*Chlorella salina*		*Nannochloropsis salina*		*Dunaliella salina*		Tetraselmis *chuii*		F (p)
	Ch_{Cont}	Ch_1	N_{Cont}	N_1	D_{Cont}	D_1	T_{Cont}	T_1	
Carbo	37.05 ± 3.54	63.31^{a} ± 1.92	31.03 ± 0.97	59.30^{a} ± 2.80	31.32 ± 3.20	60.90^{a} ± 5.10	28.15 ± 3.10	48.70^{b} ± 5.10	7.852* (0.009)
t (p)	11.294* (<0.001)		16.524* (<0.001)		8.510* (0.001)		5.964* (0.004)		
Protein	45.40 ± 3.40	57.89 ^{a} ± 6.44	36.00 ± 8.31	49.50^{bc} ±3.00	45.90 ± 1.30	54.80^{ab} ± 2.10	38.28 ± 2.23	45.70^{c} ± 3.31	5.380* (0.025)
t (p)	2.974* (0.041)		2.651 (0.057)		6.241* (0.003)		3.220* (0.032)		
A.A.	50.98 ± 0.02	103.70^{a} ±0.02.	31.55±0.05	71.294 ^{b}±0.05	70.86±0.05	103.22 c ±0.03	26.73±0.04	59.80^{d} ±0.05	9194.58* (<0.001)
t (p)	30.75* (<0.001)		9.74* (<0.001)		8.89* (<0.001)		9.29* (<0.001)		
E.A.A.	21.98±0.04	48.82^{a} ±0.05	15.35±0.02	38.08 b ±0.04	32.27±0.03	61.60 c ±0.04	10.79±0.03	27.44^{d} ±0.05	3091.11* (<0.001)
t (p)	7.28* (<0.001)		9.34* (<0.001)		9.43* (<0.001)		4.66* (<0.001)		
F.A.	7.74 ± 0.98	16.34^{a} ± 3.98	7.52 ± 4.30	12.59^{ab}± 2.05	3.67 ± 1.35	12.18^{ab} ± 2.50	4.58 ± 1.32	10.66^{b} ± 2.13	2.265 (0.158)
t (p)	3.637* (0.022)		1.845 (0.139)		5.187* (0.007)		4.192* (0.014)		
PUSFA	2.45 ± 0.74	6.13^{a} ± 1.01	3.43 ± 0.79	4.41 ^{b} ± 0.87	1.30 ± 0.56	5.09^{ab} ± 0.30	1.08 ± 0.01	4.13^{b} ± 1.03	3.240 (0.082)
t (p)	5.091* (0.007)		1.443 (0.222)		10.290* (0.001)		5.127* (0.007)		
C226	1.87 ± 1.70	4.71^{a} ± 1.30	2.95 ± 0.87	3.73^{a} ± 1.22	0.70 ± 0.40	4.33^{a} ± 2.02	0.49 ± 0.13	3.39^{a} ± 1.01	0.509 (0.687)
t (p)	3.749* (0.020)		0.903 (0.411)		3.054* (0.038)		4.924* (0.008)		

Carbo, carbohydrate; A.A, amino acid; E.A.A, essential amino acid; F.A., fatty acid; PUSFA, polyunsaturated fatty acid; t(p), level of significance using t-test.

an average survival rate of 33.4% for benthic *Solea senegalensis* over 19 days under natural photoperiod, temperature 12-20°C and salinity 35.

The high mortality may be attributed to a number of factors, including the availability and nutritional adequacy of live food provided to the larvae. Zaki et al. (1998) studied the relationship between survival rate and feeding regimes in *Solea vulgaris* larvae, and reported a survival rate of 46.9% after two months. They proposed that the high larval mortality of *S. vulgaris* may be attributed to availability and nutritional adequacy of live food provided to the larvae at their different stages of growth.

D. labrax 25 - 60 dph fed with *Artemia* metanauplii enriched with (Ch_1) had higher survival and growth rates. Under these conditions growth of larvae represented by mean TL increased from 8.7±2.2 mm at age 25 dph to 35.5±1.4 mm at the end of the trial (60 dph). The mean increment in TL day^{-1}increased progressively from 0.4, 0.5 and 1.0 mm during the period 25-35, 35-45 and 45-55 dph, respectively. Near the end of the trial, 55 - 60 dph, the mean increment in TL day^{-1} increased to 1.6 mm. Therefore, the pro-gressive increase in length day^{-1} during the period 25-60 dph can be attributed to the availability and nutritional adequacy of food provided to the juvenile at their different growth stages.

B. plicatilis and *A. salina* were the main food used in rearing all marine fish larvae during the initial period of their life i.e. from 5 - 15 dph for *B. plicatilis* and from 10-25 dph with newly hatched *A.* nauplii (Firat et al., 2003). Rotifers *B. plicatilis* are regarded as living food capsules for trans-ferring nutrients to fish larvae (Lubzen et al., 1989). Several authors described the technique of larval feeding using *B. plicatilis* and *Artemia* (Lubzen et al., 1987, 1989; Barclay and Zeller, 1996; Brown, 2002).

The biochemical composition of Ch_1, D_1, $N_1$and T_1 and Ch_{cont} D_{cont}, N_{cont} and T_{cont} used to enrich *B. plicatilis* and *A. salina* nauplii to be used as live food for *D. labrax*, was 57.9, 54.8, 49.5 and 45.7 µgml^{-1} culture and 45.4, 45.9, 36.0 and 38.3 µgml^{-1} culture protein, and the total carbo-hydrate was 63.3, 60.9, 59.3 and 48.7 and 37.1, 31.3, 30.0 and 28.2 gml^{-1} culture, respectively, Therefore Ch_1 followed D_1, apparently increased protein and carbo-hydrate synthesis, which in turn increased protein level in body content greater than the other algal species. These results are in agreement with Wong and Ho (1977) who suggested that *chlorella* sp. is efficient at conversion of nitrogen to protein.

Taking into account the greater content of essential amino acid in Ch_1 and D_1, Coloso (1995) studied the requirements of phenylalanine for juvenile growth and found that increasing phenylalanine in the fish diets, increased survival rates from 79.2 to 91.7%. Srivastava et al. (1995) concluded that phenylalanine and methionine were from protein bound amino acids and they decreased in the developmental stages of cultured and wild salmon suggesting their importance in developmental stages. Also Ronnestad et al. (1998) concluded that when growing eggs and larvae of sea bass, the free amino acids appeared to be a significant energy substrate during these stages. Jacobsen and smith (1968) concluded that methionine (as free amino acid or in simple peptide form) was utilized in protein synthesis.

These results match our results obtained for the high survival rate (79.5 and 73.8%) for sea bass larvae fed on live food (*B. plicatilis* and newly hatched *Artemia*) enriched with Ch_1 and D_1which contained the greater amount of protein, amino acid and essential amino acid. From these results, $Ch_1$and D_1 used to enrich the feeding regime for *D. labrax* larvae during the rearing period could be considered the better formulated diet which provides nutrition requirements in young growing stages of marine species. The results for total fatty acid content and poly-unsaturated fatty acid fractions showed significant increased in Ch_1 compared to other algal species followed D_1. Concerning omega (ω_3)-poly-unsturated fatty acid DHA content significantly increase in Ch_1 and D1 to 4.7 and 4.3 μgml^{-1} culture respectively compared to other algal species.

Castell et al. (1994) revealed that DHA is an important component for developing nervous systems in both invertebrates and vertebrates and is an essential fatty acid for normal development. Watanabe (1993) said that enriching rotifers and *Artemia* nauplii with DHA prior to feeding those to larval fish helped to provide this essential fatty acid for the fish larvae during a critical phase of their development. They also showed that enrichment of *Artemia* nauplii with eicosapentaenoic acid (EPA $C_{20:5}$) did not provide the larvae with the essential DHA and many types of marine fishes are apparently incapable of elongating the EPA to DHA. Additionally, feeding rotifers and *Artemia* nauplii with DHA results in EPA enhancement providing eicosanoids which positively enhance immunocompetence in fish larvae. Castell et al. (1994) concluded that *Artemia* and rotifers are capable of readily retro converting $C_{22:6}$ (n-3) to $C_{20:5}$ (n-3) through a process of β-oxidation. These results confirm those obtained by Barclay and Zeller (1996) and also agree with our opinion since the results of their study suggested that brief feeding of rotifers and *Artemia* nauplii with DHA-rich microalgae may provide the best strategy for n-3HUFA enrichment of live food organisms used in aquaculture.

Watanabe (1979) found that DHA (ω_3-PUFA) are essential fatty acids for marine fishes and speculated that their high levels are derived from marine organisms known to contain high amounts of ω_3-PUFA. D' Abramo (2002) showed that lipid and essential fatty acids especially DHA (PUFA) played an important role in nutritional physiology and was reflected in good growth and survival for the marine fish larvae that often require the addition of carbohydrates.

Conclusion

We conclude that *Chlorella salina*, when cultured in the experimental medium Ch_1, achieved better growth and survival rates for *D. labrax* larvae when they were fed on live food (*B. plicatilis* and *Artemia* nauplii) enriched with this alga followed by *D. salina*. This may be due to its provision of the important metabolites essential for the growth of marine fish larvae, such as protein, fatty acids especially omega-3 fatty acid fractions necessary for growth and development.

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