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Full Length Research Paper

Effect of enriched *Brachionus plicatilis* and *Artemia salina* nauplii by microalga *Tetraselmis chuii* (Bütcher) grown on four different culture media on the growth and survival of *Sparus aurata* larvae

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The growth, developmental stages and survival rates of *Sparus aurata* larvae fed with *Brachionus plicatilis* and *Artemia salina* nauplii enriched by microalga *Tetraselmis chuii* were studied. Two experiments were carried out; the first concerning with culturing the microalga (*T. chuii*) in four different media, then using these cultures for enrichment of the rotifer (*B. plicatilis*) and brine shrimp (*A. salina*) nauplii). The second experiment was considered the application of enriched *B. plicatilis* and *A. salina* were used for feeding *S. aurata* larvae. Mean growth and survival rate of larvae were used for evaluation of the best medium. Erdschriber medium was considered the best algal medium compared with the other media used because the cell density were the highest (24.5 X 10^6 cell.ml^{-1}) on the 11 day (late logarithmic/early stationary phase of growth), the highest survival and greatest growth rates of *S. aurata* larvae were noticed. The total fatty acids in *T. chuii* especially the polyunsaturated fatty acid were represented by content 5.5% [arachidonic acid (ARA)], 4.8% [eicosapentaenoic acid (EPA)] and 5.0% [docosahexaenoic acid (DHA)] to the total fatty acids. The higher production of total fatty acids in *B. plicatilis* fed on *T. chuii* grown in Erdschriber medium was due to the accumulation of polysaturated fatty acids (PUFAs) (94.7% of the total lipids). EPA was absent, while DHA (22:6ω_3) was enhanced and constituted 657.49 µg.g^{-1} (91.4% of the total fatty acids). Saturated fatty acids were detected more than unsaturated one (63% of the total fatty acids), in case of *A. salina* fed on *T. chuii* grown in Erdschriber medium, especially the short chain fatty acids (C16:0, C18:0 and C21:0). On the other hand PUFAs constituted 22.3% of the total fatty acids, due to the presence of DHA which formed 8.0% of the total fatty acids, while ARA and EPA were absent. The results show that *B. plicatilis* and newly hatched Artemia enriched with *T. chuii* grown on was effective for good growth and survival rate of *S. aurata* larvae.

Key words: *Tetraselmis chuii*, Erdschriber medium, *Brachionus plicatilis*, *Artemia salina*, *Sparus aurata* larvae, growth rate, survival.

INTRODUCTION

One of the basic problems in aquaculture is to supply sufficient live food of the best quality at the lowest cost, upon demand. The availability of a reliable and highly nutritional larval food is one of the crucial demands at this production stage. Some microalgae strains are recognized as excellent sources of proteins, carbohydrates, lipids and vitamins, to be used as food and feed additives

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a(Leonardos and Lucas, 2000; Knuckey et al., 2006; Krienitze and Wirth, 2006). The microalgae in quaculture are of great importance because they start the food chain and its nutritional values related to their biochemical composition. Biochemical composition of the algal cells was related to its cellular compositions (Geider and La Roche, 2002). The genus *Tetraselmis* is one of the most widely used microalga in mariculture for feeding marine organisms, due to its ability to grow under a wide range of physical and chemical environmental conditions. It is also one of the preferred foods for rotifer cultures (Makridis et al., 2006). *Tetraselmis chuii* is very important due to its higher proteins, lipids, essential fatty acids and sterols. The chemical composition of Rotefer is similar to that of the algae upon which they fed (Ben-Amotz et al., 1987; Cariç et al., 1993). Rotefer (*Brachionus plicatilis*) and *Artemia* sp. are normally used because of their ease in culture, however, they are deficient in essential nutrients, especially essential long chain fatty acids such as 20:4 ω_6, 20:5 ω_3, and 22:6 ω_3 (Han et al., 2000).

Production and rearing of fish larvae have been identified as a major constraint to many aquaculture processes (Sukenik et al., 1993). *Sparus aurata* is of commercial importance and high economic and market value. The artificial spawning, larval rearing and experimental growth of *S. aurata* have been investigated by Al-Absawy (1997), Mabrouk (1999) and Zaki et al. (2007). Much effort is being devoted to developing a commercially feasible technology to produce EPA directly from microalgae (Barclay et al., 1994; Lebeau and Robert, 2003).

Lipids are major sources of metabolic energy during the embryonic and pre-feeding fish larval stages (Evans et al., 2000). Studies have shown that essential fatty acids (EFA), such as docosahexaenoic acid (DHA, 22:6ω_3), eicosapentaenoic acid (EPA, 20:5ω_3), and arachidonic acid (ARA, 20:4ω_6) are important in larval fish nutrition (McEvoy et al., 1998; Estevez et al., 1999; Sargent et al., 1999; Zaki and Saad, 2010). Recently, absolute and relative levels of DHA, EPA and ARA in the diets of marine fish larvae have received considerable attention (Sargent et al., 1999; Harel et al., 2002; Bell and Sargent, 2003), and lack of these fatty acids can result in reduced growth and survival during first feeding, as well as incomplete pigmentation of the fry (Watanabe, 1982). Addition of microalgae to rotifers as a long term enrichment technique (combined growth and ω_3 PUFA enrichment during the production phase of Rotifers) seems to be very efficient in obtaining high ω_3 PUFA content in rotifers while maintaining a normal lipid content (Rainuzzo et al., 1997). Saad (2007) proved that the alga *T. chuii* can synthesize great amount of PUFA by media modification through increasing Na Cl 1.5 times that of control.

The aim of this study was to investigate the growth and survival rates of *S. aurata* larvae reared on rotifer (*B. plicatilis*) and brine shrimp (*Artemia salina* nauplii) enriched with the green microalga *T. chuii* grown on four different media composition to describe the embryonic and larval developmental stages of *S. aurata* larvae. This information is a necessary tomaximize marine hatchery production and to select the best medium promote the growth of fish larvae.

MATERIALS AND METHODS

Microlgal culture

The microalgal strain (*T. chuii*) was cultured in filtered sterilized seawater enriched with four different culture media (Boussiba et al., 1987; Erdschriber as represented in Utex, 1993; Palanisamy et al., 1991; Guillard, 1975) with controlled conditions of temperature 20 ± f/$_2$-enriched sea water medium, before Guillard, 1975 2°C, pH 7 to 7.5 and 1000 Lux illumination in a 12 L:12 D cycle. Glass flasks of 0.5 to 2 L capacity were used for stock cultures (indoor) starting with an inoculation density of 0.3 X 10^6 cell.ml^{-1}. The density was maximized once, and the maximum growth rate was attained at 11 days (late logarithmic/early stationary phase of growth). The starter stock cultures were used intern to inoculate subcultures (carboys 20 L capacity) and then the latter were maintained to be mass produced in about 8 polyethylene bags (50 L each). The bags were enriched according to each medium constituent, and supplied with light intensity 1500 Lux with aeration through electric air blower to serve vigorous aeration. The cultured algae were used to enrich and evaluate *B. plicatilis* and *Artemia* metanauplii or also used as green water during the larval rearing period in the experiment.

Growth measurement of *Tetraselmis chuii*

Cell counts of *T. chuii* were measured using hemocytometer, and the mean number of cells per ml was obtained. The optical density of *T. chuii* was also determined spectrophotometrically at 560 nm (Wetherell, 1961) using UV/V Spekal 1300, Analytik Jana AG Spectrophotometer. Growth measurements were applied triplicate every 3 days

Rotifer culture

B. plicatilis was cultured at temperature 26°C and salinity 24 ppt in four plastic square tanks with 1.0 m^3 capacity (one tank for each treatment food regime). The cultures of rotifers were reared through long term enrichment period with *T. chuii* grown on the different 4 culture media at day light intensity starting with density 25 ind/ml and collected after 3 days at density 200 ind/ml through 50 µm mesh size plankton net and rinsed with clear sea water, and then enriched again for short term enrichments in four plastic containers with the four different cultures of the tested alga.

Brine shrimp culture

A. salina was produced by hatching brine shrimp cysts through decapsulation technique. They were incubated in sea water to hatch as described by Lavens and Sorgloos (1996). The produced nauplii were harvested, and then fed to the fish larvae of S. aurata at age of 16 day after hatching (DAH) except for the case of Erdschriber medium, the fish larvae started feeding Artemia nauplii from the day 13 after hatching due to their rapid growth. The

enrichment of Artemia metanauplii by the four different treatments started after 36 h after hatching of Artemia, then fed to larvae at age 20 DAH.

Analytical methods

Total protein and lipid contents of algal strain in different culture media were performed. Fatty acids content and their fractionations were determined in the algal cells after 11 days of culturing, in addition in enriched *B. plicatilis* and *A. salina*. Total protein was determined by the Folin-phenol method of Lowery et al. (1951). Total lipid contents were analyzed gravimetrically after extraction with chloroform–methanol (2:1) using the Folch method as modified by Bligh and Dyer (1959). Fatty acid methyl esters were analyzed using gas liquid chromatography (HP-6890 gas–liquid chromatography).

Spawning and larval rearing of *Sparus aurata*

Adult and fully ripe *S. aurata* (5 males and 7 females with total length of 26 to 30 cm and total weight of 640 to 860 g) were collected from Damietta Governrate in Egypt (E1-Ratoma fish farm, salinity 34 ppt). The fish were acclimatized for ten days in cylindrical tanks capacity 3 tons of sea water. The fish were fed with small live fish and crab about 2 to 4% of the fish weight twice daily at salinity 38 ± 2 ppt and temperature (14 to 16°C). After twelve days the fish spawned naturally. Floating fertilized ova was collected.

Twelve glass aquaria each 60 L as triplicate were used for the experiment (39 ppt water salinity and 19 ± 2°C). The density of newly hatched larvae was about 20 larvae/L. The aquaria were cleaned daily, also about 70% of its water and the dead larvae were removed. The pH range was 8.3 ± 2, ammonia and nitrite component were always not exceed 0.012 mg^{-1}. Illumination was about 800 lux for 24 h, very gentle aeration was conducted.

Larval rearing and developmental stages of *S. aurata* from fertilized eggs until 28 DAH was described. The feeding regime for *S. aurata* larvae started at age 4^{th} day after hatching when mouth was open using *B. plicatilis* followed by *Artemia* nauplii and meta nauplii enriched on *T. chuii* (150 $X10^3$ $cell.ml^{-1}$) as described by FAO (1999) based on the four different treatment adopting a feeding Schedule regime (Table 1).

Growth, survival rates of *Sparus aurata* larvae

The growth measured as (HL: Head length, TrL: Trunk length, TaL: Tail length and WL: Width length), survival rates were monitored. Ten larvae per aquarium were taken at days (4, 8, 13) after hatching. While, at day 18, 23 and day 28 after hatching the morphometric measurements were determined.

Statistical analysis

Statistical tests were performed using SPSS Inc. program version 15 (2006). Data were represented as mean ± standard deviation of three replicates. Data were analyzed using overall one-way analysis of variance (ANOVA) and, when differences observed were significant at P ≤ 0.05, the means were compared by LSD test.

RESULTS

Significant increase in the cell counts of *T. chuii* at P ≤ 0.05 was obtained in Erdschriber medium (24.5 x1 0^6 $cell.ml^{-1}$) on 11 days age (late logarithmic/early stationary phase of growth), than the other three medium (Figure 1). The results of one way ANOVA revealed that there was no significant difference in the mean cell count of *T. chuii* on the four culture media at P ≤ 0.05 (Table 2) on the 11 days age. As regarded with optical density, there was parallel relationship with cell counts (Figure 2), while the analysis of variance showed a significant difference in the optical density of the four different cultured media (Table 2).

Significant differences at P ≤ 0.05 in the total protein and lipid content of *T. chuii* throughout the experimental period occurred among the media (Table 2). As shown in Figure 3, total protein attained higher values in the Erdschriber medium. The results show that there were significant differences between protein content of *T. chuii* grown in Erdschriber medium and Boussiba or $f/_2$ media. On the other hand, the results obtained for total lipids showed significant increase (P ≤ 0.05) in Erdschriber medium than in the other three media (Figure 4).

Results in Table 3 showed that the maximum fatty acids contents in *T. chuii* was found with Erdschriber medium, 33.19 $\mu g.g^{-1}$ (35.5% of the total fatty acids content), 36.5 $\mu g.g^{-1}$ (39.1%) and 23.7 $\mu g.g^{-1}$ (25.4%), respectively for saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFAs) and polyunsaturated fatty acids. The highest percentage of PUFAs in *T. chuii* was grown in Erdschriber medium due to the presence of ARA (20:4 ω_3), EPA (20:5 ω_3) and DHA (22:6 ω_3). ARA was represented by 5.5%, EPA by 4.8% and DHA by 5.0% of the total fatty acids.

Composition of the fatty acids in Rotifera (*B. plicatilis*) feed on *T. chuii* grown on four different culture media is represented in Table 4. The higher total lipid content was recorded when rotifers fed on *T. chuii* grown on Erdschriber medium (718.99 $\mu g.g^{-1}$). The higher production of total fatty acids was due to the accumulation of PUFAs (94.7%) of the total lipids. EPA was absent, while DHA (22:6ω_3) was enhanced and constituted 657.49 $\mu g.g^{-1}$ (91.4% of the total fatty acids).

The results represented in Table 5 showed that the fatty acids composition of *A. salina* nauplii fed on *T. chuii* grown on Erdschriber medium had higher fatty acids content (1859.02 $\mu g.g^{-1}$). Saturated fatty acids were presented more than unsaturated ones (63% of the total fatty acids), especially the short chain fatty acids (16:0, 18:0 and 21:0). On the other hand, PUFAs constituted 22.3% of the total fatty acids, due to the presence of DHA which formed 8.0% of the total fatty acids, while ARA and EPA were absent.

The morphometric measurements of *S. aurata* larvae enriched with rotifers and Artemia fed by the alga *T. chuii* grown on four different culture media from day 18 to 28 after hatching were determined (Table 6). The results show that the maximum growth of *S. aurata* larvae fed on *T. chuii* grown on Erdschriber medium, the maximum measurements reached 2.08, 1.95, 5.68 and 1.72 mm for HL, TrL, TaL and WL, respectively at the end of the

Table 1. Schedule of *S. aurata* larvae fed on Rotifer (*B. plicatilis*) and *A. salina* nauplii fed on *T. chuii* grown in four different culture media.

Age larvae (DAH)	Culture media							
	Palanisamy		Erdshcriber		Boussiba		f/2	
	Rotifer	Artemia	Rotifer	Artemia	Rotifer	Artemia	Rotifer	Artemia
0-3	0	-	0	-	0	-	0	-
4-6	5-10	-	5-10	-	5-10	-	5-10	-
7-10	15-20	-	15-20	-	15-20	-	15-20	-
11-13	25	-	25	0.5*	25	-	25	-
14-16	25	0.5*	20	1.0	30	-	30	-
17-20	20	1.0	20	1.5	25	0.5*	25	0.5*
21-24	15	1.5	15	2.0	20	1.0	20	1.0
25-28	10	2.0	10	3.0	15	1.5	15	1.5

*means feeding Artemia newly hatched nauplii only. DAH, Days after hatching.

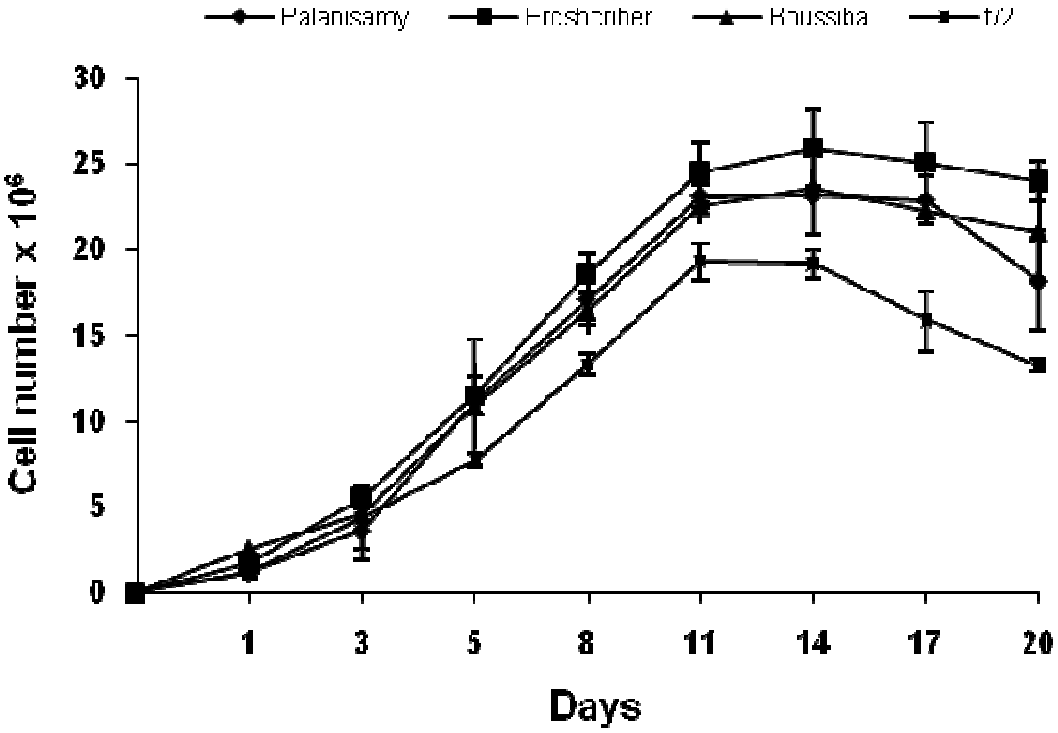

Figure 1. Growth of *T. chuii* (cell number x 10^6) under four different culture media for 20 days culturing. Data are the mean ± standard deviation of three replicates.

Table 2. Analysis of variance of cell number, optical density, total protein and lipid content of *T. chuii* under four different culture media in late logarithmic phase/early stationary phase of growth (11 day age).

Parameter	Culture media				
	Palanisamy	Erdschriber	Boussiba	f/2	F
Cell number	23.13 ^{a} ± 0.68	24.53 ^{a} ± 1.77	22.53 ^{a} ± 1.05	19.27 ^{a} ± 1.12	10.02*
Optical density	0.20 ^{a} ± 0.03	0.237 ^{b} ± 0.03	0.17 ^{c} ± 0.02	0.12 ^{d} ± 0.01	14.25*
Total protein	6.09 ^{a} ± 0.42	6.33 ^{a} ± 0.21	3.56 ^{b} ± 1.19	3.42 ^{b} ± 0.43	16.3*
Total lipid	0.06 ^{a} ± 0.002	0.14 ^{b} ± 0.02	0.06 ^{c} ± 0.003	0.05 ^{d} ± 0.01	47.1*

*, Statistically significant at $p \leq 0.05$; different letters indicate statistical differences between groups (ANOVA, $P \leq 0.05$).

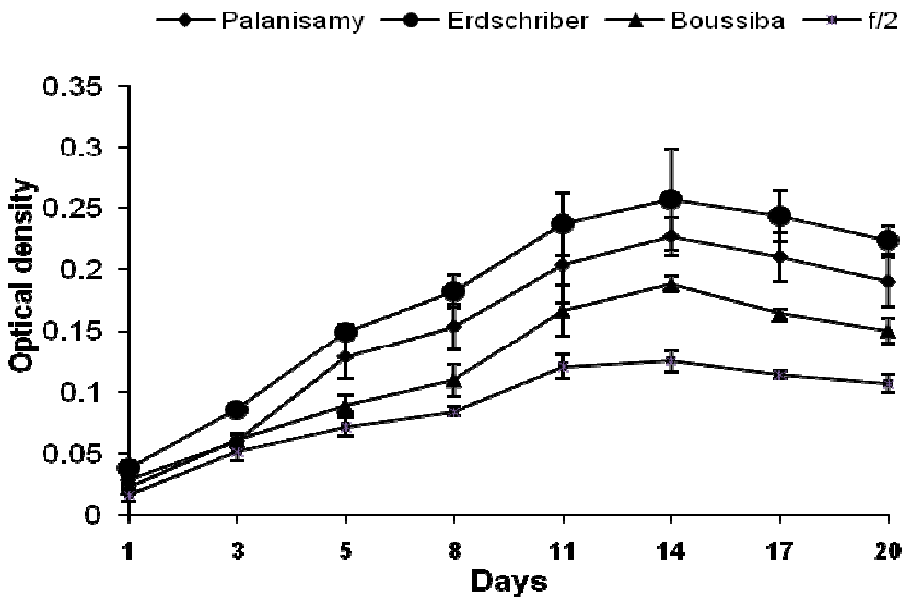

Figure 2. Growth of *T. chuii* (optical density) under four different culture media for 20 days culturing. Data are the mean ± standard deviation of three replicates.

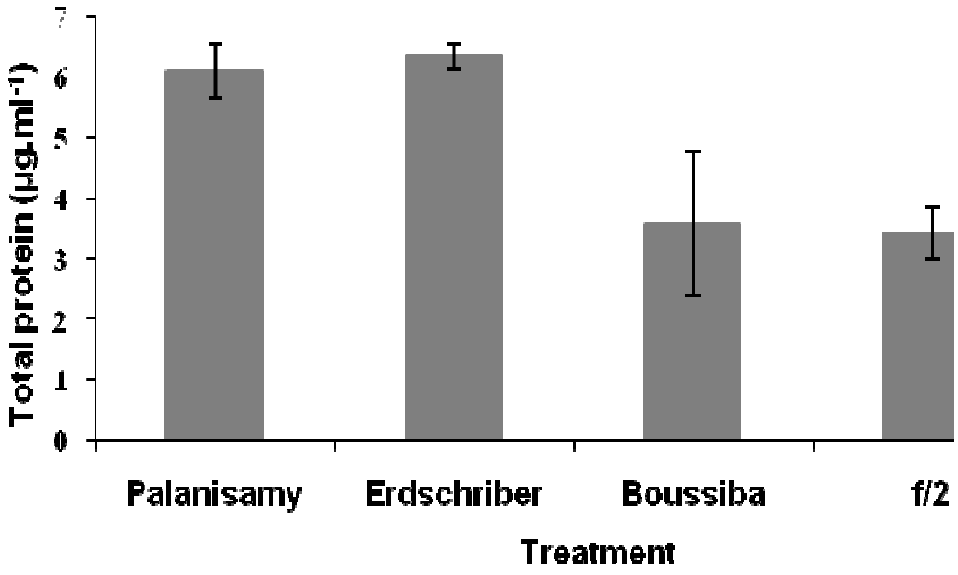

Figure 3. Total protein content in *T. chuii* (µg.ml^{-1}) under four different culture media in late logarithmic phase/early stationary phase of growth. All bars representing a mean ± SD. marked with different superscripts differ significantly with respect to each other ($P \leq 0.05$).

experiment (28 day old). The feeding regime of *S. aurata* larvae fed on *B. plicatilis* and *A. salina* enriched by *T. chuii* grown on four different culture media is represented in Table 7. The results indicate that the mean total length of the larvae enriched by *T. chuii* cultured on Erdschriber medium exhibited the maximum mean body length at age 28 DAH (9.71 mm) extremely higher than that obtained in Palanisamy medium (7.42 mm), Boussiba (6.31 mm) and $f/_2$ medium (5.60 mm).

Statistical analysis showed that there were significantly differences of larval length among all the treatments on 28 DAH (Table 7). On the other hand, the rate of increment per day in length was reached highest value (0.32 mm) in case of Erdschriber medium when used for culturing *T. chuii* as enrichment alga than that obtained for Palanisamy medium (0.25 mm) followed by Boussiba

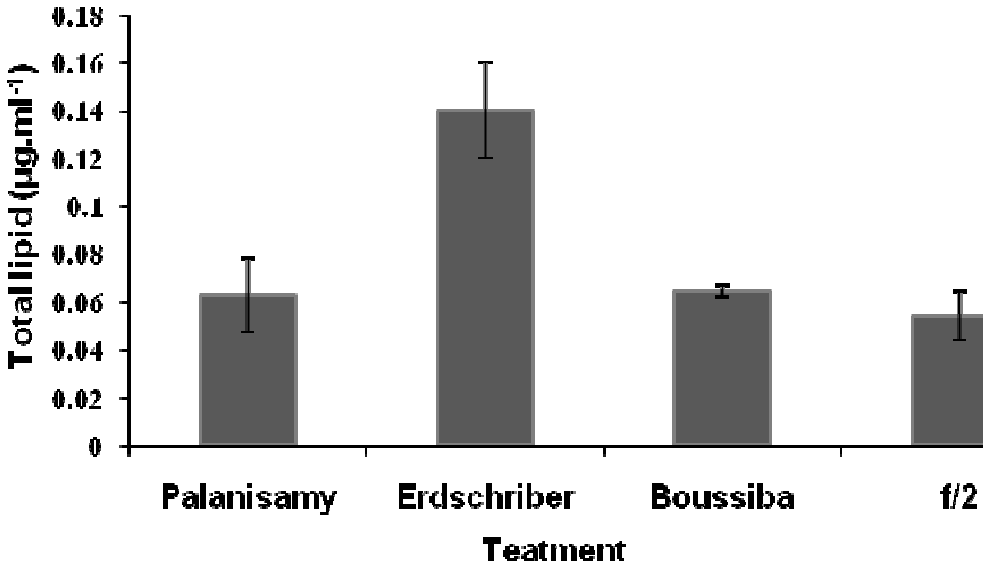

Figure 4. Total lipids content in *T. chuii* (µg.ml^{-1}) under four different culture media in late logarithmic phase/early stationary phase of growth. All bars representing a mean ± SD.

Table 3. Fatty acids composition (as µg.g^{-1}) of *T. chuii* in the four culture media in late logarithmicphase/early stationary phase of growth.

Fatty acid	Palanisamy	Culture media		f/2
		Erdschriber	Boussiba	
SFA				
C 6:0	0.51	0.30	0.36	0.11
C 8:0	0.17	0.07	0.09	0.08
C 10:0	0.07	-	-	0.06
C 12:0	0.22	0.21	0.23	0.25
C 13:0	1.27	1.48	1.33	1.91
C 14:0	0.27	2.74	0.16	2.48
C 15:0	0.61	0.81	0.66	0.79
C 16:0	7.45	25.24	2.73	21.75
C 17:0	0.11	0.88	0.25	0.13
C 18:0	0.60	0.65	0.27	0.90
C 20:0	0.17	-	-	-
C 21:0	0.44	0.81	0.34	-
Sum	11.89	33.19	6.42	28.46
MUFAs				
C 14:1	0.48	0.63	0.62	0.78
C 15:1	0.40	0.59	0.43	0.92
C 16:1	0.24	20.98	0.21	13.86
C 17:1	-	0.22	-	0.09
C 18:1	3.44	14.08	2.81	4.52
C 22:1	0.24	-	-	-
Sum	4.8	36.5	4.07	20.17
PUFAs				
C 18:2	0.96	7.05	1.88	1.75

Table 3. Continue.

C 18:3	0.16	1.95	0.20	0.57
C 20:2	-	0.48	-	0.17
C 20:4 (ARA)	0.38	5.12	0.77	1.38
C 20:5 (EPA)	0.59	4.45	0.52	1.18
C 22:6 (DHA)	2.62	4.65	4.06	5.54
Sum	4.71	23.7	7.43	10.59
Total fatty acids (µg.g-1)	21.4	93.39	17.92	59.22

Table 4. Fatty acid composition (µg.g^{-1}) of enriched *B. plicatilis* feed on *T. chuii* grown in four different culture media.

Fatty acid	Palanisamy	Culture media		f/$_2$
		Erdschriber	Boussiba	
SFA				
C 6:0	0.06	0.47	0.22	0.81
C 8:0	1.96	0.07	0.31	0.31
C 10:0	-	-	0.04	0.13
C 11:0	0.13	-	0.15	-
C 12:0	0.39	0.21	0.37	0.47
C 13:0	2.51	1.24	2.29	2.77
C 14:0	0.46	0.51	0.44	0.94
C 15:0	0.22	0.77	0.15	0.92
C 16:0	5.74	15.73	6.79	17.2
C 17:0	0.97	0.21	3.57	0.18
C 18:0	1.83	6.77	1.96	6.47
C 20:0	0.06	0.73	0.19	0.79
C 21:0	-	0.41	1.01	0.53
C 23:0	-	-	-	2.58
C 24:0	-	1.29	-	-
Sum	14.33	28.41	17.49	34.1
MUFAs				
C 14:1	1.01	0.48	0.91	1.23
C 15:1	0.95	0.43	0.84	0.15
C 16:1	0.46	0.15	0.41	0.49
C 17:1	0.11	0.36	-	0.66
C 18:1	-	1.24	0.18	1.69
C 20:1	-	-	-	0.29
C 22:1	-	7.35	0.71	0.93
Sum	2.53	10.01	3.05	5.44
PUFAs				
C 18:2	-	1.49	0.32	2.33
C 18:3	-	1.75	0.01	0.81
C 20:2	-	-	-	0.46
C 20:4 (ARA)	-	0.33	-	0.56
C 22:2	1.29	19.51	5.79	18.89
C 22:6 (DHA)	59.21	657.49	305.01	43.24
Sum	60.5	680.57	311.13	66.29
Total fatty acids (µg.g-1)	77.36	718.99	331.67	105.83

Table 5. Fatty acid composition (µg.g^{-1}) of enriched *A. salina* nauplii fed on *T. chuii* grown in four different culture media.

Fatty acid	Culture media			
	Palanisamy	Erdschriber	Boussiba	f/2
SFA				
C 6:0	9.56	21.71	20.18	10.2
C 8:0	8.54	2.73	2.44	6.3
C 10:0	2.51	3.83	0.72	3.2
C 11:0	6.86	1.4	1.41	9.33
C 12:0	1.48	2.46	1.98	4.71
C 13:0	6.63	8.66	11.03	17.96
C 14:0	10.71	14.19	14.49	1.03
C 15:0	2.42	3.82	4.68	6.96
C 16:0	183.44	411.57	189.56	168.78
C 17:0	14	14.29	1.13	5.4
C 18:0	177.16	291.49	143.11	121.68
C 20:0	3.4	56.4	2.79	5.02
C 21:0	7.02	328.34	-	7.84
C 22:0	-	9.53	78.75	368.41
Sum	433.73	1170.42	472.27	368.41
MUFAs				
C 14:1	4.19	5.01	11.94	4.49
C 15:1	4.59	5.72	4.92	3.39
C 16:1	24.43	36.88	26.75	3.91
C 17:1	5.40	4.93	13.11	1.62
C 18:1	111.7	154.59	818.17	25.1
C 20:1	-	29.86	4.50	-
C 22:1	31.39	36.12	25.37	-
Sum	181.7	273.11	904.76	38.51
PUFAs				
C 18:2	155.85	245.18	153.37	26.01
C 18:3	4.70	8.36	3.64	2.37
C 20:2	13.56	10.91	9.53	-
C 20:3	2.72	2.10	7.75	-
C 20:4 (ARA)	0.46	-	5.55	-
C 20:5 (EPA)	1.47	-	-	31.58
C 22:1	-	-	25.37	-
C 22:2	-	-	46.77	5.90
C 22:6 (DHA)	17.44	148.94	31.20	33.78
Sum	196.2	415.49	283.18	99.64
Total fatty acids (µg.g-1)	811.63	1859.02	1660.21	506.56

(0.20 mm) and f/2 medium (0.18 mm).

The main stages of embryonic larval developmental stages of *S. aurata* larvae are illustrated in photos 1-14. Survival percentage was significantly increased in larvae fed with *B. plicatilis* and *A. salina* enriched with *T. chuii* alga cultured on Erdschriber medium, it reached 41% after 28 days after hatching, while it was 33.3, 22.5 and 16.7% for Palanisamy, Boussiba and f/2 media, respectively. There was significant difference among the four treatments which observed in the survival rate on the 28 DAH (Table 8).

DISCUSSION

The evaluation of algae as live food for fish larvae is generally based on the selection of species that sustain the maximum growth, survival and development. This

Table 6. Morphometric measurements of *S. aurata* larvae fed in the four feeding regimes of *T. chuii* grown in four different cultured media.

Age (day)	HL	TrL	TaL	WL
Palanisamy				
18	1.34±0.06	1.38 ±0.03	2.78±0.23	0.93 ±.12
23	1.47±0.06	1.49±0.01	3.22±0.29	1.17 ±0.15
28	1.7±0.17	1.69±0.01	4.03±0.06	1.38 ±0.07
Erdschriber				
18	1.59±.08	1.57±0.22	3.39±0.19	1.07±0.21
23	1.94±0.22	1.72±0.28	4.44±0.23	1.39±0.16
28	2.08±0.14	1.95±0.21	5.68±0.34	1.72±0.08
Boussiba				
18	1.4±0.1	1.07±0.05	3.03±0.59	1.02±0.1
23	1.53±0.05	1.18±0.03	2.49±0.06	1.15±0.05
28	1.57±0.21	1.37±0.12	3.37±0.35	1.26±0.05
f/2				
18	0.98±0.14	0.90±0.06	2.05±0.13	0.87±0.06
23	1.25±0.06	1.20±0.03	2.26±0.03	1.02±0.03
28	1.30±0.15	1.26±0.03	3.04±0.49	1.23±0.05

HL, Head length; TrL, trunk length; TaL, tail length; WL, width length.

Table 7. Average total length (mm) of *S. aurata* larvae fed on the four feeding regimes of *T. chuii* grown on four different cultured media.

Age (day)	Culture media			
	Palanisamy	Erdschriber	Boussiba	f/2
0	$2.3^{a} \pm 0.10$	$2.^{a} \pm 0.10$	$2.0^{a} \pm 0.10$	$2.0^{a} \pm 0.10$
8	$3.03^{a} \pm 0.05$	$3.66^{b} \pm 0.01$	$2.91^{a} \pm 0.11$	$2.72^{a} \pm 0.33$
13	$3.94^{a} \pm 0.03$	$5.05^{b} \pm 0.03$	$3.53^{a} \pm 0.01$	$3.30^{a} \pm 0.21$
18	$5.00^{a} \pm 0.15$	$6.55^{b} \pm 0.4$	$4.30^{a} \pm 0.2$	$3.92^{a} \pm 0.32$
23	$6.18^{a} \pm 0.32$	$8.10^{b} \pm 0.34$	$5.20^{a} \pm 0.1$	$4.71^{a} \pm 0.17$
28	$7.42^{a} \pm 0.11$	$9.71^{b} \pm 0.9$	$6.31^{c} \pm 0.55$	$5.60^{d} \pm 0.38$

Data are the mean ± standard deviation of three replicates; different letters indicate statistical differences between groups (ANOVA, $P \leq 0.05$).

study suggests that the different chemical compositions of the culture media used in the experiments induced distinct biochemical profiles of *T. chuii*. In the present study, significant differences in protein and lipids content of *T. chuii* throughout the experimental period occurred among the different media. Total protein attained higher values in the Erdschriber medium than in Palanisamy, Bossiba and $f/_2$ medium on the 11 days. This may be due to the faster consumption of nitrogen from the medium (Lourenço et al., 1997). Most of the cell nitrogen is in the protein form; nitrogen availability can affect the synthesis and accumulation of cell constituents, such as pigments, proteins, carbohydrates, amino acids, nucleic acids and lipids (Utting, 1985). Microalgal species can vary significantly in their nutritional value, and this may also change under different culture conditions (Brown et al., 1997).

Saad (2007) concluded that the algal cultures in the exponential growth phase contain more protein, while cultures in the stationary phase have more carbohydrates and lipid. Certain microalgal species exposed to nitrogen limitation shift their metabolism from biomass production and start accumulating lipids (Meng et al., 2009). The lipid content can double or even triple in microalgae exposed to certain nitrogen conditions (Hu et al., 2008). Also Zaki and Saad (2010) observed that the increase of NaCl in the medium by 1.5 times than that of control which leads to increase survival percentage of *Dicentrarchus labrax* larva from 22.35 to 30%, and

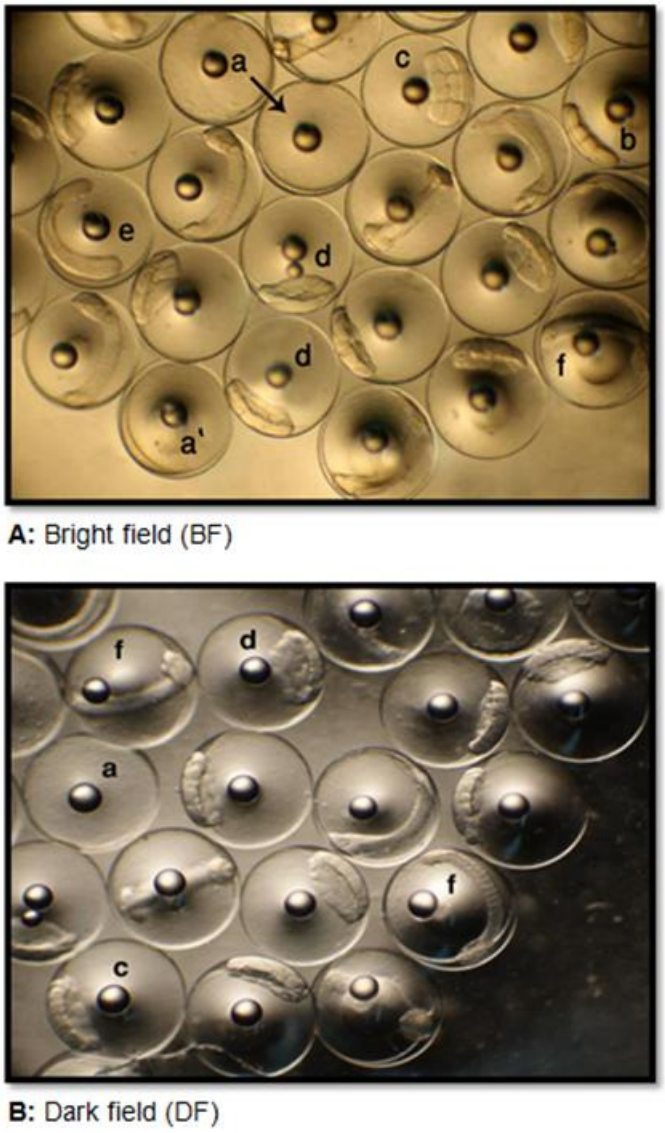

Photo 1. Embryonic developmental stages inside ova of *Sparus aurata* (4.5X). a, Ripe ova; á, germinal disk stage (25 ± 5 min) after fertilization; b, four cells blastomers stage; c, eight cells blastomers stage; d, Morula stage (diameter 1.28mm) after 3 ± 0.2 h after fertilization e, beginning of gastrula stage; f, full formed embryo inside ova (hatching of embryo took 36 ± 12 h after fertilization).

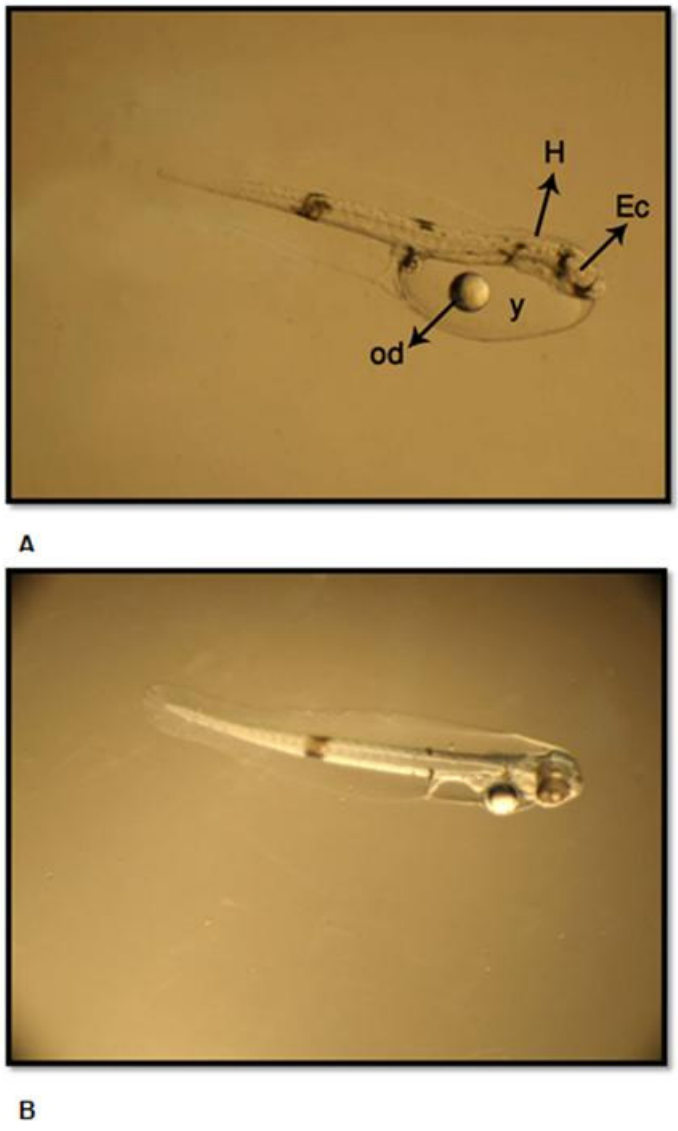

Photo 2. Pre-larval developmental stages of *Sparus aurata* (4.5X) showing: A, Newly hatched embryo pre-larva 0 day after hatching (DAH) presents incomplete organs and apparatus. The eyes are not pigmented and still not functional. The mouth still not open. The yolk is very abundant. Feeding is exclusively endogenous. (H, Heart; Ec, eye cup; Od: oil droplet; Y, yolk); B, Pre-larval at 3 (DAH). The mouth and eye are not yet functional. All the yolk has been resorbed.

increase mean total length from 8 to 10 mm, while the increase in length/day was 0.5 mm/day (0.3 mm/day in control). These results were in accordance to the present study, which represented that polyunsaturated fatty acids content (EPA, DHA) in *T. chuii* were always higher in Erdschriber medium and constituting 5.5 and 4.8% of the total fatty acids, respectively. This may be due to the inorganic nitrogen compounds (NO_3 in Erdschriber medium) which induced remarkable variations in the pH of the cultures. These observations agreed with other authors (Fabregas et al., 1989; Saad, 2007). Most of the studies on the dietary effect of manipulation on growth and pigmentation had been focused on ω_3 PUFA, probably due to their predominance in marine fish (Bell et al., 2003; Villalta et al., 2005). Other studies had indicated the importance of ω_6 PUFA on pigmentation, in particular ARA (Bell and Sargent, 2003) which is an essential fatty acid (EFA) for marine fish.

EPA, DHA and ARA are effective for good growth and survival of *S. aurata* larvae; however, the effect of these acids on survival is better than on growth, this in accordance with study on the flat fish species (Gapasin and Duray, 2001). The fatty acids composition of rotifers

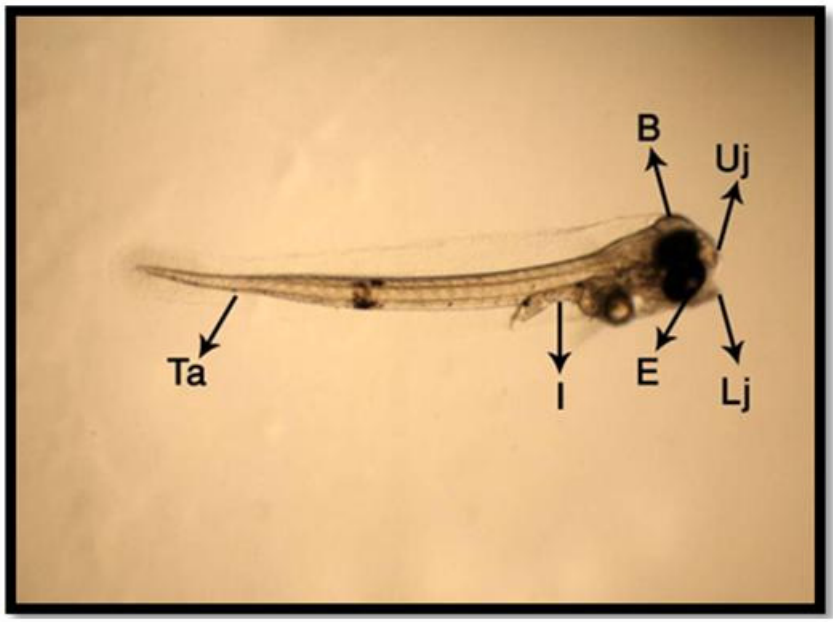

Photo 3. Larva at age 4 (DAH) 4.5X the mouth and eye are completely functional. Bright field (BF). (B, Brain; Uj: upper jaw; Lj, lower haw; E, eye; I, intestine; Ta, tail).

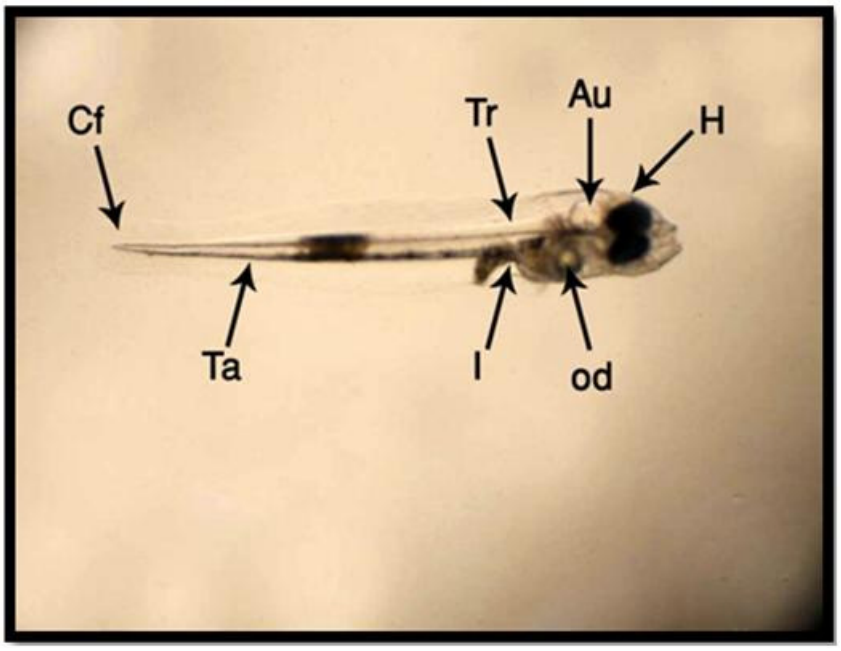

Photo 4. Larva at age 5 (DAH) 4.5X fed with partial exogenous feeding. Bright Field (BF). (H, Head; Au, audiutary; Tr, trunk; Cf, caudal fin; Od, oil droplet; I, intestine; Ta: tail).

is closely related to that of the diets used, and short-term feeding of rotifers with algae will shift the fatty acid composition towards that of the algal species used (Reitan et al., 1993). The results of the present study showed that the higher production of total fatty acids in rotifers fed on *T. chuii* grown on Erdschriber medium (1859.02 µg.g-1) was due to the accumulation of PUFAs (94.7% of the total lipids). EPA was absent, while DHA (22:6ω_3) was enhanced and constituted 657.49 µg.g^{-1} (91.4% of the total fatty acids). In contrast to the present

Photo 5. Larva at age 7 (DAH) 4.5X fed with enriched Rotifer. Dark field (DF).

Photo 6. Larva at age 9 (DAH) 4.5X fed with enriched Rotifer. Dark Field (DF).

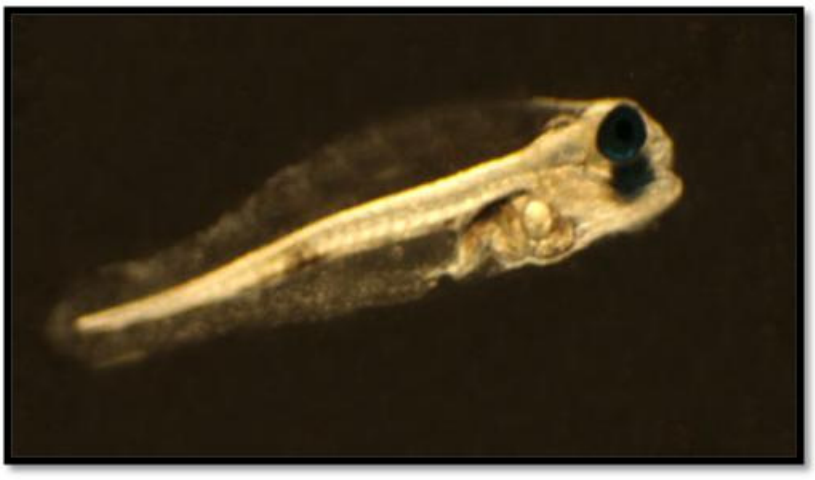

Photo 7. Larva at age 13 (DAH) 3.5X fed with *Rotifer* + *Artemia* nauplii. Dark Field (DF).

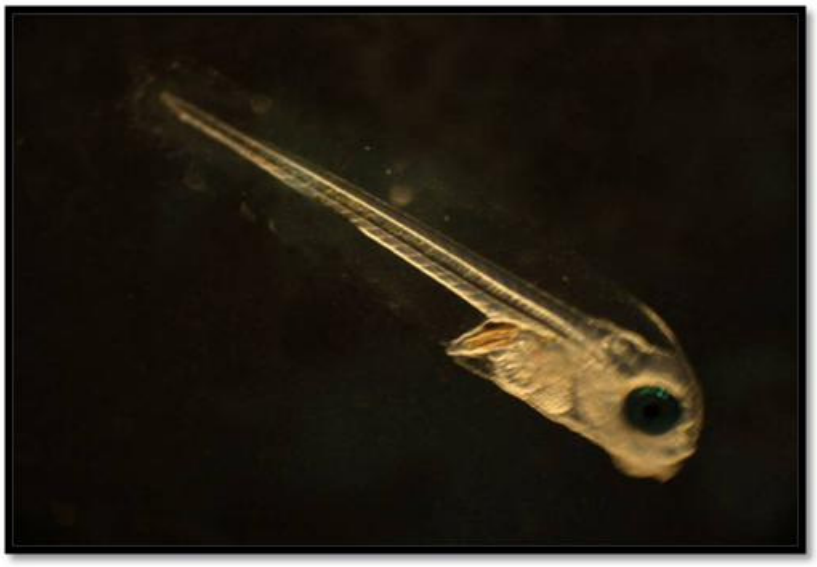

Photo 8. Larva at age 15 (DAH) 3.5X fed with Rotifer + *Artemia* nauplii. Dark Field (DF).

Photo 9. Larva at age 17 (DAH) 3.5X fed with enriched *Artemia* nauplii + enriched Rotifer. Dark field (DF).

results, El-Dakar *et al.* (2001) cultured *T. chuii* on Walne medium, and concluded that, EPA was found and constituting 5.11% of the total fatty acids, while DHA was absent in *B. plicatilis* fed on *T. chuii*. This may be related to the differences in chemical composition of the culture medium used for algal growth. The survival rate of *D. labrax* larvae fed on rotifers enriched by *T. chuii* was 21.20% (El-Dakar et al., 2001), and the survival rate and growth of *D. labrax* larvae were improved as EPA content increased in their feed (algae or rotifers). The same trend was found in the present results, where DHA, EPA and DHA: EPA ratio of *T. chuii* and *B. plicatilis* had significant effects on the growth and survival of *S. aurata* larvae. High dietary levels of DHA, relative to EPA, with ratio up to 10:1 in rotifers promoted growth in Atlantic cod (Gi Park et al., 2006).

Our results show that the morphometric measurements for the larvae were varied according to the feed regime,

Photo 10. Larva at age 20 (DAH) 3.0X fed with enriched *Artemia* meta nauplii. Bright field (BF).

Photo 11. Larva at age 20 (DAH) 3.0X fed with enriched *Artemia* meta nauplii. Dark field (DF) (m, mouth; I, intestine).

and the maximum growth of *S. aurata* larvae fed on *T. chuii* grown on Erdschriber medium, where its reached 2.08, 1.95, 5.68 and 1.72 mm for HL, TrL, TaL and WL, respectively at the end of the experiment (28 DAH). These results coincided with those obtained by Firat et al. (2003) on *Dentex dentex* fish larvae.

Also the present study showed that the fatty acid composition of *A. salina* nauplii fed on *T. chuii* grown on Erdschriber medium had higher fatty acids content (1859.02 µg.g^{-1}). Saturated fatty acids were presented more than unsaturated ones (63% of the total fatty acids), especially the short chain fatty acids (16:0, 18:0 and

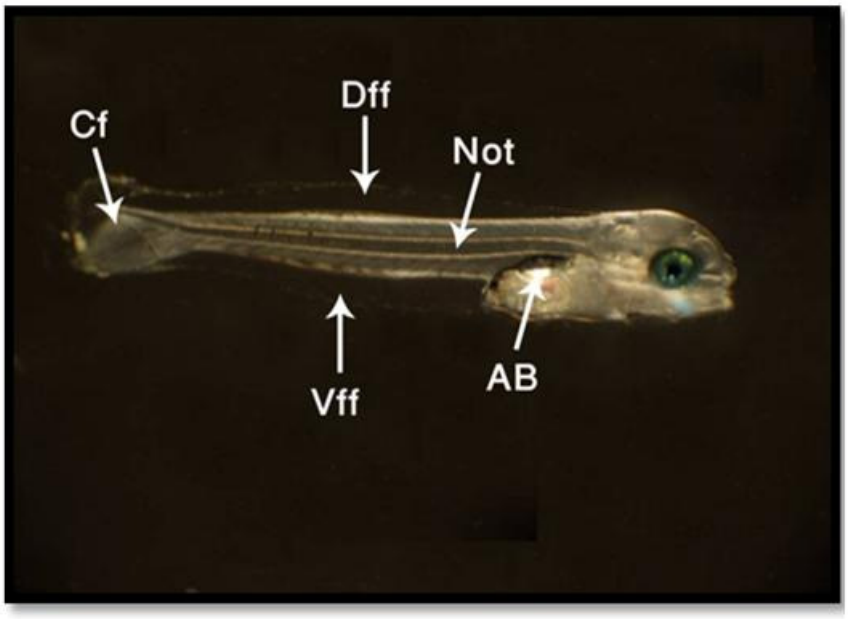

Photo 12. Larva at age 23 (DAH) 2.5X fed with enriched *Artemia* meta nauplii. Dark field (DF) (Not, notocord; Dff, dorsal fin fold; AB, air bladder; Vff, ventral fin fold); Cf, caudal fin).

Photo 13. Larva at age 25 (DAH) 2.5X fed with enriched *Artemia* meta nauplii (Orange color). Bright field (BF).

21:0). On the other hand PUFAs constituted 22.3% of the total fatty acids, due to the presence of DHA which formed 8.0% of the total fatty acids, while ARA and EPA were absent. This agreed with Thompson et al. (1993) who found that diets with higher percentages of the saturated fats were more benefit for the rapid growth of larvae, because energy is released more efficiently from saturated fats than unsaturated fats. Linoleic acid (18:2 ω_6) and linolenic acid (18:3 ω_3) are thus essential dietary fatty acids, which can easily be converted by fish larvae via a series of desaturation and elongation reactions to very long-chain (C20 and C22 PUFAs), for example, the principal ω_3 PUFAs, eicosapentaenoic acid (EPA, 20:5 ω_3) and docosahexaenoic acid (DHA, 22:6 ω_3), and the ω_6 PUFA, arachidonic acid (ARA, 20:4 ω_6) (Jobling and Bendiksen, 2003; Wallis et al., 2002).

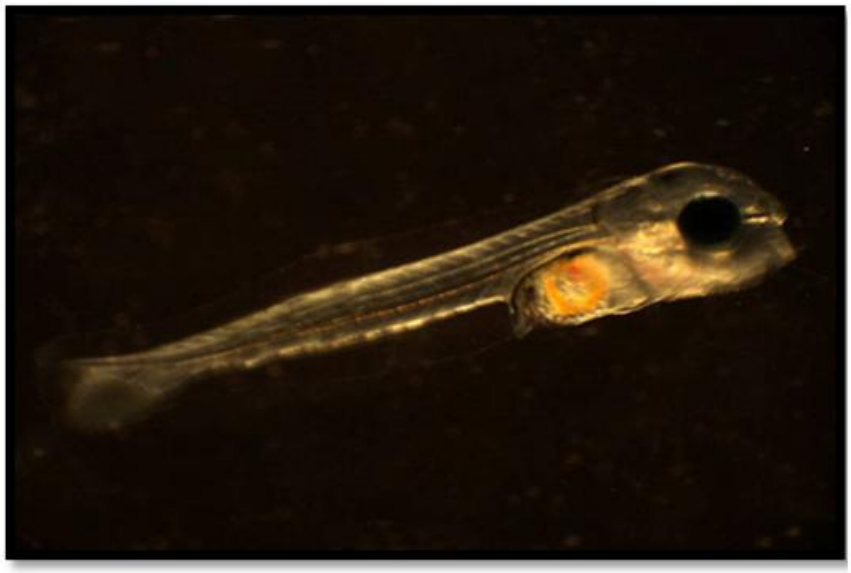

Photo 14. Larva at age 28 (DAH) 2.0X fed with enriched *Artemia* meta nauplii (Orange color). Dark field (DF).

Table 8. Percentage of survival (%) of *S. aurata* larvae fed on *T. chuii* grown on four different cultured media.

Age (day)	Culture media			
	Palanisamy	Erdschriber	Boussiba	f/2
0	100	100	100	100
8	$91.5^{a} \pm 2$	$95.3^{b} \pm 1$	$94.5^{b} \pm 1$	$89.5^{a} \pm 1$
13	$78.0^{a} \pm 1$	$80.0^{a} \pm 2$	$75.0^{b} \pm 1$	$68.0^{c} \pm 1$
18	$49.0^{a} \pm 1$	$55.0^{b} \pm 1$	$40.0^{c} \pm 1$	$35.5^{d} \pm 1$
23	$35.2^{a} \pm 2$	$49.0^{b} \pm 2$	$30.7^{c} \pm 1$	$23.8^{d} \pm 1$
28	$33.3^{a} \pm 1$	$41.0^{b} \pm 1$	$22.5^{c} \pm 2$	$16.7^{d} \pm 2$

Data are the mean ± standard deviation of three replicates; different letters indicate statistical differences between groups (ANOVA, $P \leq 0.05$).

Conclusion

This study suggest that *T. chuii* grown on Erdschriber medium promoted better for fish larval growth and the fatty acids composition of *B. plicatilis* and *A. salina* were reflected in the corresponding fatty acids of algae they fed on. Therefore, algal species rich in ω_3 fatty acids may cover the ω_3-highly unsaturated fatty acids (HUFAs) requirements of larvae that important to obtain the high survival, growth and quality of fish larvae. So enrichment of *B. plicatilis* and *A. salina* with *T. chuii* which commonly used in Egyptian marine hatcheries was effective in improving the nutritional value of rotifers for best growth and survival of *S. aurata* larvae.

Abbreviations

PUFAs, Polyunsaturated fatty acids; **DHA,** docosa-hexaenoic acid; **EPA,** eicosapentaenoic acid; **ARA,** arachidonic acid; **EFA,** essential fatty acids; **SFA,** saturated fatty acids; **HUFAs,** highly unsaturated fatty acids; **DAH,** day after hatching.

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