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 conducted to study 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 10⁶ 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 polysaturated fatty acid 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 (22:6ω₃) 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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The microalgae in aquaculture 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 Rotifer is similar to that of the algae upon which they fed (Ben-Amotz et al., 1987; Carič et al., 1993). Rotifer (*Brachionus plicatilis*) and *Artemia* 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 \( \omega_6 \), 20:5 \( \omega_3 \), and 22:6 \( \omega_3 \) (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, 22:6\( \omega_3 \)), eicosapentaenoic acid (EPA, 20:5\( \omega_3 \)), and arachidonic acid (ARA, 20:4\( \omega_6 \)) 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 \( \omega_3 \) PUFA enrichment during the production phase of Rotifers) seems to be very efficient in obtaining high \( \omega_3 \) 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 to maximize marine hatchery production and to select the best medium promote the growth of fish larvae.

**MATERIALS AND METHODS**

**Microalgal culture**

The microalgal strain (*T. chuii*) was cultured in filtered sterilized seawater enriched with four different culture media (Boussiba et al., 1987; Erdschreiber as represented in UTEX, 1993; Palanisamy et al., 1991; Guillard, 1975) with controlled conditions of temperature 20 ± 1°C-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 10^4 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 (Wetherill, 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 rotifiers 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 Erdschreiber 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 to 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 L⁻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 4th day after hatching when mouth was open using B. plicatilis followed by Artemia nauplii and meta nauplii enriched on T. chuii (150 X10⁶ 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 x 10⁶ 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 µg.g⁻¹ (35.5% of the total fatty acids content), 36.5 µg.g⁻¹ (39.1%) and 23.7 µg.g⁻¹ (25.4%), respectively for saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA) 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 ω3), EPA (20:5 ω3) and DHA (22:6 ω3). 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) 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 µg.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 (22:6ω3) was enhanced and constituted 657.49 µg.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 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 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.

<table>
<thead>
<tr>
<th>Age larvae (DAH)</th>
<th>Palanisamy</th>
<th>Erdshcriber</th>
<th>Boussiba</th>
<th>f/2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rotifer</td>
<td>Artemia</td>
<td>Rotifer</td>
<td>Artemia</td>
</tr>
<tr>
<td>0-3</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>4-6</td>
<td>5-10</td>
<td>-</td>
<td>5-10</td>
<td>-</td>
</tr>
<tr>
<td>7-10</td>
<td>15-20</td>
<td>-</td>
<td>15-20</td>
<td>-</td>
</tr>
<tr>
<td>11-13</td>
<td>25</td>
<td>-</td>
<td>25</td>
<td>0.5*</td>
</tr>
<tr>
<td>14-16</td>
<td>25</td>
<td>0.5*</td>
<td>20</td>
<td>1.0</td>
</tr>
<tr>
<td>17-20</td>
<td>20</td>
<td>1.0</td>
<td>20</td>
<td>1.5</td>
</tr>
<tr>
<td>21-24</td>
<td>15</td>
<td>1.5</td>
<td>15</td>
<td>2.0</td>
</tr>
<tr>
<td>25-28</td>
<td>10</td>
<td>2.0</td>
<td>10</td>
<td>3.0</td>
</tr>
</tbody>
</table>

*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).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Palanisamy</th>
<th>Erdshcriber</th>
<th>Boussiba</th>
<th>F/2</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell number</td>
<td>23.13 ± 0.68</td>
<td>24.53 ± 1.77</td>
<td>22.53 ± 1.05</td>
<td>19.27 ± 1.12</td>
<td>10.02*</td>
</tr>
<tr>
<td>Optical density</td>
<td>0.20 ± 0.03</td>
<td>0.237 ± 0.03</td>
<td>0.17 ± 0.02</td>
<td>0.12 ± 0.01</td>
<td>14.25*</td>
</tr>
<tr>
<td>Total protein</td>
<td>6.09 ± 0.42</td>
<td>6.33 ± 0.21</td>
<td>3.56 ± 1.19</td>
<td>3.42 ± 0.43</td>
<td>16.3*</td>
</tr>
<tr>
<td>Total lipid</td>
<td>0.06 ± 0.002</td>
<td>0.14 ± 0.02</td>
<td>0.065 ± 0.003</td>
<td>0.05 ± 0.01</td>
<td>47.1*</td>
</tr>
</tbody>
</table>

*, Statistically significant at p ≤ 0.05; different letters indicate statistical differences between groups (ANOVA, P ≤ 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 ≤ 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<sup>-1</sup>) 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<sup>-1</sup>) of *T. chuii* in the four culture media in late logarithmic phase/early stationary phase of growth.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Palanisamy</th>
<th>Culture media</th>
<th>Erdschriber</th>
<th>Boussiba</th>
<th>f/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C 6:0</td>
<td>0.51</td>
<td>0.30</td>
<td>0.36</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>C 8:0</td>
<td>0.17</td>
<td>0.07</td>
<td>0.09</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>C 10:0</td>
<td>0.07</td>
<td>-</td>
<td>-</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>C 12:0</td>
<td>0.22</td>
<td>0.21</td>
<td>0.23</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>C 13:0</td>
<td>1.27</td>
<td>1.48</td>
<td>1.33</td>
<td>1.91</td>
<td></td>
</tr>
<tr>
<td>C 14:0</td>
<td>0.27</td>
<td>2.74</td>
<td>0.16</td>
<td>2.48</td>
<td></td>
</tr>
<tr>
<td>C 15:0</td>
<td>0.61</td>
<td>0.81</td>
<td>0.66</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>C 16:0</td>
<td>7.45</td>
<td>25.24</td>
<td>2.73</td>
<td>21.75</td>
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<tr>
<td>C 16:1</td>
<td>0.11</td>
<td>0.88</td>
<td>0.25</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>C 17:0</td>
<td>0.60</td>
<td>0.65</td>
<td>0.27</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>C 20:0</td>
<td>0.17</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>C 21:0</td>
<td>0.44</td>
<td>0.81</td>
<td>0.34</td>
<td>-</td>
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</tr>
<tr>
<td>Sum</td>
<td>11.89</td>
<td>33.19</td>
<td>6.42</td>
<td>28.46</td>
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<table>
<thead>
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<th>MUFAs</th>
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<tbody>
<tr>
<td>C 14:1</td>
<td>0.48</td>
<td>0.63</td>
<td>0.62</td>
<td>0.78</td>
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</tr>
<tr>
<td>C 15:1</td>
<td>0.40</td>
<td>0.59</td>
<td>0.43</td>
<td>0.92</td>
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<tr>
<td>C 16:1</td>
<td>0.24</td>
<td>20.98</td>
<td>0.21</td>
<td>13.86</td>
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<tr>
<td>C 17:1</td>
<td>-</td>
<td>0.22</td>
<td>-</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>C 18:1</td>
<td>3.44</td>
<td>14.08</td>
<td>2.81</td>
<td>4.52</td>
<td></td>
</tr>
<tr>
<td>C 22:1</td>
<td>0.24</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Sum</td>
<td>4.8</td>
<td>36.5</td>
<td>4.07</td>
<td>20.17</td>
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</tbody>
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<table>
<thead>
<tr>
<th>PUFAs</th>
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</tr>
</thead>
<tbody>
<tr>
<td>C 18:2</td>
<td>0.96</td>
<td>7.05</td>
<td>1.88</td>
<td>1.75</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Continue.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Palanisamy</th>
<th>Culture media</th>
<th>Boussiba</th>
<th>f/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 18:3</td>
<td>0.16</td>
<td>1.95</td>
<td>0.20</td>
<td>0.57</td>
</tr>
<tr>
<td>C 20:2</td>
<td>0.17</td>
<td>0.48</td>
<td>-</td>
<td>0.17</td>
</tr>
<tr>
<td>C 20:4 (ARA)</td>
<td>0.38</td>
<td>5.12</td>
<td>0.77</td>
<td>1.38</td>
</tr>
<tr>
<td>C 20:5 (EPA)</td>
<td>0.59</td>
<td>4.45</td>
<td>0.52</td>
<td>1.18</td>
</tr>
<tr>
<td>C 22:6 (DHA)</td>
<td>2.62</td>
<td>4.65</td>
<td>4.06</td>
<td>5.54</td>
</tr>
<tr>
<td>Sum</td>
<td>4.71</td>
<td>23.7</td>
<td>7.43</td>
<td>10.59</td>
</tr>
<tr>
<td>Total fatty acids (µg.g-1)</td>
<td>21.4</td>
<td>93.39</td>
<td>17.92</td>
<td>59.22</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Palanisamy</th>
<th>Culture media</th>
<th>Boussiba</th>
<th>f/2</th>
</tr>
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<tbody>
<tr>
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<td>0.07</td>
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</tr>
<tr>
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<td>-</td>
<td>0.04</td>
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<tr>
<td>C 11:0</td>
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<td>-</td>
<td>0.15</td>
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</tr>
<tr>
<td>C 12:0</td>
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<td>0.21</td>
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<td>2.77</td>
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<tr>
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<td>0.51</td>
<td>0.44</td>
<td>0.94</td>
</tr>
<tr>
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<td>0.22</td>
<td>0.77</td>
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<td>0.92</td>
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<tr>
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<td>0.97</td>
<td>0.21</td>
<td>3.57</td>
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<tr>
<td>C 18:0</td>
<td>1.83</td>
<td>6.77</td>
<td>1.96</td>
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<td>0.73</td>
<td>0.19</td>
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<tr>
<td>C 21:0</td>
<td>-</td>
<td>0.41</td>
<td>1.01</td>
<td>0.53</td>
</tr>
<tr>
<td>C 23:0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.58</td>
</tr>
<tr>
<td>C 24:0</td>
<td>-</td>
<td>1.29</td>
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<td>-</td>
</tr>
<tr>
<td>Sum</td>
<td>14.33</td>
<td>28.41</td>
<td>17.49</td>
<td>34.1</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Culture media</th>
<th>Palanisamy</th>
<th>Erdschriber</th>
<th>Boussiba</th>
<th>f/₂</th>
</tr>
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<tbody>
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<td></td>
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<tr>
<td>C 6:0</td>
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<td>9.56</td>
<td>21.71</td>
<td>20.18</td>
<td>10.2</td>
</tr>
<tr>
<td>C 8:0</td>
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<td>8.54</td>
<td>2.73</td>
<td>2.44</td>
<td>6.3</td>
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<tr>
<td>C 10:0</td>
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<td>2.51</td>
<td>3.83</td>
<td>0.72</td>
<td>3.2</td>
</tr>
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<td>6.86</td>
<td>1.4</td>
<td>1.41</td>
<td>9.33</td>
</tr>
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<td>C 12:0</td>
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<td>1.48</td>
<td>2.46</td>
<td>1.98</td>
<td>4.71</td>
</tr>
<tr>
<td>C 13:0</td>
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<td>6.63</td>
<td>8.66</td>
<td>11.03</td>
<td>17.96</td>
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<td>10.71</td>
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<td>3.82</td>
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<td>189.56</td>
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<td>14.29</td>
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<td>177.16</td>
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<td>121.68</td>
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<tr>
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<td>3.4</td>
<td>56.4</td>
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<td>5.02</td>
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<td>7.84</td>
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<td>433.73</td>
<td>1170.42</td>
<td>472.27</td>
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<td><strong>MUFA</strong>s</td>
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<tr>
<td>C 14:1</td>
<td></td>
<td>4.19</td>
<td>5.01</td>
<td>11.94</td>
<td>4.49</td>
</tr>
<tr>
<td>C 15:1</td>
<td></td>
<td>4.59</td>
<td>5.72</td>
<td>4.92</td>
<td>3.39</td>
</tr>
<tr>
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<td>24.43</td>
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<td>1.62</td>
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<td>C 18:1</td>
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<td>154.59</td>
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<td>-</td>
<td>29.86</td>
<td>4.50</td>
<td>-</td>
</tr>
<tr>
<td>C 22:1</td>
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<td>36.12</td>
<td>25.37</td>
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<tr>
<td>Sum</td>
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<td>181.7</td>
<td>273.11</td>
<td>904.76</td>
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<td><strong>PUFA</strong>s</td>
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<td>155.85</td>
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<td>26.01</td>
</tr>
<tr>
<td>C 18:3</td>
<td></td>
<td>4.70</td>
<td>8.36</td>
<td>3.64</td>
<td>2.37</td>
</tr>
<tr>
<td>C 20:2</td>
<td></td>
<td>13.56</td>
<td>10.91</td>
<td>9.53</td>
<td>-</td>
</tr>
<tr>
<td>C 20:3</td>
<td></td>
<td>2.72</td>
<td>2.10</td>
<td>7.75</td>
<td>-</td>
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<tr>
<td>C 20:4 (ARA)</td>
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<td>-</td>
<td>5.55</td>
<td>-</td>
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<td>C 20:5 (EPA)</td>
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<td>-</td>
<td>31.58</td>
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<td>C 22:1</td>
<td></td>
<td>-</td>
<td>-</td>
<td>25.37</td>
<td>-</td>
</tr>
<tr>
<td>C 22:2</td>
<td></td>
<td>-</td>
<td>-</td>
<td>46.77</td>
<td>5.90</td>
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<tr>
<td>C 22:6 (DHA)</td>
<td></td>
<td>17.44</td>
<td>148.94</td>
<td>31.20</td>
<td>33.78</td>
</tr>
<tr>
<td>Sum</td>
<td></td>
<td>196.2</td>
<td>415.49</td>
<td>283.18</td>
<td>99.64</td>
</tr>
<tr>
<td>Total fatty acids (µg.g⁻¹)</td>
<td>811.63</td>
<td>1859.02</td>
<td>1660.21</td>
<td>506.56</td>
<td></td>
</tr>
</tbody>
</table>

(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
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

---

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

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

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.

<table>
<thead>
<tr>
<th>Age (day)</th>
<th>Culture media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palanisamy</td>
<td>Erdschriber</td>
</tr>
<tr>
<td>0</td>
<td>2.3 ±0.10</td>
</tr>
<tr>
<td>8</td>
<td>3.03 ±0.05</td>
</tr>
<tr>
<td>13</td>
<td>3.94 ±0.03</td>
</tr>
<tr>
<td>18</td>
<td>5.00 ±0.15</td>
</tr>
<tr>
<td>23</td>
<td>6.18 ±0.32</td>
</tr>
<tr>
<td>28</td>
<td>7.42 ±0.11</td>
</tr>
</tbody>
</table>

Data are the mean ± standard deviation of three replicates; different letters indicate statistical differences between groups (ANOVA, *P* ≤ 0.05).
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. chiui* 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 $\omega_3$ PUFA, probably due to their predominance in marine fish (Bell et al., 2003; Villalta et al., 2005). Other studies had indicated the importance of $\omega_6$ 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...
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 (22:6ω₃) was enhanced and constituted 657.49 µg.g⁻¹ (91.4% of the total fatty acids). In contrast to the present

**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 jaw; 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, auditory; Tr, trunk; Cf, caudal fin; Od, oil droplet; I, intestine; Ta, tail).
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).
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,
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
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 \(\omega_6\)) and linolenic acid (18:3 \(\omega_3\)) 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 \(\omega_3\) PUFAs, eicosapentaenoic acid (EPA, 20:5 \(\omega_3\)) and docosahexaenoic acid (DHA, 22:6 \(\omega_3\)), and the \(\omega_6\) PUFA, arachidonic acid (ARA, 20:4 \(\omega_6\)) (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.

<table>
<thead>
<tr>
<th>Age (day)</th>
<th>Palanisamy</th>
<th>Erdschriber</th>
<th>Boussiba</th>
<th>f/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>91.5 ± 2</td>
<td>95.3 ± 1</td>
<td>94.5 ± 1</td>
<td>89.5 ± 1</td>
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<td>13</td>
<td>78.0 ± 1</td>
<td>80.0 ± 1</td>
<td>75.0 ± 1</td>
<td>68.0 ± 1</td>
</tr>
<tr>
<td>18</td>
<td>49.0 ± 1</td>
<td>55.0 ± 1</td>
<td>40.0 ± 1</td>
<td>35.5 ± 1</td>
</tr>
<tr>
<td>23</td>
<td>35.2 ± 2</td>
<td>49.0 ± 1</td>
<td>30.7 ± 1</td>
<td>23.8 ± 1</td>
</tr>
<tr>
<td>28</td>
<td>33.3 ± 1</td>
<td>41.0 ± 1</td>
<td>22.5 ± 2</td>
<td>16.7 ± 2</td>
</tr>
</tbody>
</table>

Data are the mean ± standard deviation of three replicates; different letters indicate statistical differences between groups (ANOVA, P ≤ 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 ω3 fatty acids may cover the ω3-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.

REFERENCES

Utex (1993). The culture collection of algae at the University of Texas at Austin. J. Phycol., 29(2).