
The effect of lipid supplementation on growth and fatty acid composition of Tapes philippinarum spat

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Abstract

The present study investigated the possible use of emulsions as an artificial lipid supplement to live algal for seed of the Manila clam *Tapes philippinarum*. The uptake and assimilation of an emulsion, rich in docosahexaenoic acid (DHA, 22:6n – 3) and eicosapentaenoic acid (EPA, 20:5n – 3) and fed at concentrations of 20 and 40% of the algal dry weight, were verified analytically by fatty acid analysis of the animals and their diets. Dietary requirements for n – 3 highly unsaturated fatty acids (HUFAs) were examined by supplementing *Dunaliella tertiolecta*, which contains no polyunsaturated fatty acids longer than 18:3n – 3 and *Tetraselmis suecica*, which contains EPA but only trace amounts of DHA. An algal mixture of *Isochrysis galbana* (clone T-Iso) and *Chaetoceros muelleri* (1:1, on dry weight basis) was used as the control diet. After 4 weeks, the lipid supplementation resulted in a significant increase of the DHA level in the seed compared to the animals fed non-supplemented *Dunaliella* (from 9.5 to 19.8 and 22.0% at a supplementation of 20 and 40%, respectively) or *Tetraselmis* (from 3.4 to 24.8 and 26.9%, at a supplementation of 20 and 40%, respectively) diet. Feeding solely *D. tertiolecta* resulted in a significantly lower daily growth rate (DGR) compared to animals fed *T. suecica* or the mixed algal diet. Lipid supplementation improved the DGR of the clams fed *D. tertiolecta* while hardly any effect could be detected in those fed *T. suecica*. The poor nutritional value of *D. tertiolecta* was indicated by the continuous decrease of the DGR and resulted in a DGR that was no longer significantly different from the starved ones at the end of the experiment. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Bivalve; Lipid; Fatty acid; Algal supplement; *Tapes philippinarum*

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1. Introduction

The intensive rearing of bivalves still relies on the massive production of unicellular algae (Coutteau and Sorgeloos, 1992) especially for growing young spat which represent the largest biomass in a commercial hatchery (Manzi and Castagna, 1989). The high cost and unpredictable culture success of algae has inspired the development of artificial diets such as microcapsules (Langdon and Waldock, 1981), mixed diets (Trider and Castell, 1980), yeast based diets (Coutteau et al., 1994c), lipid microspheres (Robinson, 1992a,b; Coutteau et al., 1994a, 1996; Heras et al., 1994) and liposomes (Parker and Selvovitch, 1986) to substitute or supplement live algal diets. Several studies have illustrated the importance of lipids in bivalve nutrition (Hein et al., 1991; Thompson et al., 1996; Wiktors et al., 1996). The n = 3 highly unsaturated fatty acids (HUFAs) eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) have been reported to be essential for optimal growth for at least some species of juvenile bivalves (Langdon and Waldock, 1981; Waldock and Holland, 1984; Hurd et al., 1989; Chu and Greaves, 1991). Nevertheless, fatty acid requirements of bivalves are still unclear partly due to the lack of suitable experimental diets. Many studies have compared growth and survival of animals fed different monospecific or mixed algal diets. However, care should be taken with the interpretation of these results since interspecific differences such as digestibility, toxicity, cell size and various nutritionally important components other than fatty acids may interfere. This can be avoided by supplementing algal diets with encapsulated or emulsified lipids. Lipid emulsions are off-the-shelf supplements that are widely used in shrimp and fish hatcheries and have potential to standardize and optimize algal diets currently used in bivalve hatcheries. Coutteau et al. (1994a, 1996) illustrated the uptake and assimilation of lipid emulsions by larval Ostrea edulis and juvenile Placopecten magellanicus.

The present study investigated the possible use of emulsions as an artificial lipid supplement to live algae for seed of the Manila clam Tapes philippinarum. The uptake and assimilation of an emulsion were verified analytically by fatty acid analysis of the animals and their diets. Dietary requirements for n = 3 HUFAs (especially DHA and EPA) were examined by supplementing Dunaliella tertiolecta, which contains no polyunsaturated fatty acids longer than 18:3n-3 and T. suecica, which contains EPA but only trace amounts of DHA. A mixed algal diet of Isochrysis galbana (clone T-iso) and Chaetoceros neogracile (1:1, on dry weight (DW) basis) which is known to support good growth (Coutteau et al., 1994b), was used as the control diet.

2. Materials and methods

2.1. Diets

I. galbana (clone T-iso), C. neogracile, D. tertiolecta and T. suecica were grown in 41 batch cultures in filtered seawater (0.2 μm) enriched with Walne medium (Coutteau, 1996). Cultures were mixed by aeration and maintained under continuous light conditions at a temperature of 25°C. Cell concentrations of the cultures were counted with a haemocytometer. The algae were harvested and sieved. The weight of the four algal species was sampled.

The experimental emulsion (DGR), obtained from Tinamenor, Spain 7 days prior to the experiment, contained 50% lipids (78% polyunsaturated fatty acids, 22% monounsaturated and 0.01% and 0.32% of WW). The feeding was carried out by a peristaltic pump.

2.2. Culture of the spat

Juvenile Manila clams T. philippinarum were obtained from Tinamenor, Spain 7 days prior to the experiment. Distilled water was used to prepare the silo in which an air–water lift culture was used. Aeration was regulated by a peristaltic pump and the latter refilled with 5 μl/day during a period of 4 weeks.

The spat were stocked at an initial density of 5,000 clams/m2. Every week, the animals were harvested, weighted, and restocked. The DGR was calculated according to the formula:

\[ \text{DGR} = \frac{\text{weight of day } 1 - \text{weight of day } n}{\text{weight of day } 1} \]

with n = 7, WW, respectively, and the amount of food was adjusted to 10% of WW (Coutteau et al., 1994b):

\[ \text{DGR}_{\text{dry }} = \frac{\text{DW algal diet}_{\text{dry }} \times n}{\text{DW of day } 1} \]

with n = day of the week; (DW algal diet) was assumed to be 10% calculated from the growth of cell-1 of the algae used in the Tapes philippinarum = 23.18. D. tertiolecta, determined by filtering known volumes of glassfiber filters (Whatman GF-C solution, the filters were dried (relDGR) was calculated as a proportion of the mixed algal diet.

2.3. Experimental design

A mixed diet of I. galbana, C. neogracile, T. suecica and D. tertiolecta was used as a weight-specific ration of 1% (Coutteau et al., 1994b).
haemocytometer. The algae were harvested in the exponential phase. Every week, each of the four algal species was sampled for lipid analysis.

The experimental emulsion Em50D, prepared by INVE Technologies (Baasrode, Belgium), contained 50% lipid on wet weight (WW) basis, water, emulgators, antioxidants, preservatives and liposoluble vitamins (A, C, D and E, respectively, 0.18, 0.08, 0.013 and 0.32% of WW). The fatty acid composition is given in Table 2.

2.2. Culture of the spat

Juvenile Manila clams (T. philippinarum), initial live weight 1 ± 0.1 mg, were obtained from Tinamener, Spain and acclimatized to the experimental conditions during 7 days prior to the experiment. During this holding period they were fed a mixture of I. galbana (clone T-Iso) and C. neogracilis at a ratio of 1% algal dry weight per wet weight of clams (DW·WW -1·day -1). The system for rearing spat consisted of 24 aquaria placed in a water bath of 21°C. Each 5 l aquarium contained one down-welling silo in which an air–water lift carried water up from the aquarium into the silo and one point aeration to keep the food in suspension (Coutteau et al., 1994b). Three times a week, the spat were washed with a jet of tap water, the silos and aquaria were cleaned and the latter refilled with 5-μm filtered seawater. The animals were fed twice a day during a period of 4 weeks.

The spat were stocked at an initial concentration of 0.75 g wet weight (WW) per silo. Every week, the animals were harvested, rinsed with tap water, blotted dry with paper towel, weighed and restocked at 0.75 g WW per silo. The daily growth rate was calculated according to the formula:

$$DGR(\%\cdot day^{-1}) = \left[ (WW_t/WW_0)^{1/n} - 1 \right] \times 100$$

with \(n\) = 7, \(WW_t\), respectively, \(WW_0\) = WW at day 7, respectively, day 1. Each day the amount of food was adjusted to keep the food ration constant according to the formula (Coutteau et al., 1994b):

$$(DW\text{ algal diet})_{day}^n = (DW\text{ algal diet})_{day}^{0} \times (1 + DGR/100)^{n-1}$$

with \(n\) = day of the week; (DW algal diet)_{day}^{0} = 0.0075 g; DGR = daily growth rate which was assumed to be 10% in the first week of the experiment and thereafter calculated from the growth during the previous week. The cellular dry weight (pg cell -1) of the algae used in the calculations were: I. galbana (clone T-Iso) = 14.1, C. neogracilis = 23.18, D. tertiolecta = 48.8 and T. suecica = 168. Algal DW were determined by filtering known volumes of algal suspension on pre-weighed and combusted glassfiber filters (Whatman GF/C, 1 μm). After rinsing with 0.5 M ammonium formate solution, the filters were dried for 24 h at 60°C and weighed again. The relative DGR (relDGR) was calculated as a percentage of the DGR observed for clams receiving the mixed algal diet.

2.3. Experimental design

A mixed diet of I. galbana (clone T-Iso) and C. neogracilis (1:1 on DW basis) fed at a weight-specific ration of 1% (algal dry weight per WW of seed per day,%DW·WW -1).
2.5. Statistical analysis

Statistical analysis of the data was conducted using Tukey’s Honest Significant Difference (HSD) test. All means were checked by Bartlett’s test for homogeneity of variances.

3. Results

3.1. Growth

The average DGR and the related growth results for 4 weeks are given in Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Week</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iso + Chet</td>
<td>8.140.49&lt;sup&gt;y&lt;/sup&gt;</td>
<td>7.820.21&lt;sup&gt;y&lt;/sup&gt;</td>
<td>6.970.34&lt;sup&gt;y&lt;/sup&gt;</td>
<td>8.060.56&lt;sup&gt;y&lt;/sup&gt;</td>
<td>100.0</td>
</tr>
<tr>
<td>Starv</td>
<td>2.340.51&lt;sup&gt;y&lt;/sup&gt;</td>
<td>1.990.98&lt;sup&gt;y&lt;/sup&gt;</td>
<td>1.900.48&lt;sup&gt;y&lt;/sup&gt;</td>
<td>1.430.38&lt;sup&gt;y&lt;/sup&gt;</td>
<td>100.0</td>
</tr>
<tr>
<td>Dun</td>
<td>4.930.04&lt;sup&gt;y&lt;/sup&gt;</td>
<td>3.370.76&lt;sup&gt;y&lt;/sup&gt;</td>
<td>3.120.32&lt;sup&gt;y&lt;/sup&gt;</td>
<td>3.350.13&lt;sup&gt;y&lt;/sup&gt;</td>
<td>60.6</td>
</tr>
<tr>
<td>Dun +20Em</td>
<td>6.650.15&lt;sup&gt;y&lt;/sup&gt;</td>
<td>4.840.96&lt;sup&gt;y&lt;/sup&gt;</td>
<td>2.080.35&lt;sup&gt;y&lt;/sup&gt;</td>
<td>0.780.19&lt;sup&gt;y&lt;/sup&gt;</td>
<td>81.7</td>
</tr>
<tr>
<td>Dun +40Em</td>
<td>7.180.08&lt;sup&gt;y&lt;/sup&gt;</td>
<td>5.440.14&lt;sup&gt;y&lt;/sup&gt;</td>
<td>3.010.16&lt;sup&gt;y&lt;/sup&gt;</td>
<td>0.940.02&lt;sup&gt;y&lt;/sup&gt;</td>
<td>95.6</td>
</tr>
<tr>
<td>Tet</td>
<td>8.350.95&lt;sup&gt;y&lt;/sup&gt;</td>
<td>6.901.27&lt;sup&gt;y&lt;/sup&gt;</td>
<td>5.021.09&lt;sup&gt;y&lt;/sup&gt;</td>
<td>6.520.35&lt;sup&gt;y&lt;/sup&gt;</td>
<td>102.6</td>
</tr>
<tr>
<td>Tet +20Em</td>
<td>8.160.56&lt;sup&gt;y&lt;/sup&gt;</td>
<td>7.080.42&lt;sup&gt;y&lt;/sup&gt;</td>
<td>5.520.47&lt;sup&gt;y&lt;/sup&gt;</td>
<td>6.696.14&lt;sup&gt;y&lt;/sup&gt;</td>
<td>100.2</td>
</tr>
<tr>
<td>Tet +40Em</td>
<td>7.610.27&lt;sup&gt;y&lt;/sup&gt;</td>
<td>6.720.61&lt;sup&gt;y&lt;/sup&gt;</td>
<td>6.218.91&lt;sup&gt;y&lt;/sup&gt;</td>
<td>7.380.74&lt;sup&gt;y&lt;/sup&gt;</td>
<td>93.5</td>
</tr>
</tbody>
</table>

The values represent the average DGR ± standard deviation of three replicate aquaria, followed by the reIDGR (%) in the next row. Values for the average DGR with the same letter within a column are not significantly different (Anova, Tukey HSD Test, P < 0.05).

For abbreviations: see text.

day<sup>-1</sup> is known to support good growth for juvenile T. philippinorum (Cuetteau et al., 1994b) and was used as a reference diet (Iso + Chet). Starvation was used as a negative control (Starv). D. tertiolecta (Dun) and T. suecica (Tet), fed at a feeding ration of 1%, were supplemented with emulsion Em50D to give 20 (Dun +20Em and Tet +20Em) and 40% (Dun +40Em and Tet +40Em) lipid expressed as percent of total algal DW (Table 1). All treatments were run in triplicate aquaria.

2.4. Fatty acid analysis

Fatty acid methyl esters (FAME) were prepared by direct transesterification with 10% acetyl chloride in methanol (Lepage and Roy, 1984) and analyzed in a Chrompack CP9001 gas chromatograph equipped with an autosampler. Injection was done on column into a very polar 50 m capillary column, BPX70, with a diameter of 0.32 mm and a layer thickness of 25 µm connected to a 2.5 m deactivated precolumn. The carrier gas was H2 and the detection mode FID. The oven temperature was set to increase the initial temperature from 85 to 150°C at a rate of 20°C min<sup>-1</sup>, from 150 to 152°C at 0.1°C min<sup>-1</sup>, from 125 to 174°C at 0.7°C min<sup>-1</sup>, from 174 to 180°C at 10°C min<sup>-1</sup> and to stay at 180°C for 2 min. Identification was based on standard reference mixtures (Nu-Chek-Prep, U.S.A.). Integrations and calculations were done with the software program ’Maestro’ (Chrompack).

### Table 2

<table>
<thead>
<tr>
<th>Fatty acid composition (%) of total fatty acids</th>
<th>Tagus phillipinorum</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>19.70±0.21</td>
</tr>
<tr>
<td>16:0</td>
<td>11.96±0.13</td>
</tr>
<tr>
<td>16:1 n−7</td>
<td>4.50±0.11</td>
</tr>
<tr>
<td>16:4 n−3</td>
<td>5.10±0.13</td>
</tr>
<tr>
<td>18:1 n−9</td>
<td>17.50±0.31</td>
</tr>
<tr>
<td>18:1 n−7</td>
<td>1.90±0.13</td>
</tr>
<tr>
<td>18:2 n−6</td>
<td>6.30±0.23</td>
</tr>
<tr>
<td>18:3 n−6</td>
<td>0.40±0.03</td>
</tr>
<tr>
<td>18:3 n−3</td>
<td>9.00±0.21</td>
</tr>
<tr>
<td>18:4 n−3</td>
<td>9.20±0.13</td>
</tr>
<tr>
<td>20:1 n−9</td>
<td>6.30±0.13</td>
</tr>
<tr>
<td>20:4 n−6</td>
<td>5.00±0.11</td>
</tr>
<tr>
<td>22:5 n−3</td>
<td>3.70±0.13</td>
</tr>
<tr>
<td>22:6 n−3</td>
<td>11.10±0.13</td>
</tr>
<tr>
<td>Total saturated fatty acids</td>
<td>32.90±0.31</td>
</tr>
<tr>
<td>Total monoenoic fatty acids</td>
<td>23.60±0.33</td>
</tr>
<tr>
<td>Total n−3 PUFA</td>
<td>30.50±0.23</td>
</tr>
<tr>
<td>Total n−6 PUFA</td>
<td>14.00±0.31</td>
</tr>
<tr>
<td>Total n−3 n−6</td>
<td>2.90±0.21</td>
</tr>
<tr>
<td>DHA/EPA</td>
<td>11.25±0.13</td>
</tr>
</tbody>
</table>

Values are the means of three replicates.

<sup>1</sup>Minor components (present at levels
<sup>1</sup>1. Tagus philippinorum (clone T-100).
<sup>1</sup>2. C. prostralis.
<sup>1</sup>3. D. tertiolecta.
<sup>1</sup>4. T. suecica.
<sup>1</sup>5. Levels < 0.1%.
<sup>1</sup>6. Total saturated fatty acids.
<sup>1</sup>7. Total monounsaturated fatty acids.
<sup>1</sup>8. Total n−3 polyunsaturated fatty acids.
<sup>1</sup>9. Total n−6 polyunsaturated fatty acids.
2.5. Statistical analysis

Statistical analysis of the data included one way analysis of variance (ANOVA) and Tukey’s Honest Significant Difference (HSD) Test. The homogeneity of the variances of means was checked by Bartlett’s Chi-square test.

3. Results

3.1. Growth

The average DGR and the relDGR of the spat fed the various diets or starved during 4 weeks are given in Table 1. The mixed algal diet of *L. galbanus* (clone T-iso) and *C. megalosiphon* were used in the lipid supplementation tests with *T. philippinarum* spat.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Iso&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Chue&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Dui&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Test&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Em50D&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>19.70 (2.3)</td>
<td>23.60 (3.3)</td>
<td>1.90 (1.1)</td>
<td>2.80 (0.3)</td>
<td>0.80 (1.1)</td>
</tr>
<tr>
<td>16:0</td>
<td>11.90 (1.3)</td>
<td>11.70 (4.3)</td>
<td>19.20 (5.5)</td>
<td>20.80 (2.3)</td>
<td>15.50 (0.5)</td>
</tr>
<tr>
<td>16:1n-7</td>
<td>4.50 (1.1)</td>
<td>18.40 (6.3)</td>
<td>1.80 (1.1)</td>
<td>5.60 (1.1)</td>
<td>1.60 (0.0)</td>
</tr>
<tr>
<td>16:4n-3</td>
<td>9.40 (0.6)</td>
<td>9.60 (0.6)</td>
<td>8.30 (0.6)</td>
<td>1.10 (0.0)</td>
<td>1.10 (0.0)</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>15.70 (3.3)</td>
<td>0.50 (1.1)</td>
<td>7.10 (3.1)</td>
<td>20.40 (5.5)</td>
<td>16.40 (0.0)</td>
</tr>
<tr>
<td>18:1n-7</td>
<td>1.90 (1.1)</td>
<td>0.30 (1.1)</td>
<td>1.70 (3.1)</td>
<td>2.50 (0.0)</td>
<td>1.10 (0.1)</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>6.30 (2.3)</td>
<td>0.80 (1.1)</td>
<td>13.60 (1.1)</td>
<td>2.00 (3.3)</td>
<td>2.70 (0.1)</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>0.40 (0.0)</td>
<td>2.70 (1.1)</td>
<td>4.80 (0.0)</td>
<td>1.60 (0.0)</td>
<td>1.60 (0.0)</td>
</tr>
<tr>
<td>18:4n-3</td>
<td>9.10 (2.3)</td>
<td>33.00 (1.3)</td>
<td>12.40 (3.3)</td>
<td>0.20 (0.0)</td>
<td>0.20 (0.0)</td>
</tr>
</tbody>
</table>

Values are the means of three replicates followed by the standard deviation between brackets.

<sup>1</sup>M. Cuss et al., *L. galbanus* (clone T-iso).

<sup>2</sup>T. megalosiphon.

<sup>3</sup>D. fortunata.

<sup>4</sup>T. necrana.

<sup>1</sup>Levels ≤ 0.1% = 21:5n-3.

<sup>2</sup>Total saturated fatty acids.

<sup>3</sup>Total monounsaturated fatty acids.

<sup>4</sup>Total n-3 polyunsaturated fatty acids.

<sup>5</sup>Total n-6 polyunsaturated fatty acids.
supported best growth with a DGR ranging from 7.0 to 8.1% and was used as a reference to evaluate the weekly DGR in the other diets. During starvation, the DGR declined from 2.34 to 0.43%. Already in the first week, the DGR of the clams fed solely *D. tertiolecta* (Dun) was significantly lower than the control treatment (Iso + Chaet). The supplementation of 20% lipids to the *D. pulicaria* diets resulted in an increase of the relDGR from 60.6 to 81.7%, corresponding with a DGR which was no longer significantly different from the control treatment. The relDGR increased further to 95.6% when 40% lipids were supplemented, resulting in a DGR which was significantly higher than the DGR of clams fed sole *D. tertiolecta*. The DGR of the lipid supplemented *D. pulicaria* diets (Dun + 20Em and Dun + 40Em) remained higher during the whole culture period compared to the DGR in the non-supplemented *D. pulicaria* diet, although significant differences were only detected in the first and third week at a supplementation level of 40%. The DGR of all the *D. pulicaria* diets showed a continuous decline in the DGR during the experimental culture period. As a result, none of the three *D. pulicaria* diets (Dun, Dun + 20Em and Dun + 40Em) showed a significantly higher DGR than the starved animals in the last week of the experiment. The DGR of the clams fed solely *T. suecica* was significantly higher compared to those fed solely *D. tertiolecta* during the whole culture period. The supplementation of *T. suecica* with 20 or 40% lipid never resulted in a significant increase of the DGR. Except for week 3, the DGR in the three *T. suecica* diets was never significantly different from the control diet. In contrast to the animals fed *D. tertiolecta*, the relDGR remained stable after the first week.

3.2. Fatty acid composition

The fatty acid composition of the 4 algal species and the emulsion are given in Table 2. The major fatty acids in *J. galbana* (clone T-Iso) were 14:0, 16:0, 18:1n−9, 18:3n−3 and 22:6n−3. The main fatty acids in *C. neogracile* were 14:0, 16:0, 16:1n−7, 18:4n−6 and 20:5n−3. *D. tertiolecta* contained no PUFA more unsaturated or longer than 18:3n−3 but the proportions of C18 PUFA were much higher than in the other algal species. Other major fatty acids were identified as 16:0, 16:4n−3, 18:1n−9 and 18:2n−6. The main difference between the two green algae was the presence of 20:4n−6, traces levels of DHA and especially the abundance of the EPA in *T. suecica*. Furthermore *T. suecica* contained high levels of 16:0, 16:4n−3, 18:1n−9, 18:3n−3 and 18:4n−3. The fatty acid profile of the emulsion was dominated by DHA (44.8%), EPA (6.8%), 16:0 (15.6%) and 18:1n−9 (16.8%).

Notes to Table 3:
Values are the means of two replicates followed by the standard deviation between brackets.
*Minor components (present at levels < 1% in all treatments) were not included in the table.
*For abbreviations: see text.
*Total saturated fatty acids.
*Total monounsaturated fatty acids.
*Total n−3 polyunsaturated fatty acids.
*Total n−6 polyunsaturated fatty acids.
Table 3

Fatty acid composition (% of total fatty acids) of T. philippinarum spat fed an algal diet of *D. tertiolecta* or *T. suecica* with or without the supplementation of 20 and 40% of a DHA-rich emulsion, a mixed algal diet of *L. galbana* (clone 1) and *C. neogracile* or starved during 4 weeks.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Treatment^b</th>
<th>Iso + Chlam</th>
<th>Starv</th>
<th>Dun</th>
<th>Dun + 20Em</th>
<th>Dun + 40Em</th>
<th>Tet</th>
<th>Tet + 20Em</th>
<th>Tet + 40Em</th>
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<td>14:0</td>
<td></td>
<td>12.51 %</td>
<td>15.00%</td>
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<td>15.00%</td>
<td>15.00%</td>
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<tr>
<td>16:0</td>
<td></td>
<td>19.61 %</td>
<td>20.50 %</td>
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<tr>
<td>16:1n-7</td>
<td></td>
<td>11.20 %</td>
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<td>13.00%</td>
<td>13.00%</td>
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<tr>
<td>18:0</td>
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<tr>
<td>18:1n-9</td>
<td></td>
<td>10.10 %</td>
<td>10.10 %</td>
<td>10.10 %</td>
<td>10.10 %</td>
<td>10.10 %</td>
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<tr>
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<td>2.00 %</td>
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<td></td>
<td>3.00 %</td>
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<tr>
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<tr>
<td>20:5n-3</td>
<td></td>
<td>7.00 %</td>
<td>7.00 %</td>
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<td>7.00 %</td>
<td>7.00 %</td>
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<td>7.00 %</td>
</tr>
<tr>
<td>22:4n-6</td>
<td></td>
<td>8.00 %</td>
<td>8.00 %</td>
<td>8.00 %</td>
<td>8.00 %</td>
<td>8.00 %</td>
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</tr>
<tr>
<td>22:5n-3</td>
<td></td>
<td>3.00 %</td>
<td>3.00 %</td>
<td>3.00 %</td>
<td>3.00 %</td>
<td>3.00 %</td>
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<tr>
<td>Total sat^a</td>
<td></td>
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<tr>
<td>Total mono^b</td>
<td></td>
<td>18.90 %</td>
<td>18.90 %</td>
<td>18.90 %</td>
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<td>18.90 %</td>
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<tr>
<td>PUFA^b</td>
<td></td>
<td>14.00 %</td>
<td>14.00 %</td>
<td>14.00 %</td>
<td>14.00 %</td>
<td>14.00 %</td>
<td>14.00 %</td>
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<tr>
<td>DHA/EPA^c</td>
<td></td>
<td>1.10 %</td>
<td>1.10 %</td>
<td>1.10 %</td>
<td>1.10 %</td>
<td>1.10 %</td>
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</tbody>
</table>

^a^ Data for total n-6 and total n-3 were calculated as the sum of the percentage of all fatty acids.

^b^ Data are means of three replications ± SD (n = 3).

^c^ DHA/EPA was calculated as 20:5n-3/20:4n-6.
In general, the fatty acid composition of the algae was reflected in that of the animals (Tables 2 and 3), for example, the higher levels of 14:0, 16:1n-7, 20:4n-6 and 22:5n-6 in the control diet resulted in higher levels of these fatty acids in the clams compared to the animals fed D. tertiolecta or T. suecica. Similarly, the abundance of 18:2n-6 in D. tertiolecta (13.6%) resulted in a higher proportion of this fatty acid in the spat (5.4%) compared to the spat fed the control diet (2.2%) or T. suecica (2.9%). On the other hand, the levels of 18:4n-3 in the spat fed T. suecica (2.7%) were higher than in the animals fed D. tertiolecta (0.5%) but it did not fully reflect the high level of this fatty acid present in T. suecica (8.1%) compared to D. tertiolecta (0.8%). Similarly, the level of 18:3n-3, which was twice as high in D. tertiolecta than in T. suecica, was not clearly expressed in the fatty acid composition of the animals. On the other hand, the absence of DHA in T. suecica resulted in a decrease in the DHA level in the animals fed this alga (3.4%) compared to clams fed the control diet (8.5%). The spat fed with D. tertiolecta, which did not contain any HUFA, clearly showed a lower EPA level compared to animals fed T. suecica or the control diet (respectively, 2.8, 11.3 and 7.7%) whereas the levels of 22:5n-6 and particularly DHA (2.8 and 9.5%) were much higher compared to the animals fed T. suecica (1.0 and 3.4%). The supplementation of 20 or 40% lipids to a diet of D. tertiolecta resulted in a marked increase of the DHA level from 9.5% in the non-supplemented algal diet to 19.8 and 22.0%, at a 20 and 40% lipid supplement, respectively, whereas the EPA level in the animals was not affected. As a result, the DHA/EPA ratio drastically increased from 3.4 (Dun) to 6.5 (Dun + 20Em) and 7.4 (Dun + 40Em). Due to the combined effect of increasing total n-3 PUFA and decreasing total n-6 PUFA levels the n-3/n-6 PUFA ratio increased from 1.8 to 3.0 and 3.4 at 20 and 40% supplementation, respectively (Table 3). The level of 22:5n-3 and especially DHA and the total n-3 PUFA increased when 20 or 40% lipids were supplemented to the T. suecica diet (Table 3). Since the supplementation of the emulsion resulted in a sharp increase of the DHA content while the EPA level in the animals decreased, the DHA/EPA ratio changed from 0.3 (Tet) to 3.0 (Tet + 20Em) and 3.4 (Tet + 40Em). The higher n-3 PUFA levels in the animals that received a 20 or 40% lipid supplement was accompanied by a decline in the amount of PUFA from the n-6 family. As a result, the n-3/n-6 ratio changed from 3.6 (Tet) to 6.7 and 7.1 in the Tet + 20Em and Tet + 40Em treatments, respectively (Table 3). For Dunaliella as well as Tetraselmis fed clams, further increase of the lipid supplement from 20 to 40% affected the level of most fatty acids to a lesser extend compared with the changes between the non-supplemented algal diet and a 20% supplement (Table 3).

4. Discussion

Limited live weight increases in starved bivalves have been observed by several authors and probably resulted from the buildup of shell material at the expense of initial reserves and/or the natural food supply still present in the seawater (Langdon and Waldock, 1981). Despite its high lipid content, the growth of the animals fed solely D. tertiolecta was significantly lower than those fed T. suecica or the mixed algal diet. The low food value of D. tertiolecta has been demonstrated in previous studies with juvenile and larval bivalves (Langdon and Walker, 1992; Delaunay et al., 1993) and is especially DHA and EPA. The DHA-rich lipid emulsion to D. tertiolecta 6.3% EPA but no DHA, resulted in T. suecica and supplementing 20% of animals. This was in agreement with the usage gelatin/acaia encapsulated liposomes and D. tertiolecta fed to Cossartia and other Dunaliella fed clams receiving 10% lipid. On the other hand, Langdon and Waldock (1981) found when 40% oyster lipid extract or 20% of 20 and 9%, respectively, increased the lipids in the microcapsules and the hard clam Mercenaria, either n-3 HUFA rich or deficient.

Previous studies, under natural fatty acid composition of the diet, the composition of T. philippinarum present experiment, the supplementing the DHA levels was evident in the spats of T. suecica. Similar effects were seen with C. rotula when a Tetrasselmis or Dunaliella microcapsules. On the other hand, a similar emulsion as the one used for the increase in the DHA levels from juvenile P. maculata (Langdon and Waldock, 1996). Interestingly, the supplementing absent in T. tertiolecta and present in Dunaliella. EPA proportions in the Dunaliella Tetrasselmis treatments compared selective retention of DHA in starved EPA by DHA in Tetrasselmis. In accordance with previous studies of 20:4n-6 could be related to the production of eicosanoids. Studi
and larval bivalves (Langdon and Waldock, 1981; Enright et al., 1980a; Wikfors et al., 1992; Delaunay et al., 1993) and is generally attributed to the absence of n-3 HUFA, especially DHA and EPA. The DGR of T. philippinarum increased by supplementing a DHA-rich lipid emulsion to D. tertiolecta. Feeding solely T. suecica, which contained 6.3% EPA but no DHA, resulted in higher DGR compared to the DHA enriched D. tertiolecta and supplementing 20 or 40% lipids only slightly affected the DGR of the animals. This was in agreement with the results of Langdon and Waldock (1981) who used gelatin/acidia encapsulated lipids (DHA or oyster lipid extract) to supplement D. tertiolecta led to Crassostrea gigas. In the present experiment, the DGR of the Danvillea fed clams receiving 20% lipid was further enhanced by supplementing 40% lipid. On the other hand, Langdon and Waldock (1981) reported a decreased growth when 40% oyster lipid extract or 10% DHA was supplemented compared to a supplement of 20 and 5%, respectively. However, the efficiency with which the supplemented lipids in the microcapsules and the emulsion are ingested and assimilated by the spat is not known. Langdon and Waldock (1981) suggested that either EPA or DHA are likely to meet the n-3 HUFA requirements of the animals, although a mixture of both may possibly be slightly better to obtain optimum growth rates. Enright et al. (1986b) found that a monospecific diet, grown under conditions that promoted higher algal DHA levels, resulted in higher growth rates in juvenile O. edulis. Similarly, Thompson and Harrison (1992) found a positive correlation between larval growth and survival of C. gigas and the DHA level in Thalassiosira pseudonana cultured under various conditions. However, when feeding the DHA rich algae I. galbana (clone T-iso), similar growth rates were obtained for the juvenile scallop P. magellanicus (Coutteau et al., 1996) and the hard clam Mercenaria mercenaria (Hurd et al., 1989), by supplementing either n-3 HUFA rich or deficient lipids.

Previous studies, under natural as well as experimental conditions, illustrated that the fatty acid composition of the diet did, at least to some extent, affect the fatty acid composition of T. philippinarum (Beninger and Stephan, 1985; Porteres, 1991). In the present experiment, the supplementation of the emulsion resulted in a drastic increase of the DHA levels in the spat compared to the animals fed solely D. tertiolecta or T. suecica. Similar effects were demonstrated by Langdon and Waldock (1981) in juvenile C. gigas when a Tetraselmis or Danvillea diet was supplemented with DHA containing microcapsules. On the other hand, the supplementation of I. galbana (clone T-iso) with a similar emulsion as the one used in this experiment, resulted in a less pronounced increase of the DHA levels from 17.9 to 19.4% and 20.77 to 28.11% in the total lipids of juvenile P. magellanicus and larval O. edulis, respectively (Coutteau et al., 1994a, 1996). Interestingly, supplementing an emulsion containing 6.8% EPA, which was absent in D. tertiolecta and present at a level of 6.3% in T. suecica, did not affect the EPA proportions in the Danvillea treatments and resulted in decreased levels in the Tetraselmis treatments compared to the animals fed the non-supplemented diets. The selective retention of DHA in starved and Danvillea fed clams and the replacement of EPA by DHA in Tetraselmis fed clams indicated a major importance of DHA, in accordance with previous studies (Coutteau et al., 1996) whereas the selective retention of 20:4n-6 could be related to the role of this fatty acid as a precursor for the production of eicosanoids. Studies with 14C-labelled linoleic (18:2n-6) and respec-
tively linolenic acid (18:3n-3) showed that the oyster *C. virginea* (Chu and Greaves, 1991) and the yellow clam *Mesodesma matroides* (De Moreno et al., 1976) did not have the ability to further elongate and desaturate these exogenous fatty acids to arachidonic acid (20:4n-6) and EPA or DHA, respectively. Although Waldeck and Holland (1984) showed some 14C incorporation in EPA and DHA (less then 1%) in C. gigas fed 14C-labelled *D. tertiolecta* and *T. suecica*, the elongation and desaturation activity is probably too low to fulfill the essential fatty acid (EFA) requirements in juvenile bivalves indicating that they must be supplied through the diet. Furthermore, 18:3n-3, 18:2n-6 and 18:1n-9 which were abundant in *T. suecica*, and especially in *D. tertiolecta*, may competitively inhibit the conversion of EPA to DHA since they all compete for the delta-6 fatty acid desaturase (Sargent et al., 1993, 1997). Thompson and Harrison (1992) suggested that there exists a threshold value for the EPA content beyond which more essential fatty acids will not improve growth and survival in larval bivalves. The same hypothesis may be valid for the DHA requirements of juvenile bivalves whereas the situation for EPA is less clear. Sargent et al. (1997) argue that a possible negative effect of a relative excess of EPA over DHA in larval fish feeds may be due to the established role of EPA to competitively depress the production of eicosanoids from arachidonic acid. Recently, Thompson et al. (1996) found that the growth rate of larval *C. virginea* was negatively correlated with the EPA content in their algal diet.

It has been documented that variations in the culture conditions of microalgae influence their biochemical and fatty acid composition which in turn can affect their nutritional and energetic value as a food for bivalves (Gallagher and Mann, 1982; Utting, 1986; Whyte, 1987; Thompson and Harrison, 1992; Durstan et al., 1993; Thompson et al., 1996). In this respect, the use of inexpensive off-the-shelf emulsions as a supplement to algal diets may be useful to standardize and improve the lipid quality and quantity of various algal diets commonly fed in commercial hatcheries. Furthermore, emulsions can be a cost-effective tool to improve the food value of easily growing algal species which are lacking certain essential fatty acids.

Apart from the possibility that the lipid supplement did not fully meet the fatty acid requirements of *T. philippinarum*, e.g., with respect to the optimal n-3/n-6 and DHA/EPA/20:4n-6 proportions, it should be mentioned that the present results suggest that the lack of MUFA is probably not the only factor responsible for the low food value of *D. tertiolecta*. Indeed, it could not be explained why the DGR of the animals fed *D. tertiolecta* in combination with the lipid supplements continuously decreased throughout a 4-week culture period. Although it has been demonstrated that *D. tertiolecta* can be digested and assimilated by juvenile bivalves (Peirseon, 1983; Waldock and Holland, 1984), algal metabolites may inhibit growth (Ward and Targrett, 1989). Several studies have investigated the nutritional requirements of bivalves by feeding various non-specific or mixed algal diets (Epifanio, 1982; Gallagher and Mann, 1982; Enright et al., 1986a,b; Lang and Milician, 1986; Wikfors et al., 1992; Albertososa et al., 1993; Delaunay et al., 1993; Knauer and Southgate, 1996; Thompson et al., 1996). However, it remains difficult to correlate the gross biochemical and/or fatty acid composition of algae with their food value since it is impossible to take all the interspecific differences between different algal diets into account. Furthermore, certain minor components such as vitamins, respect, lipid supplementation of algae, nutrition studies and contribute to requirements in various stages of bivalves' artificial diet which could eliminate.
minor components such as vitamins and minerals may play an important role. In this respect, lipid supplementation of algal diets could be a useful alternative for further lipid nutrition studies and contribute to a better understanding of the specific fatty acid requirements in various stages of bivalves. Ultimately, the aim to develop an adequate artificial diet which could eliminate the need for live algae remains.

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References


Lepage, G., Roy, C.C., 1984. Improved recovery of fatty acids through direct transesterification without prior extraction or purification. J. Lipid Res. 25, 1391–1396.


