Comparison of *Isochrysis galbana* and *Chlorella* sp. microalgae on growth and survival rate of European flat oyster (*Ostrea edulis*, Linnaeus 1758) larvae

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Influence of microalgal feeding with *Isochrysis galbana* and *Chlorella* sp. on the growth and survival rate of larvae of European flat oyster, *Ostrea edulis*, was investigated. Larvae were reared with four food regimes: *I. galbana*, *Chlorella* sp., mixture of both and unfed for 16 days. After 16 days, it was observed that the larvae reached the umbo stage with a mean size in length 220±4.12 µm and 219±5.76 µm (initial length, 159 µm) (P > 0.05). Larvae were fed on *I. galbana* and mixture diet. At this stage, survival rate was 30% and 24%, respectively (P > 0.05). Growth and survival rates for the feeding regimes were significantly higher than the *Chlorella* sp. and unfed groups (P < 0.05).

**Keywords:** *Ostrea edulis*, Flat Oyster, Larvae, Feeding, Microalgae

**Introduction**

Bivalve culture relies on juvenile collection from the natural environment but the spats can only be collected during a short period of the year in many locals. Therefore, commercial oyster growers are dependent on reliable supply of hatchery produced juveniles for stocking purposes. Generally, oyster hatcheries minimize total mortality by attempting to maximize larval growth and success at metamorphosis. One of the most important aspects of hatchery operations is feeding.

The cultivation of microalgae as food for the commercial rearing of marine animals is of critical importance in the mariculture industry. Marine unicellular algae have been widely and directly utilized in all growth stages of bivalves as the principal source of food for rearing. Early culture research indicated that certain phytoplankton species were better than others rations for larvae. Minute naked flagellates belonging to the groups Chlamydomonaceae, Cryptomonadaceae and Chrysomonadaceae have been used for swimming bivalve larvae. Present study is to compare the effect of *Isochrysis galbana* and *Chlorella* sp. on growth and survival rates of *O. edulis* larvae.

**Materials and Methods**

Adult oysters were collected from natural beds at the end of April from Mersin Bay, Izmir, Turkey. Oysters were transported to the laboratory and kept at 17°C during the night. Adult oysters were induced to spawn by thermal stimulation (17°C-27°C) for 20 minutes. Healthy veliger larvae were obtained after thermal stimulation of adult oysters. Larvae were transferred in to 1 l erlenmeyer flask where they were cultured, with three replicates. Four growth groups were conducted. Larvae were fed on *I. galbana*, *Chlorella* sp., equal mixture of *I. galbana* and *Chlorella* sp., and unfed (control group). Veliger (D-hinge) larvae were reared at a stocking density of 10 larvae ml⁻¹ for 16 days. Aeration was supplied via air diffusers. Temperature and sea water salinity were 18.5°C to 25°C and 36 psu to 37psu, respectively.

The unicellular algae *I. galbana* and *Chlorella* sp. were grown using a standard batch-culture regime in 1 µm filtered sea water at 22 ± 2 °C and 20-25 psu. With two-species diet (*I. galbana* and *Chlorella* sp.), each algae was added at 50% of the cell density for feeding oyster.

The culture medium was changed every 48 h, at which time samples were taken for observation, larval count and morphometric measurements. At least 50 larvae were taken from each erlenmeyer flask before recording length, width and morphological changes. Shell length (anterior-posterior) and width (dorsa-ventral) of larvae were measured by an ocular micrometer. Mortality was
assessed by counting the number of alive larvae in a sample under the microscope. Daily instantaneous growth rates (K) were calculated using the following formula:

\[ \text{Daily instantaneous growth rate (K)} = \frac{(\ln L_1 - \ln L_0)}{\Delta t} \]

Where L is measured as the length after a specified time of t days (L₁) and at the start of experiment (L₀), ∆t is time (in days).

Data were analyzed by one-way ANOVA to examine the effect of microalgal species on the larval growth rate and daily increase in shell length. Significant differences between the groups were determined by Tukey test. Survival data was arcsine transformed before statistical treatment and was analyzed by Chi-square (χ²). These statistical analyses were performed with SPSS program version 13.0 for windows.

Results

The experiment started with the larvae 159±3.28 µm in length. Growth of larvae in I. galbana and I. galbana+Chlorella sp. groups were better and faster than Chlorella sp. and unfed groups (Fig. 1). At the end of experiment, larvae (I. galbana and I. galbana+Chlorella sp. groups) reached umbo stage with a mean shell length of 220±4.12 µm and width of 200±2.51 µm. After first three days, growth of the unfed larvae ended while growth continued in the fed groups (Fig. 1). But larvae in Chlorella sp. group could not reach umbo stage and stayed at 175±1.12 µm in length and 156±1.03 µm in width.

Larval developments of I. galbana and I. galbana+Chlorella sp. groups, which were observed from veliger to umbo larvae stages, were same for 16 days. Results I. galbana and I. galbana+Chlorella sp. groups indicated that the growth of larvae did not differ significantly (P > 0.05). Similarly the highest daily mean length increments were observed I. galbana and I. galbana+Chlorella sp. groups, with 3.81 µm/day and 3.75 µm/day, respectively. On the other hand, Chlorella sp. and unfed groups showed poor increments with 2 µm/day and 1µm/day.

At the end of the experiment, the best growth rate (K) was observed in the groups fed I. galbana and I. galbana+Chlorella sp., 0.0202±0.0058 and 0.0200±0.0008 respectively (Table 1). Growth rate (K) was 0.0059±0.0001 in unfed group while the best daily growth rate was observed on the 3rd day in all groups (P > 0.05). However, slowest growth rate was determined on the 9th day for all groups (P > 0.05).

The highest survival rate at the end of the experiment was 30% in larvae fed the I. galbana. High mortality was observed in Chlorella sp. group at the 3rd day. However, the highest mortality was determined in unfed group on the 16th day (P < 0.05). Survival rate of larvae in I. galbana group was higher than Chlorella sp. and unfed groups on through umbo stage (Fig. 2).

Discussion

Environmental factors, such as temperature and food availability, are closely related to reproductive performance in bivalves. For Crassostrea gigas Lannan et al. (1980) demonstrated that larval production is related to gonadal development of parental oysters and that this involved environmental and heritable components. Quality and quantity of gametes were affected by broodstock condition. Knowledge of the general condition of animals is important to obtain gametes in the optimum state of development. In the present study, initial average size of O. edulis veliger larvae was about 159 µm in
larval feeding of bivalve mollusks such as good performance.

length in Izmir, Turkey, while it was 131 µm in the Mediterranean coast, France, 195 µm in Galicia, Spain, 170 µm in Conwy, United Kingdom. The variability of geographical characteristics such as water temperature, salinity, food quality and quantity and also genetic situation of adult oysters may be of significance in larval size.

Nutrition is the dominant factor influencing growth of O. edulis larvae. Several marine unicellular algae that are used routinely in mollusk hatcheries lead to positive effect of larval growth and survival. The content of polyunsaturated fatty acids of O. edulis has widely been used in mollusk hatcheries lead to positive effect of larval growth and survival. The content of polyunsaturated fatty acids of O. edulis has widely been used in

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>I. galbana</th>
<th>Chlorella sp.</th>
<th>I. galbana+ Chlorella sp.</th>
<th>Unfed</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.0479±0.0043</td>
<td>0.0215±0.0004</td>
<td>0.0592±0.0062</td>
<td>0.0275±0.008</td>
</tr>
<tr>
<td>6</td>
<td>0.0256±0.0022</td>
<td>0.0118±0.0028</td>
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<td>0.0039±0.0007</td>
</tr>
<tr>
<td>9</td>
<td>0.0121±0.0064</td>
<td>0.0076±0.0009</td>
<td>0.0069±0.0002</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
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<td>0.0084±0.0039</td>
<td>0.0152±0.0033</td>
<td>0.0087±0.0046</td>
</tr>
<tr>
<td>13</td>
<td>0.0167±0.0032</td>
<td>0.0191±0.0022</td>
<td>0.0361±0.0056</td>
<td>0.0057±0.0003</td>
</tr>
<tr>
<td>16</td>
<td>0.0107±0.0062</td>
<td>0.0088±0.0005</td>
<td>0.0061±0.0006</td>
<td>0</td>
</tr>
<tr>
<td>1-16</td>
<td>0.0202±0.0058</td>
<td>0.0114±0.0014</td>
<td>0.0200±0.0008</td>
<td>0.0059±0.0001</td>
</tr>
</tbody>
</table>

Table 1—Daily instantaneous growth rate (K) at feeding trials (P > 0.05)

Fig. 2—Survival rate of O. edulis larvae during experiments (Iso: Isochrysis galbana, Ch: Chlorella sp.)


