Artificial breeding of the Japanese pearl oyster *Pinctada fucata martensii* in hatcheries has usually been carried out using microalgae as food. Microalgae such as *Pavlova lutheri* and *Chaetoceros calcitrans* are suitable diets for the growth of pearl oyster larvae and spat; however, microalgal culture requires the establishment of air-conditioned facilities, a high labor input and, therefore, is a high-cost technique. An artificial diet as a substitute for algae would be very beneficial for artificial breeding. Several studies have been done on artificial diets for oyster breeding. Numaguchi and Nell have demonstrated that an artificial diet using gelatin–acacia microcapsules are a useful supplementation for the growth of Sydney rock oysters *Saccostrea commercialis* larvae. The present study determines whether gelatin–acacia microcapsules are useful for the growth of pearl oyster spat.

Gelatin–acacia microcapsules were prepared using a modified method of Langdon and Waldock. The reagents and equipment required for preparing the gelatin–acacia microcapsules are: cod liver oil, acacia powder (42 g of acacia powder dissolved in 1 L of distilled water), gelatin powder (9.2 g of gelatin powder of beef skin dissolved in 200 mL of distilled water and mixed while heating on a hot plate; this reagent is required for each preparation), and 1 N HCl and 1 N NaOH as reagents. The equipment includes: a hot plate with a magnetic stirrer, homogenizing apparatus, ultrasonic wave apparatus, and a refrigerator.

The procedure for the preparation of the gelatin–acacia microcapsules is as follows:

1. Cod liver oil (10 g) is placed into a 1-L beaker.
2. A volume of 200 mL of the acacia solution and 200 mL of the gelatin solution are added to the 1-L beaker containing cod liver oil.
3. These solutions are then homogenized for 1–2 min using homogenizing apparatus. The resultant solution forms an emulsion.
4. This emulsion is treated with the ultrasonic wave apparatus to further reduce the particle size.
5. Repeat stages 3 and 4 several times on the treated emulsion.
6. The pH of the solution is altered to pH 3.9 by adding 1 N HCl. This solution is heated to 40°C using a hot plate while mixing with a magnetic stirrer for 40 min.
7. After the 40 min, the pH of the solution is altered to pH 9.3 by adding 1 N NaOH.
8. The emulsion is poured into 2 L of chilled distilled water.
9. The solution is refrigerated for more than 2 h, allowing the solution to separate into an oily fluid (surface layer) and water (lower layer). (This procedure can be encouraged by centrifugation at 2000 r.p.m. for 20 min.)
10. Collect the oily fluid of the surface layer by pipette or suction pump. It is this oily fluid which contains a lot of the gelatin–acacia microcapsules. The size of the microcapsules can be controlled by the quantity of the ultrasonic wave treatment. For the diet of bivalves, a suitable microcapsule size is approximately 5 μm.

Pearl oyster spat used were produced by the Pearl Oyster Seed Production Center, Nagasaki Pearl Fisheries Cooperative Association. Spat were obtained approximately 3 months after being fertilized artificially in the hatchery. Average hinge length of each spat was 3.5 mm. Ten spat were allo-
Table 1  Effects of gelatin–acacia microcapsule (GAM) concentration on the growth of pearl oyster spat over 11 days

<table>
<thead>
<tr>
<th>Diets</th>
<th>Concentration (particles/mL)</th>
<th>Initial (0 day)</th>
<th>Final (11th day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfed</td>
<td>0</td>
<td>3.44 ± 0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.38 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GAM&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3.66 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.73 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GAM</td>
<td>5 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3.69 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.03 ± 0.40&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>GAM</td>
<td>2 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3.40 ± 0.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.74 ± 0.57&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>GAM</td>
<td>1 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>3.63 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.85 ± 0.39&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>*</sup>GAM size was 5.6 ± 1.9 μm (mean ± SD, n = 30).

Values are the mean ± SD (n = 10), values within a column with different superscripts were significantly different (Duncan multiple range test, P < 0.05)

Table 2  Effect of supplementation of gelatin–acacia microcapsule (GAM) to an algal diet on the growth of pearl oyster spat over 11 days

<table>
<thead>
<tr>
<th>Diets</th>
<th>Concentration (cells or particles/mL)</th>
<th>Initial (0 day)</th>
<th>Final (11th day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alga&lt;sup&gt;a&lt;/sup&gt;</td>
<td><em>I. galbana 3 x 10&lt;sup&gt;4&lt;/sup&gt;</em></td>
<td>3.45 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.12 ± 0.54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alga</td>
<td><em>I. galbana 2 x 10&lt;sup&gt;3&lt;/sup&gt;</em></td>
<td>3.66 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.98 ± 0.30&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alga + GAM&lt;sup&gt;†&lt;/sup&gt;</td>
<td><em>I. galbana 2 x 10&lt;sup&gt;3&lt;/sup&gt; + GAM 1 x 10&lt;sup&gt;3&lt;/sup&gt;</em></td>
<td>3.41 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.18 ± 0.29&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alga + GAM</td>
<td><em>I. galbana 2 x 10&lt;sup&gt;3&lt;/sup&gt; + GAM 5 x 10&lt;sup&gt;3&lt;/sup&gt;</em></td>
<td>3.79 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.73 ± 0.33&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alga + GAM</td>
<td><em>I. galbana 2 x 10&lt;sup&gt;3&lt;/sup&gt; + GAM 2 x 10&lt;sup&gt;4&lt;/sup&gt;</em></td>
<td>3.38 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.53 ± 0.27&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alga + GAM</td>
<td><em>I. galbana 2 x 10&lt;sup&gt;3&lt;/sup&gt; + GAM 1 x 10&lt;sup&gt;5&lt;/sup&gt;</em></td>
<td>3.41 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.82 ± 0.59&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Alga was *Isochrysis galbana*.

<sup>†</sup>GAM size was 5.6 ± 1.9 μm (mean ± SD, n = 30).

Values are the mean ± SD (n = 10). Values within a column with different superscripts were significantly different (Duncan multiple range test, P < 0.01).

cated to each 2-L beaker aquarium with seawater filtered using 1 μm cartridge filters. During the experiment, water temperature was kept at 24°C and salinity ranged 30–32 ppt. Feeding trials were carried out over 11 days. Hinge length of each spat was measured at the beginning and end of the feeding experiment using a stereoscopic microscope with a micrometer.

In experiment 1, the spat were either not fed (control) or fed gelatin–acacia microcapsules at concentrations of 1 x 10<sup>3</sup>, 5 x 10<sup>3</sup>, 2 x 10<sup>4</sup>, and 1 x 10<sup>5</sup> particles/mL per day (Table 1). There were no significant dietary effects (P < 0.05) between the unfed controls and the treatments fed gelatin–acacia microcapsules, which indicates that pearl oyster spat cannot grow by being fed only gelatin–acacia microcapsules. This may be because a diet of only microcapsules is insufficient in other essential nutrients (e.g. carbohydrate, protein, etc.) for the growth of pearl oyster spat.

In experiment 2, spat were fed the alga *Isochrysis galbana* at concentrations of 2 x 10<sup>3</sup> and 3 x 10<sup>4</sup> cells/mL per day. The low-density algal diet (*I. galbana*; 2 x 10<sup>3</sup> cells/mL per day) was also supplemented with microcapsules at four treatment concentrations of 1 x 10<sup>3</sup>, 5 x 10<sup>3</sup>, 2 x 10<sup>4</sup>, and 1 x 10<sup>5</sup> particles/mL per day (Table 2). The addition of microcapsules at 5 x 10<sup>3</sup> and 2 x 10<sup>4</sup> particles/mL per day to the low-density algal diets produced a significant (P < 0.01) improvement in the growth of pearl oyster spat. However, the high-density supplementation of gelatin–acacia microcapsules (1 x 10<sup>5</sup> particles/mL per day) inhibited the growth of the pearl oyster spat.

These results show that supplementing the low-density algal diet with gelatin–acacia microcapsules enhances the growth of the pearl oyster spat. Numaguchi and Nell have obtained similar results for the growth rate of Sydney rock oyster larvae that were fed gelatin–acacia microcapsules with a low-density algal diet. In their experiment, the gelatin–acacia microcapsules contained cod liver oil, which is rich in unsaturated fatty acids (C22:6n3 and C20:5n3). It has been demonstrated previously that long-chain unsaturated fatty acids are very important essential fatty acids for the growth of oyster larvae and spat. The results of the present study indicate that pearl oyster spat also require unsaturated fatty acids for their growth. However, it was observed that excess gelatin–acacia microcapsules inhibited the growth of the pearl oyster spat. It is thought that high concentrations of gelatin–acacia microcapsules may inhibit ingestion by the pearl
oyster spat or that overfeeding with gelatin–acacia microcapsules may induce a physiological influence in the pearl oyster spat. Nevertheless, in all cases, it is considered that the pearl oyster spat may require a suitable concentration of unsaturated fatty acids.

I am grateful to Dr J Nell (New South Wales Fisheries, Brackish Water Fish Culture Research Station of Australia) for his advice on the preparation of microcapsules and to Dr T Horii (National Research Institute of Fisheries Science of Japan) for statistical analyses of the data. This investigation was supported in part by a grants-in-aid from the Ministry of Agriculture, Forestry and Fisheries in Japan.

REFERENCES