Salinity–temperature and nutritional effects on the setting rate of larvae of the tropical oyster, *Crassostrea iredalei* (Faustino)

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**Abstract**

The combined effects of salinity–temperature and feeding (algal species and density) on the setting rate of *Crassostrea iredalei* larvae were studied. Six salinities (5, 10, 15, 20, 25 and 30‰) were tested at five temperatures (24, 27, 30, 33 and 36°C). A separate experiment using various algal densities (0, 25, 50, 75, 100 and 125 × 10³ cells ml⁻¹) of *Isochrysis galbana*, *Chaetoceros calcitrans* and mixtures of both was conducted. The optimum salinity–temperature conditions were 20°C and 30°C, which supported the highest mean percentage (± S.D.) larval settlement rate of 31.4 ± 3.4%. The use of different species of microalgae had a significant effect (*P* < 0.05) on the rate of larval settlement. The highest settlement rate was recorded for mixed algae (11.3%), but this was not significantly different from *I. galbana* (10.0%) (*P* > 0.05), irrespective of density. *Ch. calcitrans* produced 9.5% larval settlement rate, which also was not significantly different from *I. galbana* (*P* > 0.05). The effect of algae densities, irrespective of species, was that the highest set of 24.5% occurred at 100 × 10³ cells ml⁻¹. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Salinity; Temperature; Nutrition; Oyster; Larvae; Setting; *Crassostrea iredalei*

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1. **Introduction**

Intrinsic factors such as heredity and larval age, and extrinsic factors such as nutritional history, water parameter and the physical and chemical characteristics of the substrate have been reported to influence the settlement of most bivalve larvae (Hadh...
field, 1984). Among these, salinity and temperature represent two important ecological factors that influence the biology of the sensitive planktonic stages of estuarine organisms, such as the oyster larvae. Both these factors are easily measured, manipulated and controlled compared to most other factors.

Oysters of the genus *Crassostrea* in general are considered to be euryhaline organisms (Quayle and Newkirk, 1989). They have the ability to adapt well to temperature fluctuations (Angell, 1986). Although temperature changes within the tropical or subtropical zone may appear to have much less influence on the biology of bivalves, controlled laboratory experiments are still required to determine the effects of temperature, including effects with other parameters such as salinity. According to Dekshenieks et al. (1993), oyster larvae generally exhibit a wide tolerance of temperature fluctuations, but these may have serious effects on their physiology.

Researchers have studied the effects of temperature and salinity on the development and growth patterns of bivalve species in the wild and in the laboratory (e.g., Paul, 1980; Tettelbach and Rhodes, 1981; Kalyanasundram and Ramamoorthi, 1986). Studies in temperate countries have been conducted in the laboratory to examine the combined effects of salinity and temperature on the larval settlement of *C. gigas* (Lund, 1971; Henderson, 1983); the effects of salinity on settlement of *C. virginica* larvae (Hidu and Haskin, 1971); and the effects of temperature on settlement of *C. gigas* larvae settlement (Cooper and Shaw, 1984; Scholz et al., 1985). In Malaysia, there is one recent study on the effects of salinity on *C. belcheri* settlement by Tan (1993). The present study aimed at examining the combined effects of salinity and temperature on the settlement of larvae of the tropical oyster, *C. undulatus*, under hatchery conditions for the purpose of optimising spat production.

Currently, the use of live feed in the commercial production of bivalve larvae, such as oyster, scallop and clams, is reduced by the transfer of spat as soon as possible from the hatchery to the nursery. This is because of the operational costs involved in mass production of microalgae for the hatchery. To further decrease costs of microalgal production in oyster hatcheries, the larval stages are fed with microalgae until they reach the eyed stage. Immediately after this, they may be transported to be set elsewhere, which is referred to as ‘remote setting’. From then onwards, the eyed larvae will be either partially or, in some cases, exclusively fed on natural phytoplankton. Whether metamorphosing larvae need to be given a nutritional supplement at this stage, and in what quantity, are current issues, especially if the setting period is prolonged. In the temperate Pacific oyster (*C. gigas*), larval setting is allowed for 48 h (Henderson, 1983; Supan, 1987; Jones and Jones, 1988); but for some tropical oysters, such as *C. belcheri* (Tan, 1993) and *C. undulatus* (Devakie et al., 1993), observations indicate that larval settlement is staggered over 96 h.

Several researchers have expressed differences in opinion concerning the need to feed metamorphosing bivalve larvae. Lipovsky (1991) found that it was not necessary to feed metamorphosing *C. gigas* larvae, probably because their velum tends to degenerate and they are unable to filter food particles during this period. However, Paynter et al. (1993) stressed that *C. virginica* larvae are unable to metamorphose without food. A recent study by Baker and Mann (1994) showed that *C. virginica* larvae are able to feed at all stages of metamorphosis and setting, except during the cementation process. Since
the settlement period allowed for Pacific oysters is about 2 days, it is possible that the larvae can depend on their lipid reserves during settlement without feeding (Elston, 1991). In the case of some tropical oyster larvae, however, since the observed larval setting period is about 4 days, it is likely that the synchrony and rate of settlement during this duration will differ. There is need to determine appropriate species of microalgae and densities suitable for feeding. Thus, this study aimed to determine if the setting rate during this period can be maximised by feeding.

2. Materials and methods

2.1. Combined effects of salinity and temperature on oyster larvae setting

Oyster broodstock (C. iredalei) were procured from a nearby oyster farm at Batu Lintang, Kedah in Peninsular Malaysia. The spawning and larval production were carried out in the hatchery of the Fisheries Research Institute (FRI), Penang. Eyed larvae > 250 μm in length, were used for the experiment.

The experimental procedure was based on the methods used by Henderson (1983) and Tan (1993). Six levels of salinities (5, 10, 15, 20, 25 and 30%) were used at five temperatures (24, 27, 30, 33, and 36°C). The range of temperatures chosen was based on the water temperature monitored from the oyster setting tanks in the hatchery at the FRI. Between 1995 and 1996, the minimum and maximum water temperatures recorded were 25.5°C and 35°C, respectively. The salinities were obtained by diluting 1 μm cartridge filtered and ultraviolet treated seawater from the hatchery with distilled water. The experiment was conducted with four replicates of each treatment, with 1-l beakers containing the respective salinity levels placed in temperature controlled (±0.5°C) water baths. One water bath was used for each temperature. The beakers were lined with colourless sheet of high density polyethylene (HDPE) of 1 mm thickness to avoid larvae setting on the beaker sides. A white tile measuring 5 cm² was placed at the bottom of each beaker to serve as a substrate for the larvae to set upon. Every beaker was stocked with 1000 oyster larvae, i.e., an initial density of 1 larvae ml⁻¹. Aeration (slight bubbling) was provided by means of an adjustable portable aerator. A complete water change was made on the second day, and the respective salinity and temperature levels were maintained.

Mixed algae, Chaetoceros calcitrans and Isochrysis galbana (50:50 ratio), were fed at a rate of 70 × 10³ cells ml⁻¹ (feeding density maintained during the larval rearing period).

Larval settlement rate (%) was assessed based on the number of larvae set on the tile and the plastic sheet. The tile and plastic sheet were rinsed very gently in a bucket of filtered seawater to remove any unset or dead larvae. Unset larvae were considered not competent after the 96-h period.

2.2. Effect of feed type and density on oyster larvae setting

The experimental design was based on the method described by Henderson (1983). The two most commonly cultured microalgae for mollusc culture at the FRI hatchery are
I. galbana and Ch. calcitrans. Setting rates of oyster larvae were determined using individual species and mixed algae (50:50 ratio) as feed. Six densities of microalgae were used: 25, 50, 75, 100 and $125 \times 10^3$ cells ml$^{-1}$ and a control (without feed) for individual and mixed species. The algal stock solution was first diluted with distilled water to lower the salinity to 20% before determining the algal density with a Coulter Counter Model Z1. The various densities were then calculated based on the initial algal count in the stock.

The experiment was carried out in 1-l beakers (six replicates for each density) lined with high density polyethylene sheet of 1 mm thickness at room temperature ($30 \pm 1\, ^{\circ}C$). A white tile (substrate) was placed at the bottom of the beaker as in the earlier experiment. Initial larvae density in each beaker was 1 larvae ml$^{-1}$. The experiment was carried out for 4 days to allow larval settlement and a total water change was made after 48 h. A volume of water was removed daily from each beaker. This was equal to the volume of algal solution to be added. The volume of algae to be added daily to each beaker was based on the algal counts determined each day. Care was taken not to siphon out larvae while reducing the water volume of the beaker by fitting the siphon tube with a small nylon filter. Larval settlement rate (%) was determined as in the previous experiment.

2.3. Statistical analyses

A two-factor ANOVA was used to determine if interactions occurred between salinity and temperature effects, and between algal species and density effects. Larval sets from different treatments were assessed by One-way ANOVA, while comparisons of their means were conducted using the Tukey’s honesty significant differences (HSD) method. The Statistix 4.0 Program (Analytical Software, 1992) was used for analysis. All

![Graph](image-url)

Fig. 1. Response–surface estimation (at 5% intervals) of mean percentage settlement of larvae of C. iredalei for different temperature and salinity combinations.
percentage values were transformed to arcsine values prior to analysis to normalise the data, based on Snedecor and Cochran (1989).

3. Results

3.1. Combined effects of salinity and temperature on larval setting

*C. iredalei* larvae were found to tolerate wide ranges of salinity and temperature within the limits selected for the experiment. Larval settlement was observed at all levels of salinity and temperature. Some larvae were still swimming or crawling, even after 96 h, while others were dead. At salinities below 15%, however, the immediate response of most of the larvae was to settle at the bottom of the beaker before resuming swimming or crawling. Fig. 1 shows the rate of larval settlement in relation to temperature and salinity in the form of response–surface curves. Response–surface estimation was plotted by graphically transforming the standard deviation about the percentage mean settlement for each interaction of the variables shown in Table 1. At each point of variable interaction, a corresponding percentage response curve was connected to the positive standard deviation of the means at 5% contour intervals. The response–surface...
Table 3
Mean percentage (%) settlement of larvae of *C. iredalei* obtained with different diets of microalgae and densities at room temperature (30 ± 1°C). Data for each treatment are means (±S.D.). The means and standard error (±S.E.) of sample means are shown for all factor levels
Note: Within columns, means with a common subscript do not differ significantly (Tukey’s HSD, *P* > 0.05).
Within rows, means with a common superscript do not differ significantly (Tukey’s HSD, *P* > 0.05).

<table>
<thead>
<tr>
<th>Microalgaes</th>
<th>Density (10^3 cells ml^-1)</th>
<th>0</th>
<th>25</th>
<th>50</th>
<th>75</th>
<th>100</th>
<th>125</th>
<th>Combined data of all densities for each diet (±S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>I. galbana</em></td>
<td>0.9± (± 0.3)</td>
<td>4.1± (± 1.5)</td>
<td>8.0± (± 2.3)</td>
<td>15.2± (± 2.4)</td>
<td>25.8± (± 5.1)</td>
<td>17.0± (± 1.8)</td>
<td>10.0 (± 3.8)</td>
<td></td>
</tr>
<tr>
<td><em>Ch. calcitrans</em></td>
<td>0.9± (± 0.3)</td>
<td>6.4± (± 1.7)</td>
<td>11.1± (± 2.3)</td>
<td>16.6± (± 3.9)</td>
<td>25.0± (± 4.1)</td>
<td>6.6± (± 0.8)</td>
<td>9.5 (± 3.5)</td>
<td></td>
</tr>
<tr>
<td>Mixed algae (50:50)</td>
<td>0.9± (± 0.3)</td>
<td>9.1± (± 1.6)</td>
<td>13.1± (± 2.6)</td>
<td>21.0± (± 1.8)</td>
<td>23.1± (± 1.4)</td>
<td>9.5± (± 2.8)</td>
<td>11.3 (± 3.4)</td>
<td></td>
</tr>
<tr>
<td>Combined data of all species</td>
<td>0.9 (± 0.0)</td>
<td>6.3 (± 1.5)</td>
<td>10.5 (± 1.5)</td>
<td>17.4 (± 1.7)</td>
<td>24.5 (± 0.8)</td>
<td>10.5 (± 3.1)</td>
<td></td>
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</tr>
</tbody>
</table>
estimation used to investigate temperature–salinity interactions here should be consid-
ered to provide estimations only and not precise set rates. From the contour, settlement
rates of 15% and above are expected between salinities of 13–28‰ and temperatures
above 24°C to below 35°C. Less than 5% settlement is expected for salinities below 8‰
and above 30‰, and at temperatures somewhat less than 24°C and above 36°C.

The two-factor ANOVA (Table 2) showed that variations in the larval settlement rate
for salinity, temperature and their interaction were significantly different (P < 0.0001).
From the results, the best point of interaction for salinity and temperature was at 20‰
and 30°C, respectively, contributing the highest set rate (± S.D.) of 31.4 ± 3.4%. There
were average set rates of 14% to 24% for salinity and temperature between 15–25‰ and
27–33°C, respectively.

3.2. Effect of feed type and density on larval setting

The highest set rates of 23.1–25.8% were obtained for 100 × 10³ cells ml⁻¹,
irrespective of algal species (Table 3). Set rates at cell density of 125 × 10³ cells ml⁻¹
ranged from 6.6% to 17.0%, while at lower cell densities of 25 to 50 × 10³ cells ml⁻¹,
set rate was less than 13%. The set rate obtained for the unfed control was negligible
(0.9%). The best set rate, irrespective of algal density was obtained for mixed algae
(11.3%), although it was not significantly different (P > 0.05) from I. galbana (10.0%).
The two-factor ANOVA (Table 4) showed that the variations in larval settlement rate
between algal species, density and their interactions were significantly different (P <
0.05). The use of Ch. calcitrans resulted in lower set rate (9.5%), but it was not
significantly different (P > 0.05) from the settlement rate of larvae fed with I. galbana.

4. Discussion

This study has shown that salinity and temperature are important factors for the
setting of larvae of the tropical oyster, C. iredalei. The setting rate was found to be
highest for salinity–temperature interaction of 20‰ and 30°C, while lower setting rates
of 14–24% were obtained for salinities ranging from 15‰ to 25‰ and temperatures
between 27°C and 33°C. Very limited work has been done on this species for the
purpose of comparison except for an observation by Tan (1993) where setting was termed high (rate not mentioned) for salinities ranging from 18% to 24% at “room” temperature. The results of the present study more or less conformed to this observation. Another associated study by Tan (1993) on a related tropical species, *C. belcheri*, also showed that optimum sets of 35.8% and 38.6% (not significantly different) were obtained at salinities of 12% and 18%, respectively, at “room” temperature (level not given). Preliminary studies on these parameters in Thailand showed difference in optimal temperature condition for setting *C. belcheri* larvae: between 26°C and 28°C and salinity between 32% and 34% (Sahavacharin, 1987). However, subsequent studies have indicated that the same species could be induced to set by reducing salinity level from 32% to 15% (Sahavacharin et al., 1988).

Lund (1971) showed that maximum settlement of larvae of the Pacific oyster, *C. gigas*, occurred at a temperature of 30°C within a salinity range of 22–34%. A study by Henderson (1983) on *C. gigas* indicated that maximum set of 35–40% could be attained at a temperature of 30°C and salinity of 30%. Although high salinity is required for the larval settlement of Pacific oysters, the above-mentioned tropical oysters tend to withstand euryhaline conditions. However, both the Pacific and tropical oyster larvae require relatively higher temperature (30°C) for optimum setting.

The success of routine production of oyster larvae in the hatchery is basically dependent on the ability to manipulate and control the culture environment. One of the areas modified in relation to the biological conditions of the rearing site is conditioning of broodstock, which is expected to result in a viable larval production for culture in the hatchery (Lipovsky, 1984). Research has shown that the environmental factors including food, affecting the parent oysters are most important to the subsequent larval populations produced and their success of setting (Creekman, 1977). This probably explains the higher setting rate (14.3–31.4%) observed at the salinity range of 15–25% and temperature range of 27–33°C, because broodstocks were obtained from an environment (at Batu Lintang, Kedah) with similar salinity and temperature regimes. Upon collection, the broodstock was spawned at salinity of 28–30% and at ambient temperature (30 ± 2°C), while larval rearing was at 20% at the same temperature condition.

The experiment on feed has shown that oyster larvae, when metamorphosing over a number of days, certainly need food (irrespective of algal species), as observed from the results where settlement in unfed beakers was negligible compared to those being fed. However, algal density played a significant role in influencing settlement rate, as has been observed by Lund (1971). Optimum settlement was observed at a density of $100 \times 10^3$ cells ml$^{-1}$ irrespective of feed species. Research on a temperate species indicated that this algal density supported good set rates. Lund (1971), who worked with *C. gigas* (1500 larvae ml$^{-1}$) found that using *I. galbana* at $100 \times 10^3$ cells ml$^{-1}$ resulted in a higher set rate of 7.8%. Subsequent study on *C. gigas* by Henderson (1983) apparently showed that there were no significant influence of the algal densities (25, 50, 75, and $100 \times 10^3$ cells ml$^{-1}$ and a control without feed) used on the set rate. However, setting was seen to deteriorate at $100 \times 10^3$ cells ml$^{-1}$. Unfed larvae could have set equally as well as the fed larvae due to the short duration allowed for setting (48 h), whereby the larvae might have depended on the lipid reserves for further development (Gallager and Mann, 1981).
From this study, it was observed that larval set increased as food density was raised to $100 \times 10^3$ cells ml$^{-1}$ but decreased as cell densities were increased further. High algal densities have been shown to result in the formation of biofilm layers that stimulate larval settlement (Tritar et al., 1992; Parsons et al., 1993). However, the effect can be detrimental at very dense algal densities where fouling debris is abundant (Henderson, 1983). Schulte (1975) termed this situation as the critical cell density, whereby the larval filtration rate is hindered if food concentrations exceed this level. This probably explains why set rates started to deteriorate at cell concentrations of $125 \times 10^3$ cells ml$^{-1}$.

References


