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Effects of storage temperature and duration on the setting and post-set spat survival of the tropical oyster, *Crassostrea iredalei* (Faustino)

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Abstract

The effects of six storage temperatures (5°C, 10°C, 15°C, 20°C, 25°C and room temperature) and six storage durations (6, 12, 24, 48, 72 and 96 h) on pre-settlement larvae of *Crassostrea iredalei* indicated that settlement rate deteriorated with time for all temperatures. The highest settlement rate (40.1%) was attained at a storage temperature of 20°C for 6 h. This was followed by 10°C and 15°C for 6 h, with mean percent sets of 35.4% and 33.5%, respectively. An above-average set of 29.5% was obtained for the control larvae (larvae directly from the rearing tanks) compared to larvae stored between 10°C and 20°C for 12 to 24 h (21.1–28.2%). Average sets obtained for storage between 10°C and 20°C was 16.6–19.7% for up to 48 h, and sets for room temperature (ca. 30°C) and 5°C for 12 h were 11.9% and 16.9%, respectively; whereas at 25°C the set rate was 10.7% for 6 h. Storage at all other levels of temperature and duration resulted in poor set rates of less than 8%. All successfully set larvae from this experiment were further kept in the hatchery for three weeks to observe their short-term post-settlement survival. Survival rates were closely related to the setting rates, whereby higher sets contributed to better survival rates. The highest survival rates, 61.3–84.8%, were recorded for larvae set at temperatures ranging from 10°C to 20°C with a storage time of up to 48 h. These levels were comparable to the control (68.0%) and 5°C for up to 12 h (68.9%). Storage at 72 h resulted in total mortality at all temperatures, except for those stored at of 10°C (51.5% survival) and 20°C (14.7%).

Keywords: Storage; *Crassostrea iredalei*; Setting; Survival; Oyster; Spat

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1. Introduction

Most marine bivalve larvae are capable of delaying metamorphosis until a suitable environment or substrate is encountered (e.g. Loosanoff and Davis, 1963; Galtsoff, 1964; Bayne, 1965). This phenomenon of delayed metamorphosis is useful for 'remote setting', which involves the storage and transportation of competent pediveligers (eyed-larvae) from hatchery to the setting areas (Holiday et al., 1991). Remote setting techniques using larvae of the Pacific oyster (*Crassostrea gigas*) are now widely practised in North America, whereby stored eyed-larvae are routinely sent to distant areas which may involve a few hours or days. Commercial oyster larval setting rates obtained from this technique may range between 20% and 80% (Henderson, 1983).

In temperate countries, larval storage for the purpose of remote setting involves a process whereby they are removed from water and stored at low temperatures (usually 5°C). They are kept in moist conditions by wrapping the larvae in a nylon cloth and wet towels and the bundles are placed in an ice chest along with blue ice packs (Jones and Jones, 1983). Several studies on temperate oyster species (*Saccostrea commercialis* and *C. gigas*) have been conducted to determine the cold storage effects on larval setting rates (Carlson, 1981; Holiday et al., 1991). Despite the cold storage conditions, temperate oyster species have been found to retain their metamorphic competence without seriously affecting their ability to later function as normal, healthy bivalve spat (Henderson, 1983).

In Malaysia, coastal inhabitants traditionally consume oysters collected from the wild. With some technical and financial input from the Bay of Bengal Program, the Fisheries Research Institute (FRI), Malaysia, identified several species of oysters, such as the slipper oyster (*C. iredalei*), the mangrove oyster (*C. belcheri*) and the rock oyster (*Saccostrea* species) as of commercial value; and suitable sites for their culture and spat collection were also identified. Oyster culture in Malaysia is a recent innovation and has shown some indication of potential. To date, some 192 small-scale fisherfolk are engaged in the culture of *C. iredalei*, involving an area of 101,416 m^2 (Anonymous, 1996). This brackishwater species is noted for its tasty creamy flesh (Devakie et al., unpubl. data) and its culture requires a salinity range of 15–25 ppt. Although, the spat supply of this species is only confined to the east coast of Peninsular Malaysia, culture operations have been very successful on the west coast (which is more noted for *C. belcheri*) through spat transplantation programs. Culturists on the west coast are thus solely dependent on spat supply from the east coast, which takes more than 24 h by road. However, spat collection (peak seasons being April to June and October to December) on the east coast are hindered by monsoons which often result in total mortality of spat (salinity often reaching 0 ppt) during the November to January period. When the spat supply was disrupted for farms on the west coast, the culturists would then resort to importing oyster spat from southern Thailand. In view of this, the FRI and the Universiti Sains Malaysia embarked on hatchery propagation and remote setting of oysters, which showed encouraging results (e.g. Ng, 1993; Tan, 1993). However, no information on storage effects on tropical oyster larvae is available, except for a study on *C. belcheri* by Tan (1993). Although there are no private oyster hatcheries in

Malaysia as yet, studies on the storage effects of the tropical oyster larvae are important for handling the larvae if hatcheries were to be set up in future.

Another important aspect to consider is the subsequent survival of set larvae storaged at low temperatures. Larval mortality rates are said to be high immediately after settlement and gradually decrease after several weeks of culture (Williams, 1991). Unlike temperate oyster species which undergo a period of 'hardening' (acclimating the spat out of water for long periods), wild oyster juveniles (2–3 cm) in Malaysia are placed directly in culture areas until they attain a commercial size, ranging between 6 and 8 cm (Devakie, 1994). Techniques of oyster seed production are well established in Malaysia and there have been sporadic attempts to expose hatchery-produced seed to wild conditions, but survival rates have yet to be determined. This preliminary study investigates effects of temperature and storage duration of tropical oyster larvae and their subsequent survival rate under laboratory conditions. The study seeks to determine appropriate conditions for remote setting purposes.

2. Materials and methods

2.1. Larval setting experiments

Oyster (*C. iredalei*) broodstock were procured from a nearby oyster farm at Batu Lintang, Kedah. Spawning and larval production were carried out in the hatchery at the Fisheries Research Institute, Penang as described by Ng (1993). Eyed-larvae or pediveliger stage, which were retained on a 200 μm nitex seive, were used for the experiment.

The experimental procedure was based on the methods used by Henderson (1983) and Tan (1993). Oyster larvae (1000 larvae) from the same spawning were bundled up in nitex screens of 180 μm mesh-size and further wrapped with towels dipped in seawater to simulate moist conditions. Sixty bundles of oyster larvae were placed in each of six Styrofoam boxes, measuring $26 \times 19 \times 20$ cm. Each box was stored in an incubator (Shel-Lab Model 2020) at either 5°C, 10°C, 15°C, 20°C or 25°C and one box was at room temperature (30 ± 1°C). As a control, larvae were not exposed to storage, but instead were introduced into the experiment beakers immediately after removal from the rearing tanks. When temperatures in the incubators had stabilized, groups of larvae were removed after 6, 12 and 24 h and then every 24 h thereafter. They were placed in observation beakers for acclimatising, before removing them to 1-l Pyrex beakers for setting. The Pyrex beakers (1 000 larvae l^{-1}) were lined with high density polyethylene (HDPE) sheets of 1 mm thickness to avoid larval settlement on the sides. A white tile measuring 5×5 cm was placed on the bottom of each beaker to serve as a substrate for enhancing larval settlement. Larval sets were conducted in quadruplicate at room temperature for every level of storage temperature and duration. The duration of larval storage was extended until no further larval settlement was observed. Larvae were allowed 96 h for settlement since prior observations had indicated that larval settlement was staggered over a period of 4 days. Larval settlement counts were taken from both the white tile and the plastic lining to represent the total number set.

Salinity for the experiment was reduced to 22 ppt (a suitable level for this tropical oyster species) by mixing with freshwater, as has been routinely practised in the hatchery (Devakie et al., unpubl. data) and slight aeration was provided by means of a portable aerator. A mixture of *Isochrysis galbana* and *Chaetoceros calcitrans* (50:50) was provided daily at a rate of 70,000 cells ml^{-1}, a feeding density maintained during the larval rearing period as found suitable by Wong (1990) and Tan (1993). Water in the experiment beakers was changed after 48 h to remove any metabolites and excess plankton, and all unset larvae were rinsed slightly and returned to the respective beakers.

2.2. Post-settlement survival rate

After larval set counts were taken, the larvae together with the respective plastic sheets and tiles were transferred into 300-l troughs for short-term culture of 3 weeks to ascertain post-settlement survival rates. The same type and amount of feed was maintained for all cultures throughout the culture period.

2.3. Satistical analyses

A two factor ANOVA was used to determine if there was interaction between storage temperature and duration. Larval sets from different treatments were assessed by one-way ANOVA, while comparison 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 analyses. All percentage values were arcsine-transformed prior to analysis to normalise the data and reduce heteroscedasticity, based on Snedecor and Cochran (1989), while untransformed values are presented in the tables.

3. Results

Larval settlement occurred at all temperatures for up to 96 h storage, except for 25°C at which settlement occurred only until 72 h (Table 1). Set rates deteriorated with

Table 1
Mean percent (%) settlement (±s.d.) of *C. iredalei* larvae; rt: room temperature (30±1°C); C: control

Duration (h)	Temperature (°C)						
	5	10	15	20	25	rt	C
6	$21.9^{b}_{c}\pm1.9$	$35.4^{a}_{ab}\pm2.9$	$33.5^{a}_{ab}\pm3.9$	$40.1^{a}_{a}\pm5.2$	$10.7^{b}_{d}\pm1.0$	$30.2^{a}_{b}\pm1.6$	$29.5_{bc}\pm3.5$
12	$16.9^{c}_{b}\pm2.9$	$29.4^{ab}_{a}\pm1.8$	$27.2^{ab}_{a}\pm2.2$	$32.3^{b}_{a}\pm2.2$	$6.9^{bc}_{c}\pm0.7$	$11.9^{b}_{bc}\pm1.9$	$29.5_{a}\pm3.5$
24	$5.6^{d}_{c}\pm1.5$	$24.7^{ab}_{ab}\pm3.3$	$21.1^{b}_{b}\pm2.1$	$28.2_{a}\pm0.8$	$5.3^{c}_{c}\pm0.7$	$7.8^{bc}_{bc}\pm1.0$	$29.5_{a}\pm3.5$
48	$1.3^{de}_{c}\pm0.3$	$19.7^{b}_{b}\pm6.4$	$16.6^{b}_{b}\pm0.8$	$18.8^{c}_{b}\pm1.1$	$3.3^{cd}_{c}\pm0.5$	$4.2^{c}_{c}\pm0.8$	$29.5_{a}\pm3.5$
72	$0.6^{e}_{c}\pm0.2$	$5.3^{c}_{b}\pm0.8$	$1.5^{c}_{c}\pm0.5$	$1.9^{d}_{c}\pm0.4$	$1.3^{d}_{c}\pm0.4$	$1.5^{d}_{c}\pm0.4$	$29.5_{a}\pm3.5$
96	$0.6^{e}_{b}\pm0.1$	$0.6^{d}_{b}\pm0.3$	$0.7^{c}_{b}\pm0.3$	$0.4^{d}_{b}\pm0.1$	0	$0.2^{d}_{b}\pm0.1$	$29.5_{a}\pm3.5$
C	$29.5^{a}\pm3.5$	$29.5^{ab}\pm3.5$	$29.5^{a}\pm3.5$	$29.5^{b}\pm3.5$	$29.5^{a}\pm3.5$	$29.5^{a}\pm3.5$	

Within columns, means with a common superscript do not differ significantly (Tukey's HSD, $P > 0.05$).
Within rows, means with a common subscript do not differ significantly (Tukey's HSD, $P > 0.05$).
Treatment with zero values were excluded from statistical analyses.

Table 2
Analysis of variance of the effect of storage temperatures and durations on the settlement of oyster (*C. iredalei*) larvae, using arcsine transformed percentage data

Source	*df*	SS	*F*	P
Temperature (A)	5	3556.84	289.81	0.000
Duration (B)	5	14,128.0	1151.14	0.000
A × B	25	1511.15	24.63	0.000
Error	108	265.098		
Total	143	19,461.1		

prolonged storage duration because all larvae stored beyond 96 h died. Set rates were divided into four categories, where values above 30% were considered as good; 21% to 30% as above average; 10% to 20% as average and below 10% as poor. Good larval sets ranging from 30.2% to 40.1% were obtained for storage temperatures of 10–20°C and at room temperature for 6 h, and only at 20°C for up to 12 h. Above-average sets were obtained for the control, 20°C, 15°C and 10°C for up to 24 h, and at 5°C for 6 h. Average sets were obtained for 5°C and room temperature up to 12 h, while poor set rates of less than 10% were obtained for all other temperatures and durations. The two factor ANOVA (Table 2) showed that the variations in larval settlement rate between storage temperatures and duration and their interactions were significantly different ($P < 0.05$).

Generally, survival at post-settlement was directly related to the set rates obtained, where higher set rates contributed to higher survival (Table 3). Larvae stored at shorter duration gave better set rates. Highest survival rate (84.8%) was obtained for larvae kept at storage temperature of 20°C for up to 24 h. Survival rates for larvae stored at 5°C for up to 12 h and at 10–20°C for up to 48 h were comparable to the control. Survival rates of larvae stored at 25°C and room temperature for up to 48 h, ranged from 22.3% to 59.7%. Of larvae stored for 72 h, only those at 10° and 20° showed survival (51.5% and 14.7%, respectively). The two factor ANOVA (Table 4) showed that variations in

Table 3
Mean percent (%) survival of post-set spat (±s.d.) of *C. iredalei*; rt: room temperature (30±1°C); C: control

Duration (h)	Temperature (°C)						
	5	10	15	20	25	rt	C
6	$69.9^{a}_{a} \pm 7.9$	$73.4^{a}_{a} \pm 10.5$	$76.3^{a}_{a} \pm 5.6$	$77.6^{a}_{a} \pm 4.7$	$59.7^{ab}_{ab} \pm 16.1$	$45.3^{b}_{b} \pm 4.1$	$68.0_{a} \pm 3.6$
12	$68.9^{a}_{b} \pm 6.7$	$79.0^{a}_{a} \pm 5.2$	$75.1^{a}_{a} \pm 4.3$	$81.6^{a}_{a} \pm 4.7$	$39.4^{b}_{c} \pm 7.0$	$31.2^{c}_{c} \pm 4.0$	$68.0_{b} \pm 3.6$
24	$1.3^{b}_{d} \pm 1.0$	$61.3^{a}_{b} \pm 6.1$	$64.9^{a}_{b} \pm 3.8$	$84.8^{a}_{a} \pm 2.2$	$35.4^{b}_{c} \pm 9.2$	$26.4^{c}_{c} \pm 3.0$	$68.0_{b} \pm 3.6$
48	0	$66.4^{a}_{a} \pm 6.6$	$62.6^{a}_{a} \pm 9.6$	$63.6^{b}_{a} \pm 3.0$	$26.8^{c}_{b} \pm 9.0$	$22.3^{c}_{b} \pm 4.0$	$68.0_{a} \pm 3.6$
72	0	$51.5^{b}_{b} \pm 3.6_{b}$	0	$14.7^{c}_{c} \pm 2.8$	0	0	$68.0_{a} \pm 3.6$
96	0	0	0	0	0	0	
C	$68.0^{a} \pm 3.6$	$68.0^{a} \pm 3.6$	$68.0^{a} \pm 3.6$	$68.0^{b} \pm 3.6$	$68.0^{a} \pm 3.6$	$68.0^{a} \pm 3.6$	

Within columns, means with a common superscript do not differ significantly ($P > 0.05$), $n = 4$.
Within rows, means with a common subscript do not differ significantly ($P > 0.05$), $n = 4$.
Treatment with zero values were excluded from statistical analyses.

Table 4
Analysis of variance of the effect of storage temperatures and durations on the short-term survival rate of settled oyster (*C. iredalei*) larvae in the hatchery, using arcsine transformed percentage data

Source	*df*	SS	*F*	P
Temperature (A)	5	15,969.7	246.88	0.000
Duration (B)	5	60,359.4	933.10	0.000
A×B	25	14,872.9	45.98	0.000
Error	108	1397.25		
Total	143	92,599.2		

post-set survival rate between the storage temperatures and durations and their interactions were significantly different at ($P < 0.05$).

4. Discussion

This study provides basic information on the storage temperature, duration and their combined effects on setting rates of larvae of the tropical oyster, *C. iredalei*. In general, larval settings deteriorated with increasing storage duration at all temperatures. Despite this negative effect of prolonged storage on larval setting, cold storage of larvae enhanced the setting rate as compared to larvae removed from the rearing tanks and immediately allowed to set (the control). Larvae stored at 20°C for 6 h resulted in the highest set rate of 40.1% followed by those stored at 10°C and 15°C with a set rate of 35.4% and 33.5%, respectively, for the same duration. Results from this study differ from those of Tan (1993) for another species of tropical oyster, *C. belcheri*, whereby the highest setting rate reported was at 15°C (21.0–25.5%) for up to 72 h and 20°C (15.5–23.1%) for up to 48 h. In this study of *C. iredalei*, set rate at 15°C for 72 h was 1.5%, while at 20°C for 48 h it was 18.8%.

The requirements of tropical oyster larvae were found to differ from those of temperate oyster species, both in terms of optimal storage temperature and duration. Pacific oyster larvae stored at 5°C exhibited no significant differences in set rates with storage duration. An increase in mean larval settlement of 38.9 to 56.1% was sustained from day 2 until day 8 (Henderson, 1983). Larvae of *C. gigas* stored in nylon wrappings at 5°C for 5–8 days were also found to increase setting rates (Carlson, 1981). For *S. commercialis* larvae, best set rates in the range of 77–85% were obtained at 11°C for up to 98 h storage, with *C. gigas* larval sets (68%) were also unaffected at a storage temperature of 6°C for up to 98 h (Holiday et al., 1991). Further evidence of the tolerance of cold storage of temperate oyster larvae prior to setting was also observed for *C. gigas* larvae dispatched from America to Malaysia (maintained at 5–7°C for up to 7 days), which resulted in a setting rate exceeding 90% (Tan, 1993).

As for the short-term survival rate of oyster spat, observations showed that survival was closely related to setting rates in that, higher setting rates resulted in higher survival. At storage temperatures of 10–20°C for 6–48 h, survival ranged from 61.3% to 84.8% and did not vary significantly ($P > 0.05$) from the control (67.9%). However, it should

also be stressed that for certain temperatures and durations, the survival rate given is only relative, as considerable numbers of spat had fallen off the plastic substrates to the bottom of the long troughs and could not be differentiated by their respective substrates. In this case, counts were only taken from the plastic and tiles where the spat were still intact. Larvae that had set at any temperature after a 72-h storage period resulted in total mortality during post-settlement period, except for those at 10°C and 20°C. These results are comparable to those of Tan (1993) in which the mean survival rate post-settlement obtained for *C. belcheri* at a storage temperature of 15°C for up to 72 h ranged from 32.5% to 87%.

5. Conclusion

Our results for *C. iredalei* supported findings by Tan (1993) for *C. belcheri* which showed that tropical oyster larvae can be safely stored at lower than normal temperatures in the range of 10–20°C for about 48 to 72 h without any detrimental effects on their settlement and post-settlement survival rates. Although a small number of larvae (1000 larvae per replicate) was used in this experiment, the results obtained constitute a guideline for commercial applications, which handle millions of larvae. This duration should allow larvae to be transported to any part of Malaysia or to the neighbouring countries in Asia by air, taking into consideration the long transit period in some cases. Very low temperatures, such as 5°C, do not enhance settlement in tropical oyster larvae as compared to the temperate species. Thus, storage at 5°C, 25°C and 30°C for less than 12 h can only be employed for settings within the hatchery or at nearby culture sites in Malaysia.

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