

The Effect of Substrate Conditioning on Larval Settlement and Spat Growth of the Cupped Oyster, *Crassostrea belcheri* (Sowerby), in a Hatchery

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ABSTRACT

Substrate conditioning is a key factor for the settlement and metamorphosis of oyster larvae. The effects of substrate conditioning on larval settlement and growth of spat of the cupped oyster, *Crassostrea belcheri*, were evaluated in three trials in a closed recirculation system. Particles prepared by grinding clean oyster shell were used as a substrate for larval settlement. In each trial, substrates were conditioned by immersion in seawater for 2, 12 and 24 h before use. The number of larvae that settled on substrates conditioned for 2 h was significantly higher ($P < 0.05$) than the number that settled on substrates conditioned for 12 and 24 h in all trials. Mean setting rates ranged from 30.44 ± 9.71 to 61.71 ± 11.28 , 7.61 ± 3.51 to 35.77 ± 1.67 and 6.21 ± 1.38 to 32.77 ± 1.57 on substrates conditioned for 2, 12 and 24 h, respectively. Differences in the increments of shell width and length among oyster spat nursed over 3 wk on substrate conditions for different lengths of time were nonsignificant ($P > 0.05$).

Keywords: *Crassostrea belcheri*, substrate conditioning, settlement, closed water recirculation system

INTRODUCTION

Cupped oyster, *Crassostrea belcheri* (Sowerby), is one of the most commercial bivalves in Thailand, and many studies have been done on its biology and culture over many years (Department of Fisheries, 1994). The spats for grow-out farms are mainly collected from natural sources, but the amount of oyster seed produced from those sources is limited and insufficient. Oyster seed production from hatcheries has been continuously developed and is the subject of great interest in Thailand. Difficulties in the culture of bivalve molluscs are mainly associated with settlement and metamorphosis. Efficient hatchery

culture of bivalve molluscs requires a simple, reliable and cost-effective means of collecting and deploying juveniles. Two important criteria for good cultch are that it be a suitable surface for larval settlement and be easy to handle. Suitable substratum must be provided for successful larval settlement and metamorphosis under hatchery conditions (Pawlik, 1992). Many studies have researched the use of collectors for setting bivalve larvae, including investigations of the substrate or collector material (Holliday *et al.*, 1993; Holliday, 1996; Zanette *et al.*, 2009), collector orientation and surface contour (Holliday, 1996; Baker, 1997) and the conditioning of collectors (Fitt *et al.*, 1990; Tritar *et al.*, 1992). The preferred settlement

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surfaces are horizontal, rough-textured substrates, covered with a microbial film, and already colonized by adults of the same species (Beiras and Widdows, 1995). However, the effect of substrate conditioning on settlement and growth appears to be species specific (Gosling, 2003). No studies have been conducted on how substrate conditioning affects the larval settlement and growth of big oyster, *C. belcheri*. The objective of the current experiment was to determine ways of maximizing larval settlement and growth of spat by conditioning the substrate. The best conditioning times found could be used to achieve higher settlement rates in *C. belcheri* larvae under hatchery conditions.

MATERIALS AND METHODS

Experimental system

A closed recirculation system was designed to determine the effect of substrate conditioning on larval settlement and spat growth of big oyster, *Crassostrea belcheri*. The system consisted of a submersible pump, a 1,500 L fiberglass tank (dimensions 1.10 × 2.20 × 0.62 m) used to hold setting units, a 105 L fiberglass tank (dimensions 50 × 70 × 30 cm) used for food storage, and 9 sets of fiberglass tanks (diameter 35 cm × high 30 cm) used as setting units. A screen with a mesh size of 180 µm was fixed by a fiberglass clamp to the bottom of each fiberglass tank.

During the experiment, the water from the food storage tank was pumped into the large tank where the setting units were placed. The water was injected so that it down-welled into each setting unit and then drained through the overflow pipe before being returned to the storage tank. The rate of water flow in each setting unit was adjusted by the valve on the inflow pipe to 0.5 L per min. Water salinity was maintained at 30 ppt over the study period.

Experimental oyster larvae

Mature *Crassostrea belcheri* oysters were collected from Kantang district, Trang province. The collected oysters were cleaned and acclimated in sand-filtered seawater (30 ppt) for two days in the hatchery at Marine Shellfish Breeding Research Unit, Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang campus, Trang province, Southern Thailand. Broodstocks were conditioned in 1,000 L fiberglass tanks with an algal mixture containing 6% of both *Chaetoceros calcitrans* and *Tetraselmis suecica* per mg oyster (dry algal weight divided by dry meat weight). Two weeks later, gametes were obtained from sacrificed oysters and the eggs fertilized using one male for four females. The fertilized eggs were stocked for further development in 500 L culture tanks at a density of 15 embryos per mL in filtered (1 µm) and UV-treated seawater. The embryos developed to D larvae within 2 d and then were reduced in density to five larvae per mL. Seawater renewal and tank cleaning were conducted every 2 d, along with the addition of antibiotics (Streptomycin and Neomycin). At each draining, larvae were sieved for grading size. D larvae were fed daily with *Isochrysis galbana* at a density of 20,000 cells per mL. When the size of larvae was greater than 100 µm, a mixed diet of *I. galbana* and *C. calcitrans* was fed daily and the quantity of algae was increased to 50,000 cells per mL (Charlermwath, 2001). After about 18 d, the pediveliger stage was reached when metamorphosis occurs (competent larvae and the presence of eyespots). Pediveligers ready to set were graded with a 250 µm sieve mesh. Pediveligers retained on the 250 µm nitex sieve were then transferred to a 10 L plastic bucket and counted prior to use in experiments. Each setting unit contained about 40,000 larvae. Three trials of the experiment were performed with a completely randomized design including each treatment in triplicate.

Substrate preparation

The particulate material used as a substrate for oyster setting was made from clean oyster shell, which had been sun dried and broken into particles using a stone mortar and pestle. The particles were graded so that only those that passed through a 500 μm screen but were retained on a 250 μm screen were used for oyster setting in the experiments. The particulate material was spread over the screen in the setting unit. Substrates were conditioned by immersion in clean seawater for 2, 12 and 24 h before use.

Settlement and growth assays

Two days after being allowed to set, the number of spat on substrate at different conditioning times was counted using a binocular dissecting microscope. The number of unattached or attached spat was expressed as a percentage of settlement ($100 \times \text{total number of larvae settled} / \text{total number of larvae}$). After assaying the settlement, the experimental system and setting units containing substrate and oyster spat were cleaned with sea water. The spat were then allowed to grow for 3 wk to determine the effect of different conditioning times on growth. During this part of the study, the direction of water flow was changed to up-well through the setting units, and the water flow rate in each setting unit was increased to 4 L per min. using the adjustable valve. The water was totally renewed every 2 d and food was added twice a day (morning and evening) at a rate of 25,000 cells per mL of *Chaetoceros calcitrans* and 25,000 cells per mL of *Tetraselmis suecica*. Twenty

samples of oyster spat from each replicate were taken weekly for measurements of shell width (dorso-ventral measurement) and shell length (antero-posterior measurement).

Statistical analysis

Data were analyzed statistically using analysis of variance (one-way ANOVA) to determine differences among conditioning times. If significant effects were present, then data were subjected to Duncan's multiple range test to analyze differences among treatment means. Statistical significance was tested at the 0.05 level.

RESULTS

Larvae settlement

Settlement rates obtained under different substrate conditioning times are shown in Table 1. Mean percentage settlement differed significantly among treatments with the highest occurring on substrates conditioned for 2 h and decreasing with increasing time of conditioning. From the three experimental trials, mean percentage set ranged between 30.44 ± 9.71 and 61.71 ± 11.28 , 7.61 ± 3.51 and 35.77 ± 1.67 and between 6.21 ± 1.38 and 32.77 ± 1.57 for conditioning times of 2, 12 and 24 h, respectively.

Spat growth

After setting, hatchery-reared spat oysters were nursed in a closed recirculation system for 3 wk. The initial mean values of shell width and length were $576.82 \pm 50.68 \mu\text{m}$ and

Table 1 Percentage of *Crassostrea belcheri* larvae which set on substrates conditioned for different lengths of time.

Experimental trial	Settlement rate (%)		
	2 h	12 h	24 h
1	30.44 ± 9.71^a	7.61 ± 3.51^b	6.21 ± 1.38^b
2	43.95 ± 3.09^a	35.77 ± 1.67^b	32.77 ± 1.57^c
3	61.71 ± 11.28^a	32.77 ± 3.55^b	25.00 ± 4.37^b

Row data labeled with different letters represent means that were significantly different ($P < 0.05$) within a trial.

$582.66 \pm 44.08 \mu\text{m}$, respectively. After nursing for 3 wk, the mean shell width and length were $1,699.12 \pm 142.75 \mu\text{m}$ and $1,525.93 \pm 107.17 \mu\text{m}$, respectively (Figures 1 and 2). No significant difference was found in the daily increment of mean shell width and length among the treatments in all trials (Figures 3 and 4).

DISCUSSION

The larvae of many marine bivalves preferentially settle and metamorphose in habitats well suited to their subsequent adult life. A wide

variety of environmental factors, particularly the nature of the substratum and the presence of some dissolved compounds, both biotic and chemical, have been found to be capable of inducing settlement and metamorphosis of larva of different species of marine bivalves (García-Lavandeira *et al.*, 2005). Many studies have shown that biofilms play a very important role in the larval settlement processes of many marine bivalves (Weiner *et al.*, 1989; Tamburri *et al.*, 1992; Pearce and Bourget, 1996; Zhao *et al.*, 2003; Peteiro *et al.*, 2007; Su *et al.*, 2007). For instance, Taylor *et al.* (1998) and Zhao *et al.* (2003) reported that the percentage of

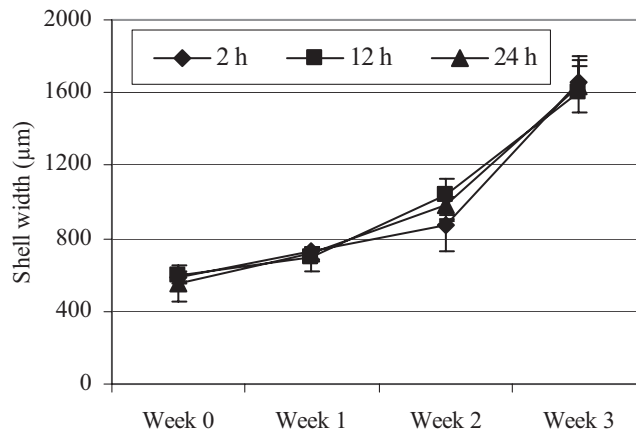


Figure 1 Shell width (mean \pm SD) of hatchery-reared spats on substrates conditioned for different lengths of time.

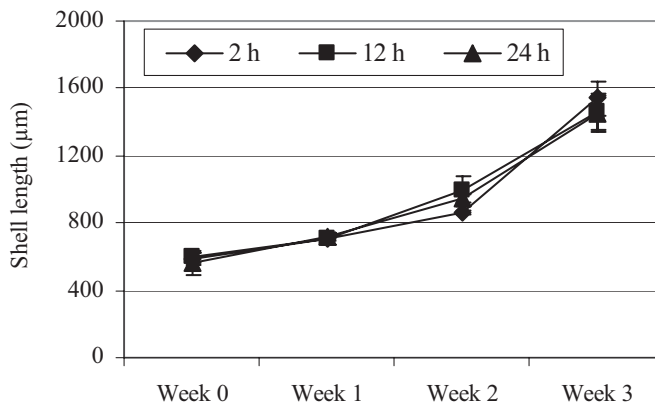


Figure 2 Shell length (mean \pm SD) of hatchery-reared spats on substrates conditioned for different lengths of time.

Pinctada maxima larvae settling on pre-conditioned collectors with biofilms was significantly higher than on collectors without biofilms. However, the presence of a biofilm layer may not have provided important chemical cues for larval settlement in the current study because the highest larval settlement occurred on substrates conditioned for the least time of 2 h. That result may have been caused by a chemical cue from dissolved substances rather than the natural biofilm. The calcium content may have provided a chemical cue causing high settlement rates because the substrate used in the current study was newly prepared from clean oyster shell. Therefore, the concentration of calcium compounds such as

Ca(OH)_2 (calcium hydroxide) and CaCO_3 (calcium carbonate) would be higher in the setting units when the substrate was conditioned for only 2 h, rather than 12 or 24 h. The investigation by Anderson and Underwood (1994) showed that calcium hydroxide had a positive effect on the settlement of Sydney rock oysters, *Saccostrea commercialis*. Zhao *et al.* (2003) reported that excess calcium at 10 and 50 mM in seawater induced larval settlement of the silver-lip or gold-lip pearl oyster, *P. maxima*. An excess of Ca^{2+} is responsible for changing the ionic potential across the larval cell membrane, triggering a cascade of neural activity in the larvae, resulting in metamorphosis (Anderson, 1996). Substrate

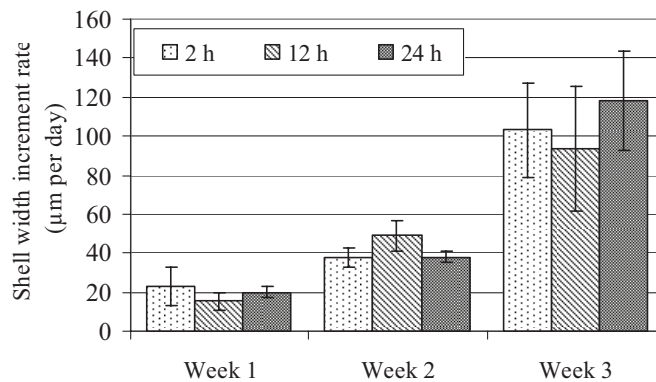


Figure 3 Daily shell width increment (vertical bars show the mean \pm SD) of hatchery-reared spats on substrates conditioned for different lengths of time.

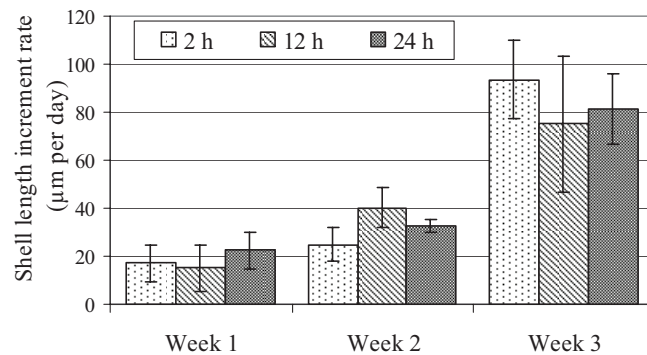


Figure 4 Daily shell length increment (vertical bars show the mean \pm SD) of hatchery-reared spats on substrates conditioned for different lengths of time.

conditioning in the current study was shown not to influence growth of oysters in the post-setting stage. O'Foighil *et al.* (1990) stated that bacteria and epifloral films on conditioned substrates were less important than suspended microalgae for early juvenile growth. They found that the Japanese scallop, *Patinopecten yessoensis*, preferentially settled on collector material conditioned with an epifloral film of diatoms, but there was no evidence that the presence of an epifloral film enhanced subsequent spat growth.

ACKNOWLEDGEMENTS

The author thanks Ms. Supatcha Dungjan and Ms. Kruewan Intasoo for help in collecting the samples, and also wishes to thank Mr. Barry Bendell for assistance in editing the manuscript. This study was funded by the Office of Higher Education Commission.

LITERATURE CITED

- Anderson, M.J. 1996. A chemical cue induces settlement of Sydney rock oysters, *Saccostrea commercialis*, in the laboratory and in the field. **Biol. Bull.** 190: 350–358.
- Anderson, M.J. and A.J. Underwood. 1994. Effects of substratum on the recruitment and development of an intertidal estuarine fouling assemblage. **J. Exp. Mar. Biol. Ecol.** 184: 217–236.
- Baker, P. 1997. Settlement site selection by oyster larvae, *Crassostrea virginica*: Evidence for geotaxis. **J. Shellfish Res.** 16: 125–128.
- Beiras, R. and J. Widdows. 1995. Induction of metamorphosis in larvae of the oyster *Crassostrea gigas* using neuroactive compounds. **Mar. Biol.** 123: 327–334.
- Chalermwath, C. 2001. **Mollusk Culture**. Rour-Keaw Publishing. Bangkok, Thailand. 253 pp.
- Department of Fisheries. 1994. **Oyster Culture**. Department of Fisheries. Ministry of Agriculture and Cooperative. Bangkok, Thailand. 49 pp.
- Fitt, W.K., S.L. Coon, M. Walch, R.R. Colwell and D.B. Bonar. 1990. Settlement behaviour of oyster larvae *Crassostrea gigas*, in response to bacterial supernatants. **Mar. Biol.** 106: 389–394.
- García-Lavandeira, M., A. Silva, M. Abad, A.J. Pazos, J.L. Sánchez and M.L. Pérez-Parallé. 2005. Effects of GABA and epinephrine on the settlement and metamorphosis of the larvae of four species of bivalve mollusks. **J. Exp. Mar. Biol. Ecol.** 316: 149–156.
- Gosling, E.M. 2003. **Bivalve Mollusks: Biology, Ecology and Culture**. Blackwell Publishing, USA. 443 pp.
- Holliday, J.E. 1996. Effects of surface orientation and slurry-coating on settlement of Sydney rock, *Saccostrea commercialis*, oysters on PVC slats in a hatchery. **Aqua. Eng.** 15: 159–168.
- Holliday, J.E., G.L. Allan, J. Frances and P. Diver. 1993. Evaluation of commercially-used collectors for Sydney rock oysters, *Saccostrea commercialis* and Pacific oysters *Crassostrea gigas*. **Aqua. Eng.** 12: 63–79.
- O'Foighil, D., B. Kingzett, G. O'Foighil and N. Bourne. 1990. Growth and survival of juvenile Japanese scallops *Patinopecten yessoensis*, in nursery culture. **J. Shellfish Res.** 9: 135–144.
- Pawlik, J.R. 1992. Chemical ecology of the settlement of benthic marine invertebrates. **Oceanogr. Mar. Biol. Ann. Rev.** 30: 273–335.
- Pearce, C.M. and E. Bourget. 1996. Settlement of larvae of the giant scallop, *Placopecten magellanicus* (Gmelin), on various artificial and natural substrata under hatchery-type conditions. **Aqua.** 141: 201–221.
- Peteiro, L.G., R. Filgueira, U. Labarta and M.J. Fernández-Reiriz. 2007. Effect of submerged time of collector ropes on the settlement capacity of *Mytilus galloprovincialis* L. **Aqua. Res.** 38: 1679–1681.

- Su, Z., L. Huang, Y. Yan and H. Li. 2007. The effect of different substrates on pearl oyster *Pinctada martensii* (Dunker) larvae settlement. **Aqua**. 271: 377–383.
- Tamburri, M.N., R.K. Zimmer-Faust and M.L. Tamplin. 1992. Natural sources and properties of chemical inducers mediating settlement of oyster larvae: A re-examination. **Biol. Bull.** 183: 327–338.
- Taylor, J.J., P.C. Southgate and R.A. Rose. 1998. Assessment of artificial substrates for collection of hatchery-reared silver-lip pearl oyster (*Pinctada maxima*, Jameson) spat. **Aqua**. 162: 219–230.
- Tritar, S., D. Prieur and R. Weiner. 1992. Effects of bacterial films on the settlement of the oysters, *Crassostrea gigas* (Thunberg, 1793) and *Ostrea edulis*, (Linnaeus, 1750) and the scallop *Pecten maximus* (Linnaeus, 1758). **J. Shellfish Res.** 11: 325–350.
- Weiner, R.M., M. Walch, M.P. Labare, D.B. Bonar and R.R. Cowell. 1989. Effects of biofilms on the marine bacterium *Alteromonas colwelliana* (LST) on set of the oysters *Crassostrea gigas* (Thunberg, 1793) and *C. virginica* (Gmelin, 1791). **J. Shellfish Res.** 8: 117–123.
- Zanette, G.B., J.F. Ferreira, F.C. Da Silva, A. Pereira and C.M.R. De Melo. 2009. Influence of the type of collector and collector preparation periods on the settling rate of the scallop *Nodipecten nodosus* L. in the laboratory. **Aqua. Res.** 40: 1451–1458.
- Zhao, B., S. Zhang and P. -Y. Qian. 2003. Larval settlement of the silver or gold-lip pearl oyster *Pinctada maxima* (Jameson) in response to natural biofilms and chemical cues. **Aqua**. 220: 883–901.