Evaluation of selected substrates for collection of hatchery reared black-lip pearl oyster (*Pinctada margaritifera*) spat

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Abstract

An experiment was conducted to investigate the effect of collector materials and position on *Pinctada margaritifera* spat attachment. Settlement on polyethylene pipes (54.6%) was significantly higher ($P < 0.05$) than on plastic baskets (25%). Significantly higher ($P < 0.05$) spat catch was recorded from the collectors which were installed on lower height of settlement tank than upper. The number of dead spat after settlement on pipe (1.4%) and basket (1.5%) were not significantly different ($P > 0.05$). Possible factors causing the pattern of settlement reveals that it could be related to the material used in collector, reaction to light and gravity force. These results indicated that polyethylene pipes positioned horizontally in the third bottom part of the tank are suitable for the settlement of *P. margaritifera* pediveliger larvae in a hatchery and may also be suitable for grow out in the ocean.

Keywords: *Pinctada margaritifera*, Settlement, Spat, Collector

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Introduction

Small spat can be obtained from hatcheries or through natural spatfall where a wide variety of substrate may be used (Quayle, 1988). The success of oyster aquaculture depends on a reliable spat source, making hatchery production an option for predictable spat supply. The production and larval rearing of pearl oyster spat in hatchery was recently established (Pit and Southgate, 2000). Earlier, the harvest of oyster spat in the Persian Gulf using spat collectors which were placed at depleted natural beds and reproductive season proved to be unsuccessful (Rameshi, 2006). Spat production through artificial propagation of oysters in order to recover the depleted natural beds has been successfully introduced (Alagarswami, 1970; Saucedo and Monteforte, 1997). In the past two decades the pearling industry has been relying more on hatchery-cultured spat (Gervis and Sims, 1992; Sims, 1993; Clarke et al., 1996; Southgate and Beer, 1997; Acosta-Salmon, 2004). However, the settlement of pediveliger larvae is highly influenced by specific physical and chemical factors of substrates (Michener and Kenny, 1991; Baker, 1997; Doroudi and Southgate, 2002; Faimali et al., 2004; Su et al., 2007; Yu et al., 2008). The percentage of metamorphosis and larval settlement along with economical and effective methods were considered to be very important and critical for aquaculture (Davis et al., 1990; Cann-Moisan et al., 2002). Minaur (1969) applied a variety of settlement substrates as spat collectors to crawling *Pinctada maxima* pediveligers including: glass, glass wool, byssus threads derived from juvenile *P. maxima*, string, nylon thread, bamboo, and bivalve shells. However, metamorphosis failed in all cases and no spat were produced. Consequently preference of type of collector was not reported. Alagarswami et al. (1983) used many kinds of materials (fibreglass, frosted and plain glass plates, glass jars, fibrillated synthetic ropes, split bamboo and coconut leaf stem) as spat collectors of *Pinctada fucata*. These authors observed that dense spat settled on the fiberglass tank bottom. However, all the collectors except glass jars showed satisfactory results in number of attached spat. Later, Taylor et al. (1998) showed that the combination of PVC slats and polypropylene ropes was suitable for the collection of *P. maxima* spat in the hatchery.

It has been shown that the position and orientation of collectors in the settlement tank could affect the settlement rate of spat of pearl oyster. Taylor et al. (1998) observed that horizontally oriented substrates are more effective than those vertically positioned. Alagarswami et al. (1983) reported that in horizontal suspensions, the upper surface of the plates had a slightly higher *P. fucata* spat density than the lower surface. Reactions of larvae to the light may justify the variation in larval settlement by changing the orientation and position of collectors. Michener and Kenny (1991) observed that oyster *Crassostrea virginica* (Gmelin) larvae preferred shaded than bright attachment sites. *Pinctada mazatlanica* (Hanley) larvae were observed to settle on dark and less reflective substrates

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(Saucedo et al., 2005). In a study conducted by Su et al., 2007, the settlement of the *P. martensi* larvae in the experiments with four different colour substrata was compared and showed that the deep colour plastic sheets (red and blue) attracted significantly more larvae than the light colour (green and yellow).

To our knowledge, work focusing on the settlement of *P. margaritifera* larvae in response to different materials has not been carried out. Therefore, the present study was carried out to investigate the efficiency of three types and two positions of collector materials on the settlement, growth and mortality of *P. margaritifera* spat.

Materials and method

This study was done in Bandar Lengeh Mollusk Research Center, in the north coast of the Persian Gulf. Black polyethylene pipes and plastic baskets were used as two substrates for larvae settlement. Polyethylene pipes were cut horizontally to make two equal parts and floated in the tank with their convex side down. Eight baskets and eight pipes were attached together alternately with rope and hung in two rows with equal distances from the surface and bottom of the tank (Figure 1). Baskets and pipes in upper height were named Basket Surface and Pipe Surface and those in lower height called Basket Deep and Pipe Deep, respectively. There were four baskets and four pipes in each height (Bo Zhang et al., 2010). Collectors were equally spaced to give the chance for spat to swim freely and settle on all collectors. All materials used for making the collectors were soaked in seawater prior to the experiment to leach out any potential toxins (Sieling, 1950). The prepared collectors were pressure cleaned with fresh water and sun dried before being placed in a 700 L fibreglass tank (Taylor et al., 1998). The tank was filled with 1µm filtered and ultra violet treated seawater before introducing the 25 days old pediveliger larvae at a density of 1 larva ml^{-1}. Spat were fed 2.5 x 10^4 cells per ml with ternary combination of microalgae using 50% of T. Iso and equal cell numbers of *Chaetoceros muelleri* and *Chaetoceros calcitrans* two times daily at 08.00 and 20.00 hrs. Water temperature and salinity were maintained at 29 ± 1°C and 35 ± 1g L^{-1}, respectively and exchanged 100% prior to morning feeding. Spat on each collector were counted 10 days after the introduction of collectors into the tank. Twenty days later, 55-day-old spat on all collectors were removed from the tank and their growth and post settlement mortality were calculated. Shell height, hinge length, and thickness of 60 randomly selected spat of each collector were measured as growth parameters.

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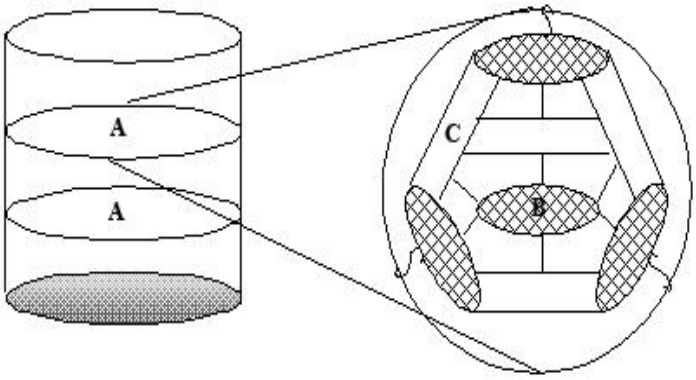

Figure 1: Schematic distribution of collectors in the settlement tank. (A) Location of baskets and pipes in the tank, (B) Baskets, and (C) Pipes

All statistical analyses were carried out using SPSS 15.0. Differences in values for biometry data of collected spat between baskets and pipes were compared using the t-test. One-way analyses of variance (ANOVA) and Tukey post-hoc comparisons were performed on mean values to determine the statistical differences ($P < 0.05$) among data sets of the number of spat on different position of collectors. Levene's Test for homogeneity was carried out to insure homogeneity assumptions are met.

Results

At the end of the experiment, 55 days old juvenile spat of *P. margaritifera* attached on the basket were found to be larger in shell height (4.55 ± 0.99 µm), hinge length (6.28 ± 1.30 µm), and thickness (0.99 ± 0.16 µm) as compared to spat attached to the pipe, however, the difference was not statistically ($P > 0.05$) significant (Figure 2). Mortality of spat on the baskets (1.5%) and pipes (1.4%) was not statistically different ($P > 0.05$). More than half of the spat (54.6%) settled on the pipe, while the baskets collected 25% of spat and 20.4% of the spat settled on the bottom or walls of the tank (Figure 6.4). Results showed that there was no significant ($P > 0.05$) difference in the number of attached spat between the different collectors on the upper row; however, it was significantly ($P < 0.05$) higher on pipes (3686 ± 159) compared to baskets (3305 ± 262) on the lower row. Collectors positioned in the lower row collected significantly greater numbers of spat compared to those installed in the upper row ($P < 0.05$).

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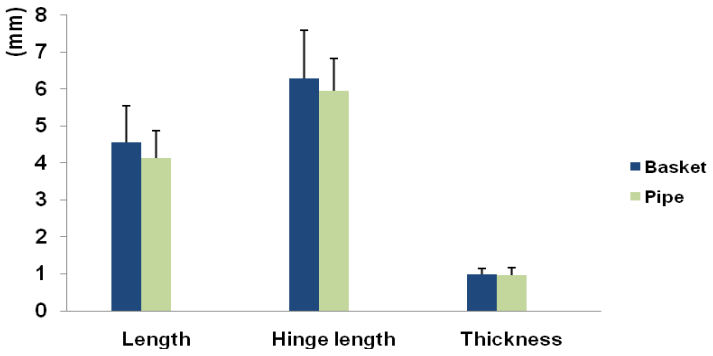

Figure 2: Shell height, hinge length and thickness (mean ± standard deviation, n=60 for each of 8 replicates) of 55 days old *Pinctada margaritifera* spat on two types of collectors. Means are not different (P < 0.05)

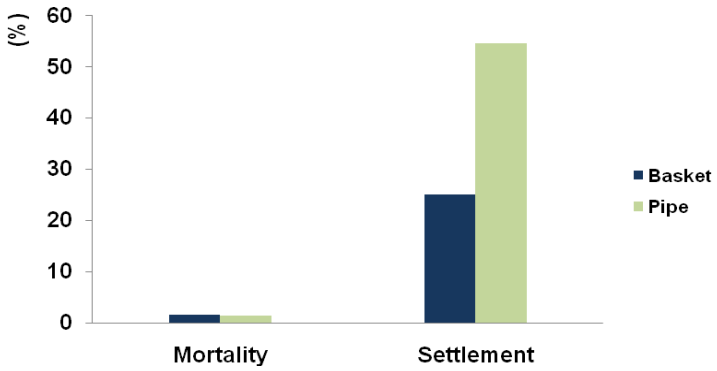

Figure 3: Performance of substrates for the settlement (%), and mortality (%) of *Pinctada margaritifera* spat. Settlement was calculated 10 days after introducing of pediveliger to the settlement tank and post settlement mortality, at the end of experiment

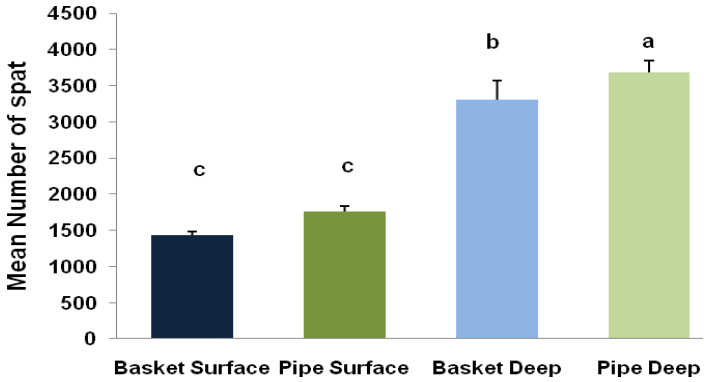

Figure 4: Mean number (± standard deviation, n=4 replicates) of *Pinctada margaritifera* spat on two types of collectors at two heights. Means with the same superscript are not significantly different (P < 0.05)

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Discussion

The use of appropriate substrates has been shown to promote improvement in the settlement of bivalve larvae both in the hatchery conditions and in the ocean (Alagarswami et al., 1983; Rose, 1994; Holliday, 1996; Taylor et al., 1998). It has been suggested that spat of pearl oyster should attain a certain size before being transferred to the ocean to minimize the stress and mortality rate (Dybdahl et al., 1990; Rose, 1990; Anon, 1991). The results of the present study showed that juvenile spat on the baskets are slightly larger than those of on polyethylene pipes. Perforated baskets may provide better access for the attached spat to floating microalgae while, spat on the polyethylene pipes were limited to smaller portions of microalgae.

Post settlement mortality of spat on collectors was not statistically different and it was negligible (below 2%). It is concluded that collectors used in this study provided good substrate for culture of spat after settlement. A great amount of spat died before settlement and accumulated at the bottom of the tank. Dead or less motile microalgae cells colonized in the bottom of the tank and deteriorated the condition of the water. Spat on the tank bottom may be subjected to more stress during water change. Polypropylene fabrics as artificial substrates to add living space of *Litopenaeus vannamei* have been successfully used (Zhang et al., 2010).

Polyethylene pipe collected greater number of *P. margaritifera* spat than plastic baskets. Taylor et al. (1998) reported similar results with PVC pipe. The large stable area of attachment offered by split pipe allows spat to place byssal threads in a radial pattern providing a stronger attachment (Taylor et al., 1997). This pattern of attachment may provide some advantages in grow out practices compared to monofilament nylon and rope which are commonly used to collect bivalve spat. Phelger and Cary (1983) reported monofilament nylon as a good collector for giant rock scallop, *Crassadoma gigantea*; however, as the spat grew the area for attachment reduced and chance for untangling increased in areas with strong currents and tides. The roughened surface of polyethylene pipes may provide better substrates for crawling pediveliger compared to smooth surfaces of plastic baskets.

The number of *P. margaritifera* spat on the collectors significantly varied with the height of the collector in the water column. Collectors in deeper parts of the tank collected greater number of spat than the shallow parts. The importance of position and orientation of collectors for the attachment of spat has been reported by many researchers (Thomson, 1950; Cranfield, 1973; Alagarswami et al., 1983; Holliday, 1996; Taylor et al., 1998). Results of this study were in accordance with the observations on *Crassostrea virginica*, the avoidance of light for the selection of settlement position (Ritchie and Menzel, 1969; Shaw and Stallworthy, 1970; Phelps, 1983). Alagarswami et al. (1987) also reported improved settlement of *P. fucata* spat in dark-colored tanks. Force of gravity could also be effective on a greater number of attached spat on lower levels of collectors. The velum of peiveliger larvae pushes to the anterior side and the animal moves with two

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mechanisms, swimming with the ciliary motion of velum and crawling with the foot. The velum disappears in the plantigrade stage before the animal start its sessile life (Alagarswami et al., 1987). It is thought that the plantigrade larvae would face difficulty to swim against the gravity without the ciliary motion of velum to reach the collectors. Very few spat attached to the undersurface of the substrates as compared to the upper surface, indicating the influence of gravity force. Taylor (1998) observed no significant differences in the number of attached spat of *P. maxima* on horizontally placed PVC slats in different heights. He has also ruled out strong effects of light and gravity on the rate of attachment of spat on substrates. Cranfield (1970) cited that turbulence caused by collector orientation and arrangement as a major factor in spatfall variation. He proposed that variation in experimental techniques was largely responsible for the conflicting reports on the settlement patterns of oyster larvae.

Based on the results of this study, texture and position of substrates are two very important conditions for increasing the number of attached *P. margaritifera* spat. Plastic baskets could be replaced with split roughened polyethylene pipes to collect *P. margaritifera* spat in the hatchery. Strong attachment of spat on pipes suggests that it could be used in the ocean.

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