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Substrate Selection for Larval Settlement and Spat Growth in the Purple Clam, *Saxidomus purpuratus* (Sowerby) in Laboratory Culture

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ABSTRACT

The purpose of this study is to determine the appropriate substrate for larval settlement and spat growth in the purple clam, *Saxidomus purpuratus* in laboratory culture. Larvae were reared with 3 different types of sediments (mud, sand, and mixed) for 46 days in settlement experiment, and settled spats were further grown in 3 types of sediments for 36 weeks in growth experiment. The density of settled spats in muddy sediments was more than 2 times higher than those in mixed or sandy sediments. But, the average size of settled spats in muddy sediments was smaller than those in mixed or sandy sediments. After 36 weeks of growth period, growth rate decreased as shell length increased. When shell length was less than 2 mm, growth rate in mixed sediments was significantly higher than that in sandy sediments. When shell length was more than 2 mm, there was no significant difference in growth rate among different substrates. Sediment type affected growth rate only when the spats were relatively small (less than 2 mm). Muddy sediments seems better for larval settlement, while mixed sediments is best for spat growth. We suggest the laboratory procedure for enhancing seedling production of *S. purpuratus.*

Keywords: *Saxidomus purpuratus*, Substrate, Larval Settlement, Spat Growth.

INTRODUCTION

In the life cycle of marine invertebrates, the most drastic changes occur during metamorphosis and settlement. The body shape changes from pelagic form to benthic form and surrounding environment also changes from water to substrates. Therefore, the ecological and biological information during both larval period and post-larval period is necessary in the aquaculture of marine animals. For aquaculturists, basic water quality (DO, pH, salinity, temperature) and food conditions are the most important factors during the larval period. But, during the post-larval period (after settlement), substrate conditions become more important than water quality. Especially for the soft bottom species including most of bivalves, type of sediments is very important for the survival and growth.

The purple clam, *Saxidomus purpuratus* (Class Bivalvia: Family Veneridae) is a local species inhabiting relatively restricted areas around Korea, Japan, and China (Wei *et al.,* 1982; Kishioka *et al.,* 1996; Choe *et al.,* 1999). *S. purpuratus* is found from intertidal to 40 m depth of subtidal areas with mixed sediments of sand, silt, and clay (Kim *et al.,* 2000). Annual yield was 7,000-9,000 MT during 1995-1999 (Kim *et al.,* 2001b). Recently, the commercial yield from the traditional exploitation of natural fisheries by divers has been declining; much attention has been concentrated to the aquaculture of this species.

Studies on *Saxidomus purpuratus* have dealt with the basic ecological aspects (Kim, 1971; Zhang *et al.,* 2004), natural yield and growth (Kim *et al.,* 2001b), reproductive biology (Kim, 1969; Ideo e*t al.,* 1995; Chung *et al.,* 1999; Kim e*t al.,* 2001a; Park *et al.,* 2003), feeding physiology (Lee *et al.,* 2002), and aquaculture (Wei *et al.,* 1982; Kishioka *et al.,* 1996;

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Kim *et al.* 2000; Choi *et al.,* 2001; Choi *et al.,* 2003).

Thanks to the cooperative efforts by the Korea Ocean Research & Development Institute (KORDI) and Taean Marine Hatchery of the National Fisheries Research & Development Institute (NFRDI), some of physical and biological conditions for artificial fertilization, embryonic development, and larval growth were appropriately established (Kim *et al.,* 2000; Choi *et al.,* 2001; Choi *et al.,* 2003). However, the rearing conditions for post-settled spats have not been well understood yet. Although observation showed that survival of *Saxidomus purpuratus* spats reared with sediments was higher than those under water-only condition, there is no information about which kind of sediments is best for *S. purpuratus* spats. Here, we set the purpose of this study to determine the optimal substrates for settlement and growth of *S. purpuratus* spats. The results obtained from this study will be directly used for enhancing laboratory technique for the *S. purpuratus* spats and further application to the local hatcheries.

MATERIALS AND METHODS

1. Preparation of larvae

Adult *Saxidomus purpuratus* were collected from a subtidal area near Geoje Island by commercial divers. Gametes were obtained by dissection (Kim *et al.,* 2000). Gonadal tissues were removed from shells and washed twice with 1-μm filtered seawater (FSW, salinity: 32 psu). Sex was determined by observing gonadal tissues under a compound microscope (× 400). Sperm or eggs were obtained by scrubbing gonads in FSW. Sperm suspension was passed through a 20-μm screen, and was kept in a 100-ml beaker. Egg suspension was passed through a 100-μm screen to remove larger particles, and eggs were collected on a 40-μm screen for smaller eggs and particles to pass through. Eggs were treated with diluted ammonia water (Choi *et al.,* 2003), and then rinsed 3 times with filtered seawater. Before fertilization, the density of egg was adjusted to 100 eggs/ml. Fertilization was achieved by mixing two suspensions. Embryos were reared in an incubator (20℃) with gentle aeration. After 2 days, they developed into D-shaped veliger larvae.

2. Settlement experiment

For the first 12 days larvae were reared under water-only condition with unialgal diet of *Isochrysis galbana* (Prymnesiophyceae). After then, 3 types of substrates, namely Mud (particle size less than 64 μm), Sand (125-300 μm), and Mix (1:1 mixture of mud and sand) were added to the aquaria. Substrates were contained in different Petri dishes so that they could be separated each other. Five Petri dishes per substrate were laid on the bottom of the aquaria. The size of larvae at the beginning of experiment was 173.5 ± 12.3 μm. Larvae were reared for additional 46 days. During the settlement experiment, temperature was 20 ± 1℃, water was renewed by half everyday, and larvae were fed the mixture of *I. galbana* and *Chaetoceros gracilis* (Bacillariophyceae). At the end of experiment, the density of settled spats for each substrate were enumerated by sieving with a 300-μm mesh screen. The size (shell length) of settled spats were measured under a stereozoom microscope (Olympus).

3. Growth experiment

After the settlement experiment, all the spats were pooled in a beaker and were divided randomly into 3 groups. From each group, 10 clams (shell length: 830-890 μm) were selected for the growth experiment. Spats from each group were transferred into a beaker with each type of sediments, and then beakers were laid on a aquaria. Growth experiment was carried out for 36 weeks. The shell length of spats reared in different sediments was measured with an electronic caliper (Mitutoyo) to the nearest 0.01 mm at a 2-week interval. Growth rate (k) was calculated as;

$$k = \ln (SL_2 / SL_1) / (T_2 - T_1)$$

where SL_1 and SL_2 were the shell length at time T_1 and T_2, respectively. During the experiment, temperature was 20 ± 1℃, water was renewed by half everyday, and spats were fed the mixture of *Isochrysis galbana* and *Chaetoceros gracilis.*

4. Statistical analyses

One-way analyses of variance (ANOVA) were performed on SPSS package to examine the effects of substrate types on the settlement and growth. Before the analyses, density and shell length data were tested for normality (Shapiro-Wilk's test) and homogeneity of variance (Bartlett's test). If at least one of the above ANOVA requirements was not met, the data were $\log_{10}$ transformed, then ANOVA was repeated. If significant F values were observed in any ANOVA tests, multiple comparisons were conducted using Tukey's HSD (Zar, 1984) to determine which means were significantly different from one another. For all analyses, a significance level of $\alpha = 0.05$ was used.

RESULTS AND DISCUSSION

1. Settlement

On the whole, the density of settled *Saxidomus purpuratus* spats ranged from 510 to 3,057 spats/m^2. The highest value was found in muddy sediment, while the lowest in sandy sediments. Although the average density of settled spats in muddy sediments (1,529 spats/m^2) was more than 2 times higher than those in mixed (713 spats/m^2) or sandy sediments (815 spats/m^2), there was no statistical significance among different sediment types (ANOVA, $p > 0.05$) due to great within-variations (Fig. 1). The coefficients of variation (CV) of spat density in muddy and mixed sediments were as high as 78% and 81%, respectively. Two explanations of these high CV are possible.

The one is that during the experimental period, some of microenvironmental conditions within the same sediment type were not homogeneous. We set experimental period as long as 46 days, which was not only sufficient time for larval settlement, but also excessive time for experimental conditions to be heterogeneous. Choi *et al.* (2001) reported that *Saxidomus purpuratus* larvae with shell length of 180-200 μm became competent and began looking for settling substrates. But, in practice, settled spats can be easily distinguishable from background sediments only when they are sufficiently larger than adjacent sediment particles. So, we should wait for more time so that spats could grow larger than 300 μm (the largest particle in this study). Unfortunately, we had no preliminary information on the growth rate of spats, we should perform somewhat longer experiment. With the data obtained from this study (see below), we could calculate the minimum daily growth increment as 11.6 μm, which indicates that the shell length exceeds 300 μm after only 2 weeks. Therefore, our experimental period was much longer than needed. During these excessive period after the settlement, there might be confounding effects which masked substrate preference of spats, such as heterogeneous colonization of microorganisms on sediment surface or localized mortality of spats.

The other explanation is that *Saxidomus purpuratus*

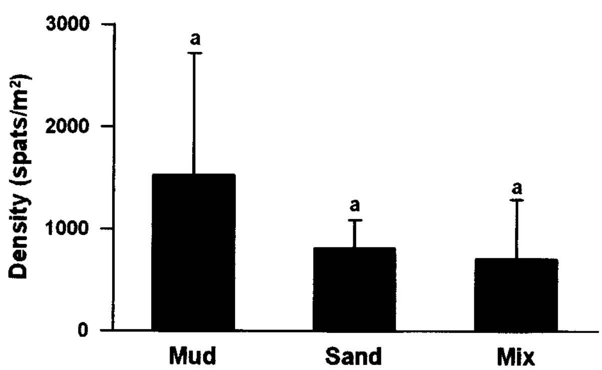

Fig. 1. Density of settled *Saxidomus purpuratus* spats in 3 different substrate types. Bar represents standard deviation (n = 5). Density was not significantly different ($p > 0.05$) among substrate types.

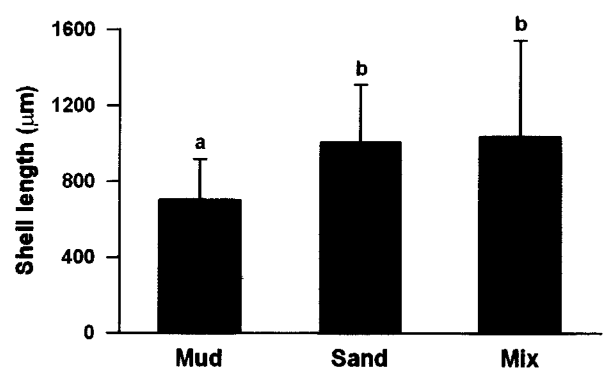

Fig. 2. Shell length of *Saxidomus purpuratus* spats in 3 different substrate types after the settlement experiment. Values with the same character are not significantly different ($p > 0.05$).

larva actually had no preference to the sediment types, and hence showing random-like distribution. However, we have no evidence from this study to ascertain if this explanation is true. In many studies, bivalve species showed preference to their specific substrate during metamorphosis and settlement (Mokady *et al.,* 1991; Pearce and Bourget, 1996; Devakie and Ali, 2002; de Montaudouin *et al.,* 2003). Thus, additional studies are needed to test if there exists substrate preference during the settlement of *S. purpuratus* larvae. Here, we can only conclude that muddy sediments seems better for the settlement of *S. purpuratus* larvae.

Unlike density, there was significant difference in the size of settled spats (ANOVA, p = 0.030; Fig. 2). After 46 days, shell length of spats increased more than 4 times. The size (mean ± SD) of settled spats in muddy sediments (707 ± 213 μm) was significantly smaller than those in mixed (1,039 ± 506 μm) or sandy sediments (1,010 ± 302 μm). The daily growth increments for muddy, sandy and mixed sediments were 11.6 μmm, 18.2 μm and 18.8 μm, respectively. The differences in shell length of spats among different sediment types seemed to be related to the extended period of settlement experiment. That is, the sediment type did affect the growth of *Saxidomus purpuratus* spats just after the settlement.

2. Growth

After 36 weeks of growth period, shell length increased ca. 25 times (Fig. 3). Shell length (mean ± SD) was highest in mixed sediments (22.9 ± 0.9 mm), followed by muddy sediments (22.5 ± 1.5 mm); lowest in sandy sediments (20.9 ± 1.6 mm). But, the

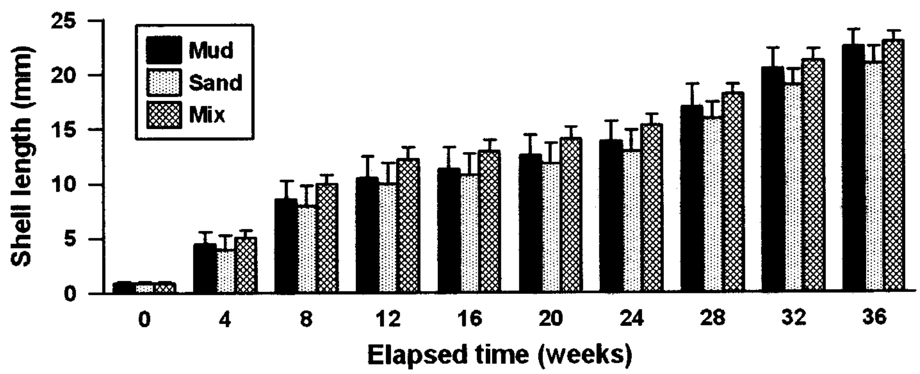

Fig. 3. Changes in shell length of *Saxidomus purpuratus* spats in 3 different substrate types during the growth experiment. Bar represents standard deviation (n = 10).

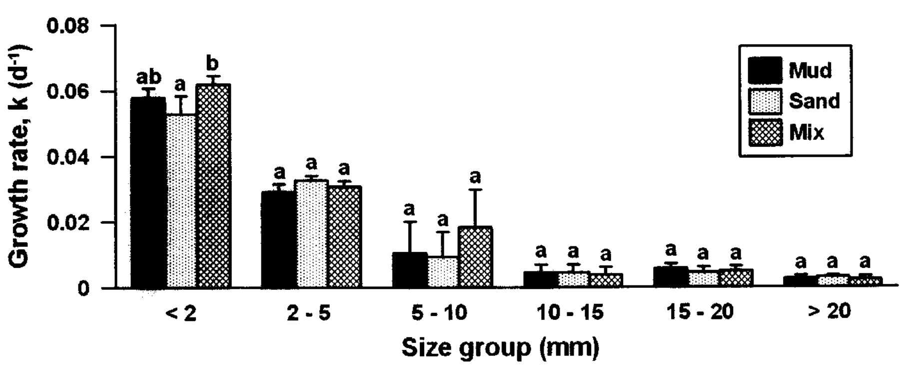

Fig. 4. Comparison of growth rates in *Saxidomus purpuratus* spats among 3 different substrate types for different size groups during the growth experiment. Values with the same character are not significantly different (p > 0.05).

differences were not statistically significant (ANOVA, $p > 0.05$). The shell length data were fitted well to the linear equation. The fitted growth equations for each substrate were as follows:

Mud: SL (mm) = 0.0778 T (days) + 2.394 ($r^2 = 0.970$)

Sand: SL (mm) = 0.0726 T (days) + 2.273 ($r^2 = 0.970$)

Mix: SL (mm) = 0.0786 T (days) + 3.370 ($r^2 = 0.955$)

From these equations, we can see that the daily growth increment is higher in the following order: mix > mud > sand. Therefore, mixed sediments seems best for the growth of *Saxidomus purpuratus* spats.

To know if there was a change in growth rate among substrate with different size (or age) of spats, individual data were re-arranged and grouped to several size groups. Growth rate (k) decreased exponentially as size increased (Fig. 4). When shell length was < 2 mm, growth rate in mixed sediments was significantly higher than that in sandy sediments (ANOVA, $p = 0.023$). But, as shell length increased more than 2 mm, there was no significant difference in growth rate among different substrates. Sediment type affected growth rate only when the spats were relatively small; the duration was less then 4 weeks. During the growth period, no mortality was recorded in all 3 sediment types. This implies that sediments is essential for the spat growth of *Saxidomus purpuratus.* Through our experiences, spats grown under water-only condition showed high mortality or low fitness. When clams are in erect position with siphons upward and foot downward supported by sediments, they are less vulnerable to the physical disturbances such as turbulences, abrupt change in water temperature or light intensity.

In summary, here we suggest the laboratory procedure for enhancing seedling production of *Saxidomus purpuratus* as follows:

(1) Veliger larvae to be reared for 12 days in water-only condition.

(2) Competent larvae to be allowed in muddy sediments for 2 weeks to settle.

(3) Settled spats to be grown in mixed sediments at least for the first 8 weeks.

With above procedure, we can expect increased survival and growth rate of *S. purpuratus* spats in the laboratory. However, these procedure does not reflect the conditions for settlement and growth in nature. To better understand the mechanisms related to the settlement in wild populations, more systematic studies for the sediment preference of *S. purpuratus* are needed.

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