The Use of Living Mussels as a Substratum for Growing Seedlings of Two Sargassum Species from the Perspective of Coastal Seaweed Bed Restoration in the East China Sea

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Abstract: Sargassum vachellianum and Sargassum horneri are the main dominant species and primary producers of seaweed beds in Chinese coastal ecosystems that play an important role in marine blue carbon sinks. However, due to the influence of frequent human activities and global climate change, the seaweed beds formed by Sargassum vachellianum and Sargassum horneri in Chinese coastal waters are gradually declining. In this study, Sargassum zygotes were cultured onto the surface of mussels and then used to repair the declining seaweed bed resources through seaweed transplantation, which was indirectly achieved based on the fast attachment of mussel byssus. The results show that zygotes of Sargassum vachellianum and Sargassum horneri could grow on the surface of mussels and, over time, the force and rate of mussel adhesion gradually increased. The adhesion of Mytilus coruscus was greater than that of Septifer virgatus mussels. After four hours, the rate of adhesion for Mytilus coruscus with a shell length of 30 mm was 100%, and the adhesion force was the highest, at 0.511 ± 0.099 N. Hence, Mytilus coruscus showed better adhesion than Septifer virgatus. After 41 days, the mean length of Sargassum horneri germlings on the surface of Mytilus coruscus grew to 5.554 ± 0.724 mm, the daily growth rate was about 0.154 mm/d, and the mean density declined by 50.59%. After 31 days, the mean length of Sargassum vachellianum germlings increased to 5.510 ± 0.763 mm, the daily growth rate was about 0.191 mm/d, and the mean density declined by 21.21%. After 2 months of development of the mussel–seaweed combinations in coastal waters, the survival rate of Sargassum horneri was 7.6 ± 0.9% and that of Sargassum vachellianum was 25.9 ± 10.5%. Hence, compared with Sargassum horneri, Sargassum vachellianum attached to Mytilus coruscus showed better development, and this system can be used to combat the decline in seaweed bed resources. In this method, mussels were used as an intermediate attachment medium to indirectly achieve the settlement of zygotes for seaweed transplantation, and, therefore, their use as a substratum serves as the basis for a novel technique for seaweed beds restoration.

Keywords: seaweed beds; zygote; mussel; adhesion; transplantation technique

1. Introduction

Seaweed beds are typical coastal ecosystems which provide an optimal environment for marine life to live and breed and play an important role in the conservation of fishery resources, marine eutrophication, and improvement of the ecological environment in coastal waters. They are a sensitive sensor for global climate warning and an important part of coastal blue carbon sinks that cannot be ignored [1,2]. Sargassum vachellianum and Sargassum horneri are the dominant species of seaweed beds in Chinese coastal ecosystems,
which are widely distributed from Liaoning (124°10′18.58″ E, 39°50′20.02″ N) to Hainan (109°59′23.41″ E, 18°24′09.86″ N) [3].

The ecosystems of Chinese coastal seaweed beds have been affected by habitat changes in recent years. Global warming, marine disasters, and frequent human activities have exacerbated the decline in coastal seaweed resources, including coastal topography, sediment thickness, eutrophication, and pollutants [4–10].

To date, the restoration of seaweed beds has typically followed three strategies: the removal of the predatory species (e.g., the culling of sea urchins) [11]; the spraying spore-filled water; and the installation of substrata for seaweed colonization (e.g., artificial reefs) [12–15]. Among these strategies, the construction of substrata has mainly been achieved by placing seaweed on a reef and binding maternal seaweeds [16]. Most studies have focused on the shape and materials of reefs as well as the strategies for culturing germings [17], and the process of zygotes transplantation has been rarely involved [18].

The technology of seaweed transplantation in Japan and the Philippines is relatively mature. Clay, limestone, and gravel can be used to culture zygotes, which are riveted or spread in near shore areas to restore seaweed beds loss, and good practical effects have been obtained [19–21]. Environmental factors such as sediment thickness, nearshore hydrodynamics, and light intensity often determine the success of seaweed bed restoration and construction [22]. The nearshore water of the East China Sea is turbid, and the slope is comparatively large. With the influence of sediment transport from the Yangtze River, marine sediments have been silted, and zygotes have been significantly affected by waves; therefore, it is difficult for them to adhere to the rock. Based on many years of experience with seaweed transplantation, we have found Mytilus coruscus and Septifer virgatus to be distributed in the intertidal and subtidal zones, which is the same as for Sargassum vachellianum and Sargassum horneri. The sediment thickness of mussels’ surfaces was less than on rock, making it suitable for zygote attachment. Taking into account the fast and strong adhesion of mussels to substratum, it had been proposed that mussels can be promising intermediates for attachment of seaweed seedlings in coastal waters. Moreover, the main aim of this study was to show that mussels could be used as intermediaries for Sargassum spp. zygote settlement on their surfaces and to determine the strength and speed of mussel adhesion to substratum to prove mussels’ use for fast attachment of seedlings in the open sea.

2. Material and Methods

2.1. Selection of Mussel Specifications

Mussels of different species and sizes were collected in May 2020 from the subtidal zone of Gouqi Island, Zhejiang Province (122°44′14.28″ E, 30°42′52.61″ N). Mussels, including Mytilus coruscus and Septifer virgatus with shell lengths ranging from 1 to 5 cm, were collected by scuba divers, and the byssal threads were cut off with a blade to ensure that the stem was not damaged. Then, the mussels were brought back to the laboratory, the sediment on the outer surface of the mussel was gently removed with a brush, and the water on the outer surface was removed using absorbent paper. The mussels have been grouped into two species groups, and the biological parameters of each individual, including shell length (L, mm), width (SW, mm), and weight (W, g), were determined. Mussels of each individual with shell lengths of approximately 20, 30, and 40 mm were selected and divided into 5 groups, i.e., A1, A2, A3, B1, and B2 (Table 1). The length and width of mussel shells were measured by using a vernier caliper (PD–151, PROKIT’S INDUSTRIES, Shenzhen, China), and weights were measured using an electronic balance (JCS100, YINGHENG, Shanghai, China).
Table 1. The biological parameters of the group with different specifications.

| Specifications | Species           | W (g)       | L (mm)      | SW (mm)      |
|----------------|-------------------|-------------|-------------|--------------|
| A1             | Mytilus coruscus  | 0.822 ± 0.154 c | 20.708 ± 1.306 c | 11.992 ± 1.117 b |
| A2             | Mytilus coruscus  | 2.203 ± 0.335 b | 30.485 ± 1.828 b | 17.028 ± 0.486 a |
| A3             | Mytilus coruscus  | 5.907 ± 0.793 a | 40.148 ± 2.356 a | 22.876 ± 0.372 a |
| B1             | Septifer virgatus | 1.113 ± 0.300 b | 19.916 ± 1.374 b | 11.290 ± 0.614 b |
| B2             | Septifer virgatus | 3.790 ± 1.051 a | 30.740 ± 1.291 a | 15.980 ± 1.991 a |

(a, b, c) indicate significant differences in one species group (p < 0.05).

2.2. Collection of Zygotes from Seaweed

Male and female seaweeds (fertilized eggs have not been released) of Sargassum vachellianum and Sargassum horneri in the subtidal zone of Gouqi Island were collected and fostered in indoor pools in May 2020. After 2–3 days, the seaweeds were rinsed with flowing seawater to remove the fertilized eggs from the receptacles. At the same time, zygotes of Sargassum horneri were collected by a 200 mesh filter and those of Sargassum vachellianum were collected using a 500 mesh filter. The collected zygotes were mixed with water to form a mixture with a high concentration of zygotes (Figure 1).  

![Figure 1](image)

**Figure 1.** Release and collection of zygotes: (a) Sargassum horneri zygotes being released from receptacles, scale bars = 1 cm, (b) zygotes water of Sargassum horneri, with red arrows indicating divided zygotes, scale bar = 100 µm.

2.3. Experimental Design

The force and rate of adhesion of the mussels to substratum were measured in an artificial culture pond. Furthermore, the type of substrate in the pond was concrete. The groups of mussels with different shell lengths (individuals in each group) were cultured in the pond (dimensions of 5 m × 3 m × 1.5 m) separately, and seawater was injected into the pond until its depth was 20–30 cm. The distance between any two mussels was 5–8 cm to avoid mussel aggregation (Figure 2). The adhesion force and rate of Mytilus coruscus and Septifer virgatus adhesion were determined at different times (after 1, 2, 4, 7, 8, 10, 12, and 24 h). The adhesion force was determined using a digital force gauge (ZP–10, AIGU, Hong Kong, China), and in natural conditions, the adhesion force of intact shells was determined using a digital force gauge (ZP–200, AIGU, Hong Kong, China) and expressed in Newtons (N). In the pond, thirty mussels were used to measure the adhesion force in each group and different groups of shells were used to measure the adhesion force of intact mussels in different time; individuals of Mytilus coruscus with shells from 20 to 30 mm were used. The measures were conducted in natural conditions in intersection of intertidal zone and subtidal zone.
Figure 2. Adhesion force measurements and Sargassum zygote adhesion: (a) placement of mussels for adhesion force measurements, where the red arrow indicates a byssal thread; (b) mussels for zygote adhesion.

On the basis of the measurements of adhesion force and the rate of mussel adhesion, the best experimental specifications were selected for Sargassum zygote adhesion, about 380–400 shells were used for zygote attachment measurements. The water containing zygotes was evenly applied on the outer surface of the mussels; the density of the zygote suspension used was \((4–5) \times 10^4 \text{ ind/mL}\). It was then cultured in an outdoor pool (dimensions of \(3 \text{ m} \times 1.5 \text{ m} \times 0.6 \text{ m}\), in which six oxygen pumps were placed. The seawater was changed once a day. The water temperature in the pool during sargassum seedling cultivation ranged from 25.3 to 28 °C (26.28 °C in average), increasing from early morning to noon and then gradually decreased to 11 PM. Average light intensity was 3200 lux, it increased from 5–6 AM to 8–11 AM, reached 20,000 lux, and then decreased after noon. The temperature and light intensity of the water were recorded using an Onset HOBO (MX2202, Onset Computer Corporation, Bourne, MA, USA). Adhesion of zygotes was observed on mussel surfaces after 3–5 days, and the mean density and length of the germlings were recorded. The initial density of Sargassum horneri germlings on the shells was 10–13 ind/cm², and the density of Sargassum vachellianum germlings was 9–10 ind/cm². During cultivation, miscellaneous seaweeds in the pond were cleaned out in time to avoid affecting the growth and development of Sargassum germlings. The mean density and length of the Sargassum horneri germlings were recorded from the 10th day and that of the Sargassum vachellianum germlings were recorded from the 5th day. Five seedlings were measured for length estimation of each Sargassum species at each measuring time. Similarly, 5 shells were used for density measurements. The length and density of zygotes were observed using an Olympus SZX7 (Olympus Corp., Tokyo, Japan), and the length and density of seedlings were measured on microphotographs obtained with a Swiftcam SC500 (SWIFT Inc., Xiamen, China). When germling’s length grows to 3–5 cm, seaweeds-mussels combinations can be transferred into the open sea. The place where surface waves have relatively less influence on it, where the slope is less than 30°, depth is about 3–5 m, and there are sediments with lithofacies, is the good site for proliferation. They were exposed in these conditions from September to November during our study period. The annual average water temperature is 17–19 °C, suitable for Sargassum vachellianum and Sargassum horneri growth. The total duration of Sargassum horneri and Sargassum vachellianum seedlings cultivation in the pond were 107 and 132 days.

2.4. Data Analysis

The data were analyzed using SPSS version 25 (SPSS Inc., Chicago, IL, USA). The data among different groups were tested for normality and homogeneity of dispersion and compared using one-way ANOVA, followed by Tukey’s post-hoc test for multiple comparisons. \(p < 0.05\) was considered to indicate statistically significant differences. Mean density and length are expressed as mean ± standard deviation. The data graphs were
generated by using Origin 2018 (OriginLab, Northampton, MA, USA). The daily growth rate (DGR) of seedlings was calculated with the following equation:

\[ DGR = \frac{H - H_0}{t} \times 100\% \]

where \( H \) is the seedling length at the end of the experiment, \( H_0 \) is the initial seedling length, and \( t \) is the time interval (days). We used the mean seedling length on first day and at the end of experiment for calculation.

3. Results

3.1. Adhesion Force and Rate of Mussels

The adhesion force and the rate of adhesion of both *Mytilus coruscus* and *Septifer virgatus* increased gradually with time (Figure 3). Within 24 h, the adhesion force of the A2 group increased from 0.348 ± 0.047 to 1.027 ± 0.072 N, and the adhesion force of B1 was the lowest, increasing from 0.033 ± 0.001 to 0.558 ± 0.085 N. The adhesion force of the A2 group was greater than that of A1 and that of B2 was greater than that of B1. From 12 to 24 h, the adhesion force of the two mussel species tended to be stable. There was a significant difference in adhesion force between 1 and 7 h for mussels with 30 mm shell length (groups A2 and B2) (\( p < 0.05 \)). We have five groups of mussels in total; for the adhesion rate of A3 group, mean length was about 40 mm, which was smaller than A1 and A2, so we did not measure the adhesion force of A3.

![Figure 3](image_url)  
*Figure 3.* Adhesion force of mussel with time. The letters a, b, c, d, and e indicate significant differences in one length group between different hours. There is no significant difference between data marked with the same letters (\( p > 0.05 \)), but differences are significant for data marked with different letters (\( p < 0.05 \)).

These results were supported by measurement of the adhesive force of intact *Mytilus coruscus* with different shell length in natural conditions (Figure 4). The adhesion of mussels with shell length of 2.4–6.2 cm is about 19.6–107.8 N. The adhesion force increases with shell length, and the relationship between them can be represented as a power function, i.e., \( y = 8.26 \times 1.29 \) (\( R^2 = 0.54 \)).
Figure 4. The relationship between shell length and adhesion force of *Mytilus coruscus*. The red line is the fit curve, the darker pink area is the 95% confidence region, and the light pink areas are the 95% prediction regions.

The amount attached to substratum individuals of *Mytilus coruscus* and *Septifer virgatus* increased with time (Figure 5). It can be observed from the figure that with an increase in time, the adhesion rate of *Mytilus coruscus* and *Septifer virgatus* increased. After one hour, the percentage of attached *Mytilus coruscus* of the A2 group was more than 80% and reached 100% after four hours. Within four hours, the percentage of attached *Septifer virgatus* of the B1 and B2 groups were less than 40%. The adhesion rate of the A3 group was lower than that of the A1 and A2 groups.

Figure 5. Comparison of adhesion rates of mussels with different specifications.

Within 24 h, the adhesion force of *Mytilus coruscus* (A2) with 30 mm shell length was greater than that of the A1, B1, and B2 groups; all individuals of *Mytilus coruscus* of 20 and 30 mm length were attached after four hours, whereas percentage of attached individuals
in other groups was less than 80% at the same time. Hence, adhesion was the best for length (30 mm) of *Mytilus coruscus* among all groups.

3.2. Adhesion and Growth of Zygotes

Due to the force and rate of *Mytilus coruscus* and *Septifer virgatus* adhesion in the above analysis, we selected *Mytilus coruscus* with a shell length of about 30 mm as the substrate for zygotes, and then conducted experiments on zygote adhesion as well as the growth of *Sargassum horneri* and *Sargassum vachellianum*. The survival rate of zygotes was 10–20 ind/cm² after one day.

After five days, rhizoids of *Sargassum horneri* were observed on the surface of the mussels (Figure 6a), and germlings were observed after 10 days (Figure 6b).

![Figure 6](image)

**Figure 6.** Adhesion and growth of germlings: (a) germlings of *Sargassum horneri* adhered on shell surface after 5 days, scale bar = 100 μm; (b) germlings of *Sargassum horneri* adhered on shell surface after 10 days, scale bar = 1 mm.

It can be observed from the growth of germlings on the outer surface of mussels that the germling mean length of the two species of seaweeds gradually increased with time, and the mean density gradually decreased (Figure 7). There was a significant difference in germling length between 10 and 21 days after cultivation (*p* < 0.05). After 41 days of cultivation, the mean length of *Sargassum horneri* germlings increased to 5.554 ± 0.724 mm and the daily growth rate was about 0.154 mm/d. In addition, after 41 days, the mean density decreased to 4.200 ± 2.588 ind/cm², i.e., a reduction of 50.59% as compared with 8.500 ± 3.109 ind/cm² on the 10th day, which is a significant difference (*p* < 0.05) (Figure 7a).

There was a significant difference in mean length between the 5th and 16th day (*p* < 0.05), and after 31 days, the mean length of the *Sargassum vachellianum* germlings increased to 5.510 ± 0.763 mm, and the daily growth rate was about 0.190 mm/d. The mean density decreased from 6.600 ± 1.817 on the 5th day to 5.200 ± 1.304 ind/cm², i.e., a reduction of 21.21%. There was no significant difference in mean density during cultivation (Figure 7b).
The growth of Sargassum horneri and Sargassum vachellianum on the outer surface of mussels is shown in Figures 8–10. On the 18th day, the second blade of Sargassum horneri was observed, the third blade was observed on the 21st day, and the fourth blade appeared on the 35th day (Figure 8). The second blade of Sargassum vachellianum was observed on the 20th day of culture, and the third blade was observed on the 25th day (Figure 9). On the 132nd day of culture, the mean length of Sargassum vachellianum germlings was approximately 9.22 cm, 7 leaves were observed, and the germlings were in the seedling stage. After 107 days of cultivation, the mean length of Sargassum horneri leaves was approximately 2.87 cm (Figure 10).
Figure 8. Growth and development of *Sargassum horneri*. (a–f) The germlings after 10, 18, 26, 31, 35, and 41 days of culture; scale bars = 1 mm.

Figure 9. Growth and development of *Sargassum vachellianum*. (a–f) The germlings after 5, 10, 16, 20, 25, and 31 days of culture; scale bars = 500 μm.

Figure 10. Mussel–seaweed combinations. (a,b) The germlings after 92 and 132 days, respectively, of *Sargassum vachellianum* cultivation; (c,d) the germlings after 100 and 107 days, respectively, of *Sargassum horneri* cultivation, scale bars = 500 μm.
When the germlings had grown to 3–5 cm and had tolerance to the environment, they were artificially sown in the offshore reef area. The combinations with fine integrality were screened out and spread on the seafloor by scuba divers and marked for regular monitoring. After two months of development of the mussel–seaweed combinations in coastal waters, the survival rate of Sargassum horneri was 7.6 ± 0.9%, and that of Sargassum vachellianum was 25.9 ± 10.5%. When proliferating these combinations elsewhere, areas with smaller reef slope, fewer predators and herbivorous animals, and low current velocity should be selected according to the actual offshore conditions.

4. Discussion

The optimum temperature for the growth of germlings was 20 °C; when the temperature increased to 25 °C, the survival and growth rate significantly decreased [23]. In our experiment, Sargassum horneri was cultured in an outdoor pool, and because of the shallow water depth, it was significantly affected by solar irradiance. The daily average water temperature was about 26.28 °C. On the 10th day of cultivation, the mean length of Sargassum horneri was just 0.922 ± 0.038 mm. Zhang (2012) cultured Sargassum vachellianum zygotes at 21 °C for 30 days, and the mean length was 5.05 mm [24]. We observed similar results in our study, with germlings having a length of 5.51 ± 0.76 mm after 31 days. Compared with those of Sargassum horneri, the germlings of Sargassum vachellianum were more tolerant to high temperature. In addition, Zhang (2018) suggested that the adhesion rate of Sargassum horneri zygotes was high when the density was in the range of 1–10 ind/cm², which was consistent with the range of 4.2–8.5 ind/cm² observed in our study [23].

Compared with the traditional method of artificial reef for seaweed restoration, the technology of mussel–seaweed combination transplantation can save more manpower and is more eco-friendly. It is expected that this new technology will be used in the restoration and construction of seaweed ecosystems. Sparidae often feed on mussels and seaweed [25,26], and, therefore, it is necessary to avoid the effect of predators when selecting the site for proliferating mussel–seaweed combinations. The combinations may move to other areas due to the influence of terrain gradient and current velocity. However, according to actual experiments, most combinations remain trapped in the crevices of reefs, and these crevices are excellent places for mussels and seaweed to grow [27,28]. By observing the process of seaweed transplantation based on attachment of Lamellibranchia, Lv (2019) found that secondary attachments of Saccharina japonica and Undaria pinnatifida occurred [29].

Overall, the technique for growing seaweed using mussels involves catching mature seaweed, collecting zygotes, pond cultivation, and proliferation in coastal waters (Figure 11). The greatest benefit of this technique is that the influence of sediment and light intensity on the growth of germlings can be effectively controlled, but possible limitations include predators and herbivorous animals, terrain gradient, and current velocity.

![Diagram](image_url)

**Figure 11.** The overall technique for seaweed bed restoration using mussels.
In this study, we showed that *Sargassum horneri* and *Sargassum vachellianum* could grow on the outer surface of mussels; however, under natural conditions, whether the growth of *Sargassum horneri* and *Sargassum vachellianum* affects the growth and filtration of mussels remains to be further studied. The tolerance of seaweed to environmental factors such as temperature and light intensity varied at different growth stages, and the best length of seaweed germlings for transplanting remains to be determined. In addition, the prosperity of seaweed is significantly affected by the velocity of the current, and whether the adhesion force of mussel byssus can withstand the influence of waves remains to be verified by experiments.

5. Conclusions

The force and rate of *Mytilus coruscus* and *Septifer virgatus* adhesion were studied, and we found that the adhesion force of *Mytilus coruscus* was greater than that of *Septifer virgatus* on mussels with the same shell length. The attachment of mussels with a 30 mm shell length was better in terms of strength and duration than that of mussels with 20 and 40 mm shell lengths. Zygotes of *Sargassum horneri* and *Sargassum vachellianum* could grow on the outer surface of mussels. After 41 days of cultivation in a pond, the mean density of *Sargassum horneri* germlings was reduced by 50.59%, the mean length increased to 5.554 ± 0.724 mm, and the daily growth rate was 0.154 mm/d. After 31 days of cultivation, the mean density of *Sargassum vachellianum* decreased by 21.21%, the mean length increased to 5.510 ± 0.763 mm, and the daily growth rate was about 0.191 mm/d. The growth effect of *Sargassum vachellianum* germlings was better than that of *Sargassum horneri*.

In this study, we verified the feasibility of transplanting subtidal seaweeds through mussel–seaweed combinations for the restoration of seaweed beds. In addition, the effects of predators, velocity of the current, and lithofacies gradient on mussel attachment should be considered when sowing in coastal waters.

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