Objectives:

The subcontractor is to improve seagrass transplanting techniques, to establish a seagrass transplanting demonstration and a sustaining seagrass donor supply sites.

Expected Outputs：

The subcontractor is expected to deliver a technical report demonstrating the following:

- Improve transplanting techniques of seagrass based on the protection of seagrass at the early replanting stage, and establish a seagrass transplanting demonstration;
- Establish a sustaining donor supply at semi-closed ponds for seagrass transplant without disturbance of the current seagrass meadows;
- Establish a seagrass transplanting demonstration site.

Time：

Mid-October 2017- mid March, 2019

Report outline:

Background

Improvement of seagrass replanting techniques

- Seed Sorting Technology
- Seed Germination Promoting Technique
- Improved Transplanting Technique in the sea
- Culture of Donor Plants through land-based culture system of green house
- Culture of Donor Plants through aquaculture pond

Conclusion and prospects

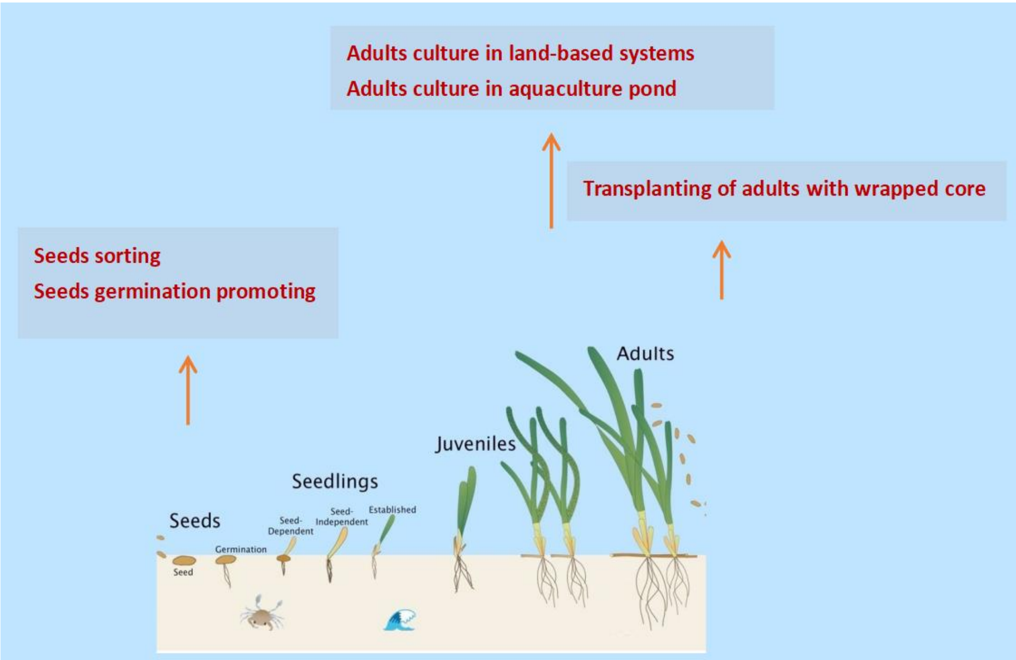

Fig.1 Seagrass transplant technology at different stages of its life history.

Seagrass transplanting report and establishment of improved techniques of replanting seagrass

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As the important habitats of fishery species, seagrass meadows provide shelter and food for fish, and support the fisheries recruitment and sustainability. However, seagrass in the world has increasingly declined under the background of climate change and human activities, and faster in recent years. In China, the similar results on seagrass has been found in China coastal waters, the seagrass loss has led to key habitats loss of fishery species (e.g. spawning ground, nursing ground), further bring serious influences on recruitment of fishery resources. So, it is necessary and urgent thing to recover seagrass beds for the conservation of fishery species in coastal waters. In the present, the transplanting seagrass is mainly from the existing seagrass meadow by the digging methods, which often cause the low survival rate of seagrass, as well as the low rebuilding options. The transplanting techniques of seagrass is a very important content in YSLME-II phase, so this report summarized the results of seagrass transplanting techniques and the seagrass transplanting demonstration.

Background

There are a large number of seagrass ecosystems on the coast of the tropics to the temperate environments. They provide breeding habitats and food sources for many marine organisms. Seagrass bed can increase the surrounding biodiversity, the primary productivity and the water body transparency. They are the engineers of the ecosystem and one of the three typical ecosystems of the ocean. Costanza et al. (1997) estimated the ecological and economic value of various ecosystems and they found that the ecological and economic value of coastal ecosystems accounts for more than 30% of the world's total ecosystems, while half of the contribution comes from seagrass beds.

Under the influence of natural factors and human activities, seagrass distribution has declined. Waycott et al. (2009) conducted a comprehensive assessment of 215 research work around the world and found that from 1980 to 2006, seagrass habitats has been disappeared at a rate of 110 km^2 each year. The area of seagrass has been disappeared by 29% since 1979. In China, the decline of seagrass beds is also serious. However, due to insufficient research and historical data , the degree of seagrass decline only stays at the description stage. Human activity is the main cause of the degradation, especially the destructive digging and aquaculture activities. Sanggou Bay is one of the main distribution area of seagrass in the north of China. Before the 1980s, the seagrass bed dominated almost all the intertidal zones along the Rongcheng coast. There were many species in seagrass beds, such as echinoderms, shellfish and fish. However since the 1980s, the distribution of seagrass beds has shrunk rapidly, and the biomass of marine life unique to seagrass beds has also been greatly reduced. At present, seagrass beds remaining in Sanggou Bay can only be seen in Chudao and Bahe Port, and there are only sporadic places in other places.

The restoration and reconstruction of seagrass beds have received more and more attention in the past 2-3 decades. Because seagrass beds can be restored in a short period of time through seed or rhizome breeding, and researchers in the world are exploring effective seagrass transplant recovery techniques. Current seagrass bed recovery techniques include seed method, adult transplantation method and habitat restoration method (Martins et al., 2005; Orth et al., 2006; Park et al., 2007).

The adult transplantation method is one of the commonly used methods. The more effective transplantation techniques reported include the sod method, coring method and rhizome method (Goodman et al., 1995; Fonseca et a., 1998; Zhang et al., 2013). For the rhizome method, the collection of shoots is not as labor-intensive as the sod and coring method and sediment were seldom collected along with the rhizome and shoots, so the method of fixation of the transplanting unit include hand-broadcast method (Phillips et al., 1973), staple method (Liu et al., 2015) and the framework method (TERFS) (Short et al., 2002). However, in the intertidal zone with strong current, fixation of the transplanting unit and expansion of seagrass is still difficult. Therefore, effective maintenance of transplanting unit is more critical to achieve the success of transplant and restoration.

For seagrass can produce a large number of seeds during the sexual reproductive season, and collection of seagrass seeds commonly cause no damage to the donor sites, so the use of seed has become a common method for the restoration of the seagrass beds. The current seed restoration method is to collect seeds and dispersing directly to the sea area to be restored, while have limitations of bottlenecks such as low natural germination rate, long germination cycle and low seedling establishment rate (Orth Et al., 2009), so its effectiveness needs to be further improved.

In recent years, the use of greenhouse to cultivate the seeds and seedlings of seagrass has been initially explored. This method can start from the seed, culture the seedlings to the transplantable specifications, and improve the survival rate of the seedling. However, due to the high cost of greenhouse building and long time culture for the dormancy of seagrass seeds and low growth rate of seagrass seedlings, more improvement is still needed. Also the seeds are easy mildew for the metamorphism, damage and low vigor of seeds, so the seed sorting the preliminary work of artificially cultivating the seedlings.

Based on the above limitation, the project aimed to developed an improved method for seagrass transplantation in intertial and subtial area, to establish an transplant demonstration of eelgrass, which is the one of the most widely distributed species in the world. This project was carried out eelgrass (*Zosterea marina* L.) in Rongcheng Chudao area (Sanggou Bay, Shandong Province). This area once covered by eelgrass, but now there are a lot of bared area. Mainly, a low cost green house for eelgrass culture were established, an effective adult shoot culture method, seed sorting method, seed germination method.

Table 1. Project Schedule

Period	**Project Schedule**
2017.10-2017.12	Seed Sorting Technology Establishment
2017.10-2018.03	Improvement of Seed Germination Promotion Technique
2017.12-2018.06	Early Seedling Cultivation
2019.03-2019.08	Seagrass Transplantation in the Sea and Establishment of Demonstration
2019.03-2019.08	Eelgrass culture in the land-based culture system
2019.08	Report Submission

1. Seagrass Technical Report

1.1 The establishment of seagrass seed sorting technique

1.1.1 Seed collection

Seed collection occurred in mid-July (the best time for seed collection) on the shore of Chudao, Sanggou Bay, Shandong Province. The eelgrass flowering shoots were collected and put in a 20-mesh screen bag, and the bag was then suspended in the sea. To prevent the bag from floating on the water, 1 kg stones were put in the mesh bags so that the mesh bag was completely submerged. At the beginning of early October, the mixture of seeds and propagules in the bags were brought back to the laboratory, and the propagules were removed, and then, the seed material was obtained.

1.1.2 Seed sorting technology

The seeds were divided into those with a diameter larger than 1.8 mm and those with a diameter smaller than 1.8 mm by a sieve with a pore size of 1.8 mm. Then, a 2.19 mol/L sucrose solution was used to divide the seeds into diameters larger than 1.8 mm that float (A), diameters larger than 1.8 mm that sink (B), diameters less than 1.8 mm that float (C), diameters less than 1.8 mm that sink (D), and control seeds without any treatment (E). Thirty seeds of each of the above seed types (A, B, C, D, and E) were placed into a petri dish. The petri dishes were covered with mesh, and the five treated groups were placed in a recirculating water system. During the experiment, the germination of the seeds in each petri dish was recorded every 5 days. The germination rate (%) = number of germinated seeds/total number of seeds × 100. The water was changed every 2 days for a total of 60 days.

1.1.3 Results of the seed sorting technology

The seed germination rate was obtained after 60 days. The germination rate of the control group (E) was only 16%. The germination rates were as follows: D> B> E> A> C. No significant difference was found between groups B and D (p>0.05), while the difference was significant between groups B and D and among groups A, C, and E (p<0.05). The results showed that the optimum seed germination rate of eelgrass can be obtained by using 1.8 mm mesh and 2.19 mol/L sucrose solution.

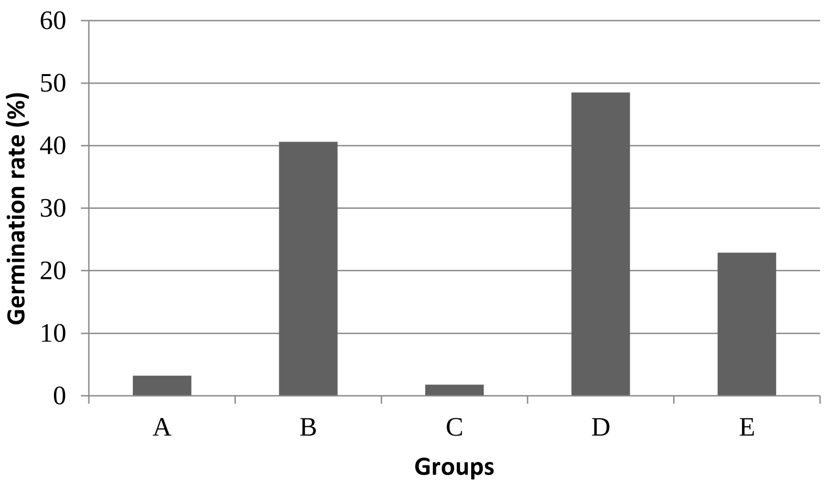


Fig.1 Seed germination of eelgrass *Zostera marina*. L. A: seeds with diameter larger than 1.8 mm and that float on the surface of the water; B: seeds with diameter larger than 1.8 mm that sink

in the water; C: seeds with diameter less than 1.8 mm and that float on the surface of the water; D: seeds with diameter less than 1.8 mm that sink in the water; and E: the control groups.

1.2 Technology to promote seed germination

1.2.1 The collection of eelgrass seeds:

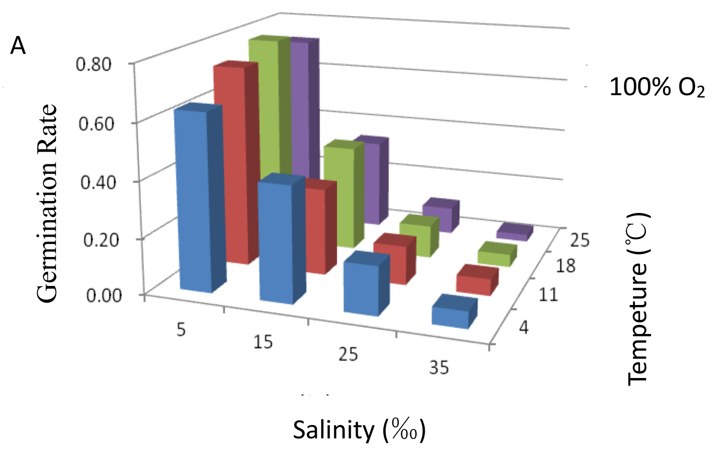
The collection method was the same as that above, in Section 1.1.1.

1.2.2 Seed germination conditions:

Different temperature gradients: 4°C, 11°C, 18°C and 25°C; different dissolved oxygen concentrations: normal dissolved oxygen and 0%, 40% and 75% of the normal dissolved oxygen; and different salinities: 5‰, 15 ‰, 25 ‰ and 35 ‰ were set for every 30 seeds as a group and for 3 replicates of each group. The experiments were conducted at different temperatures, dissolved oxygen concentrations and salinities for a total of 100 days.

1.2.3 Results:

The results show that salinity significantly affected the seed germination rate ($p<0.05$)(Fig. 2). The seed germination rate of eelgrass under low salinity was much higher than other treatments, while temperature and dissolved oxygen did not significantly affect the seed germination rate.



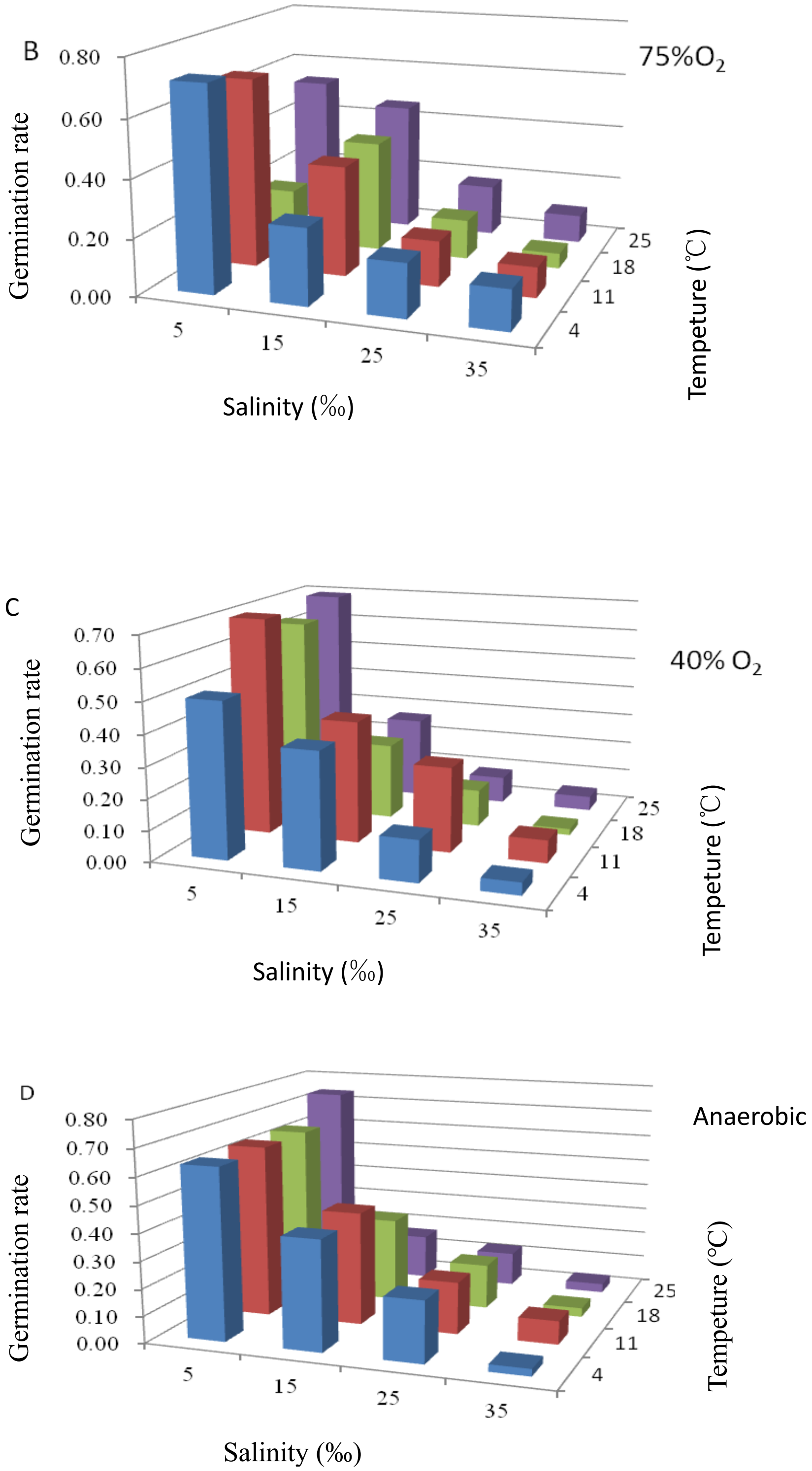
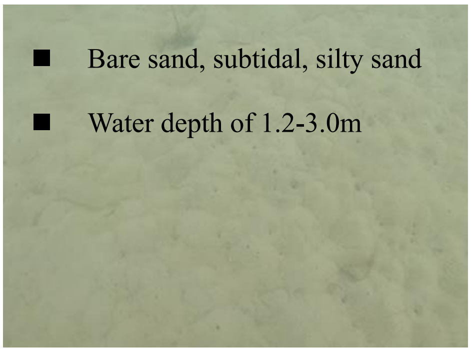


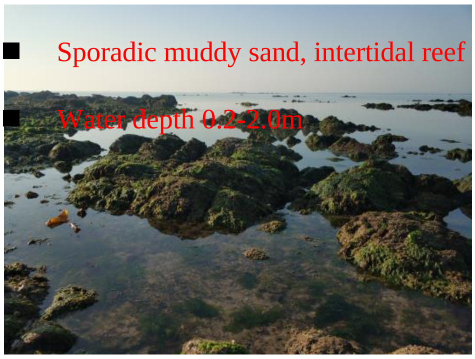
Fig. 2 The seed germination rate of eelgrass seeds at different temperatures, salinities and oxygen concentrations.

1.3 Technology of Transplanting Eelgrass with Wrapped Core

1.3.1Drawing on the method of tree transplanting, the roots and the soil are tied up with straw rope to avoid the loss of the donor soil and ensure transplant success. The sediment of large eelgrass blocks was wrapped with litter rope, dried kelp leaves or waste mesh to slow down the loss the sediment from the donor site and to extend the time for root's acclimation to the transplant site, thereby improving the restoration success.

Two sites with different sediment and environmental types were selected. One is subtidal bare sand and the other one is intertidal rocky reef environment with silty sand.





1.3.1 Transplanting of eelgrass in the subtidal bare sand

1.3.1.1 Material and method

Transplantation time: April 21-22, 2019

Transplantation method: Eelgrass litter and kelp leaves were used to wrap the sediment of eelgrass block. Eelgrass in the donor site was used as a control. A total of 3550 shoots were transplanted.

Measurement method: On June 16, 2019, 30 shoots were randomly selected and collected for height and leaf width measurement; the number of reproductive plants was counted in situ; digital photos were taken, and image processing software was used for automatic or semi-automatic coverage and area measurement.

1.3.1.2 Results

The eelgrass shoots grew significantly from the beginning of transplantation with the mean height grew from 21.2cm to 39.2cm ($p<0.05$). The transplanted shoots finished sexual reproduction process with a mean of 21.4 seeds per reproductive shoot. The area of the blocks increased by 6.5% and coverage increased by 28.5%.

Table 2 Measurement of the transplanted shoot in subtidal bare sand in Chudao Island, Sanggou Bay.

	Transplantation site	Donor site（Control）
Plant initial height (cm)	21.2±4.3	21.2±4.3
Plant height (cm)	39.2±4.9	72.7±8.6
Initial blade width (mm)	3.10±0.32	3.10±0.32
Blade width (mm)	3.56±0.55	5.66±0.87
Number of reproductive plants (ind/m^2)	26	79
Reproductive shoot height (cm)	46.8±9.2	89.3±11.5
Area change (%)	↑6.5±3.3	0
Number of seeds / reproductive shoot	21.4±4.5	38.9±6.9
Coverage change (%)	↑28.5±6.4	↑56.7±5.6

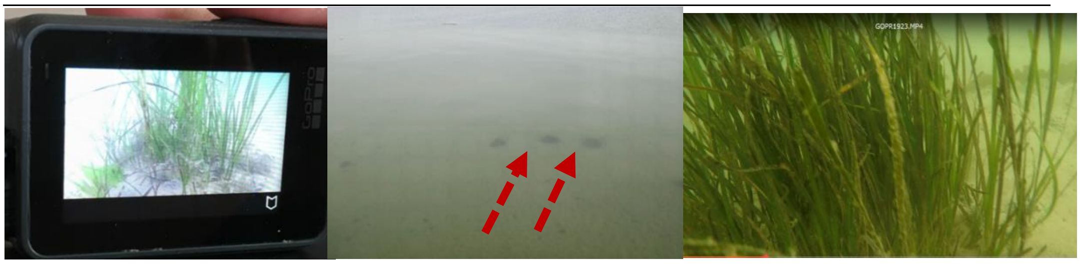


Fig. 3　The transplanted eelgrass in the subtidal bare sand in June 2019

1.3.2 Transplantation of eelgrass in intertidal reef environment

1.3.2.1 Material and method

Transplantation time: March 22-23, 2019

Transplantation method: Eelgrass litter and kelp leaves were used to wrap the sediment of eelgrass block. Eelgrass in the donor site was used as a control. A total of 4950 shoots were transplanted.

Measurement method: On June 16, 2019, 30 shoots were randomly selected and collected for height and leaf width measurement; the number of reproductive plants was counted in situ; digital photos were taken, and image processing software was used for automatic or semi-automatic coverage and area measurement.

1.3.2.2 Results

The shoot height of transplanted eelgrass was significantly increased, reaching 66.2cm, which was close to the plant height of the donor site (P>0.05). Eelgrass shoots finished sexual reproduction process with a mean of 31.2 seeds per reproductive shoot. The area of the blocks increased by 22.9% and coverage increased by 41.6%.

Table 3 Measurement of the transplanted shoot in reef environment in Chudao Island, Sanggou Bay.

	Transplantation site	Donor site（Control）
Plant initial height (cm)	18.5±3.4	18.5±3.4
Plant height (cm)	66.2±9.5	72.7±8.6
Initial blade width (mm)	3.10±0.32	3.10±0.32
Blade width (mm)	5.60±0.61	5.66±0.87
Number of reproductive shoots (ind/m2)	53	79
Reproductive shoot height (cm)	76.1±14.3	89.3±11.5
Area change (%)	↑22.9±5.6	0
Number of seeds / reproductive shoot	31.2±8.7	38.9±6.9

Coverage change (%)	↑41.6±8.3	↑56.7±5.6

Fig. 3 The transplanted eelgrass in the intertidal reef in June 2019

1.4 Land-Based Systems for Eelgrass Culture

1.4.1 Establishment of the Land-Based Culture Systems

In March 2019, we began the construction of the culture systems. The location was selected at the south of Chudao, in Sanggou Bay. The culture system includes: the main body green house, culture pool, water supply system, and drainage system. Pool size: 1.4 m×2.0 m×0.9 m, a total of six pool were constructed. The water supplying the system was pumped from the Nanni Bay, with the annual average temperature of 12.4℃.

Fig. 4 Establishment of the land-based system for eelgras culture

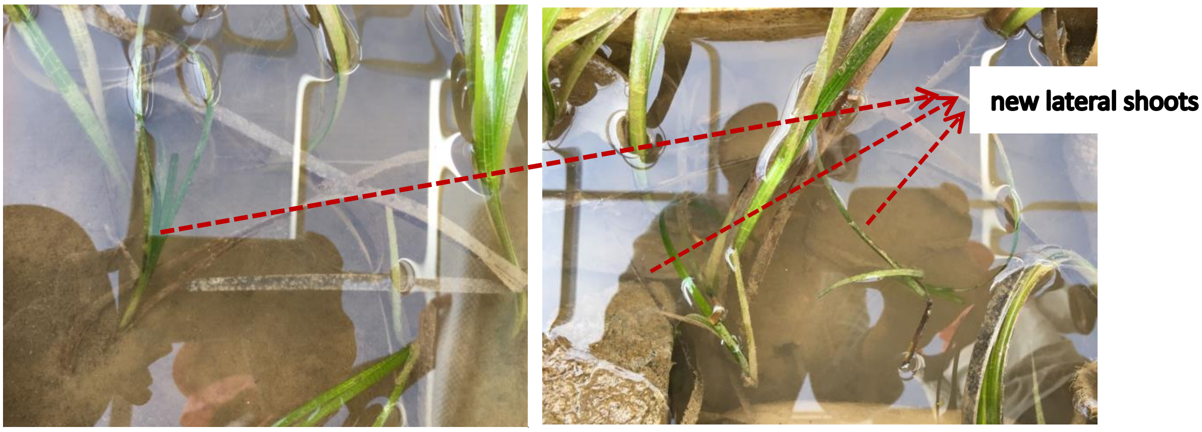
1.4.2 Development of adult shoots culture method:

Source of bottom material: silty sand from the land. Method: eelgrass shoots were collected from Sanggou Bay, the sediment were washed, and shoot with 3-5 rhizome node were put in plastic box/ basket with the silty sand from the land. 6 shoots were put in each box.The number of shoots, the height and width of the leaves were measured every month.

Results

Preliminary results showed that after one month, eelgrass showed significant growth with a survival rate of 89.4% and an average of 1.57 new plants per box.

	Cultured (June)	Cultured (July)	August
Plant height (cm)	50.2±3.6	43.5±5.6	41.5±6.2
Blade width (mm)	4.62±0.52	4.34±0.87	4.31±0.65
Production rate of new shoots	-	21.0±12%	57.1±13.4%



1.5 Expanding of adults shoots in aquaculture pond

Fig.4 Seagrass transplant in coastal sea cucumber pond for shoots expanding

In June 2018, 8000 eelgrass shoots were transplanted to a costal sea cucumber pond. Through the expanding of seagrass shoots in sea cucumber pond, more material were obtained (~12000 shoots) for transplanting in the sea and less destroy for the donor meadows.

2. Conclusion and prospects

Important progress has been made in this project. We established seed sorting technology, seed germination promoting technology, improved transplanting technology, land-based culture technology and culture expansion technology. The above technologies will provide important technical support for the restoration and reconstruction of seagrass in the future. From Oct. 2017 to Aug. 2019, the project complete the transplant of eelgrass in the Chudao area and established a demonstration. A total of 10000 eelgrass shoots were cultured or transplanted. The transplanted eelgrass patches increase the biodiversity by 5 more species, compared to the naked surrounding sandy or muddy patches.

In addition to our research, the improved transplantation of eelgrass has also been carrying out by the research team of Pro. Zhang from Ocean University of China using staple method in northern lagoons and by Pro. Liu from Institute of Oceanology, Chinese Academy of Science, using the method of rhizomes bound to a small elongate stone with bio-degradable cotton thread in Qingdao.

There is also a large area of seagrass distribution along the coast of Korea. The importance of seagrass is not only reflected in its ecological functions, but also important cultural values. Both China and South Korea have seagrass culture, which is also an important aspect of our country's seagrass protection and research.

Our report aimed to improve the transplanting technology and develop sustainable way for eelgrass transplant without destroy and disturbance of the present meadows. There is still much work need to be carried out for the aim and this report is just the began of the innovative work. The success of seagrass transplantation is closely related to the conditions of marine environment. Terrestrial pollution and human activities have a great impact on the transplantation and restoration of seagrass. Under the current continuous decline of seagrass in China, the improvement of marine environment and seagrass transplantation and culture techniques is both the key to the ecological functions of seagrass in coastal environment. What should be pointed out is that the Chinese government has stepped up efforts to rectify the environment and the overall coastal environment is developing in a good direction. We can foresee that the seaweed distribution will be greatly restored by the combination of improved transplantation methods and better environmental conditions.