Study on New Seagrass Bed at the Coastal Area of Yellow River Estuary in Dong Ying

Ruijia Zhou1, Sheng Zhao1,*, Donghui Xu1, Shuai Song2 and Xiaoyang Jian1
1North China Sea Environmental Monitoring Center, State Ocean Administration, Qingdao, China
2North China Sea Marine Technical Support Center, State Ocean Administration, Qingdao, China

*Corresponding author e-mail: zhaosheng@ncs.mnr.gov.cn

Abstract. Seagrass resource is an important part of the Marine ecosystem, eelgrass resources worldwide recession received extensive attention of the world. Through the tracking of Yellow River Estuary in Dong Ying, we found a piece of seaweed bed, new types of seaweed for short eelgrass (Zostera japonica Asch. et Graebn), which are mainly distributed in the intertidal zone. During the investigation, seaweed cover increases by 0.54 km² to 0.54 km². In the year to August 2015, the biomass is 322.13 g/m², stem branch density of 794 plants/m². The rise and fall of seaweed bed is closely related to the water environment changes. Through the study of multiple environmental factors influence on short eelgrass life state, we found that salinity has enormous influence on the growth of eelgrass. In the five salinity conditions which the temperature is 20 ºC, the net oxygen production and the activity of ribulose-1, 5-diphosphate carboxylase were the highest when the salinity was 19.459, and the photosynthesis intensity decreased when the salinity was higher or lower than this value. According to the survey, from 2002 to 2014, the inflow of the Yellow River Estuary increased from less than 5 billion cubic meters to nearly 30 billion cubic meters. Therefore, the increase of the sea water amount of the Yellow River and the decrease of the salinity in the estuarine waters are the important reasons for the growth of the seagrass bed.

1. Introduction

Seagrass is a monocotyledonous plant that lives in the shallow tropical and temperate waters. It can grow completely in the submerged environment with a complete system of root, stem and leaf. Zostera marina L is one kind of seaweed, which belongs to biogenic order, Potamogeton family and short eelgrass. It is mainly distributed in the coastal waters of temperate zone. This kind of seaweed generally grows on the muddy or sandy sea floor, especially in shallow water bay or estuary where the water flow is slow and the transparency of sea water is high [1]. In the coastal domain of Shandong Peninsula, there were abundant resources of short eelgrass in history. In recent decades, due to the changes of natural environment and the influence of human factors, the resources of short eelgrass in the world are in constant decline [2]. Especially in the Yellow River Estuary, the high sediment
concentration and the deterioration of the environment lead to the poor light transmittance of the sea water, which leads to the serious reduction of the distribution domain of Chlorella in this domain.

In this paper, we found and tracked the new seagrass bed in the Yellow River Estuary of DongYing in the summer of 2010 which displayed the distribution shape changed from the patch to the interconnected large-scale, and its density and distribution range were significantly expanded. We speculated that the formation of seagrass bed was related to the increase of the amount of the Yellow River entrance runoff in recent years [3]. Therefore, we investigated the biomass and distribution of the short eelgrass, classified and identified the samples based on its sequence and studied the physiological response of the short eelgrass to temperature and salinity in order to provide reference for further research and environmental assessment.

2. Method

2.1. Investigation and collection of short eelgrass
The survey was conducted from August 2010 to August 2018. The investigation was carried out in the low tide period in order to find more seaweed. The site was located in the Yellow River estuary domain of DongYing (central coordinate, 119°05'58"E, 37°50'29"N) with an domain of about 1km². In addition, we used satellite images to analyze the distribution domain change of short eelgrass in the whole Yellow River Estuary from 2001 to 2014.

2.2. Species identification of short eelgrass
We extracted the genomic DNA of samples which collected in the Yellow River Estuary of Dongying in May 2018(119°05'58"E, 37°50'29"N) using the Tiangen plant genomic DNA extraction kit. The primers, PCR reaction system and reaction conditions were refered to Kollipara's method [4]. PCR amplified products were detected by 1% agarose gel electrophoresis and positive clones were sent to Shanghai Sunny Company for sequencing. The sequence was compared with blast detection in GenBank database, and the species name, landing number and similarity rate of the most similar sequence were obtained.

2.3. Experiment on the response of short eelgrass to salinity
We used a quantitative frame of 25 cm × 25 cm to collect the samples which depth was 15 cm and we collected creeping roots, stems and leaves of all seaweeds in the column. Then we raised the samples in the incubator with light intensity of 2200lux, the temperature of 23℃, the salinity of 31.128, and the inflated air.

After 72hours, we divided samples into six groups and cultured them in seawater with gradient salinity (the percentage concentration of cultured seawater was 0%, 20%, 40%, 50%, 60%, 80%, and 100%). Then we measured the oxygen production of photosynthesis and the oxygen consumption of respiration using black and white bottle method [5].

We set up three parallel experiments in each group and measured the dissolved oxygen content in the culture water using black and white bottle method and obtained the respiration / photosynthesis intensity every two hours. In addition, we used enzyme-linked immunosorbent assay to determine the activity of ribulose-1, 5-diphosphate carboxylase in each group.

3. Discussion

3.1. Growth of Short Eelgrass
We analyzed the distribution of Chlorophyta by extracting the satellite images of the Yellow River Estuary from 2001 to 2018. The results showed that there was no short eelgrass in 2001. However, in 2003, there were two domains of short eelgrass. The central coordinates were 119°05'58"E, 37°50'29"N, 119°09'19"E, 37°48'15"N. We named them as monitoring domain 1 and monitoring domain 2, with an domain of 0.44 km² and 0.10 km².
Figure 1. The distribution of short eelgrass in the ecological domain of the Yellow River Estuary

During 2003-2018 there have been short eelgrass in this domain (except for domain 2 in 2005) and the domain has increased from 0.54km² in 2003 to 3.06km² in 2018.

Figure 2. The remote sensing monitoring of short eelgrass
The results of remote sensing monitoring were shown in Table 1. We found that there were no short eelgrass in 2001, four years with an domain of about 1km² or less, one year with an domain of about 1.5km² and three years with an domain of about 3km². The overall change trend of the growth domain of short eelgrass was shown in Fig.3. Except for the decrease of the domain in 2012 and 2016, the trend in other years was basically the same.

### Table 1. Results of remote sensing monitoring

| Time (year) | Monitoring domain | Coordinates of Center Point | Acreag (km²) | Total (km²) |
|-------------|-------------------|-----------------------------|--------------|-------------|
| 2001        | All               | Not found                   | Not found    | 0           |
| 2003        | Domain 1          | 119°05'58",37°50'29"       | 0.44         | 0.54        |
|             | Domain 2          | 119°08'19",37°48'48"       | 0.10         |             |
| 2005        | Domain 1          | 119°05'58",37°50'29"       | 0.59         | 0.59        |
|             | Domain 2          | 119°09'19",37°48'15"       | 1.00         | 1.15        |
| 2007        | Domain 1          | 119°05'58",37°50'29"       | 0.77         | 1.76        |
|             | Domain 2          | 119°08'56",37°48'17"       | 0.99         |             |
| 2010        | Domain 1          | 119°05'58",37°50'29"       | 0.71         | 1.21        |
|             | Domain 2          | 119°09'23",37°48'13"       | 0.50         |             |
| 2012        | Domain 1          | 119°05'58",37°50'29"       | 1.53         | 3.39        |
|             | Domain 2          | 119°08'54",37°48'16"       | 1.86         |             |
| 2014        | Domain 1          | 119°05'58",37°50'29"       | 0.96         | 2.83        |
|             | Domain 2          | 119°08'54",37°48'16"       | 1.87         |             |
| 2016        | Domain 1          | 119°05'58",37°50'29"       | 1.64         | 3.06        |
|             | Domain 2          | 119°08'54",37°48'16"       | 1.42         |             |

**Figure 3.** The overall change trend of the growth acreag of short eelgrass

### 3.2. Species identification of short eelgrass

In this experiment, we carried out the molecular identification based on ITS sequence. We selected eight plants from two groups of samples randomly, determined and compared their ITS sequences. The results showed that the similarity rate of ITS sequences of the two groups was over 99% which proved that they belong to the same species. Combined the blast comparison analysis of ITS sequence in GenBank database and the analysis of its morphological characteristics, we determined that the newly discovered seagrass bed in Domain 1 and Domain 2 were short eelgrass.
3.3. Effect of salinity on short eelgrass

We used black-and-white flask to measure the oxygen production of photosynthesis and the oxygen consumption of respiration and designed concentration gradients of 0%, 20%, 40%, 50%, 60%, 80% and 100% which represented actual salinity of the gradients were 0.54, 8.25, 12.67, 15.78, 19.46, 24.82 and 31.13.

Figure 5. Effect of seawater with different salinity on oxygen production / consumption of short eelgrass

The results showed that the intensity of photosynthesis and respiration of short eelgrass changed in inverted U-shape with the increase of seawater salinity. When the seawater concentration was 50% (salinity was 15.78), the net oxygen production of short eelgrass was the highest.

4. Conclusion

Historical data showed that there were abundant short eelgrass in the intertidal zone of Shandong Province in the depth of 2-5m before 1980's, and now it has been degraded and depleted. The seagrass bed found has developed from 0.54km² in 2003 to 3.05km² with an increase of 564.8%. The rapid increase area coincides with the increase flow of the Yellow River.
The black-and-white bottle and the activity experiment of ribulose-1,5-diphosphate carboxylase proved that environment with the high salt and low salt are not suitable for the growth of short eelgrass. When the salinity is in the range of 15-20, the growth is favorable. The results explained that the distribution range of short eelgrass increased rapidly with the increase of the amount of the Yellow River flowing into the sea since 2003.

Figure 6. The relationship between runoff volume of the estuarine area and the distribution acreag

Seagrass beds are important to coastal ecosystems. They can not only provide important habitats for marine organisms and food sources for many organisms, but also play an important role in the global carbon, nitrogen and phosphorus cycle. This study proved that the stable runoff in the Yellow River estuary was an important condition for the growth of seagrass bed, and provided a new idea for the management department to protect the fragile ecosystem.

Acknowledgments
This work was financially supported by Study on early warning and three-dimensional monitoring technology of green tide of Enteromorpha prolifera in the Yellow Seafund (2016YFC1402103).

References
[1] Jong-Hyeob Kim, Seung Hyeon Kim, Young Kyun Kim, Jung-Im Park, Kun-Seop Lee. Growth dynamics of the seagrass Zostera japonica at its upper and lower distributional limits in the intertidal zone [J]. Estuarine, Coastal and Shelf Science, 2016, 175.
[2] Xiaomei Zhang, Yi Zhou, Peng Liu, Feng Wang, Bingjian Liu, Xujia Liu, Hongsheng Yang. Temporal pattern in biometrics and nutrient stoichiometry of the intertidal seagrass Zostera japonica and its adaptation to air exposure in a temperate marine lagoon (China): Implications for restoration and management [J]. Marine Pollution Bulletin, 2015, 94 (1-2).
[3] R.J. Wasserman, A.K. Whitfield, S.H.P. Deyzel, N.C. James, S. Hugo. Seagrass (Zostera capensis) bed development as a predictor of size structured abundance for a ubiquitous estuary-dependent marine fish species [J]. Estuarine, Coastal and Shelf Science, 2020, 238.
[4] Kollipara K P, Singh R J, Hymowitz T. Phylogenetic and genomic relationships in the genus GlycineWilld. Based on sequences from the ITS region of nuclear rDNA. Genome, 1997, 40 (57-68)
[5] D. A. Neitzel, T. L. Page, E. G. Wolf. Multiple Bottle Holder for in Situ Measurement of Primary Productivity [J]. D. A. Neitzel; T. L. Page; E. G. Wolf, 2011, 41 (1).