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Full Length Research Paper

Annual change in photosynthetic pigment contents of *Zostera marina* L. in Swan Lake

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This study investigated the annual change in photosynthetic pigment contents of *Zostera marina* L. in Swan Lake from April 2009 to March 2010. Photosynthetic pigment of *Z. marina* L. was extracted using the soaking method and measured spectrophotometrically. The maximum value of total chlorophyll content observed was 2.40 ± 0.034 mg/g in October, while the minimum was 1.22 ± 0.028 mg/g in June. On the other hand, maximum chlorophyll a content was 1.58 ± 0.020 mg/g in October and the minimum was 0.76 ± 0.036 mg/g in March. The highest and lowest chlorophyll b content was 0.83 ± 0.016 mg/g in October and 0.40 ± 0.005 mg/g in January, respectively. Finally, carotenoid was highest at 0.39 ± 0.006 mg/g in January and least at 0.13 ± 0.004 mg/g in April. Based on seasonal analysis, chlorophyll content was higher during the late-autumn and early-winter, and lower in the summer; while carotenoid content was higher during the late-autumn and winter, and lower in early-spring and late-summer.

Key words *Zostera marina* L., Swan Lake, photosynthetic pigment, annual change.

INTRODUCTION

The eelgrass (*Zostera marina* L.) is one of the advanced flowering plants that belong to Potamogetonaceae. This seagrass is a cosmopolitan species, and is distributed mainly in temperate maritime locations, including the North Atlantic and Pacific area (Den Hartog, 1970). In China, eelgrass can be found in the coastal areas of Shandong, Liaoning and Hebei provinces (Yang and Wu, 1981). Eelgrass mainly grows in the infra-littoral zone and sub-tidal zone, and grows well in sand-mud sediment, especially in the shallow sea or estuary with low water flow and high transparency. Usually, eelgrass forms large communities in shallow waters around the coast or around islands (Sun and Wu, 1992; Ye and Zhao, 2002).

There are two kinds of photosynthetic pigments in higher plants that are distributed in the thylakoid membrane: chlorophyll and carotenoid (Pan et al., 2008). Photosynthetic pigments in leaves could transport and absorb light energy, which acts as the important material base that plants depend on for photosynthesis. The level of photosynthetic pigments is the index of a plant's growth status and the leaves' photosynthetic capacity. Chlorophyll has excellent light absorbability and mainly absorbs the infrared and the blue-violet light. Carotenoid mainly absorbs the blue-violet light, while not being able to absorb infrared light. In addition, carotenoid can decrease membrane lipid peroxidation and protect chlorophyll from irradiance injury when the light compensation point is exceeded (Beer and Waisel, 1979; Beer, 1998; Li, 2006).

The effects of environmental factors on the photosynthesis of eelgrass had been studied well. Dennison and Alberte (1986) reported that photosynthetic and growth responses are primary indices that assess eelgrass response to reciprocal transplantation between different water depth gradients. Light intensity is the primary factor affecting seagrass photosynthesis. When compared with terrestrial plants, the photosynthetic capacity of seagrass is lower than that of terrestrial shade plants (Erftemeijer et al., 1993; Vermaat and Verhagen, 1996; Xu et al., 2007). Marsh et al. (1986) studied the effect of temperature on photosynthesis of eelgrass, and found that net photosynthesis of eelgrass increased with temperature when irradiance is at the light compensation point. Photosynthetic efficiency peaked at 25 to 30°C, but

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Abbreviations: PSII, Photosystem II; **PSI,** photosystem I.

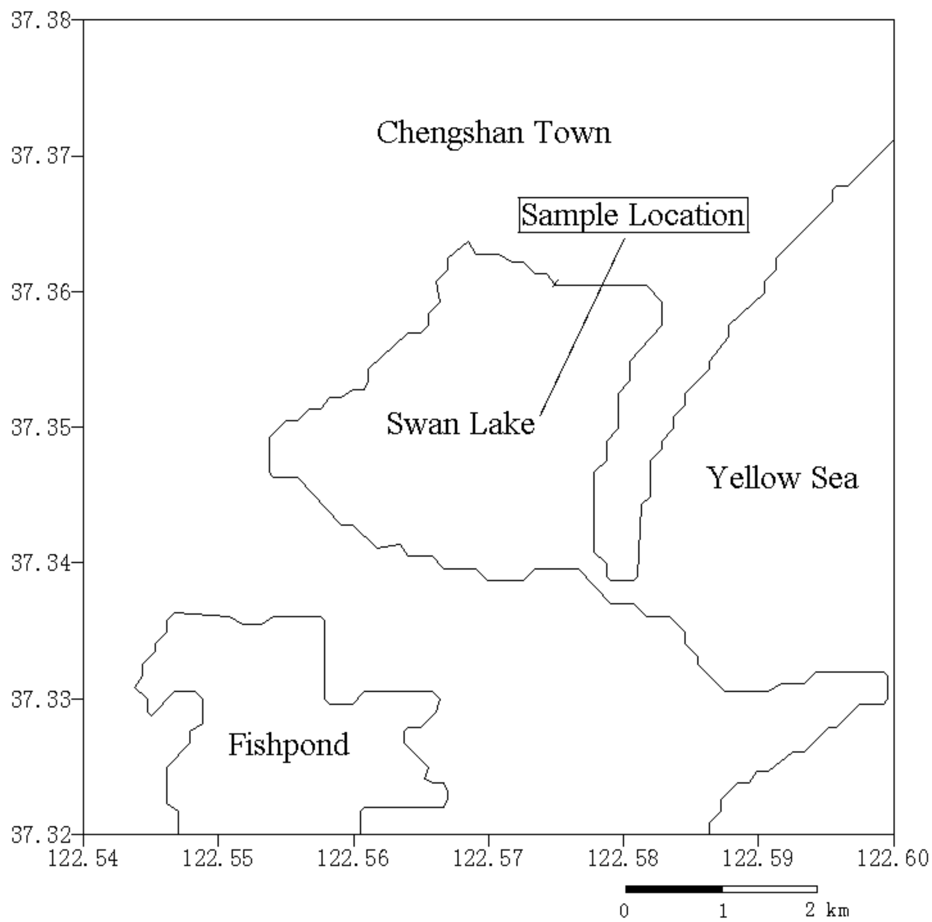

Figure 1. Geographical location of Swan Lake.

started to decrease when temperature reached 35°C. Prasad and Strzalka (1999) reported that Zn, Cd, Hg, Pb and Cu could disturb the biosynthesis of intracellular pigments, therefore leading to decreased chlorophyll content. This study also showed that heavy metal ions could take up the redox potential of photosystem II (PSII) and affect PSII by inhibiting enzyme activity and photosynthetic phosphorylation of seagrass. Several studies proved that algae can influence the photosynthesis of seagrass. In low light, algae are more competitive than seagrass because they have the advantages of simple structure, low respiration consumption and low light compensation point. In addition, eutrophication leads to increased epiphytic algae on the leaf of seagrass, which would in turn reduce the area of seagrass photosynthesis (Sand-Jensen, 1977; Mazzella and Alberte, 1986). Dennison (1987) research showed that the total chlorophyll content of eelgrass varies with season changes. Total chlorophyll content is highest in the winter and lowest in the summer and autumn. Gross photosynthesis peaks in late-summer, but net photosynthesis peaks in spring (May) due to high respiration rates at summer temperatures (Dennison, 1987). In this study, we investigated the annual change of photosynthetic pigment contents of eelgrass in Swan Lake and hope to give a valuable theoretical reference to the physiology of eelgrass photosynthesis.

MATERIALS AND METHODS

Study site and sampling period

Samples were collected monthly from the Swan Lake in Rongcheng City, Shandong Province of China from April 2009 to March 2010. Figure 1 shows the sampling location. Swan Lake is located in southeast Chengshan Town of Rongcheng City (N37.3382°-37.3588°, E122.5551°-122.5793°). It covers an area of 4.8 km^2. Swan Lake is a typical tidal inlet system and an entrance channel, 86 m wide and connects the tidal basin with the open sea (Xue et al., 2002; Jia et al., 2003).

Sample collection and treatment

30 samples were collected monthly for photosynthetic pigment measurements. Fresh, healthy leaves with no visible epiphyte colonization were selected randomly. Samples were cleaned by gauze, wrapped in tinfoil, put into a 10 ml centrifuge tube, and preserved in a liquid nitrogen jar. Tests and analyses were carried out in the laboratory.

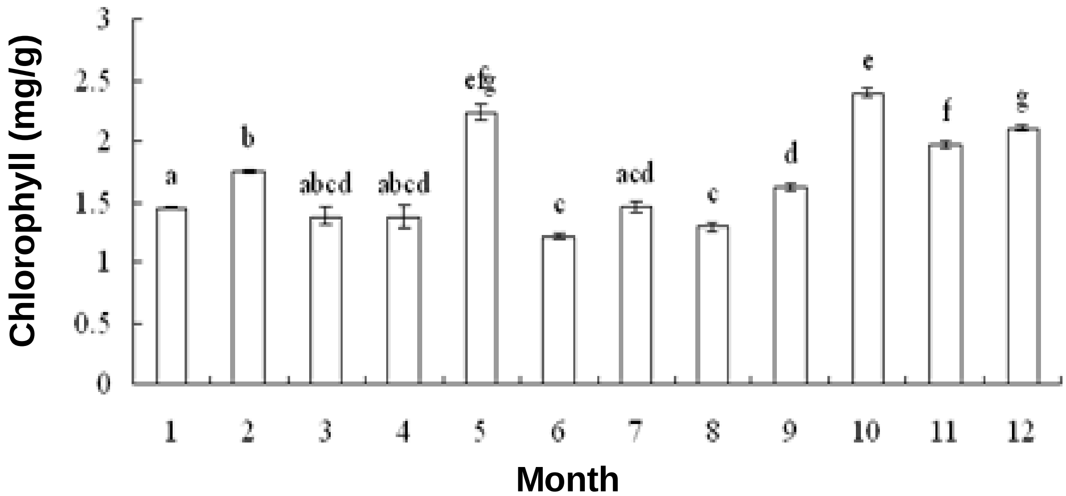

Figure 2. Annual change in total chlorophyll content of *Z. marina* in Swan Lake. Bars are mean values (n = 30) and error bars represent S.E. of the means. Different letters above error bars indicate significant differences ($P < 0.05$).

Determination method

Photosynthetic pigments were extracted in 95% alcohol using the soaking method and then measured spectrophotometrically (Xiao and Wang, 2005). Absorbance at 665, 649 and 470 nm of the resulting solution was measured with the UV spectrophotometer. The formulae used were as follows:

Chlorophyll a = $(13.95D_{665} - 6.88D_{649})V/M/1000$

Chlorophyll b = $(24.96D_{649} - 7.32D_{665})V/M/1000$

Carotenoid = $(4.08D_{470} - 11.56D_{649} + 3.29D_{665})V/M/1000$

Total chlorophyll = chlorophyll a + chlorophyll b

Where, D represents the absorbed optical density (g/ml); V represents the volume of extract (ml); and M represents the fresh weight of leaves (g). Photosynthetic pigment content was measured in mg/g.

Statistical analysis

Data were statistically analyzed using Excel and SPSS13.0 software. Statistical significance was set at P<0. 05.

RESULTS

Total chlorophyll content

Maximum total chlorophyll content was 2.40 ± 0.034 mg/g in October, while the minimum was 1.22 ± 0.028 mg/g in June. Total chlorophyll was higher in late-autumn and lower in the summer. It gradually increased from January, peaked in May, and then gradually decreased. In September, it began to increase again and became stable in October. This trend is presented in Figure 2.

Chlorophyll a content

Maximum chlorophyll a content was 1.58 ± 0.020 mg/g in October, while the minimum was 0.76 ± 0.036 mg/g in March. Chlorophyll a was higher in the winter and lower in the summer. The change trend was similar to that of total chlorophyll content, as shown in Figure 3.

Chlorophyll b content

The highest and lowest chlorophyll b content was 0.83 ± 0.016 mg/g in October and 0.40 ± 0.005 mg/g in January, respectively. Chlorophyll b was higher during the winter and the spring and lower in the summer. It gradually increased from January, peaked in May, and then gradually decreased. In September, it began to increase again, had another peak value in October, and then decreased afterwards. This trend is illustrated in Figure 4.

Carotenoid content

Finally, carotenoid content was highest at 0.39 ± 0.006 mg/g in January and lowest at 0.13 ± 0.004 mg/g in April. The trend of annual change in carotenoid content differed greatly from that of chlorophyll. It showed a tendency to be higher during the late-autumn and winter, but lower in early-spring and late-summer. It peaked in January, decreased gradually, and reached the lowest point in April. In August, it reached another low point and began to increase in September. This trend is presented in Figure 5.

DISCUSSION

Photosynthetic pigments play an important role in plant

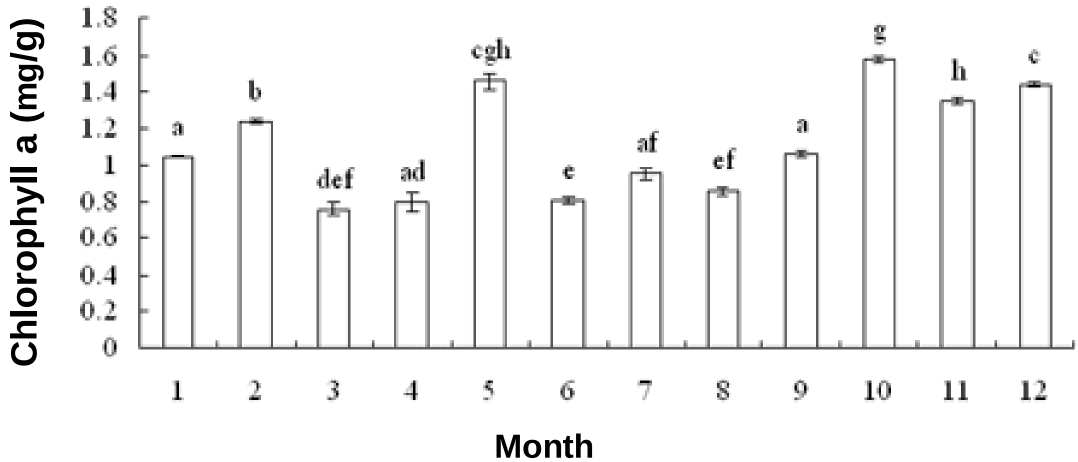

Figure 3. Annual change in chlorophyll a content of *Z. marina* in Swan Lake. Bars are mean values (n = 30) and error bars represent S.E. of the means. Different letters above error bars indicate significant differences ($P < 0.05$).

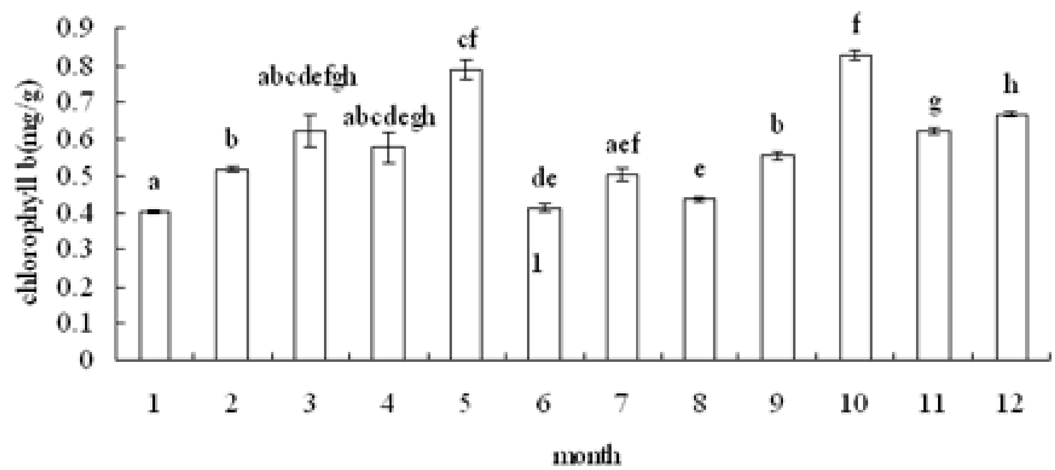

Figure 4. Annual change in chlorophyll b content of Z. marina in Swan Lake. Bars are mean values (n=30) and error bars represent S.E. of the means. Different letters above error bars indicate significant differences (P<0.05).

photosynthesis, which involves the absorption and utilization of light energy. In fact, photosynthetic pigments are the foundation of plant photosynthesis. The levels of photosynthetic pigments reflect the status of photosynthesis capability and are important index of plant physiologic characteristics (He et al., 2009). It has been found that illumination is one of the primary factors influencing plant photosynthesis. After the photoreduction reaction, the protochlorophyllide becomes transformed to chlorophyllide. Strong light stress and excess light energy can trigger the photooxidation reaction of oxygen and chlorophyll. Hypoxia can induce the accumulation of Mg protoporphyrin IX and Mg protoporphyrin methyl ester, so that chlorophyll cannot be synthesized. Almost all processes in chlorophyll biosynthesis require the participation of enzymes. Temperature affects enzyme activity, and thus affects chlorophyll synthesis. Mineral elements also have a great effect on chlorophyll synthesis. Chlorosis refers to the inability to synthesize chlorophyll and can occur when there is a lack of mineral elements, such as N, Mg, Fe, Mn, Cu and Zn. N and Mg are constituents of chlorophyll, while Fe, Mn, Cu and Zn are enzyme activators that have an indirect effect on chlorophyll synthesis (Pan et al., 2008). When a plant is subjected to the influence of environmental factors (light, temperature and mineral elements), the physiologic process will be disturbed: water in cells will be unbalanced, membrane system structure will be injured, harmful metabolites will accumulate, protein synthesis will decrease and so on. These effects will directly or indirectly affect photosynthetic pigment content, and thereby influence plant photosynthetic performance

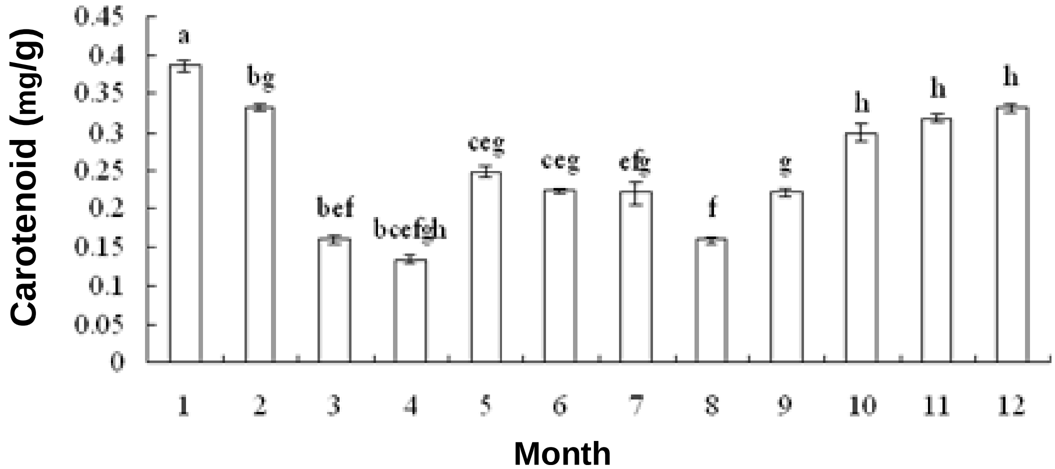

Figure 5. Annual change in carotenoid content of *Z. marina* in Swan Lake. Bars are mean values (n = 30) and error bars represent S.E. of the means. Different letters above error bars indicate significant differences ($P < 0.05$).

(Wang et al., 2009).

Annual change in chlorophyll content of *Z. marina* L. in Swan Lake

This study found that chlorophyll a, chlorophyll b and total chlorophyll contents of eelgrass in Swan Lake were higher in late-autumn and early-winter and lower in summer, which is consistent with the results reported by Dennison (1987). In addition, Dennison (1987) showed that net photosynthesis of eelgrass peaked in May, and the same was concluded in this study. Summer is the season with the strongest light illumination and the highest temperature, and is also the period when plants grow most vigorously. In summer, eelgrass leaves grow fast and leaves in surface water become exposed to strong light illumination, thus leading to the photooxidation reaction of oxygen and chlorophyll. Furthermore, high temperature stress will decrease the biosynthesis of the intermediate products of chlorophyll synthesis: 5-aminolevulinic acid and protoporphyrin IV. At the same time, high temperature stress will increase the active oxygen in eelgrass, which will lead to membrane lipid peroxidation and decreased chlorophyll content. Marsh et al. (1986) reported that net photosynthesis of eelgrass increased with temperature when irradiance reached the light compensation point. Photosynthetic efficiency peaked at 25 to 30°C, but started to decrease when temperature reached 35°C. In our study, eelgrass chlorophyll content was higher in May and October when the temperature in Swan Lake was between 15 and 19°C. However, the highest temperature in Swan Lake was about 25°C in August. Thus, the annual change in chlorophyll content of eelgrass in Swan Lake was different from the trend reported by Marsh et al. (1986). This may be attributed to the possible differences in physiological characteristics of eelgrass in various sea areas.

Annual change in carotenoid content of *Z. marina* L. in Swan Lake

Carotenoid exists in the chloroplasts and functions in light energy absorption and transformation. In addition, carotenoid can prevent chlorophyll injury due to excess light energy and is related to PSI and PSII in photosynthesis. It can prevent the outward transfer of the excited energy of chlorophyll molecules, while being able to protect excited chlorophyll molecules from photo-oxidative damage (Yu and Tang, 1998). In this study, the carotenoid content of *Z. marina* L. in Swan Lake was higher in late-autumn and winter. This is because low temperature stress may trigger the overproduction of reactive oxygen species and decrease antioxidant enzyme activities. If so, the non-enzymatic antioxidative system, which consists of ascorbic acid, carotenoid, glutathione and others, will eliminate the reactive oxygen species through direct and indirect mechanisms (Wang et al., 2009). Therefore, low temperature stress can lead to increased carotenoid content. In addition, more red light and less blue light are absorbed by the seawater, while the penetrability of the latter is stronger than the former (Ross, 1984). Winter is the season with the weakest light illumination. Carotenoid will increase in order to increase absorption of blue light for photosynthesis, thus leading to higher carotenoid content in winter. In contrast, early-spring brings increased temperature and stronger light illumination, therefore, leading to decreased carotenoid content.

In conclusion, this study found obvious seasonal changes in the photosynthetic pigment contents of *Z. marina* L. in Swan Lake. Chlorophyll content was higher during the late-autumn and early-winter, but lower in summer. In contrast, carotenoid content was higher during the late-autumn and winter, but lower in early-spring and late-summer. The findings of the research will improve the current knowledge on the physiology of photosynthesis in *Z. marina* L.

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