Phenology and morphology of the marine phanerogam *Zostera caulescens* Miki in Funakoshi Bay, northeast Honshu Island, Japan

Soha Shabaka and Teruhisa Komatsu

Ocean Research Institute, The University of Tokyo, 1–15–1, Minamidai, Nakanoku, Tokyo 164–8639, Japan
E-mail: soha@ori.u-tokyo.ac.jp

Abstract — *Zostera caulescens* Miki is distributed around the Honshu Island, Japan, and a threatened species. Its population in Funakoshi Bay on the Sanriku Coast, northeast Honshu Island, is known to be the world's longest seagrass. However, its ecological and morphological characteristics have not been well studied. Therefore, bimonthly sampling of its flowering and vegetative shoots together with its rhizomes and roots was conducted along a depth gradient in Funakoshi Bay to examine its phenology and morphology including flower structures. *Zostera caulescens* in Funakoshi Bay showed different morphology from those in central Honshu Island and the Korean Peninsula. The Leaf apex shapes were characterized by two forms: mucronate and emarginate. This study discovered that of flowering shoots was changed from an emarginate form in summer to a mucronate one in autumn and winter. Biomass and leaf area index (LAI) of flowering shoot showed higher values in summer and lower values in autumn and winter. Shoot density, biomass and LAI of flowering and vegetative shoots were lower at deeper stations. Number of flowers per flowering shoot and number of fruits per flower in Funakoshi Bay was less than in central Honshu Island. New recruits of vegetative and immature flowering shoots were observed in winter.

Key words: morphology, phenology, seagrass, shoot dynamics, *Zostera caulescens*

Introduction

Most Japanese seagrasses belong to the family Zosteraceae, and consist of *Zostera asiatica* Miki, *Zostera caespitosa* Miki, *Zostera caulescens* Miki, *Zostera marina* L., *Zostera japonica* Asch. et Graebn., *Phyllospadix iwatensis* Makino, and *Phyllospadix japonicas* Makino (Miki 1933, Aioi 1998). *Zostera caulescens* is endemic to Japanese and Korean coastal waters (Shin and Choi 1998). In Japan, *Z. caulescens* is distributed between Moura Bay at the northern tip of Honshu Island (Omori 1992) and Sagami Bay in central Honshu Island (Omori 1994).

Zostera caulescens is a dominant species in Funakoshi Bay on the Sanriku coast facing the Pacific Ocean and is known to be the world's longest seagrass due to flowering shoots reaching 7 m in length (Aioi et al. 1996, 1998, Sultana and Komatsu 2003). This species is listed in the Red Data Book of threatened marine flora in Japan (Wildlife Division, Agency of the Environment, Japan 2000), and several studies have examined its biology and morphology (Omori 1989, 1991, 1992) and its distribution (Tatsukawa et al. 1996, Komatsu et al. 2003 Sagawa et al. 2008). However, previous studies did not examine morphology and phenology such as

recruitment of flowering and vegetative shoots. The importance of *Z. caulescens* forests to the temperate coastal waters of Japan together with their threatened existence requires precise examination of its seasonal dynamics, morphology, and recruitment in Funakoshi Bay on the Sanriku coast. This study aims to provide details on the phenology and morphology of *Z. caulescens* along a depth gradient over the course of a year.

Materials and Methods

Study area

Funakoshi Bay is located on the Sanriku coast of northeast Honshu Island and opens to the Pacific Ocean in a wide mouth with the maximum depth of 30 m (Fig. 1). Three seagrass species have been observed from the southern part of the bay (Shibatanai and Komatsu in preparation), including *Z. caulescens* in a wide bed extending from depths of about 3 to 15 m (Sultana and Komatsu 2003).

Sampling methods and measurements of environmental parameters

Bimonthly sampling was carried out from June 2007 to
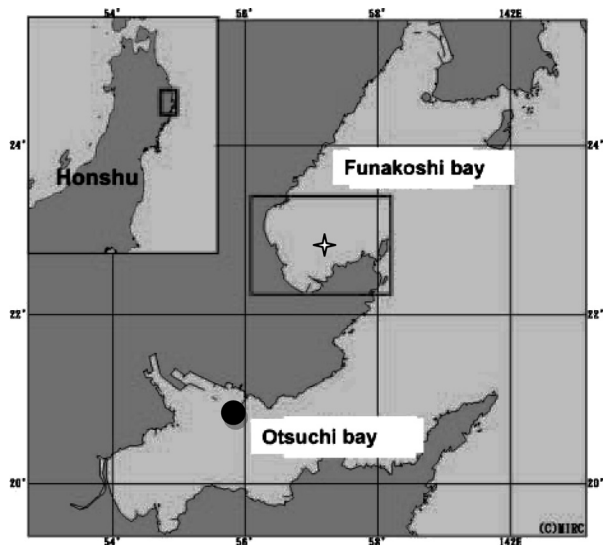
Fig. 1. Map showing the study area in Funakoshi Bay, the STD station (star), and automatic weather station in Otsuchi Bay (closed circle).

June 2008: on 30 June, 11 August, 7 October, 30 December 2007, 26 January, 25 April, and 21 June 2008. We established four stations, Stn. 1 at 6 m deep, Stn. 2 at 9 m deep, Stn. 3 at 12 m deep and Stn. 4 at 15 m deep, to cover the range of depths across which *Z. caulescens* is distributed in the bay (Fig. 1). At every station, plants were collected from three quadrats (0.04 m^2) randomly set around the station in each sampling month except in June 2007 (two quadrat samples) by SCUBA diving. In every quadrat, both above-ground parts (vegetative and flowering shoots) and below-ground parts (rhizomes and roots) of the seagrass were collected. Samples were frozen to preserve them for later laboratory measurement. Vertical profiles of water temperature and salinity were taken at Stn. 3 using a salinity-temperature-depth instrument (Model TCDKU, Alec Co.). Solar radiation reaching the sea surface was monitored at 10-min intervals using an automatic weather station (AADI) located on a raft in Otsuchi Bay neighbouring Funakoshi Bay (Fig. 1). Solar radiation data from June 2007 to June 2008 were compiled.

Morphological parameters

Shoot length, (*L*) and width (*W*) of all leaves in every shoot were measured using a scale with millimetre precision. Shape of leaf apex, number of leaves per shoot, number of internodes on a stem (vertical rhizomes), thicknesses of horizontal rhizomes, and lengths of internodes (Fig. 2) were observed, counted, and/or measured. Flowers of flowering shoots were counted. Lengths and widths of spathe and spadix attaching to a flower were measured, and number of pistils, anthers and fruits inside the spadix were counted (Fig. 2).

Shoot density and leaf area index

Vegetative and flowering shoots from each sampled quadrat were counted separately. Data of leaf length (*L*) and width (*W*) were used to calculate leaf area index (LAI) by multiplying *L* by *W* for every leaf. The LAI of each shoot was obtained by summing all LAIs of leaves attaching to the shoot. The total LAI was also calculated for each quadrat by summing the LAIs of all shoots in the quadrat.

Biomass

The biomass was measured according to the method proposed by Ott (1990). After immersion of the samples in a solution of 5% HCl for 2 hrs, the epiphytes were removed by scraping with a glass slide. The above-ground and below-ground parts were then dried separately in an oven at 60 °C until they reached constant weight (for about 2 days) and were then weighed on an electronic balance with 0.1 mg precision.

Statistical analyses

Temporal and spatial variations in shoot densities, biomass, and LAI were statistically analysed using statistical software (Statsistica ver. 7, StatSoft Inc.). Two-way analysis of variance (ANOVA) was used to test differences among stations and among seasons by grouping data by sampling date and depth. Before the analysis, the homogeneity of variance of all variables was tested using Leven's test. When significant differences were found by this test, the data were log transformed. When the transformation did not remove the heterogeneity, the analysis was performed on the raw data by setting the *F*-test alpha to 0.01 (González-Correa et al. 2007). When ANOVA showed significant differences, Tukey's *post hoc* tests were conducted to test for *a posteriori* significance differences in means.

Results

Environmental parameters

At Stn. 3, in the deepest layer, the highest temperatures were 18.1 °C in August 2007 and 18.4 °C in October 2007 while the lowest temperature was 5.5 °C in April 2008 (Fig. 3). In the deepest layer, the highest salinities were 34.05 psu in December and 33.90 psu in January 2007 and the lowest salinity was 33.39 psu in April 2008 (Fig. 4). Mean daily solar radiation showed a clear seasonal pattern. Higher radiations were observed from April to August with a peak in June, and lower ones were recorded during autumn and winter with the minima in December and January (Fig. 5).

Morphological description of *Z. caulescens*

Zostera caulescens had a leaf apex with 7 to 9 veins. Leaf apex shapes of vegetative shoots were grouped into two
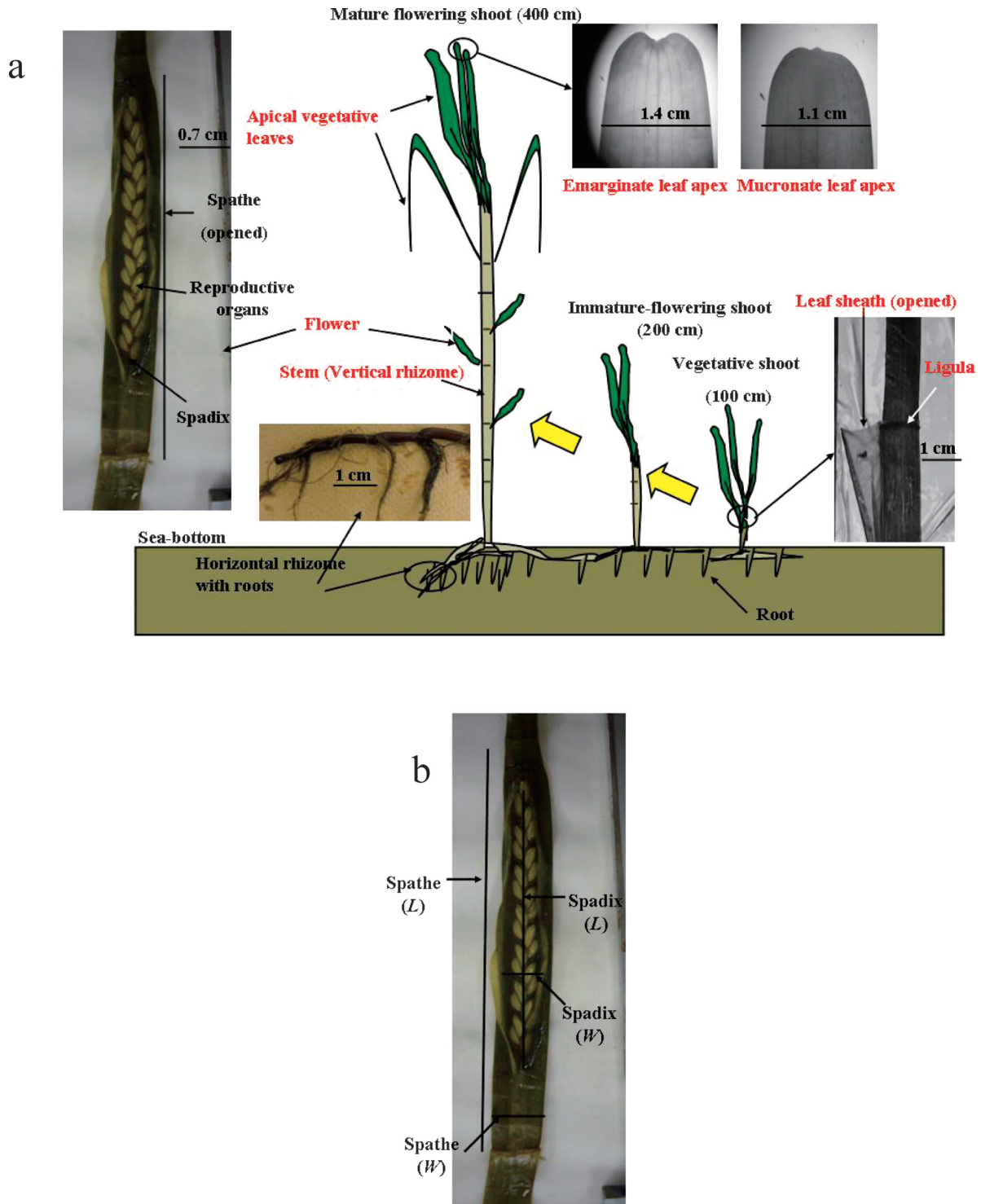
Fig. 2. Diagram showing the structure of *Zostera caulescens* (a), the positions of the measurements taken for the spathe and the spadix (b)

forms: mucronate and emarginate (Fig. 2). The two forms were found among leaves on the same shoot. The mucronate form was dominant (∼>80%) through the year. In June and August, the leaf apexes of the apical vegetative leaves on the flowering shoot were emarginate form while those of all leaves were mucronate form in autumn and winter. In April, all juvenile and intermediate leaves of the apical vegetative

leaves had the emarginated form at the leaf apex while all of adult leaves had the mucronate form.

Numbers of leaves on a vegetative shoot and apical vegetative leaves on a flowering shoot ranged between 3 and 6 with a mean value of 4 and between 3 and 7 with a mean value of 5, respectively. The mean width of vegetative leaves was between 7 and 9 mm. Apical vegetative leaves on the
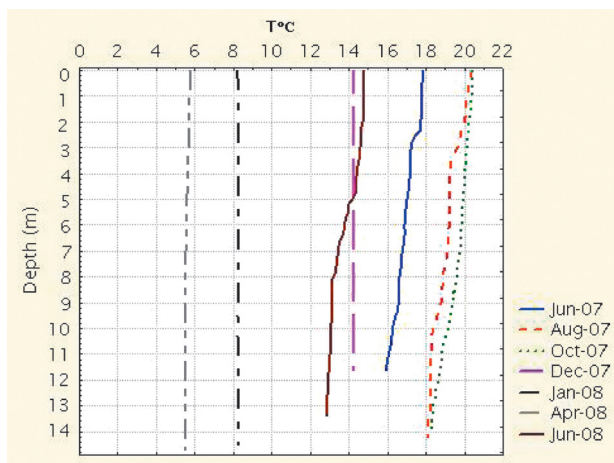
Fig. 3. Vertical water temperature profiles at Stn. 3 for each sampling period.
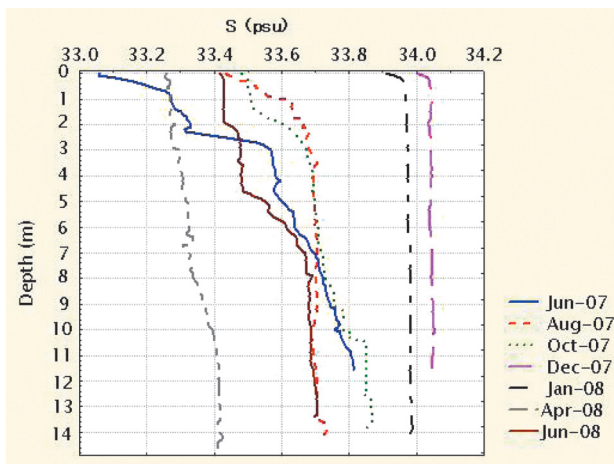
Fig. 4. Vertical salinity profiles at Stn. 3 for each sampling period.
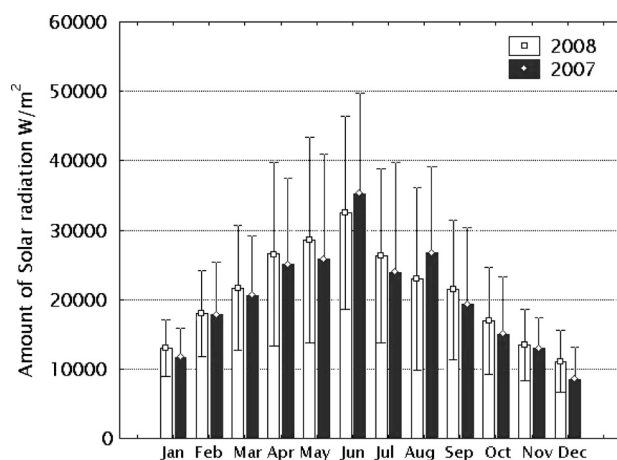
Fig. 5. Mean daily solar radiation reaching the sea surface per month (thick bars) as recorded by the automatic weather station in Otsuchi Bay from 2007 to 2008. Bars indicate standard deviations.

flowering shoot had leaf widths ranging from 12 to 14 mm. The mean number of leaves per shoot and the mean width of leaves did not show a spatial or seasonal trend. The leaves of the vegetative shoots and the apical vegetative leaves were held in a bundle by a leaf sheath with tubular, membranous and closed form, which is a common characteristic among the species of subgenus *Zostera*. The leaf sheaths varied in length between 4 and 28 cm roughly proportional to shoot length. The top of the leaf sheath covered a small leathery ligula which characterizes the family Zosteraceae. The ligula ranged from 2 to 3 mm in length (Fig. 2).

Horizontal rhizomes were creeping and cylindrical bearing several unbranched roots and one shoot at each node. The horizontal rhizomes were 2 to 6 mm thick with internode lengths between 10 and 30 mm. When vegetative shoots developed into flowering shoots, their stems became elongated (Fig. 2). The number of internodes per stem ranged between 1 to 2 internodes in the youngest flowering shoot with a total length less than 1 m and 30 in the oldest flowering shoot reaching more than 5 m long. Internodal intervals were not constant on the same stem ranging from 3 cm in its lower part near the node of the horizontal rhizome to 20 cm in its upper part near the apical vegetative leaves. .

A flowering shoot of *Z. caulescens* consisted of a long stem with flowers and apical vegetative leaves (Fig. 2). Flowers were made up of spathes with spadices carrying reproductive organs and fruits (Fig. 2). Flowering shoots with flowers were observed in June and August 2007, and in June 2008. The mean numbers of flowers per shoot were 3 ± 1 in June and August 2007, and 4 ± 2 in June 2008 with the maximum of 8 in these months. The mean lengths of flowers were 22.22 ± 6.52 cm in June, 16.65 ± 6.13 cm in August 2007, and 24.95 ± 8.72 cm in June 2008. The mean widths of flowers were 0.69 ± 0.12 cm in June, 0.72 ± 0.11 cm in August 2007, and 0.78 ± 0.20 cm in June 2008.

The mean spathe length and width of flowers were respectively 7.89 ± 1.94 cm and 0.69 ± 0.12 cm in June, 7.58 ± 1.41 cm and 0.72 ± 0.11 cm in August 2007, and 8.46 ± 3.26 cm and 0.78 ± 0.20 cm in June 2008. The mean ratios of spathe length to width were 11.32 ± 2.21 in June, 10.56 ± 1.53 in August 2007, and 10.88 ± 3.55 in June 2008.

The mean spadix lengths of flowers were 6.91 ± 1.66 cm in June, 6.46 ± 1.03 cm in August 2007, and 6.15 ± 2.17 cm in June 2008. The mean spadix widths were 0.66 ± 0.13 cm in June 2007, 0.57 ± 0.1 cm in August 2007, and 0.48 ± 0.13 cm in June 2008. The mean ratios of spadix length to width were 10.39 ± 1.97 in June, 11.52 ± 2.09 in August 2007, and 12.73 ± 3.27 in June 2008.

The mean numbers of pistils and anthers per spathe were respectively 11 ± 2 and 21 ± 7 in June 2007 and 11 ± 6 and 24 ± 6 in June 2008. The ratios of pistils to anthers were nearly constant at $1:2$. In August 2007, no reproductive organs were observed in flowers. The mean numbers of fruits

(fertilized ovaries) per spadix in June and August 2007 were 11 ± 2 and 5 ± 3, respectively. The maximum number of fruits per spadix was 14 in June 2007. No fruits were observed in June 2008.

Seasonal changes in shoot density

Two-way ANOVA showed that the mean densities of flowering shoots had significant temporal ($F_{6, 80}=4.413$, $p<0.01$) and spatial ($F_{3, 80}=7.879$, $p<0.01$) variations while those of vegetative shoots had only a significant spatial variation ($F_{3, 80}=23.584$, $p<0.01$). The mean density of flowering shoots in December 2007 was significantly lower than those in June and August 2007 and June 2008 (Tukey's test, $p>0.01$). At the shallower stations (Stns. 1 and 2), the mean flowering shoot densities in June and December 2007 were the maximum and the minimum for the year, respectively (Fig. 6a). At the deeper stations (Stns. 3 and 4), the mean flowering shoot densities in August and October 2007 were the maximum and the minimum for the year, respectively (Fig. 6a). Vegetative shoot density was higher than flowering shoot density, and did not show a specific seasonal trend (Fig. 6b).

Seasonal changes in flowering and vegetative shoot biomass

Two-way ANOVA showed that the mean biomasses of flowering and vegetative shoots had significant temporal variation ($F_{6, 80}=6.331$, $p<0.01$ and $F_{6, 80}=4.316$, $p<0.01$, respectively) and significant spatial variation ($F_{3, 80}=8.121$, $p<0.01$ and $F_{3, 80}=14.527$, $p<0.01$, respectively). Tukey's test showed that the mean biomasses of flowering shoots in June and August 2007 were significantly higher than those in December 2007 and January 2008 ($p<0.01$). The maximum mean flowering shoot biomasses at 1, and Stns. 2, 3 and 4 were found in June 2007 and in August 2007, respectively (Fig. 7a). At all stations, the mean flowering shoot biomass had the minimum in December 2007 (Fig. 7a). Tukey's test showed that the mean biomass of vegetative shoots in January 2008 was significantly lower than the values in August and October 2007; however, it did not show a seasonal trend (Fig. 7b).

Seasonal changes in biomass of below-ground parts

Two-way ANOVA showed that the mean biomass of below-ground parts had significant temporal ($F_{6, 80}=5.914$, $p<0.01$) and spatial ($F_{3, 80}=20.481$, $p<0.01$) variations. Despite the significant temporal variation in the mean biomass of the below-ground parts, there was no seasonal pattern (Fig. 7c). Tukey's test showed that the mean biomass of below-ground parts in August 2007 was significantly higher than those in June and December 2007 ($p<0.01$).
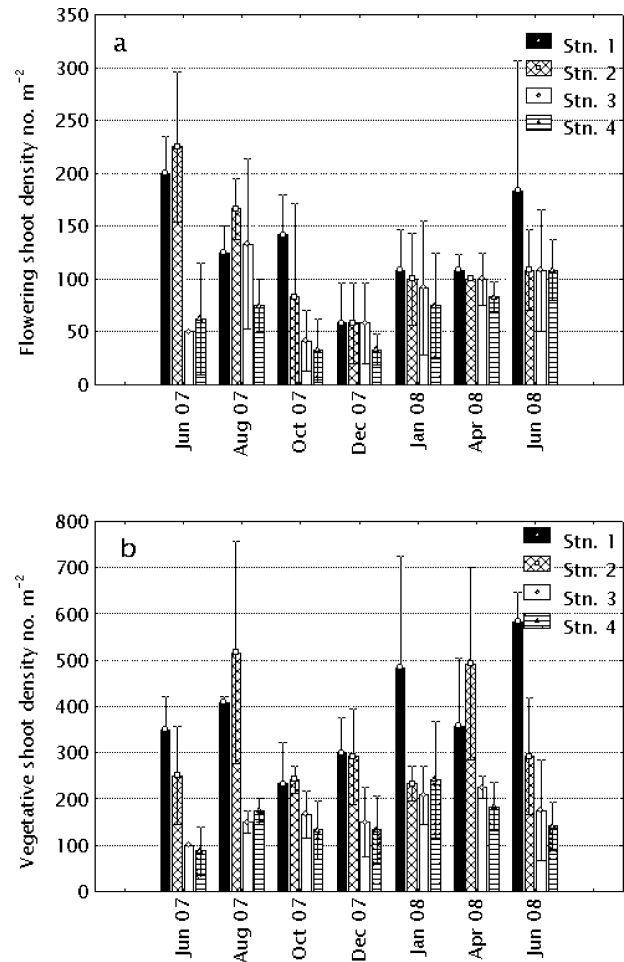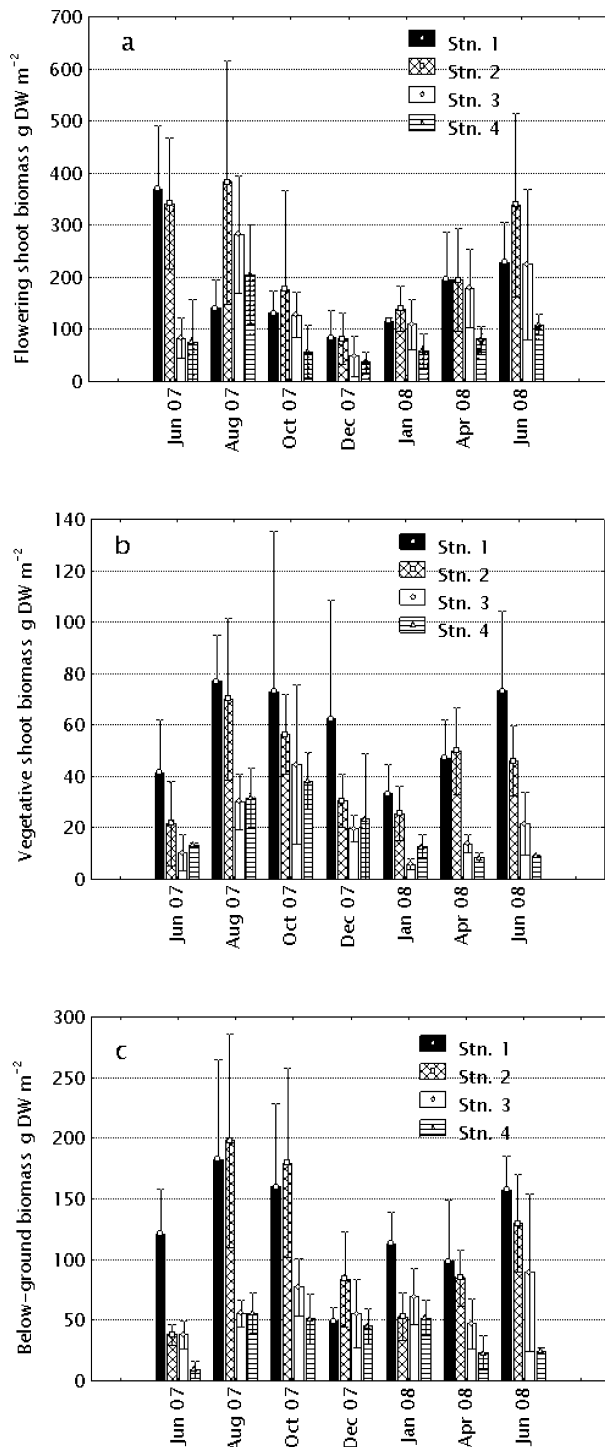

Fig. 6. Mean shoot densities of flowering (a) and vegetative (b) shoots of *Zostera caulescens* by station. Bars indicate standard deviations.

Seasonal changes in leaf area index

Two-way ANOVA showed that the mean LAI per unit area of both flowering and vegetative shoots had significant temporal ($F_{6, 80}=10.637$, $p<0.01$ and $F_{6, 80}=3.263$, $p<0.01$, respectively) and spatial ($F_{3, 80}=6.999$, $p<0.01$ and $F_{3, 80}=18.291$, $p<0.01$, respectively) variations. Tukey's test indicated that the mean LAIs per unit area of the apical vegetative leaves in June and August 2007 were significantly higher than those in October and December 2007 and in January 2008 ($p<0.01$). The mean LAIs at Stns. 1 and 2 reached the maxima in June and August 2007, respectively, and minima in October and December 2007, respectively (Fig. 8a). The mean LAIs at Stns. 3 and 4 were the maxima in August 2007 and minima in October and December 2007, respectively (Fig. 8a). The LAIs of the vegetative shoots did not show a seasonal pattern (Fig. 8b) and moreover, Turkey's test did not give significant results (Tukey's test, $p<0.01$).
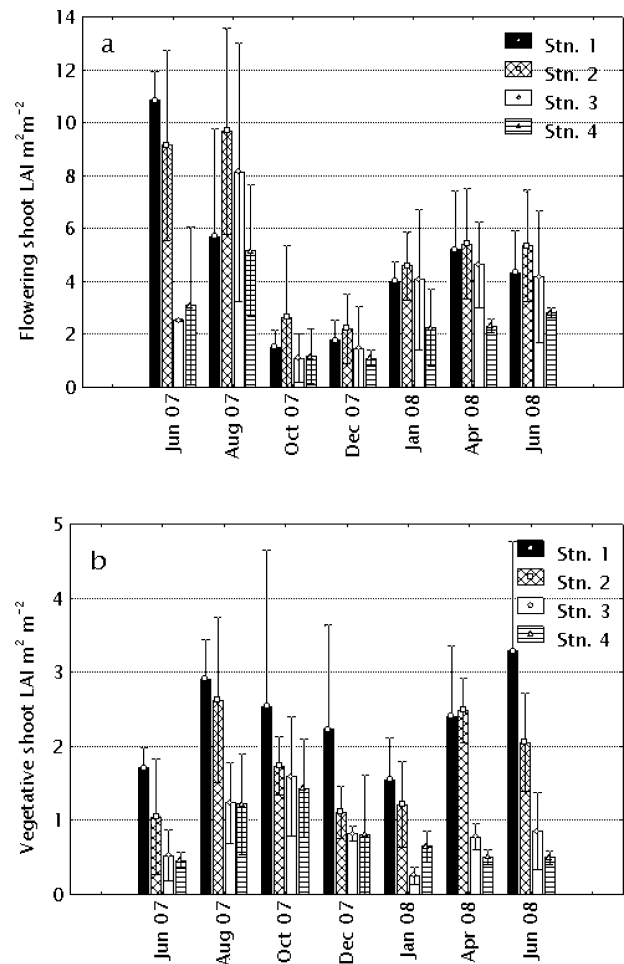
Spatial changes in shoot density, biomass, and leaf area index

The mean density and biomass of flowering shoots and

Fig. 7. Mean biomass of flowering (a) and vegetative (b) shoots and below-ground parts (c) of *Zostera caulescens* by station. Bars indicate standard deviations.
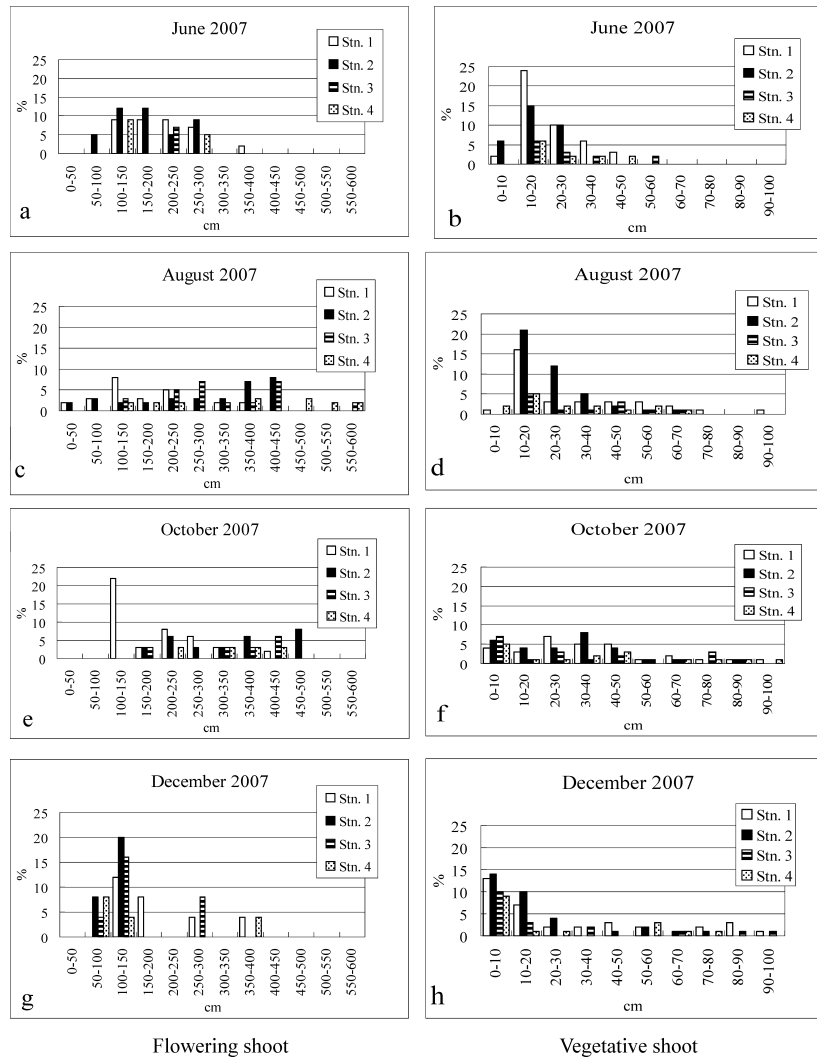

Fig. 8. Mean LAI of apical shoots on flowering (a) and vegetative (b) shoots of *Zostera caulescens* by station. Bars indicate standard deviations.

Stns. 1 and 2 (Tukey's test, p<0.01).

Frequency distributions of flowering shoot lengths

In June 2007, flowering shoots were between about 70 and 400 cm in length. Shoots less than 100 cm in length could be regarded as immature because of less occurrence of flowering shoots under this size class and occupied only 5% of all flowering shoots. Flowering shoot sizes did not show specific spatial trends in June 2007. Grouping the data in this month showed that more than 90% of flowering shoots were between 100 and 300 cm in length (Fig. 9). In August 2007, the longest flowering shoots reached 600 cm at Stns. 3 and 4 (Fig. 9). Small flowering shoots less than 100 cm were distributed at Stns. 1 and 2 while longer flowering shoots (more than 400 cm) were found only at Stns. 3 and 4 (Fig. 9). Immature shoots ranging between 43 and 134 cm in length made up about 22% of the flowering shoots, and all of flowering shoots more than 134 cm in length were mature. In October 2007, flowering shoots of the smallest size class (100 to less than 150 cm in length) were dominant at Stn. 1 while those over 350 cm in length were abundant at deeper

the mean LAI of the apical vegetative leaves per unit area at Stn. 4 was significantly lower than at Stns. 1 and 2 (Tukey's test, p<0.01). The mean density, biomass, and LAI of vegetative shoots per unit area at Stns. 3 and 4 were significantly lower than at Stns. 1 and 2 (Tukey's tests, p<0.01). The mean biomass of below-ground parts decreased with increasing depth. Those at Stns 3 and 4 were significantly lower than at

Flowering shoot

Vegetative shoot

Fig. 9. Frequency distributions of *Zostera caulescens* shoot length from June 2007 to December 2007. Data for flowering and vegetative shoots are presented on the left side (a, c, e, and g) and right side (b, d, f, and h), respectively.

stations where the maximum length reached 500 cm (Fig. 9). In October 2007, 36% of flowering shoots between 102 and 255 cm in length were immature. In December 2007 and January 2008, all flowering shoots were immature with length modes between 100 and less than 150 cm in December 2007 and between 100 and less than 200 cm in January 2008 (Figs. 9 and 10). In both months, immature shoots did not exceed 400 cm in length. In April 2008, flowering shoots were also less than 400 cm in length, although larger size classes between 200 and less than 400 cm made up higher percentages of the total shoots (>55%) than in the previous two months (<20%) (Figs. 9 and 10). In June 2008, flowering shoots increased in length to reach the maximum of 500 cm at Stn. 3 with the mode of size classes being between 100 and less than 200 cm (Fig. 10). In June 2008, the immature flowering shoots represented 67% of all flowering shoots and ranged from 53 to 335 cm in length. From January to June in 2008, flowering shoots longer than 300 cm were distributed at Stns. 3 and 4 while the mode of size classes at Stn. 1, the shallow-

est station, was less than 300 cm (Fig. 10).

Frequency distributions of vegetative shoot lengths

The maximum size of vegetative shoots was 100 cm in length, and their lengths did not show specific spatial distribution (Figs. 9 and 10). The mode of size classes was between 10 and 20 cm (Figs. 9 and 10). The maximum lengths of vegetative shoots in the 100 cm size class occurred in August, October, and December 2007 while size classes smaller than 10 cm regarded as new recruits increased in percentage at all stations from October 2007 to January 2008 (Figs. 9 and 10).

Discussion

Generally, seagrasses exhibit morphological plasticity in response to the variation in environmental parameters. For example, Backman (1991) identified five ecotypes of *Z.*

19
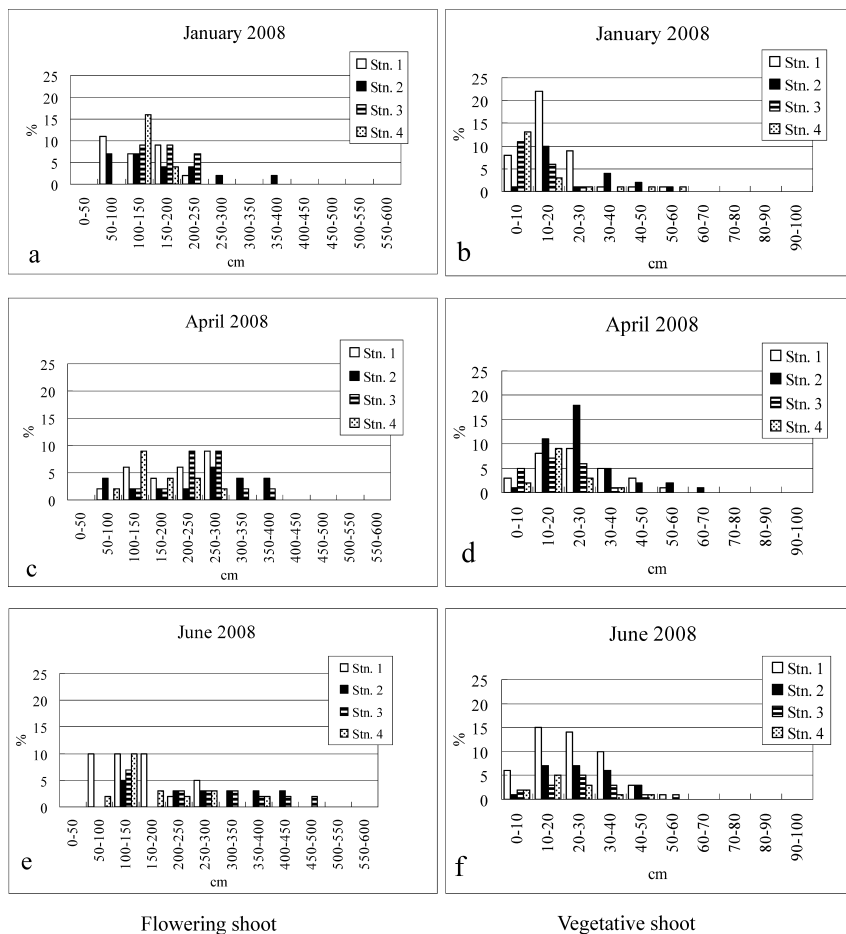
Fig. 10. Frequency distributions of *Zostera caulescens* shoot length from January 2008 to June 2008. Data for flowering and vegetative shoots are presented on the left side (a, c, and e) and right side (b, d, and f), respectively.

marina in the North American Pacific Coast. The species of the subgenus *Zostera* have similar morphology. Therefore, it is important to examine the morphological characteristics of these species in their different geographical distributions. Particularly in their mixed beds, it is necessary to avoid misidentification in order to monitor their dynamics on a species level for their conservation. The species of the subgenus *Zostera* are distinguished by shape of the leaf apex, number of veins, width of leaves, morphology of the reproductive shoots (Omori 1989, Shin and Choi 1998, Kuo and Den Hartog 2001), and rhizome morphology (Omori 1996).

Zostera caulescens is clearly distinguished from other *Zostera* species by the apical vegetative leaves at the terminal end of its flowering shoot while other *Zostera* species have spadix at the terminal end (Omori 1989). The examination of the spathes of *Z. caulescens* in the present study showed that this species belongs to the long spathe type with length-width ratios of spathes between 11.0 –and 13.1, which are different from the wide spathe type of *Z. asiatica* (8.7) and the slender one of *Z. caespitosa* and *Z. marina* (11.7–19.1) (Omori 1992). However, taxonomic classification of these species will be difficult due to their similar morphology of

spathes when their reproductive organs are absent.

Form of the leaf apex is a robust key in distinguishing *Zostera* species (Shin and Choi 1998). In the present study, the vegetative form of *Z. caulescens* had both the emarginate and mucronate leaf apexes, which were different from the obtuse and rounded form of *Z. marina* and truncate form of *Z. asiatica* (Shabaka and Komatsu in preparation). These two species coexisted with *Z. caulescens* in Funakoshi Bay (Shibatani and Komatsu in preparation). The shapes of leaf apex of the apical vegetative leaves on the flowering shoot of *Z. caulescens* showed a seasonal variation. Apexes of all the leaves were emarginate in summer (flowering season) and mucronate in autumn and winter. Because water temperature in summer was almost the same as in autumn, water temperature is not responsible to the seasonal change in leaf apex shapes. This seasonal change of leaf apex morphology might be related to the seasonal variation of light or some physiological process.

Leaf apex forms of this species are varied in different geographical localities. In Odawa Bay, central Honshu Island, the apical vegetative leaves had the mucronate form in May and June (Omori 1989). The seasonal variation in leaf apex

morphology was not clarified by Omori (1989) due to the lack of observations outside the flowering season. The present study showed that the leaf apex of the apical leaves was emarginate in the flowering season different from that recorded in Odawa Bay. Going outside Japan, the leaf apex shapes of Korean *Z. caulescens* were grouped into two forms. One form is either mucronate or broadly obtuse leaf apex with 9 to 13 veins of apical vegetative leaves of flowering shoots, and the other is either broadly obtuse or truncate leaf apex with 9 to 11 veins of vegetative shoots (Shin and Choi 1998). Korean *Z. caulescens* shows different leaf apex forms from those in Funakoshi Bay. The lack of the seasonal observation of Korean *Z. caulescens* prevents us from comparing temporal changes in leaf apex forms between the two localities.

Another important morphological characteristic is the leaf width. In central Honshu Island, the mean width of the apical leaves in Sagami Bay ranged between 1.35 and 1.6 cm and that in Odawa Bay varied between 1.4 and 1.8 cm (Omori 1989, 1994). The leaf widths of the Korean *Z. caulescens* varied between 1 and 1.6 cm for both apical leaves and vegetative shoots (Lee and Lee 2003). In the present study, the mean widths ranged from 0.7 to 0.9 cm in vegetative shoots and from 1.2 to 1.4 cm in apical vegetative leaves of flowering shoots.

Concerning the horizontal rhizome morphology, the thickness of Korean *Z. caulescens* rhizomes varied between 0.3 and 0.45 cm, and the length of internodes varied between 2 and 4 cm (Shin and Choi 1998). In Otsuchi Bay neighbouring Funakoshi Bay, the thickness of the horizontal rhizomes of *Z. caulescens* was between 0.5 to 0.6 cm and the internode lengths was 1.5 to 0.3 cm (Omori 1996), which is near to the values in the present study. However, the present study observed thicker rhizomes and shorter internodes than that of Korean *Z. caulescens*.

Considering morphological differences among the localities of *Z. caulescens* above-mentioned, environmental parameters such as water temperature besides genetics are important factors that influence their morphology. Water temperature in Odawa Bay was higher than in Funakoshi Bay ranging between 11.5°C in January to 25°C in August. Water temperature in Sagami Bay was about by 9°C higher than in Funakoshi Bay during the flowering season from April to June (Omori and Aioi, 2000). Water temperatures along the Korean Peninsula had a higher range (10–25°C) (Lee and Lee 2003) similar to that in Odawa Bay. It could be speculated that lower water temperature in Funakoshi Bay resulted in narrower leaves and thicker horizontal rhizomes with shorter internodes by slower growth rate. Although *Z. caulescens* is found in waters all along the Korean coast (Lee and Lee 2003), neither Shin and Choi (1998) nor Lee and Lee (2003) reported exact locations where they obtained their *Z. caulescens* samples. Thus, it makes our discussion

conservative due to lack of environmental information of Korean population.

The flowering shoot of *Z. caulescens* is a characteristic feature of this species not only because of its apical vegetative leaves but also its unique height. Our results showed that the maximum *Z. caulescens* shoot lengths at 6 m deep (Stn. 1) did not exceed 450 cm. Considering tidal fluctuations and wave heights, 450 cm is the longest shoot length that could remain under the sea surface in shallow areas as pointed out by Sutlana and Komastu (2003). In deeper bottom under low light environment, *Z. caulescens* develops long flowering shoots to obtain better light condition for photosynthesis (Sultana and Komatsu 2003). Therefore, the apical vegetative leaves are important for *Z. caulescens* to live in deeper bottom. According to Yamaki and Ogawa (2009), *Z. caulescens* seedlings germinate at lower irradiance and higher salinities than *Z. marina* which grows usually on shallower bottom. In this way, *Z. caulescens* adapt to live in deeper bottom under lower light irradiance.

The present study showed that above-ground biomass, shoot density, and LAI per unit area were reduced with depth. Reduction of biomass and density of seagrass along bottom depths is mainly controlled by the light available to the plant (Duarte 1991). Dennison and Alberte (1985) also explain how the light environment controls spatial distribution of biomass, shoot density, specific leaf area, leaf chlorophyll content and production rates of *Z. marina* along a depth gradient by conducting a field experiment using underwater lamps and light shading screens on shallow and deep *Z. marina*. Enríquez and Pantoja-Reyes (2005) stated that reduction in shoot density and above-ground biomass during low light conditions is probably an adaptation to reduce the self-shading within the canopy. Since *Z. caulescens* is distributed on the bottom from 3 m to 15 m deep in Funakoshi Bay, it is natural that the light environment controls these spatial distributions.

In Funakoshi Bay, the below-ground biomass of *Z. caulescens* showed a reduction with depth. In deeper bottom, *Z. caulescens* invests energy to increase shoot length near surface layer where light is abundant. Consequently, it allocates less energy to develop below-ground parts. Sultana and Komatsu (2002) explained another reason for the reduction of below-ground biomass with depth. They revealed that the ratio of below-ground to above-ground biomass was decreased with bottom depth. This means that root system providing mechanical support against the drag forces exerted by waves and currents is not so necessary in deeper bottom where the waves and currents are weaker than in shallower bottom.

Apart from the spatial distributions of biomass, shoot density and LAI per unit area, those of *Z. caulescens* flowering shoots in Funakoshi Bay showed a clear seasonal pattern with the maxima in summer and the minima in winter similar

to the seasonal change of temperate seagrasses as pointed out by Duarte (1989). In Funakoshi Bay in October, the mean biomass and LAI per unit area of *Z. caulescens* flowering shoot were reduced due to decrease while the water temperature in October was as high as that in summer. Although the lowest temperature was recorded in April, the mean biomass and LAI per unit area were gradually increased. Therefore, light seems to be the major factor controlling the biomass and LAI of *Z. caulescens* flowering shoots in Funakoshi Bay, northern Honshu Island.

Total above-ground biomass of *Z. caulescens* at the shallowest station (6 m) can be compared with those of *Z. marina* at a comparable depth at different localities. In Otsuchi Bay, the maximum and minimum above-ground biomass of eelgrass at 5 m deep were respectively 370 g DW m^{-2} in June and 30 g DW m^{-2} in January (Iizumi et al. 1996) while those at 5 m deep in Odawa Bay, central Honshu Island were respectively 91 g DW m^{-2} in May and 4 g DW m^{-2} in December (Aioi 1980). In Iida Bay, central Honshu Island, the Sea of Japan, the maximum and minimum above-ground biomass at 4–6 m deep were 170 g DW m^{-2} in July and 20 g DW m^{-2} in November, respectively (Taniguchi and Yamada 1979). In the present study, the maximum and minimum mean total above-ground biomass of *Z. caulescens* were 410±143 g DW m^{-2} in June 2007 to 146±74 g DW m^{-2} in December 2007. Above-ground biomass of *Z. caulescens* in Funakoshi Bay was greater than those of *Z. marina* in Honshu Island. Because *Z. marina* prefers shallow depths, bottom depths at 5 to 6 m deep are situated as lower limit of distribution. Therefore the above-ground biomass of *Z. marina* at 4–6 m deep is less than that of *Z. caulescens* at 6 m deep in Funakoshi Bay.

Reproduction is very important for seagrasses to maintain and enlarge their distributions. Fruits are one of efficient reproductive method for subgenus *Zostera* (Yamaki and Ogawa 2009). The number of *Z. caulescens* flowers in Funakoshi Bay was less than in Odawa Bay (10–20) (Omori 1989), which was greater than the maximum of 8 in Funakoshi Bay. Moreover, the number of fruits per spathe varied between 14 and 18 in Odawa Bay (Omori 1992) greater than the maximum of 14 in Funakoshi Bay. Besides fruits, vegetative reproduction is a secure reproductive method for seagrasses. Our study showed that new vegetative shoots of less than 10 cm in length were observed at all stations in December 2007 and January 2008 with higher percentages of occurrence than in other months. New immature shoots less than 1 m in length in December 2007 and January 2008 occupied higher percentages than in other months. All the flowering shoots in both months were immature. These findings suggest that both flowering and vegetative shoots of *Z. caulescens* recruited in winter. The formation of new recruits during the period of reduced photosynthesis in winter depends on the consumption of carbohydrates reserved in

below-ground parts during summer (Lee and Dunton 1996, 1997). New recruits of vegetative and immature flowering shoots enable seagrass to maintain and expand their spatial distribution in the following months by rhizome expansion. Thus, in Funakoshi Bay, the reduced reproductive effort of *Z. caulescens* using fruits indicates that the vegetative growth is more important reproduction method to maintain its population.

This study has clarified the morphology and dynamics of *Z. caulescens*, an important species at risk of extinction. Continued monitoring of the phenology of *Z. caulescens* distribution, dynamics, and reproduction is required to better understand seagrass dynamics and to conserve or restore this endangered species correctly.

Acknowledgements

References

Aioi, K. 1980. Seasonal changes in the standing crop of eel-grass (*Zostera marina* L.) in Odawa Bay, central Japan. Aquat Bot. 8: 343–354.

Aioi, K. 1998. On the Red List species of Japanese seagrasses. Aquabio. 20: 7–12. (In Japanese with an English abstract).

Aioi, K., Komatsu, T., and Morita, K. 1996. Giant seagrass, *Zostera caulescens* Miki, discovered in Funakoshi, Iwate Prefecture. Bull. Japan. Soc. Fish. Oceanogr. 60: 7–10. (In Japanese with an English abstract).

Aioi, K., Komatsu, T., and Morita, K. 1998. The world's longest seagrass, *Zostera caulescens* from northeastern Japan. Aquat. Bot. 61: 87–93.

Backman, T. W. H. 1991. Genotypic and phenotypic variability of *Zostera marina* on the west coast of North America. Ca. J. Bot. 69: 1361–1371.

Dennison, W. C. and Alberte, R. S. 1985. Role of daily light period in depth distribution of *Zostera marina* (eelgrass). Mar. Ecol. Prog. Ser. 25: 51–61.

Duarte, C. M. 1989. Temporal biomass variability and production biomass relationships of seagrass communities. Mar. Ecol. Prog. Ser. 51: 269–276.

Duarte, C. M. 1991. Seagrass depth limits. Aquat. Bot. 40: 363–377.

Enríquez, S. and Pantoja-Reyes, N. I. 2005. Form-function analysis of the effect of canopy morphology on leaf self-shading in the seagrass *Thalassia testudinum*, Oecologia. 145: 235–243.

Iizumi, H. 1996. Temporal and spatial variability of leaf production of *Zostera marina* L. at Otsuchi Bay, northern Japan. *In* Seagrass biology: Proceeding of an international workshop, Rottnest Island, Western Australia, 25–29 January 1996. Kuo, J.,

Phillips, R. C., Walker, D. I. and Kirkman, H. (eds.), pp. 143–148, Faculty of Science, The University of Western Australia, Nedlands.

González-Correa, J. M., Bayle Sempere, J. T., Sánchez-Jerez, P. and Valle, C. 2007. *Posidonia oceanica* meadows are not declining globally. Analysis of population dynamics in marine protected areas of the Mediterranean Sea. Mar. Ecol. Prog. Ser. 336: 111–119.

Komatsu, T., Igarashi, C., Tatsukawa, K., Sultana, S., Matsuoka, Y., and Harada, S. 2003. Use of multi-beam sonar to map seagrass beds in Otsuchi Bay on the Sanriku Coast of Japan. Aquat. Living Res. 16: 223–230.

Kuo, J. and Den Hartog, C. 2001. Seagrass taxonomy and identification key. *In*. Global seagrass research methods. Short F. T., and Coles R. G. (ed.). pp. 31–58, Elsevier Science B. V., Amsterdam.

Lee, K. S. and Dunton, K. H. 1996. Production and carbon reserve dynamics of the seagrass *Thalassia testudinum* in Corpus Christi Bay, Texas, U.S.A. Mar. Ecol. Prog. Ser. 143: 201–210.

Lee, K. S. and Dunton, K. H. 1997. Effects of *in situ* light reduction on the maintenance, growth and partitioning of carbon resources in *Thalassia testudinum* Banks ex Kijnig. J. Exp. Mar. Biol. Ecol. 210: 53–73.

Lee, K. S. and Lee, S. Y. 2003. The seagrasses of the Republic of Korea. *In*: World Atlas of Seagrasses. Green, E. P. and Short, F. T. (eds). pp. 193–198, UNEP. University of California Press, Berkeley, U.S.A.

Miki, S. 1933. On the seagrass of Japan. I) *Zostera* and *Phyllospadix*, with special reference to morphological and ecological characters. Bot. Mag. Tokyo. 47: 824 – 862.

Omori, Y. 1989. Morphology of the flowering-shoot and the leaf of *Zostera caulescens* Miki and *Zostera asiatica* Miki (Zosteraceae). Sci. Rept. Yokosuka City Mus. 37: 55–59. (In Japanese with an English abstract).

Omori, Y. 1991. Peculiarity of the flowering-shoot of *Zostera caulescens* (Zosteraceae). Sci. Rept. Yokosuka City Mus. 39: 45–50. (In Japanese with an English abstract).

Omori, Y. 1992. Geographical variation of the size of spadix and spathe and the number of flowers among the four species of the subgenus *Zostera* (Zosteraceae). Sci. Rept. Yokosuka City Mus. 40: 69–74. (In Japanese with an English abstract).

Omori, Y. 1994. Seasonal changes of the reproductive shoot of *Zostera caulescens* (Zosteraceae) in Sagami Bay, central Japan.

Sci. Rept. Yokosuka City Mus., 42: 65–69. (In Japanese with an English abstract).

Omori, Y. 1996. Rhizome morphology of the subgenus *Zostera* (Zosteraceae). Sci. Rept. Yokosuka City Mus. 44: 55–62. (In Japanese with an English abstract)

Omori, Y. and Aioi, K. 2000. Seasonal changes of the erect shoot of *Zostera caulescens* Miki (Zosteraceae) in Sanriku Kaigan, northern Honshu. Sci. Rept. Yokosuka City Mus. 47: 67–72. (In Japanese with an English abstract).

Ott, J. A. 1990. Biomass. *In* Seagrass research methods. Philips, R. C. and McRoy, C. P. (ed.). pp. 55–60, UNESCO.

Sagawa, T., Mikami, A., Komatsu, T., Kosaka, N., Kosako, A., Miyazaki, S., and Takahashi, M. 2008. Mapping seagrass beds using IKONOS satellite image and side scan sonar measurements: a Japanese case study. Int. J. Remote Sens. 29: 281–291.

Shin, H. and Choi, H. K. 1998: Taxonomy and distribution of *Zostera* (Zosteraceae) in eastern Asia, with special reference to Korea. Aquat. Bot. 60: 49–66.

StatSoft Inc. 2004. STATISTICA, Data analysis software system version 7. www.statsoft.com.

Sultana, S. and Komatsu, T. 2002. Preliminary study on shoot density and biomass of seagrass *Zostera caulescens*, in Funakoshi Bay off Sanriku Coast, Japan. Otsuchi Mar. Sci. 27: 23–27.

Sultana, S. and Komatsu, T. 2003. Changes in shoot density, biomass and leaf area of seagrass *Zostera caulescens* Miki from summer to autumn in Funakoshi Bay off Sanriku Coast, Japan. Otsuchi Mar. Sci. 28: 91–98.

Taniguchi, K. and Yamada, Y. 1979. Vertical distribution and natural life history of *Zostera marina* Linné and some species of seagrass in Iida Bay of the Noto Peninsula on the Honshu, Japan Sea coast. Bull. Jap. Sea Reg. Fish. Res. Lab. 30: 111–122. (In Japanese with an English abstract).

Tatsukawa, K., Komatsu, T., Aioi, K., and Morita, K. 1996. Distribution of seagrasses off Kirikiri in Funakoshi Bay, Iwate Prefecture, Japan. Otsuchi Mar. Res. Cent. Rep. 21: 38–47. (In Japanese).

Wildlife Division, Agency of the Environment, Japan. 2000. Threatened wildlife of Japan: Red Data Book; vascular plant, Japan. Wildlife Research Centre, Tokyo, 662 pp+pl 16.

Yamaki, K. and Ogawa, H. 2009. Characterisation of germination and seedling production of *Z. marina* and *Z. caulescens*. Coast. Mar. Sci. 33: 46–53.