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Characterization of germination and seedling production of *Zostera marina* and *Z. Caulescens*

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Abstract — Efficient recovery of seed germination and growth were investigated to determine the optimum combination of salinity and temperature in two seagrass species, *Zostera marina* L. and *Z. caulescens* Miki. Flowering shoots of *Z. marina* were collected at the depths of 0.5–3 m from Shin-nase in Sagami Bay and Nami-ita in Okirai Bay, while those of *Z. caulescens* were collected at the depths of 3–8 m from Tenjin-jima in Sagami Bay and Nami-ita in Okirai Bay. Seeds of *Z. marina* obtained from fructified spadices showed germination induction by soaking and keeping them in freshwater under dark condition at 20°C for 24 hours. This pretreatment improved germination rate by 90%. When the seeds were kept in above 5 psu, the germination rate was significantly dropped to less than 30%. While the germination rate of *Z. caulescens* was above 60% by the pretreatment with freshwater or low salinity less than 10 psu under dark condition at 20°C for 24 hours.

The minimum irradiance requirement for the growth of seedlings of *Z. marina* and *Z. caulescens* was found to be in 12 and 5 μmol photons $m^{-2} s^{-1}$, respectively. The effects of salinity and irradiance on seed germination rate were different in these two seagrass species. The difference in the effects clearly reflect the natural habitat conditions in which the plants grow.

Key words: seagrass, *Zostera marina*, *Zostera caulescens*, germination, germling production, irradiance, temperature, Sagami Bay, Okirai Bay

Introduction

Seagrasses are marine angiosperms that play important roles in the coastal ecosystem of the subarctic, temperate and tropical areas. They have very high primary productivity, and the areas with abundant seagrass provide spawning, nursing, or sheltering places for fish. They have the potential to absorb much nutrient from seawater that could prevent eutrophication in coastal waters. In recent years, seagrass beds have rapidly been disappearing not only in Japan, but also worldwide because of reclamation in shallow areas with abundant seagrass or the reduction of water transparency caused by water pollution (Short and Wyllie-Echeverria l996). It has been reported that as much as 70% of seagrass beds in 1960 had disappeared in the Seto Inland Sea by 1990 (Ministry of Environment of Japan 1994, Okaichi et al. 1996, Komatsu 1997). Several reports pointed out that seagrass bed preservation and restoration are very important and urgent issues for the environmental conservation and coastal resource management (Duarte and Chisacano 1999, Edmund and Frederick 2003, Orth et al. 2006). In Japan two seagrass species, *Z. marina* and *Z. caulescens*, form broad seagrass beds in shallow waters. The former grows in 0.3 to 5 m depth and the latter grows in 3 to 17 m depth (Aioi et al. 1996, 1998, Tatsukawa et al. 1996, Komatsu and Tatsukawa 1998, Omori and Aioi 2000, Nakaoka and Aioi 2001, Komatsu et al. 2003, Sagawa et al. 2008,). Also *Z. caulescens* is listed as an endangered species in the Red Data Book of Japan (Ministry of the Environment of Japan 2000).

Many seagrass bed restoration projects have been tested worldwide, but almost all the projects did not succeed because of very limited growth rate in seagrass due to environmental deterioration, insufficient ecological information and the inherent problems of seagrass transplanting technology (Bosworth and Short 1993, Dan et al. 1998, Moore et al. 1996, Tamaki et al. 2002). Direct transplanting technique of seagrass plants, for example, may cause irreversible damage to a donor seagrass bed from where young or fully grown plants have been collected, even though the technique provides better survival rate in transplanted plants than that of seeds directly sowed on the ground (Park and Lee 2007). Since it is known that genetic diversity is different in each region even the distance between them is short (Tanaka et al. 2002), any transplantation of seagrass collected from one colony far from the restoration site is not recommended to

avoid genetic disturbances (Ministry of the Environment, 2004). Considering such problems and issues, a cultivation system to produce germlings from seeds of seagrass must be established not only to make seagrass bed restoration, but also to keep genetic biodiversity. Development of new restoration techniques applicable in any site or species will be of enormous help for future coastal management program (Yamaki et al. 2007).

Coastal environmental conditions such as; temperature, wind, current and others change or fluctuate rapidly. There is a scarce of information on harvesting techniques for seed collection of *Zostera* species. The current practice is the use of flowering shoots for seed collection in *Z. marina*, where the flowering shoots are incubated in a container to collect the dropped seeds from the spadixes (Hoostmans et al. 1987). However, it is difficult to apply this method for the seed collection of *Z. caulescens* because the length of this species reaches about 7 m (Aioi et al. 1998, Sultana and Komatsu 2002, 2003) and is therefore too big to be kept in a container. Hence, there is really a need to develop a new seed collection technique for this purpose.

This study aims to investigate and develop the followings: (a) to develop the seed collection and storage methods used for restoration of seagrass beds of *Z. marina* and *Z. caulescens*, (b) to improve the germination rate of these two kinds of seeds, and (c) to determine the optimum conditions for germling growth.

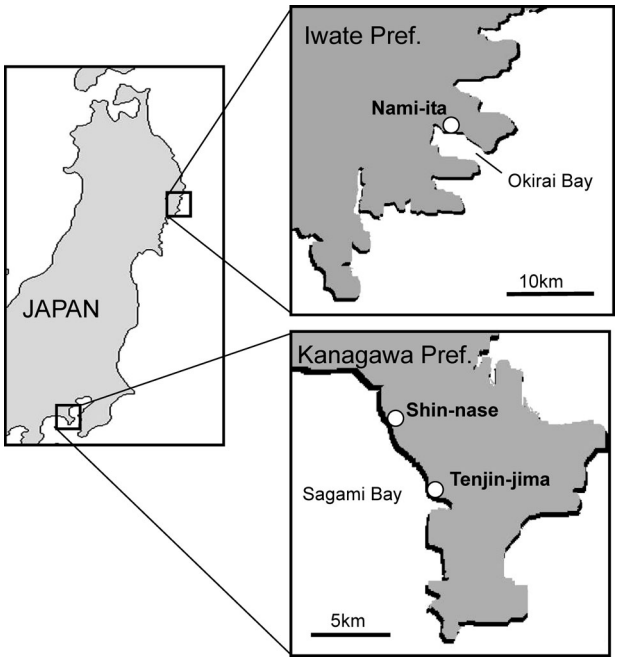

Fig. 1. Map showing locations of seed collections.

Table 1. Maturity stages of condition of the spadix of *Z. caulescens*

Stage	condition
A	pre-bloom
B	full-bloom (exposed stigmas and ruptured pollen-sacs)
C	pollinated and fructified

Materials and Methods

Flowering shoot and seed collection

Zostera marina

Approximately 200 flowering shoots with 1 m length on average were collected from seagrass beds in Shin-nase facing Sagami Bay, Kanagawa Prefecture in June 2005, and in Nami-ita facing Okirai Bay, Iwate Prefecture in August 2005. Seagrass beds at both sites were in the bottom depth range of 0.5–3 m. Seawater temperature at Shin-nase is higher than at Nami-ita (Fig. 1). Flowering shoots with fructified spadices from each site were separately incubated and cultured in a 500 *l* container filled with natural seawater (33 psu) kept at 20°C and set at a place in direct sunshine in an open laboratory to where there was direct sunlight. After 3 weeks of incubation, four to five thousands of ripen seeds were selected for seed germination experiments by specific gravity method mentioned below.

Zostera caulescens

Approximately 150 flowering shoots with 3–4 m were collected from Tenjin-jima, Yokosuka, Kanagawa Prefecture in June 2005 and from Nami-ita, Iwate Prefecture in August 2005 (Fig. 1). Only plants with fructified spadices were incubated in 500 *l* container, because the length of flowering shoots was too long to culture in it. They were sorted into three following stages according to their maturity, stage A: pre-bloom, stage B: full-bloom, stage C: pollinated and fructified (Table 1). Each stage with 170 spadices was dipped in a 50 *l* container filled with aerated natural seawater (33 psu) under the following conditions; 20°C, 1,000 μmol photons $m^{-2} s^{-1}$ and 12L : 12D photoperiod. Dropped seeds from spadix on the bottom of container were collected at every week for five weeks incubation. Collected seeds were selected by the specific gravity method. Ripen seeds were selected for seed germination experiments by specific gravity method mentioned below.

Selection and storage of ripened seeds

For the selection of ripened seeds from the both species, sodium chloride solution adjusted in 1.2 as specific gravity was used according to Kawasaki et al. (1988). Ten grams of selected seeds in each stage were placed in a 200 ml glass vial containing 150 ml of sterilized seawater with 20 g of granular activated charcoal. They were stored in a dark condition for 2 months at 20°C. During the storage, both steril-

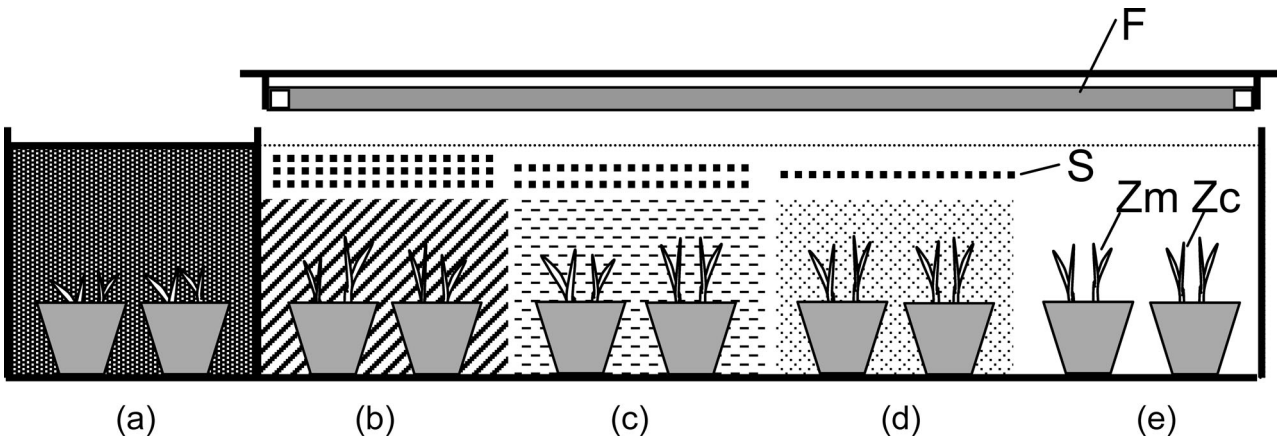

Fig. 2. Experimental setup of light intensities for the growth response of *Z. marina* and *Z. caulescens*.
(a) 0 μmol photon $m^{-2}\,s^{-1}$, (b) 2 μmol photons $m^{-2}\,s^{-1}$, (c) 5 μmol photons $m^{-2}\,s^{-1}$, (d) 12 μmol photons $m^{-2}\,s^{-1}$, (e) 45 μmol photons $m^{-2}\,s^{-1}$.
S: shading net, F: fluorescent lamp (40 W×5), Zm: seagrass (*Z. marina*), Zc: seagrass (*Z. caulescens*).

ized seawater and granular activated charcoal were replaced every week, and germinated or rotten seeds were removed daily (Yamaki et al. 2007).

Germination induction

To determine the best combination of salinity and temperature for the induction of seed germination in *Z. marina* and *Z. caulescens*, the following series were prepared in salinity and temperature; 8 salinities (0, 5, 10, 15, 20, 25, 30, and 33 psu as control) and 4 temperatures (5, 10, 15, and 20°C). Salinity adjustments were done to the filtered and autoclaved seawater with distilled water. Five seeds of each species were placed in a well filled with 4 ml of seawater adjusted in each salinity condition using multiwell plate with 12 wells. Three replicates were prepared and placed in a dark incubator for two weeks at 20°C. After two weeks of incubation, germinated seeds were counted in each well, as the seed germination of *Z. marina* is defined as split of outer seed coat (Arasaki 1950).

Germling growth in response to light intensity

Observation was done on the growth rate of young germlings of *Z. marina* and *Z. caulescens* seeds collected from Shin-nase and Tenjin-jima respectively, in response to light intensity. Ten seeds of each species were sowed at 2 cm in depth from the surface of potting soil in vinyl pot (9 cm in diameter and 10 cm height), which was composed of 70% of river sand with 0.2 mm in diameter and 30% of bark compost with 34% carbon content and 1.7% nitrogen content. These pots were pre-immersed in freshwater for several hours according to the result of germination induction experiment. Experimental setups are in Fig. 1. After pre-treatment of the seeds, the pots were moved in 22 psu seawater keeping 10°C for 2 weeks under the following five light conditions with 12L : 12D photoperiod using five tubes of 40 W fluorescent lamp (Vita-Lite, Light-Sources Inc.); 0 (dark), 2, 5, 12, and 45 μmol photons $m^{-2}\,s^{-1}$. Light intensity was regulated using plastic shade net (Fig. 2). Through the experiment, the occurrence of coleoptiles and leaves were observed and their sizes were measured every week. Growth reponses of cotyledon and leaves to light intensity were expressed as the total length of the leaves, and the result for *Z. caulescens* is shown in Fig. 3.

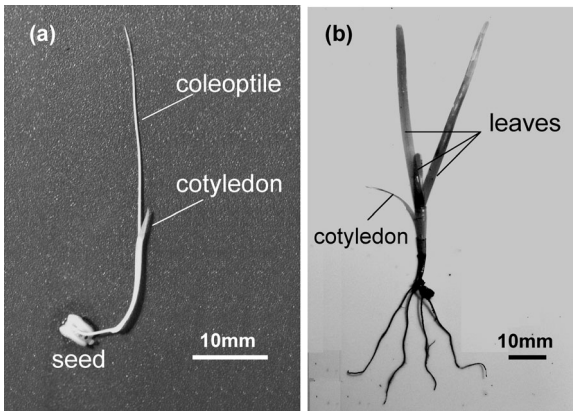

Fig. 3. Young germings of *Z. caulescens*.
(a) 1 week after germination. (b) 6 weeks after germination.

Statistics

To establish the effects of salinity, temperature, and seed collection site on germination induction, and also to know the effects of light intensity and culture periods to the germling growth of *Z. marina* and *Z. caulescens*, the results of culture experiments were tested for statistical significance with three-way analysis of variance.

Results

Seed collection and storage

About 6,000 seeds of *Z. marina* were collected from the bottom of 500 *l* container after three weeks incubation and 200 flowering shoots with fructified spadices were each collected from Shin-nase, Kanagawa Prefecture and Nami-ita, Iwate Prefecture. About 5,000 seeds were selected by specific gravity method, but almost all of them were ripened. These were then kept in a dark condition for 2 months at 20°C. Only 0.5% of them germinated, while other samples did not and putrefied seed was not observed in the stored seeds. For the seed collection of *Z. caulescens* from Tenjin-jima, Kanagawa Prefecture and Nami-ita, Iwate Prefecture, the same method and condition used to *Z. marina* were applied. The collected number of seeds in each stage (Table 1) is as follows; 30 seeds in stage A, 192 seeds in stage B, and 1,080 seeds in stage C. Among them, 6 seeds in stage A, 130 seeds in stage B and 1,070 seeds in stage C were selected by specific gravity method, and all of them were mixed and stored for two months under the same conditions as the seeds of *Z. marina*. During the storage only 0.3% of them were germinated while others did not, and putrefied seed was not observed in the stored seeds.

Germination induction

Seed germination rates of *Z. marina* and *Z. caulescens* in different temperature-salinity conditions are shown in Fig. 4. Both seeds of *Z. marina* from Shin-nase and Nami-ita germinated in all of the tested salinities and temperatures. The highest germination rate of 90% were obtained in 0 psu (distilled water) at 20°C, but the rates in both salinity and temperature were decreased from 90% or below and fall below 30% at 33 psu and 5°C accordingly. For the effects of salinity and temperature on the seed germination of *Z. caulescens* collected from Tenjin-jima and Nami-ita, salinity and temperature series were prepared similarly with *Z. marina*. The seeds collected from both sites showed the same pattern of germination rate. The above 60% rate was shown in the ranges of 5 and 10 psu and 5 and 20°C, but the rates outside this range were lower than 60% and significantly decreased to 30%. Table 2 shows statistical test results of the germination induction experiments. Significant effect of various tem-

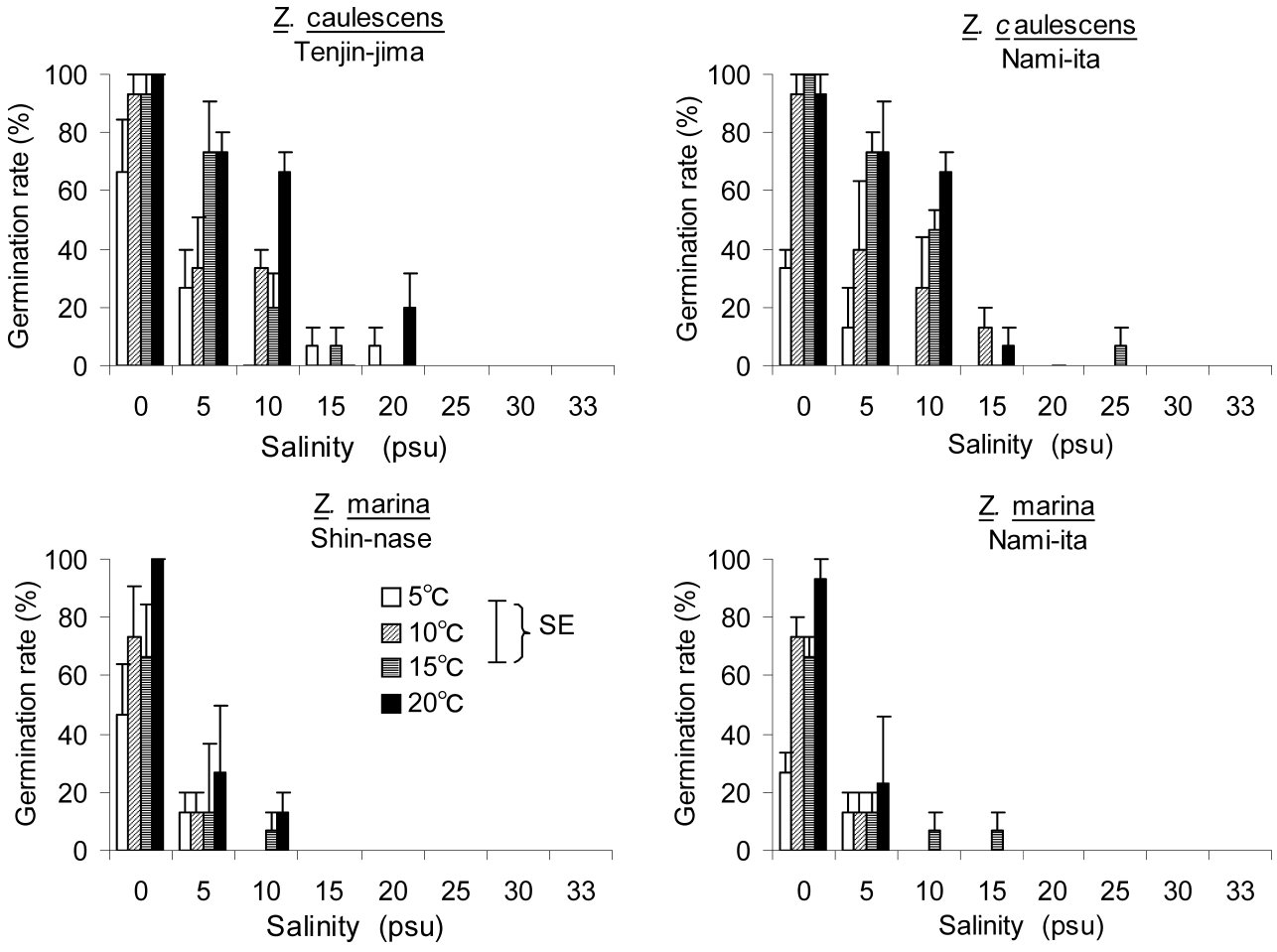

Fig. 4. Seed germination rates of *Z. marina* and *Z. caulescens* collected from Sin-nase, Nami-ita and Tenjin-jima under different salinity and temperature conditions.

peratures and salinities on germination was found ($P<0.005$) in both species, respectively. However, regional difference in the germination was not found in both species. Significant interaction between temperatures and salinities was detected. Moreover, significant effect of various salinities on germination ($P<0.005$) was also found between these two species.

Germling growth to light intensity

Growth response of coleoptiles of *Z. marina* and *Z. caulescens* to light intensity is shown in Fig. 5. For *Z. marina*, coleoptiles showed evident growth in two weeks culture after their germination. When the coleoptiles were kept for 3–4 weeks in dark condition, their length reached to 35–45 mm. On the other hand, lengths in light condition were 15–20 mm shorter than those in the dark. For *Z. caulescens*,

Table 2. Statistical analysis of germination induction of *Z. marina* and *Z. caulescens*.

	Z. marina	*Z. caulescens*
Temperature (Te)	<0.005	<0.005
Salinity (Sa)	<0.005	<0.005
Regional (Re)	n.s.	n.s.
Interactions		
Te×Sa	<0.005	<0.005
Te×Re	n.s.	n.s.
Sa×Re	n.s.	n.s.

n.s.=not significant

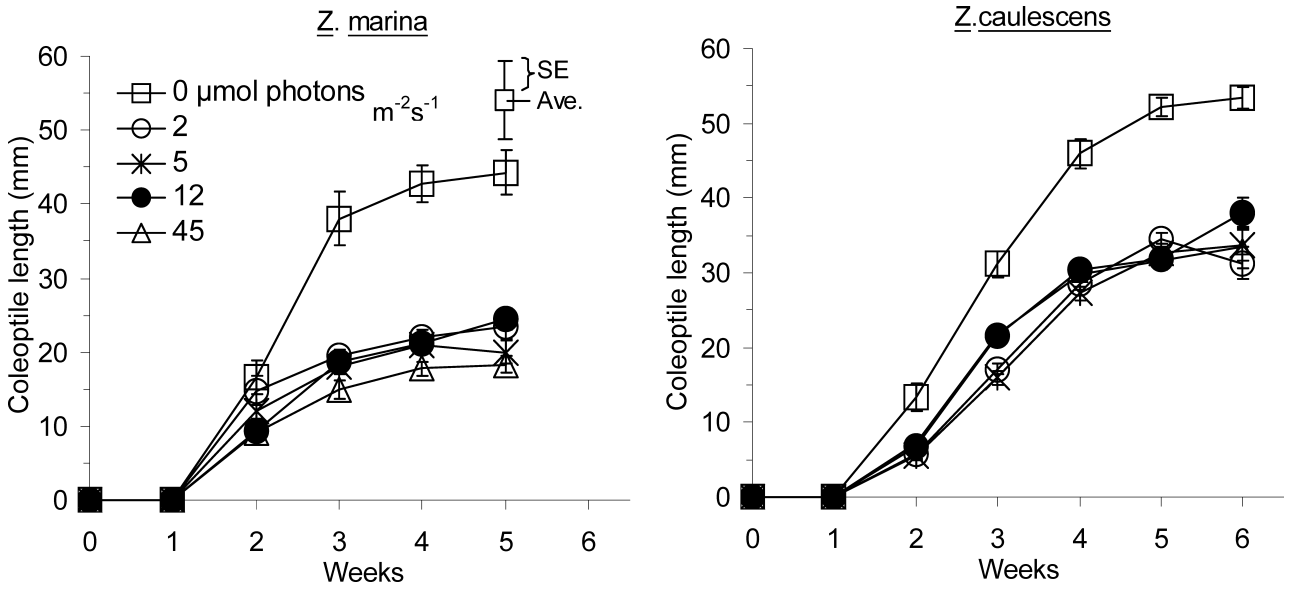

Fig. 5. Growth of cotyledons of *Z. marina* and *Z. caulescens* under different light intensities.

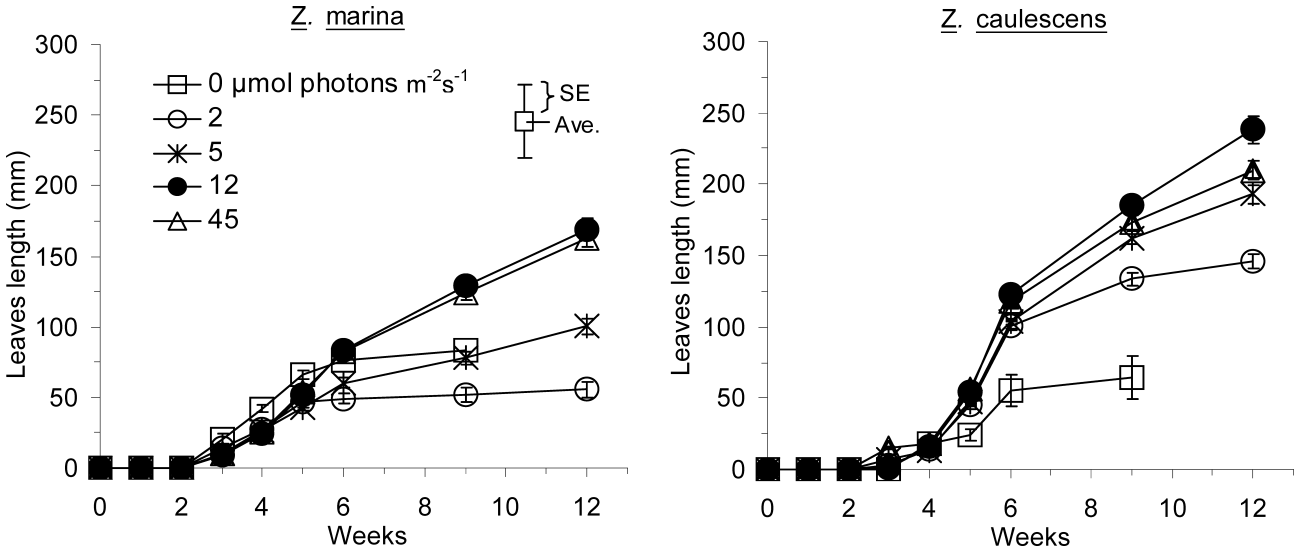

Fig. 6. Leaf growth of *Z. marina* and *Z. caulescens* under different light intensities.

Table 3. Results of statistical analysis of germling growth of *Z. marina* and *Z. caulescens* to light intensity. Levels of significant differences of the different factors and interactions.

	Coleoptile	Leaves
Species (Sp)	<0.005	<0.005
Light intensities (Li)	<0.005	<0.005
Culture periods (Cu)	<0.005	<0.005
Interactions		
Sp×Li	n.s.	<0.005
Sp×Cu	<0.005	<0.005
Li×Cu	<0.05	<0.05

n.s.=not significant

coleoptiles showed evident growth in two weeks culture after their germination. When the coleoptiles were kept for 4–5 weeks in dark condition, their length reached to 50–60 mm. On the other hand, those in light condition were 30–40 mm than those in the dark. All of coleoptiles fell off in both light and dark conditions after 6 or 7 weeks culture.

Growth response of the leaves of both species to light intensity are depicted (Fig. 6). The leaves of *Z. marina* grew to about 50 mm after 5 weeks culture. They grew rapidly under irradiance of 12–45 μmol photons $m^{-2} s^{-1}$ and reached to 150–180 mm in length after 12 weeks culture. It was observed the translucent leaves in germlings cultured 5 weeks under low irradiance of 5 μmol photons $m^{-2} s^{-1}$ and 12 weeks in dark condition. With respect to *Z. caulescens*, the maximum length of the leaves exposed to 12 μmol photons $m^{-2} s^{-1}$ reached to 230 mm after 12 weeks culture, but their growth exposed to 5 and 45 μmol photons $m^{-2} s^{-1}$ was lower than that exposed at 12 μmol photons $m^{-2} s^{-1}$, the average lengths were 190 mm at 5 μmol photons $m^{-2} s^{-1}$ and 210mm at 45 μmol photons $m^{-2} s^{-1}$. Almost all of the leaves exposed to 2 μmol photons $m^{-2} s^{-1}$ did not grow or growth was retarded even those grown in dark condition. The leaves lacked chlorophyll and were translucent until 10 weeks. This phenomenon was also observed with that in *Z. marina*. Table 3 shows the results of statistical test on the germling growth to light intensities, species as well as culture periods. The growth response of the leaves of *Z. marina* and *Z. caulescens* to light intensity was found ($P<0.005$) was significantly different. Moreover, there was a significant interaction between light intensities and species as indicated in growth response of leaves ($P<0.005$).

Discussion

Results of this study proved the efficiency of seed collection of *Z. marina* and *Z. caulescens* using their flowering shoots with fructified spadices. This is a very convenient way to collect large amount of ripe seeds dropped on the bottom of container without deteriorating the donor seagrass beds of both species, even the flowering shoots with unfructified spadices. The reason for this is that fructified spadices with leaves can produce and utilize photosynthetic products to seeds as long as leaves remains in fructified spadices. This result suggests that it is possible to see many fructified spadices with leaves which float on the sea in the natural habitat and all of them were detached at the basal part of the spadix. Also, the floating spadices are carried to other places from the original place by waves or currents and distribute the ripe seeds other areas. It was reported that the germination rate was improved an incision was done to the seed coat of *Z. capricornii* under low temperature and salinity (Conacher et al., 1994), or when these soaked with diluted seawater in the range of 1 and 10 psu (Hoostmans et al. 1987). These results support our findings that the seed germination rate of *Z. marina* was enhanced distilled water (0 psu), and that of *Z. caulescens* was enhanced under diluted seawater in the range of 0 and 10 psu at 20°C, 70% and 80% respectively.

However, rates less than 30% were observed in *Z. marina* from Shin-nase, Sagami Bay and Nami-ita. These results suggest that such higher germination rate was induced to break the dormancy in the seed by freshwater treatment, and it is not rare in terrestrial plant seeds. *Z. marina* grow in shallow coastal area or estuary in which salinity is very variable in low and such low salinity condition sometimes continue for a long period time. If the seeds of *Z. marina* do not have a potential to tolerate low salinity condition, they could not survive or grow. On the other hand, *Z. caulescens* grow in deeper area than *Z. marina*, where the salinity is higher than that in estuary or shallow coastal area. Hence, *Z. caulescens* showed higher germination rate of 70% or more compared with that of *Z. marina* in the range of 5 and 10 psu, but the rate was lower when the salinity is beyond beyond this range. In natural habitat, budding seeds were observed on fructified spadices of *Z. marina* by Arasaki (1950), and he proposed that seeds were esposed to low salinity condition and absorbed water. His observation supports our results. In contras to Churchill (1992) who proposed that the seed germination of seagrass is enhanced in low temperature or anaerobic conditions. But our results on the different temperature regime did not show better germination rate under low temperature. The different result obtained in our experiment maybe due to the fact that these were conducted under aerobic condition. Pretreatment by freshwater to the seeds of *Z. marina* and *Z. caulescens* is very efficient in promoting or inducing their seed germination without any consideration of culture period and amounts. The results will be used as new technology for the germling production of *Zostera* species.

From the results of this study, it is suggested that germinated seeds of *Z. marina* and *Z. caulescens* have some poten-

tial to acclimatize to low irradiance because of the coleoptile growth. Arasaki (1950) mentioned that coleoptile is an important organ as light sensor to start germination for the seeds of *Zostera* species. All the seeds used for the germination and germling growth experiments did not developed a coleoptile at the early stage of germination, but the seeds developed a coleoptile remained up to the second leaf come out in germling. For the germling growth of *Z. marina*, it is necessary to give them above 12 μmol photons $m^{-2} s^{-1}$, but 0.5 mol photons $m^{-2} d^{-1}$ is enough for the survival of the germling. The minimum daily cumulative irradiance to the seagrass bed in nature is reported to be 1.5 mol photons $m^{-2} d^{-1}$ (Yamaki et al. 2006). Also Joanne (2001) reported the minimum light requirement to young germlings of *Z. marina* collected from natural seagrass bed is 3.3 mol photons $m^{-2} d^{-1}$. These reports do not support our results, but suggest that young germlings of *Z. marina* and *Z. caulescens* can grow in low light conditions, especially *Z. caulescens* under 5 μmol photons $m^{-2} s^{-1}$ as minimum irradiance requirement. Because the habitat of *Z. caulescens* is deeper than that of *Z. marina*. However, plants of *Z. marina* and *Z. caulescens* in Sagami Bay have been observed in shallow coastal area growing at 2–3 m depth in the same place or sometimes mixed together since a few years ago through our recent field observation. Such phenomenon may cause deterioration of photic zone by water pollution in the coastal area, and as the results plants of *Z. caulescens* were pushed to the narrow range of their habitat.

Conclusions

Seed collection, germination, germling growth of *Z. marina* and *Z. caulescens* were investigated. For the seed collection it was very convenient to keep only fructified spadices in container filled with filtered natural seawater. The conditions for seed germination and germling growth of *Z. marina* and *Z. caulescens* varied widely, which reflected their vertical and horizontal distribution, especially in the estuary. The seed germination of both species was enhanced by the pretreatment with low salinity in the range of 0 and 10 psu, especially 0 psu (distilled water) for *Z. marina* and 0 to 10 psu for *Z. caulescens*. Seed germination of *Z. marina* was induced to keep them in distilled water at 20°C for two weeks under dark condition and very high germination rate, above 90%, for *Z. marina* was yielded by this treatment. For *Z. caulescens* above 70% of rate was obtained at 20°C for two weeks under dark condition. For the growth of germlings of *Z. marina* and *Z. caulescens*, the minimum irradiance requirement was observed in *Z. marina* at 12 μmol photons $m^{-2} s^{-1}$ and in *Z. caulescens* at 5 μmol photons $m^{-2} s^{-1}$, respectively. Results of this study will be used not only for rehabilitation and restoration of seagrass beds, but also as important technology for environmental conservation in coastal areas.

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References

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

Aioi, K., Komatsu, T. and Morita, K. 1998. The world tallest seagrass, *Zostera caulescens*, from north-eastern Japan. Aquat. Bot. 61: 87–93.

Arasaki, S. 1950. Studies on the ecology of *Zostera marina* and *Z. nana*. Bull. Jpn. Soc. Fish. 16: 70–76. (in Japanese with English abstract)

Bosworth, W. and Short, F. T. 1993. Port of New Hampshire mitigation plan. For the New Hampshire Commercial Marine Terminal Development Project in Portsmouth, New Hampshire. 98 pp.

Churchill, A. C. 1992. Growth characteristics of *Zostera marina* seedlings under anaerobic conditions. Aquat. Bot. 43: 379–392.

Conacher, C. A., Pointer, I. R., Butler, J., Pun, S. and Tree, D. J. 1994. Germination, storage and viability testing of seeds of *Zostera capricorni* Aschers. From a tropical bay in Australia. Aquat. Bot. 49: 47–58.

Dan, A., Izumi, Y., Mori, K., Hirokawa, A. and Terawaki, T. 1998. Flourishing of *Zostera marina* L. by artificial seeding on sediment stabilizer mat. Fish Eng. 35: 37–42.

Duarte, C. M. and Chiscano, C. L. 1999. Seagrass biomass and production: a reassessment. Aquat. Bot. 65: 159–174.

Edmund, P. G. and Frederick, T. S. 2003. Word Atlas of Seagrasses. University of California Press, 298 pp.

Hoostmans, M. J. M., Vermaat, J. E. and van Vierssen, W. 1987. Seed-Bank development, germination and early seedling survival of two seagrass species from The Netherlands: *Zostera marina* L. and *Zostera noltii* Hornem. Aquat. Bot. 28: 275–285.

Joanne, C. B. and Scott, W. N. 2001. Responses of eelgrass *Zostera marina* seedlings to reduced light. Mar. Ecol. Ser. 223: 133–141.

Kawasaki, Y., Iituka, T., Goto, H., Terawaki, T., Watanabe, Y. and Kikuti, K. 1988. Study on the technique for *Zostera* bed creation. Cent. Res. Inst. Electric Power Industry, Abiko Res. Lab. Rep. U14: 1–231. (in Japanese with English abstract)

Komatsu, T. 1997. Long-term changes in the *Zostera* bed area in the

Seto Inland Sea (Japan), especially along the coast of Okayama Prefecture. Oceanol. Acta. 20: 209–216.

Komatsu, T. and Tatsukawa, T. 1998. Mapping of *Zostera marina* L. beds in Ajino Bay, the Seto Inland Sea, Japan, by using echo-sounder and global positioning systems. J. Res. Oceanogr. 23: 39–46.

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 Resor. 16: 223–230.

Ministry of the Environment. 1994. Natural environment protection, primary survey report No. 4, 400 pp. (in Japanese)

Ministry of the Environment. 2000. Threatened wildlife of Japan: Red data book, 2nd ed. Japan wildlife research center, Tokyo, 678 pp. (in Japanese)

Ministry of the Environment. 2004. Notes concerning restoration of seagrass Bed, 100 pp. (in Japanese)

Moore, K. A., Neckles, H. A. and Orth, R. J. 1996. *Zostera marina* (eelgrass) growth and survival along a gradient of nutrients and turbidity in the lower Chesapeak Bay. Mar. Ecol. Ser. 142: 247–259.

Nakaoka, M. and Aioi, K. 2001. Ecology of seagrasses *Zostera* spp. (Zosteraceae) in Japanese waters: A review. Otsuchi Mar. Sci. 26: 7–22.

Okaichi, T., Komori, S. and Nakanishi, H. 1996. The resource of biomass and environment in the Seto Inland Sea. Kouseisya Kouseikaku, Tokyo, 272 pp.

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 English abstract)

Orth, R. J., Carruthers, T. J. B., Dennison, W. C., Duarte, C. M., Fourqurean, J. W., Heck, K. L., Hughes, A. R., Kendrick, G. A., Kenworthy, W. J., Olyarnik, S., Short, F. T., Waycott, M. and Williams, S. L. 2006. A global crisis for seagrass ecosystems. Biosci. 56: 987–996.

Park, J. I. and Lee, K. S. 2007. Site-specific success of three transplanting methods and the effect of planting time on the establishment of *Zostera marina* transplants. Mar. Poll. Bull. 54: 1238–1248.

Sagawa, T., Mikami, A., Komatsu, T., Kosaka, N., Kosako, A., Miyazaki, M. 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.

Short, F. T. and Wyllie-Echeverria, S. 1996. Natural and human induced disturbance in seagrass. Environ. Conserv. 23: 17–27.

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. 2002. Change in shoot density, biomass and leaf area of *Zostera caulescens* Miki from summer to autum in Funakoshi Bay of the Sanriku Coast, Japan. Otsuchi Mar. Sci., 28: 91–98.

Tamaki, H., Tokuoka, M., Nishijima, W., Terawaki, T. and Okada, M. 2002. Deterioration of eelgrass, *Zostera marina* L. meadows by water pollution in the Seto Inland Sea, Japan. Mar. Poll. Bull. 44: 1253–1258.

Tanaka, N., Omori, Y., Nakaoka, M. and Aioi, K. 2002. Gene flow among populations of *Zostera caespitosa* Miki (Zosteraceae) in Sanriku Coast, Japan. Otsuchi Mar. Sci. 27: 17–22.

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: 489–494. (in Japanese)

Yamaki, K., Shimbo, Y., Tanaka, M., Mitomi, R. and Ogawa, H. 2006. Study on expansion of eelgrass bed and new colony formation by seedlings transplant. Annual J. Coast. Engineering, JSCE. 53: 1006–1010. (in Japanese with English abstract)

Yamaki, K., Shimbo, Y., Tanaka, Koshikawa, Y., Lim, B. K., Nakamura, H. and Ogawa, H. 2007. Field study on the characteristic of the eelgrass bed under the severe wave condition and development of an erosion—Proof base for its transplantation. Annual J. Coast. Engineering, JSCE. 54: 1081–1085. (in Japanese with English abstract)