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Physicochemical Habitability Conditions for the Genus *Halophila* in Nakagusuku Bay, Japan

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Field observations were conducted to investigate the effects of physicochemical conditions on the distribution of the genus *Halophila* in subtropical Nakagusuku Bay (Okinawa, Japan). Four species belonging to the genus *Halophila* (*H. ovalis*, *H. major*, *H. nipponica*, and *H. decipiens*) were found growing in the study area. *H. nipponica* was most frequently shown in Nakagusuku Bay and *H. decipiens* was encountered less frequently, while *H. major* was mainly present at the same sites as *H. ovalis*. Statistical analysis using physicochemical data obtained at each observational site revealed that the distribution patterns of *H. ovalis* and *H. major* were independent of nutrient conditions, but were strongly affected by surface wave motion. The occurrence of *H. ovalis* was confirmed up to a depth of 8.3 m, and *H. decipiens* occurred below a depth of 9 m with *H. nipponica* where the organic content was high and wave motion was smallest among the observational sites. *H. nipponica* showed a preference for relatively high sedimentation and large amounts of organic materials and its distribution was independent of flow conditions. *H. major* grew together not only with *H. ovalis* but also with *H. nipponica*.

Keywords: Spatial distribution of *Halophila* genus; Physicochemical conditions; Field observation; Statistical analysis.

1. Introduction

Seagrasses form submersed meadow communities that are among the most productive on earth [1]. Therefore, they are recognized as important components of tropical and subtropical coastal ecosystems, and have been regularly investigated both qualitatively and quantitatively. In this respect, Costanza et al. [2] indicated an economic value of US$19004 ha^{-1} yr^{-1} for 17 seagrass beds, surpassed only by estuaries and swamps/floodplains and higher than those of coral reefs, shelf, tidal marsh/mangroves, lakes/rivers, and forests.

Halophila, *Halodule*, and *Cymodocea* are the most common genera in tropical and subtropical waters, and have been the subject of many studies [3]. Ralph [4] studied the photosynthetic response of *H. ovalis* and concluded that its optimum photosynthetic range was from 25°C to 30°C. Beer et al. [5] investigated adaptation strategies of some tropical seagrasses with respect to their abilities to grow in the upper intertidal region, and demonstrated the pH compensation point of three seagrass species in the tropical intertidal region. As discussed above, many studies regarding tropical and subtropical seagrass dynamics have been conducted and quantified the effects of biochemical conditions, such as temperature, salinity, desiccation, light intensity, pH, and nutrients. On the other hand, the effects of physical conditions, such as mean flow velocity, wave amplitude, and wave period, have also been documented [6], but the majority of such studies were limited to temperate species (e.g., *Zostera marina*), whereas there have been few investigations of tropical and subtropical species.

As Hydrocharitaceae are well-known as a complex taxonomic challenge mainly due to their high morphological plasticity [7], the Japanese taxonomy of *Halophila* had been confusing. For this concern, Uchimura et al. [3] reassessed the species diversity in Japan and recommended the following four species: *H. decipiens*, *H. major*, *H. ovalis*, and *H. nipponica*. Here, we present field observations from a subtropical bay in Okinawa, Japan, to investigate the effects of physicochemical conditions on the distribution of the genus *Halophila*, following the classification recommended by Uchimura et al. [3].

2. Materials and Methods

2.1. *In situ measurement and analytical methods*

Field observations were conducted intermittently avoiding a rough weather at 9 observational sites (see Fig.1 and Table 1) in Nakagusuku Bay, Okinawa, Japan, from June 2005 to August 2008. The environmental parameters observed in this study were water temperature, salinity, depth, and flow velocity at 15 cm above the sea floor. Water temperature and salinity were measured using self-registering measurement instruments (COMPACT-CT, Alec Electronics Co., Ltd.) at 1-min or 10-min intervals. Depth was also measured using self-registering pressure measurement instruments (COMPACT-TD, Alec Electronics Co., Ltd.) at the same time intervals as water temperature and salinity. Horizontal flow velocity components were measured using self-registering electromagnetic velocimeters

(COMPACT-EM, Alec Electronics Co., Ltd.) at 0.5-s intervals with a 1-hour burst time. Wave amplitude and wave period were calculated using zero-up crossing method, and each significant value was defined as an average of the top one-third.

Table 1 Descriptions of observational sites and the distribution of *Halophila* species in the study area. (The symbols of “++”, “+”, and “-“ mean “abundant”, “scarce”, and “not found”, respectively.)

station	latitude	longitude	mean depth	mean velocity	significant wave-velocity amplitude	significant wave period	*H. ovalis*	*H. major*	*H. nipponica*	*H. decipiens*
1	26°17'19.4''N	127°50'17.0''E	10.3 m	1.4	2.3	10.2	-	-	++	-
2	26°17'21.3''N	127°50'16.5''E	8.3 m	2.1	3.4	10.1	+	++	+	-
3	26°17'22.6''N	127°50'17.0''E	7.8 m	1.8	3.3	9.6	-	-	-	-
4	26°17'26.6''N	127°50'17.0''E	6.0 m	2.1	4.4	9.1	+	+	-	-
5	26°17'31.0''N	127°50'17.0''E	5.3 m	2.6	4.6	9.0	+	++	-	-
6	26°18'50.8''N	127°51'50.3''E	9.7 m	2.6	1.7	13.7	-	-	+	+
7	26°17'30.7''N	127°49'05.2''E	4.0 m	2.8	4.8	9.2	+	+	+	-
8	26°17'32.3''N	127°51'31.9''E	11.8 m	2.1	2.4	12.1	-	-	++	-
9	26°16'40.6''N	127°50'18.9''E	12.7 m	1.7	2.5	12.3	-	+	-	-

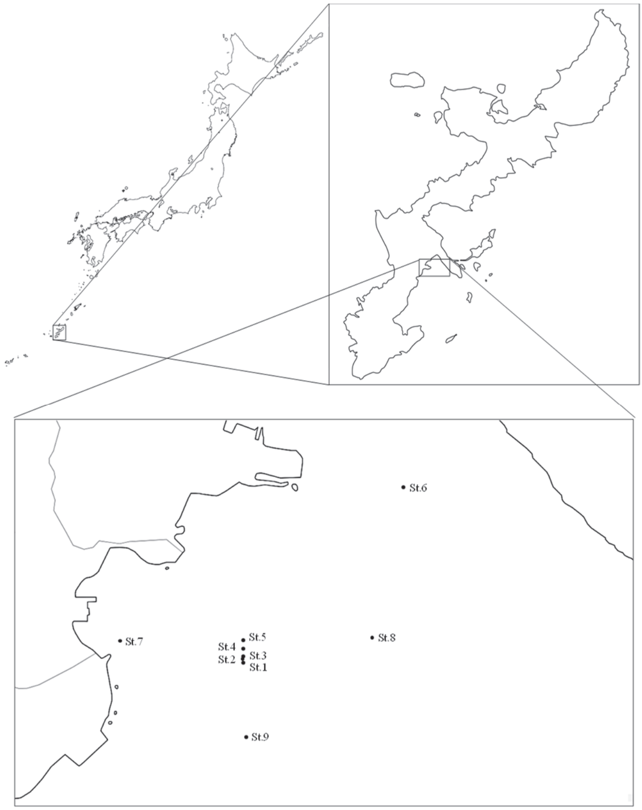


Fig. 1 Location of sampling sites.

Seagrass samples were obtained by SCUBA diving. Some shoots were desiccated in silica gel for later DNA extraction, and the remaining portions were transported to the laboratory for morphological examinations. DNA was extracted using a commercial kit (DNeasy plant mini kit, Qiagen) and protocol. The ribosomal ITS region including the 5.8S gene was selected for PCR analysis. Amplification and sequencing primers, amplification of the ITS regions of the rDNA, and automated sequencing were performed as described previously [8].

Water quality in the overlying water and the pore water and sediment quality were also measured sporadically. Sea water in the bottom layer was sampled by SCUBA diving using acid-washed polypropylene bottles taking care not to suspend the sediment. A portion of the sampled sea water was immediately filtered using a disposable filter with a pore size of 0.45 μm (Minisalt SM16555K, Saltrius K.K.). Filtrated and non-filtrated samples were bottled separately in acid-washed polypropylene bottles and stored in a cooler. Sediment was also sampled by SCUBA diving using acid-washed polypropylene bottles, and stored in a cooler. After field observation, samples were transported to the laboratory and a portion of the sediment was centrifuged in a box filled with N_2 gas to separate pore water. Obtained pore water was filtered using a disposable filter with a pore size of 0.45 μm (Minisalt SM16555K, Saltrius K.K.). All water and sediment samples were stored at –25°C in a freezer until chemical analysis as described below.

Ammonium (NH_4), nitrite (NO_2), nitrate (NO_3), and soluble reactive phosphorus (SRP) of filtrated water samples were analyzed by the indophenol, colourimetric, cadmium reduction-colourimetric, and ascorbic acid methods [9] using a spectrophotometer (TRAACS800, Bran–Luebbe). Total nitrogen (TN) and phosphorus (TP) concentrations of filtrated and non-filtrated water samples were analyzed by the cadmium reduction–colourimetric and ascorbic acid methods, respectively, after persulphate digestion. Particulate total nitrogen (PTN) and phosphorus (PTP) concentrations were calculated by subtracting dissolved total nitrogen (DTN) and phosphorus (DTP) concentrations from TN and TP concentrations, respectively. Dissolved organic nitrogen (DON) and phosphorus (DOP) concentrations were also calculated by subtracting the sum of inorganic nitrogen fractions (i.e., NH_4, NO_2, and NO_3) and SRP concentrations from DTN and DTP concentrations, respectively.

Acid volatile sulphide (AVS) content in the sediment was measured by the titration method using sodium thiosulphate solution, after sulphide was distilled from the sediment under acid conditions by addition of 20% sulphuric acid solution. Sediment chemical oxygen demand (COD) was analyzed by the alkaline potassium permanganate method. Kjeldahl TN content in the sediment was analyzed by the macro-Kjeldahl method [9], and TP content in the sediment was analyzed by the ascorbic acid method after nitric acid–perchloric acid digestion. Loss on ignition (LOI) of the sediment was calculated by the weight difference of the sample before and after heating at 600°C for 2 h. Total manganese and total iron contents in the sediment were measured with atomic absorption photometry (Z-2000, Hitachi Ltd.), after decomposition using hydrochloric acid and nitric acid solution. Total organic carbon (TOC) was measured using a CHN analyzer (JM10, J-Science Lab Co., Ltd). Grain size composition was also measured by sieving method.

2.2. *Data analysis*

The obtained flow velocity data were statistically analyzed using the statistical software (Ekuseru-Toukei 2006 for Windows, Social Survey Research Information Co., Ltd.). First, Bartlett's test of homogeneity of variance was used to test the hydrodynamic similarity between the observational sites using hydrodynamic data, i.e., mean flow velocity, wave velocity amplitude, and wave period, calculated from flow velocity data that were measured continuously using self-registering electromagnetic velocimeters. If the assumption of equality of variance was rejected, the Kruskal–Wallis test was performed. Moreover, principal component analysis was carried out using nutrient concentrations in the bottom layer and the pore water and sediment quality.

It is rather difficult to quantitatively consider growth rates and/or abundances of *Halophila* species based on our observational results. Therefore, in this study, we analyzed based on whether each individual exist there or not and its relationship with the environmental conditions.

3. Results

3.1. *Distribution of Halophila*

Uchimura et al. [10] reported that the genus *Halophila* includes four distinct species in Japan: *H. ovalis*, *H. major*, *H. nipponica*, and *H. decipiens*. All of these species were found growing in Nakagusuku Bay. *H. ovalis* was found at Stns. 2, 4, 5, and 7. *H. major* was present at the same sites as *H. ovalis*, but also at Stn. 9. *H. major* especially dominated Stns. 2 and 5. *H. nipponica* was found frequently in Nakagusuku Bay and was confirmed at multiple sites in our observation, but not at Stns. 3, 4, 5, or 9. *H. decipiens* was encountered less frequently except for Stn. 6. Overall, the distribution of *Halophila* species in our observational period was rather steady and is briefly summarized in Table 1.

3.2. *Observational results and statistical analysis*

Water temperature fluctuated between 17.1°C and 30.1°C, and there were no obvious differences among the observational sites. Salinity showed a steady value from 33.0 psu to 33.6 psu at all sites. The sediment sampled from Stns. 6 and 9 contained larger portion of fine particles and Stn. 3 was rather coarse (Fig.2).

As the assumption of equality of variance was rejected for all hydrodynamic data in Bartlett's test, the Kruskal–Wallis test was performed and summarized as below. The results of mean velocity analyses showed that Stn. 1 was the slowest site, at which the mean flow velocity was 1.4 cm s^{-1}, followed by Stn. 9 (1.7 cm s^{-1}) and Stn. 3 (1.8 cm s^{-1}). Stns. 2, 4, and 8 were intermediate sites (2.1 cm s^{-1}), and there were no differences among them. Mean flow velocity at Stns. 5 and 6 were higher (2.6 cm s^{-1}) with no difference between them, and that at Stn. 7 was highest (2.8 cm s^{-1}) (see also Fig.3(a)). Concerning significant wave amplitude, that at Stn. 6 (1.7 cm s^{-1}) was smallest, and the values at Stns. 1, 8, and 9 (about 2.4 cm s^{-1}) were also rather small. Those at Stns. 2 and 3 were larger (about 3.4 cm s^{-1}) and were indistinguishable. Those at Stns. 4, 5, and 7 were largest (about 4.6 cm s^{-1}) (see also Fig.3(b)). Significant wave periods at Stns. 4, 5, and 7 were the shortest (about 9.1 s) and were indistinguishable, followed by Stn. 3 (9.6 s). The values at Stns. 1 and 2 were intermediate (about 10.2 s) and there was no significant difference between them. Those at Stns. 8 and 9 were longer (about12.2 s) with no difference between them, and that at Stn. 6 was the longest (13.7 s) (see also Fig.3(c)).

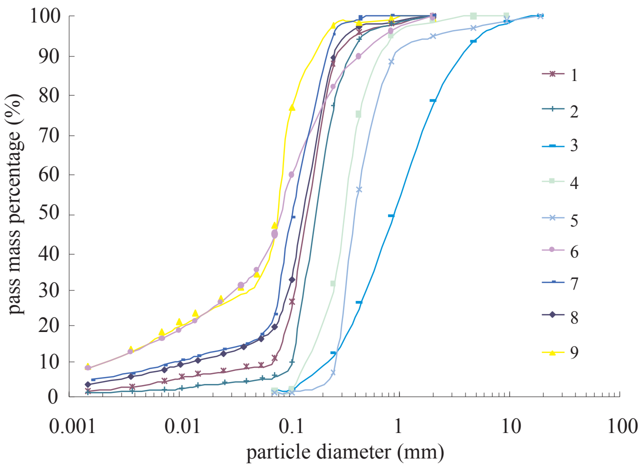
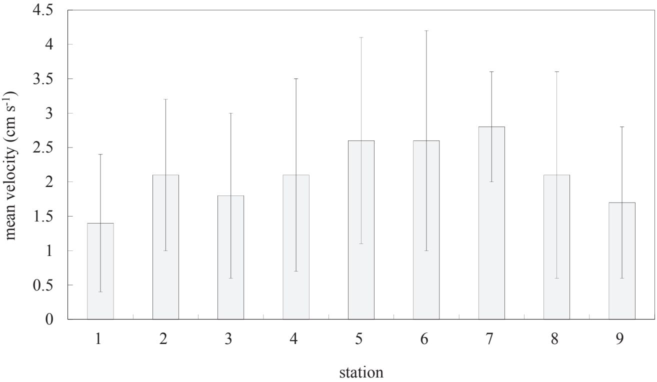
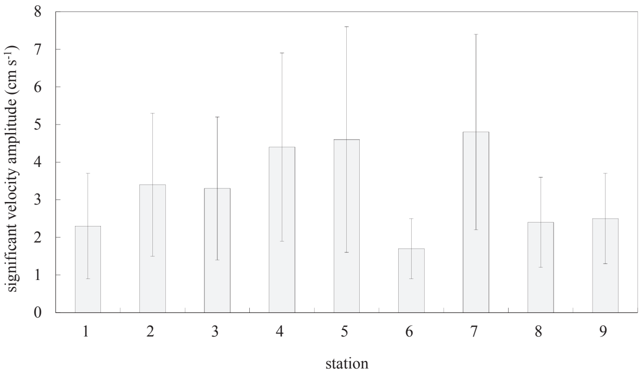


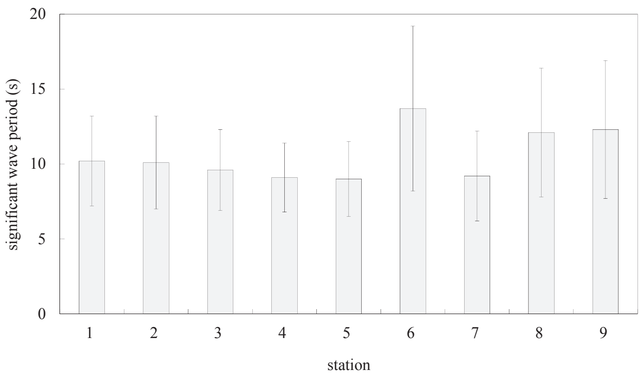
Fig. 2 Pass mass percentage of the sampled sediment.



(a) mean velocity



(b) significant velocity amplitude



(c) significant wave period

Fig. 3 Hydrodynamic properties.

Principal component analysis regarding nutrient concentrations in the overlying water and the pore water, and sediment quality showed that the first principal component was the most significant with a contribution of 98.65% (see Fig. 4). Fig. 5 shows characteristic vectors and factor loadings of the first principal component. According to Fig. 5 (a), the characteristic vector of NH_4 concentration in the pore water showed the largest value, followed by those of NO_3 and SRP concentrations in the pore water. Moreover, Fig. 5 (b) shows that factor loadings of nutrient concentrations in the pore water had large positive values and those of organic matter, iron, and manganese content in the sediment had large negative values. Principal component scores are shown in Fig. 6. Principal component scores of Stns. 2, 6, 7, and 8 showed negative values, and those of Stns. 3, 4, and 5 showed large positive values.

4. Discussion

4.1. *Statistical analysis*

From the results of principal component analysis, factor loadings of nutrient concentrations in the pore water showed large positive values and those of organic matter, iron, and manganese content in the sediment showed large negative values for the first principal component. In addition, principal components scores of Stns. 2, 6, 7, and 8 showed negative values, and those of Stns. 3, 4, and 5 had large positive values. Therefore, in this paper, Stns. 2, 6, 7, and 8 were considered as sites with relatively high sedimentation tendencies containing large amounts of organic materials. On the other hand, Stns. 3, 4, and 5 were considered to have relatively high degradation tendencies with less sedimentation and richer nutrient conditions. Moreover, from the results of the Kruskal–Wallis test, Stns. 2, 3, 4, 5, and 7 were regarded as sites strongly affected by surface wave motion. In contrast, the other sites were less affected by surface waves. Mean flow velocities at Stns. 1 and 9 were slower, but those at Stns. 6 and 8 were intermediate and higher, respectively.

4.2. *Distribution of Halophila in Nakagusuku Bay*

The distribution patterns of *H. ovalis* and *H. major* were independent of the principal component score, i.e., nutrient conditions were not responsible for their distributions in the Awase area. On the other hand, they occurred at sites strongly affected by surface wave motion. [11] tested the effects of siltation on Southeast Asian seagrass community structure and biomass, and revealed that *H. ovalis* is the next most sensitive to siltation compared to *Enhalus acoroides*. Our results

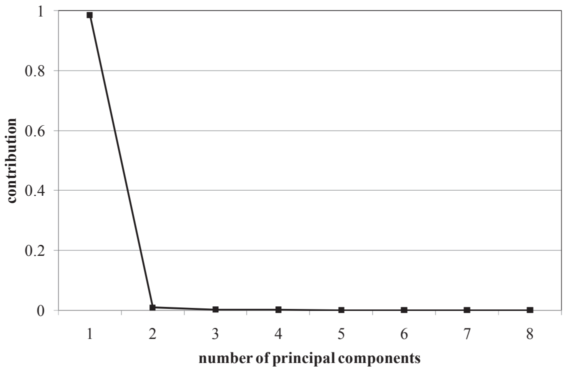
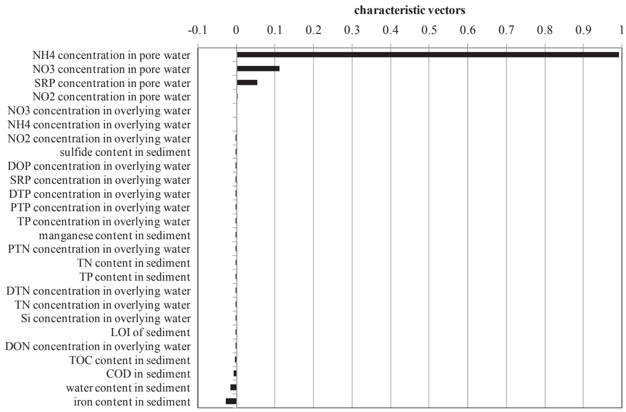
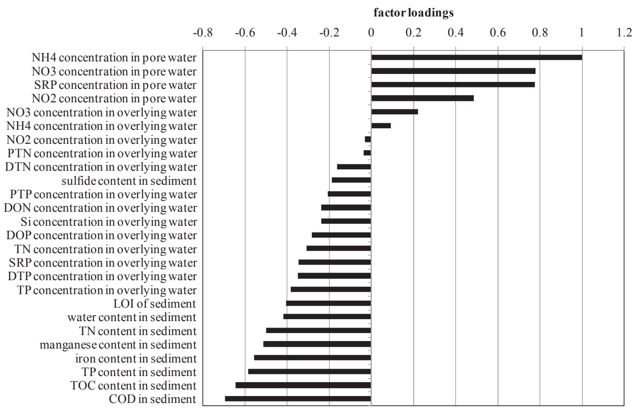


Fig. 4 Scree plot of contribution as a function of principal components.



(a) characteristic vectors



(b) factor loadings

Fig. 5 Results of the first principal component.

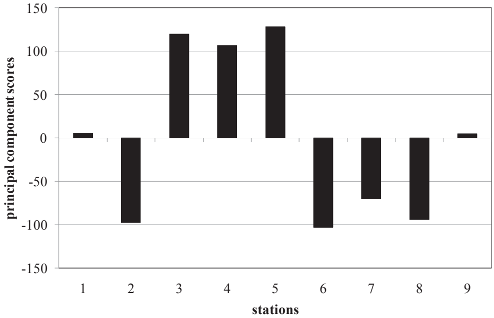


Fig. 6 Principal component scores.

agreed with their findings, but may also mean that the plants required high levels of light intensity for their growth [e.g. 12].

According to Kuo and Kirkman [13], *H. ovalis* and *H. decipiens* may grow together. However, Hulings [14] reported that *H. ovalis* occurs in a depth range of 0.5 to 2 m and there was no occurrence below a depth of 2 m, and some groups have shown that *H. decipiens* occurs in a depth range of 5 to 100 m [15]. At our observational site, the occurrence of *H. ovalis* was confirmed up to a depth of 8.3 m (Stn. 2), and *H. decipiens* occurred below a depth of 9 m (Stn. 6) with *H. nipponica* where the organic content was high and wave motion was smallest among our observational sites. These results were consistent with those reported previously [16].

H. nipponica also occurs over a wide area, but is not found at sites with high principal component scores. This suggests that *H. nipponica* has a preference for relatively high sedimentation and large amounts of organic material and its growth is independent of flow conditions. Uchimura et al. [8] reported that *H. nipponica* forms isolated small patches or is associated with *H. major* (the species described as *H. japonica* and *H. euphlebia* in their paper have been re-described as *H. nipponica* and *H. major*, respectively). According to our observations, *H. major* was associated only with *H. ovalis* at sites with high principal component scores (Stns. 4 and 5), and grew together not only with *H. ovalis* but also with *H. nipponica* at sites with low principal component scores (Stns. 2 and 7).

H. nipponica leaves found in Awase area usually had a round shape, but some had a narrow shape only at Stn. 2. Short [17] reported that seagrass morphology is closely linked to available nutrient resources. However, we could not determine the cause of the leaf deformation, and this remains to be examined in a future study.

5. Conclusions

Field observations regarding water temperature, salinity, flow velocity, water quality, sediment quality, and pore water quality were conducted to investigate the effects of physicochemical conditions on *Halophila* genus distribution in a subtropical coastal area in Japan. Nine observation sites were statistically distinguishable from each other, and their physicochemical properties were compared with *Halophila* genus distribution.

We have to note that the existence or abundance of seagrass species in coastal area must be strongly affected by catastrophic events such as the rough weather and a typhoon, which cannot be considered from our observation. Although preferable physicochemical conditions for four species of *Halophila* were briefly summarized in this paper, further studies based on physiological aspects are required to elucidate the details of these relationships.

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References

1. B. W. Touchette and J. M. Burkholder, Overview of the physiological ecology of carbon metabolism in seagrasses, *J. Exp. Mar. Biol. Ecol.* **250**, 169 (2000).
2. R. Costanza, R. d'Arge, R. d. Groot, S. Farber, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R. V. O'Neill, J. Paruelo, R. G. Raskin, P. Sutton and M. v. d. Belt, The value of the world's ecosystem services and natural capital, *Nature* **387**, 253 (1997).
3. M. Uchimura, E. J. Faye, S. Shimada, S. Arai, T. Inoue and Y. Nakamura, A re-evaluation of the taxonomic status of *Halophila euphlebia* Makino (Hydrocharitaceae) based on morphological features and ITS sequence data, *Bot. Mar.* **49**, 111 (2006).
4. P. J. Ralph, Photosynthetic response of laboratory-cultured *Halophila ovalis* to thermal stress, *Mar. Ecol. Prog. Ser.* **171**, 123 (1998).
5. S. Beer, M. Mtolera, T. Lyimo and M. Björk, The photosynthetic performance of the tropical seagrass *Halophila ovalis* in the upper intertidal, *Aquat. Bot.* **84**, 367 (2006).
6. M. S. Fonseca and M. A. R. Koehl, Flow in seagrass canopies: The influence of patch width, *Estuar., Coast. Shelf Sci.* **67**, 1 (2006).

7. V. X. Nguyen, M. Detcharoen, P. Tuntiprapas, U. Soe-Htun, J. B. Sidik, M. Z. Harah, A. Prathep and J. Papenbrock, Genetic species identification and population structure of *Halophila* (Hydrocharitaceae) from the Western Pacific to the Eastern Indian Ocean, *BMC Evol. Biol.* **14**, 92 (2014).
8. M. Uchimura, E. J. Faye, S. Shimada, G. Ogura, T. Inoue and Y. Nakamura, A taxonomic study of the seagrass genus *Halophila* (Hydrocharitaceae) from Japan: Description of a new species *Halophila japonica* sp. nov. and characterization of *H. ovalis* using morphological and molecular data, *Bull. Nat. Sci. Mus. Tokyo, Ser. B* **32**, 129 (2006).
9. APHA-AWWA-WEF: Standard Methods for the Examination of Water and Wastewater, 20th edition. American Public Health Association/American Water Works Association/Water Environment Federation, New York, USA (1998).
10. M. Uchimura, E. J. Faye, S. Shimada, T. Inoue and Y. Nakamura, A reassessment of *Halophila* species (Hydrocharitaceae) diversity with special reference to Japanese representatives, *Bot. Mar.* **51**, 258 (2008).
11. J. Terrados, C. M. Duarte, M. D. Fortes, J. Borum, N. S. R. Agawin, S. Bach, U. Thampanya, L. Kamp-Nielsen, W. J. Kenworthy, O. Geertz-Hansen and J. Vermaat, Changes in community structure and biomass of seagrass communities along gradients of siltation in SE Asia, *Estuar., Coast. Shelf Sci.* **46**, 757 (1998).
12. S. Beer and M. Björk, Measuring rates of photosynthesis of two tropical seagrasses by pulse amplitude modulated (PAM) fluorometry. *Aquat. Bot.* **66**, 69 (2000).
13. J. Kuo and H. Kirkman, *Halophila decipiens* Ostenfeld in estuaries of southwestern Australia, *Aquat. Bot.* **51**, 335 (1995).
14. N. C. Hulings, The ecology, biometry and biomass of the seagrass *Halophila stipulacea* along the Jordanian coast of the Gulf of Aqaba, *Bot. Mar.* **22**, 425 (1979).
15. F. E. C. de Oliveira, J. Pirani and A. M. Giulietti, The Brazilian seagrasses, *Aquat. Bot.* **16**, 251 (1983).
16. E. Rasmussen, The wasting disease of eelgrass (*Zostera marina*) and its effects on environmental factors and fauna, in *Seagrass Ecosystems: A Scientific Perspective*, eds. C. P. McRoy and C. Helfferich (M. Dekker, New York, 1977) pp. 1-52.
17. F. T. Short, Effects of sediment nutrients on seagrasses: Literature review and mesocosm experiment, *Aquat. Bot.* **27**, 41 (1987).