The following supplements accompany the article

Seed dispersal in the seagrass *Zostera marina* L. is mostly within the parent bed in a protected bay

Shinya Hosokawa*, Masahiro Nakaoka, Eiichi Miyoshi, Tomohiro Kuwae

*Corresponding author: hosokawa@pari.go.jp

Marine Ecology Progress Series 523: 41–56 (2015)

Supplement 1. Geographic characteristics of Kurihama Bay, Japan, relative to nearby coasts

Figure S1 shows the site of the present study (E3) in Kurihama Bay and the surrounding coastal area. There is an eelgrass bed along the Hashirimizu coast near Kurihama Bay (E1 in Fig. S1; Tanaka et al. 2011). There is probably eelgrass along the Tomyosaki coast (E2), because Tanaka et al. (2011) reported sampling rafting shoots along the coast and the authors of the present study observed patches of eelgrass there.

In addition to these three sites, there are seven other coastal sites near Kurihama Bay (Fig. S1). Sand samples were collected at all 10 of these sites. Approximately 50 g of sand was collected from the top 3 cm of sand from a spot close to the water's edge at low tide. Samples were dried and grain sizes of the dried samples were measured by using a sonic sieving particle-size analyzer (RPS-205; Seishin Enterprise, Tokyo, Japan).

Sand-grain size ranged over the same order of magnitude at all sites (Table S1) and was relatively low at Nobi (N3), Tsukuihama (N4), Miura (N5), and Kaneda (N6). The grain-size at Kurihama Bay had approximately the same distribution as that at other eelgrass sites.

LITERATURE CITED

Tanaka N, Demise T, Ishii M, Shoji Y, Nakaoka M (2011) Genetic structure and gene flow of eelgrass *Zostera marina* populations in Tokyo Bay, Japan: Implications for their restoration. Mar Biol 158:871–882

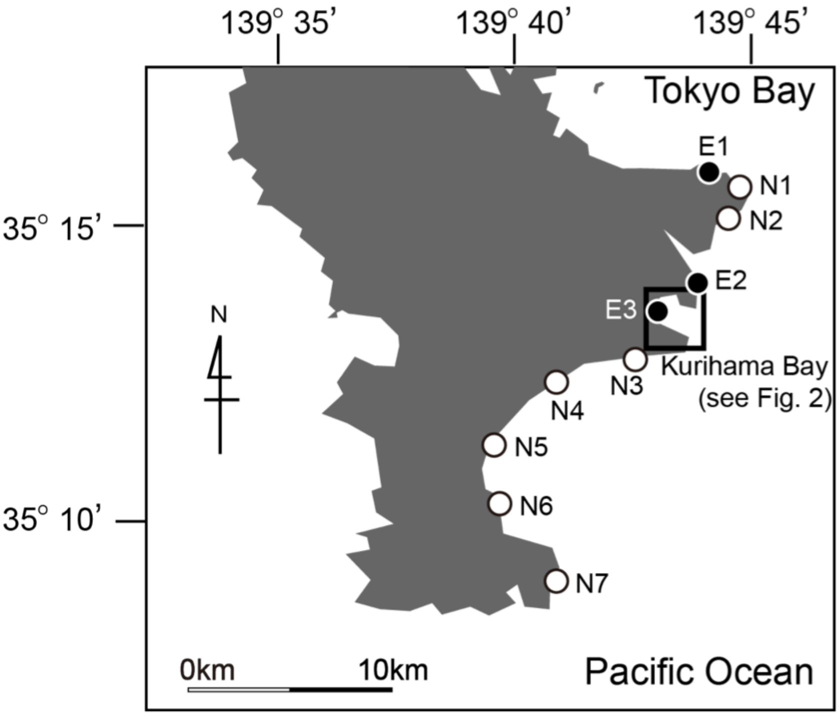

Fig. S1. Location of the study area (E3) and the sandy areas around the study site in Kurihama Bay, Kanagawa, Japan. Closed and open circles indicate sites where eelgrass beds were either observed or not, respectively. Information related to each site is presented in Table S1.

Table S1. Sand-grain sizes at the shoreline in the study area and at sandy areas around the study site in Kanagawa, Japan. The location of each site is shown in Figure S1. D25, D50, and D75 are sieve mesh opening sizes that pass 25%, 50%, and 75% of the total sand mass, respectively.

Site	Locality (Site name, City)	Sand-grain size (mm) D50 (D25–D75)	Notes
E1	Hashirimizu, Yokosuka	0.55 (0.37–1.44)	40,000 m^2 of bed (Tanaka et al. 2011)
E2	Tomyosaki, Yokosuka	0.81 (0.68–1.20)	Drifting shoots were observed by Tanaka et al. (2011). Patches were observed by the authors of the present study.
E3	Kurihama, Yokosuka	0.52 (0.34–1.40)	Approximately 6000 m^2 of bed was observed in this study.
N1	Kannonzaki, Yokosuka	1.15 (0.83–1.48)	No report whether eelgrass is present or not. However, the authors of the present study think that small bed might be present.
N2	Tatarahama, Yokosuka	0.79 (0.29–1.31)	No report whether eelgrass are present or not.
N3	Nobi, Yokosuka	0.37 (0.26–0.64)	No report whether eelgrass are present or not.
N4	Tsukuihama, Yokosuka	0.17 (0.14–0.21)	No report whether eelgrass are present or not.
N5	Miura, Miura	0.20 (0.16–0.29)	No report whether eelgrass are present or not.
N6	Kaneda, Miura	0.24 (0.19–0.46)	No report whether eelgrass are present or not.
N7	Oura, Miura	0.57 (0.39–2.27)	No report whether eelgrass are present or not.

Supplement 2. Mathematical derivations and results of statistical analyses.

Generalized linear model for buoyancy tests. The full model for the proportion of floating spathes is described by the following equation:

$$\log_e\left(\frac{P_{\mathrm{m1},i}}{1-P_{\mathrm{m1},i}}\right)=\mathbf{X}_{\mathrm{m1},i}\mathbf{a}_{\mathrm{m1}}, \quad \text{(S2.1)}$$

where $P_{\mathrm{m1},i}$ is the proportion of floating spathes i, and $\mathbf{a}_{\mathrm{m1}}$ is a vector of parameters for explanatory variables. $\mathbf{X}_{\mathrm{m1},i}$ is a vector of explanatory variables, described as:

$$\mathbf{X}_{\mathrm{m1},i}=\begin{bmatrix}1 & Seed_i & Type_i & Year_i & Seed_i\times Type_i & Seed_i\times Year_i & Type_i\times Year_i\end{bmatrix}, \quad \text{(S2.2)}$$

where $Seed_i$ is the number of seeds in spathe i, $Type_i$ is a status observation recorded as $Type_i = 0$ for harvested spathes and $Type_i = 1$ for detached spathes. $Year_i$ is the year of the test, which is recorded as $Year_i = 0$ for spathes tested in 2009 and $Year_i = 1$ for spathes tested in 2010.

The response variable $Y_{\mathrm{m1},i}$ is the status observation of spathe buoyancy, recorded as 0 for sinking spathes and 1 otherwise. The Bernoulli error distribution is applied as the probability distribution of $Y_{\mathrm{m1},i}$. Estimated parameters for the probability of floating spathes are shown in Table S2.

Generalized linear mixed model for the proportion of separated spathes and rhipidia washed up along the shoreline of Kurihama Bay, Japan. The full model for the proportion of separated spathes washed up along the shoreline is described by the following equation:

$$\log_e\left(\frac{P_{\mathrm{m2},j}}{1-P_{\mathrm{m2},j}}\right)=\mathbf{X}_{\mathrm{m2},j}\mathbf{a}_{\mathrm{m2}}+u_j, \qquad \text{(S2.3)}$$

where $P_{\mathrm{m2},j}$ is the proportion of separated spathes or rhipidia along transect line j, $\mathbf{X}_{\mathrm{m2},j}$ is a vector of fixed factors, $\mathbf{a}_{\mathrm{m2}}$ is a vector of parameters for fixed factors, and u_j is a random factor. $\mathbf{X}_{\mathrm{m2},j}$ is described as:

$$\mathbf{X}_{\mathrm{m2},j}=\left[1 \quad Date_j \quad Year_j \quad Date_j\times Year_j\right], \qquad \text{(S2.4)}$$

where $Date_j$ is the date that diaspores along the shoreline were collected, and $Year_j$ is the year of investigation, recorded as $Year_j = 0$ for 2009 and $Year_j = 1$ for 2010. u_j ($j = 1, \ldots, n_j$) is independent and Gaussian distributed with mean 0 and variance σ_u^2 (Broström & Holmberg 2011). σ_u is called a "scale parameter" in the function 'glmmML' in the R statistical package (R Core Team 2013).

The response variable $Y_{\mathrm{m2},j}$ is the number of separated spathes or spathes included in rhipidia. The binomial error distribution determined by $Y_{\mathrm{m2},j}$ and $\mathrm{N}_{\mathrm{m2},j}$, which is the total number of spathes included in all diaspores, was applied as the probability distribution of $Y_{\mathrm{m2},j}$. Estimated parameters for the proportion of spathes and rhipidia washed up along the shoreline are shown in Table S3.

Generalized linear mixed model for the density of seeds and seed-coat fragments. The full model for the density of seeds and seed-coat fragments in sediments is described by the following equation:

$$\log_e\left(Y_{\mathrm{m3},k}\right)=\mathbf{X}_{\mathrm{m3},k}\mathbf{a}_{\mathrm{m3}}+r_k, \qquad \text{(S2.5)}$$

where $Y_{\mathrm{m3},k}$ is the density of seeds or seed-coat fragments at sampling point k, $\mathbf{X}_{\mathrm{m3},k}$ is a vector of fixed factors, $\mathbf{a}_{\mathrm{m3}}$ is a vector of parameters for fixed factors, and r_k is a random factor. $\mathbf{X}_{\mathrm{m3},k}$ is described as:

$$\mathbf{X}_{\mathrm{m3},k} = [1 \quad Date_k \quad Vegetation_k \quad Date_k \times Vegetation_k], \qquad \text{(S2.6)}$$

where $Date_k$ is the date that seeds or seed-coat fragments buried in the sediment were sampled, and $Vegetation_k$ is a status observation recorded as $Vegetation_k = 0$ for sampling outside of vegetated areas or $Vegetation_k = 1$ for sampling within a vegetated area. r_k ($k = 1, \ldots, n_k$) is independent and Gaussian distributed with mean 0 and variance σ_k^2 (Broström & Holmberg 2011). σ_k is called a "scale parameter" in the function 'glmmML' in the R statistical package (R Core Team 2013).

The Poisson error distribution was applied as the probability distribution of $Y_{\mathrm{m3},k}$. Estimated parameters for the density of seeds and seed-coat fragments are shown in Table S4.

LITERATURE CITED

Broström G, Holmberg H (2011) Generalized linear models with clustered data: Fixed and random effects models. Computational Statistics and Data Analysis 55:3123–3134.

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL, http://www.R-project.org/.

Table S2. Results of a generalized linear model for the proportion of spathes with positive buoyancy. Explanatory variables are the number of seeds per spathe (Seed; discrete), spathe type (Type; categorical, either detached or harvested), experimental year (Year; categorical, 2009 or 2010), and the interaction terms. The parameter ‘Type’ is the effect of harvested versus detached spathes, and ‘Year’ is the effect of 2010 versus 2009. Dashes indicate explanatory variables that are not included in models.

	Intercept	Seed	Type	Year	Seed × Type	Seed × Year	Type × Year	QAICc
AIC best fit	1.40	–1.69 (*)	–0.97	–	1.56	–	–	223.6
	1.62	–1.71 (*)	–1.06	–0.31	1.58 (*)	–	–	224.9
	1.25	–1.57 (*)	–0.92	0.35	1.52	–0.27	–	225.2
	1.13	–1.68 (*)	–0.55	0.41	1.54	–	–0.79	226.6
	0.94	–1.55	–0.58	0.78	1.49	–0.25	–0.51	227.2
	–1.34 (**)	–0.19 (*)	1.92 (***)	–	–	–	–	227.6
	–1.21 (*)	–0.19 (*)	1.89 (***)	–0.26	–	–	–	229.1
	–1.51 (**)	–0.10	1.94 (***)	0.47	–	–0.29	–	229.1
	–1.78 (*)	–0.19 (*)	2.50 (**)	0.76	–	–	–1.16	230.0
	–2.07 (*)	–0.10	2.54 (***)	1.47	–	–0.28	–1.14	230.1
	–1.90 (***)	–	1.97 (***)	–	–	–	–	230.2
	–1.80 (***)	–	1.95 (***)	–0.22	–	–	–	231.9
	–2.40 (**)	–	2.60 (***)	0.89	–	–	–1.26	232.6
	0.31	–0.20 (**)	–	–	–	–	–	244.2
	0.47	–0.21 (**)	–	–0.38	–	–	–	244.8
	0.28	–0.14	–	0.17	–	–0.22	–	245.6
	–0.26	–	–	–	–	–	–	248.4
	–0.14	–	–	–0.32	–	–	–	249.4

AIC, Akaike Information Criterion; $QAIC_C$, adjusted to the bias-corrected AIC

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Table S3. Results of generalized linear mixed models for the proportion of total diaspores washed up along the shoreline of Kurihama Bay, Japan, represented by separated spathes or rhipidia. Explanatory variables are date (Date; continuous variable), year (Year; categorical variable, either 2009 or 2010), and the interaction terms. The parameter Year is the effect of 2010 versus 2009. Dashes indicate explanatory variables that are not included in models.

	Fixed effects				Random effects	QAICc
	Intercept	Date	Year	Date × Year	Scale parameter	
Proportion of separated spathes						
AIC best fit	1.18 (**)	–	–	–	1.74	34.8
	2.56 (***)	–	–2.20 (***)	–	1.24	35.0
	1.83 (**)	–1.14	–	–	1.69	37.6
	3.28 (***)	–1.25	–2.23 (***)	–	1.17	37.7
	3.14 (**)	–1.00	–2.01	–0.32	1.18	41.4
Proportion of rhipidia						
AIC best fit	–1.43 (***)	–	–	–	1.63	37.6
	–3.89 (***)	2.38 (***)	1.82 (***)	–	0.85	38.0
	–2.55 (***)	–	1.83 (**)	–	1.25	38.3
	–2.82 (***)	2.43 (**)	–	–	1.35	38.6
	–3.15 (***)	1.17	0.81	1.64	0.83	41.3

AIC, Akaike Information Criterion; $QAIC_C$, adjusted to the bias-corrected AIC

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Table S4. Results of generalized linear mixed models for the density of seeds and seed-coat fragments in the sediment. Explanatory variables are the date (Date; continuous variable), location (Vegetation; categorical variable, either within or outside of vegetation), and the interaction term. The parameter "Vegetation" is the effect of within vegetation versus outside of vegetation. Dashes indicate explanatory variables that are not included in models.

	Fixed effects				Random effects	QAICc
	Intercept	Date	Vegetation	Date × Vegetation	Scale parameter	
Seeds						
AIC best fit	0.53	–3.86 (***)	2.38 (***)	–	1.13	39.9
	1.76 (***)	–3.71 (***)	–	–	1.54	42.6
	0.60	–4.19 (*)	2.26 (**)	0.45	1.13	43.3
	–1.13	–	2.35 (**)	–	1.76	45.3
	0.08	–	–	–	2.05	45.9
Seed-coat fragments						
AIC best fit	–1.10	1.54 (*)	2.94 (***)	–	1.26	40.1
	0.23	1.66	–	–	2.04	42.7
	–1.10	1.53	2.94 (**)	0.01	1.26	43.6
	119.7	–	–	–	691.4	104.4
	87.63	–	60.1	–	691.4	106.7

AIC, Akaike Information Criterion; $QAIC_C$, adjusted to the bias-corrected AIC

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.