

# Reproduction dynamics of eelgrass (*Zostera marina* L.) populations in Ise–Mikawa Bay, central Honshu, Japan

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## Abstract

Suitable methods for the conservation and reconstruction of eelgrass populations are needed because of decreasing eelgrass populations. For this, it is important to understand the reproduction mechanism of eelgrass populations at a molecular level as well as at a macro level. In the present study, we analyzed the dynamic changes in eelgrass populations in Ise–Mikawa Bay by monitoring their distribution from 1993 to 2005 and by genetic analysis using microsatellite polymorphism. The distribution of eelgrass populations in the eastern part of Ise Bay showed a periodic shift with a cycle of 4 years, that is, eelgrass populations appeared on the bar trough, gradually moved to the inshore side of the habitat every year, and disappeared in the fourth year. Microsatellite analysis revealed that the eelgrass populations in Ise–Mikawa Bay have high genetic diversity. The eelgrass populations in Ise Bay and those in Mikawa Bay showed genetic differentiation and genetic connectivity. The importance of upstream parent populations, seed transportation among local populations and genetic diversity were discussed for the sustainability of eelgrass populations.

*Keywords:* conservation, eelgrass, genetic diversity, Ise–Mikawa Bay, microsatellite, *Zostera marina*.

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## Introduction

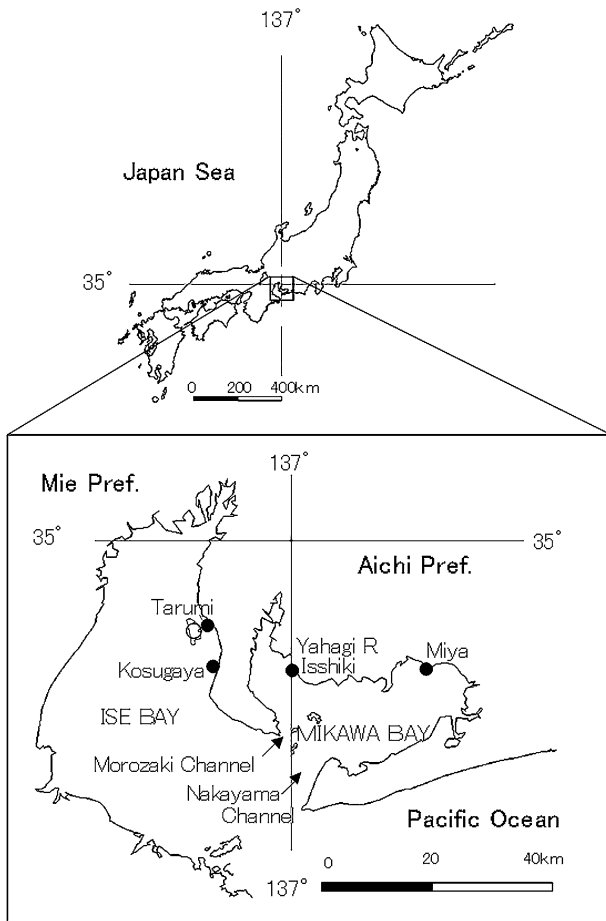
Eelgrass (*Zostera marina* L.) forms dense populations on shallow sandy and muddy bottoms in inner bay areas, and plays an important role from an ecological and fisheries standpoint (Heck *et al.* 1995). Eelgrass populations are decreasing significantly throughout the world as a result of human activities such as reclamation. Once eelgrass populations have been decreased by human activities, it is generally very difficult to restore the populations by plantation (Suzuki 2006). Eelgrass is a plant that has two breeding systems, vegetative propagation and sexual reproduction (Phillips & Menez 1988). It is unclear in many cases which reproduction mode works in each habitat. Therefore, plantation of foreign eelgrass without consideration of the genetic diversity could easily lead to

long-term disturbance of the ecosystem (Williams 2001; Hughes & Stachowicz 2004).

Ise–Mikawa Bay is a semi-enclosed bay located on Central Honshu Island, Japan (Fig. 1). The surface area of Ise Bay is 1738 km<sup>2</sup>. Large rivers, such as the Kiso River, flow into the head of the bay. Low salinity water with light density tends to flow in the surface from the bay head to the bay mouth. The surface area of Mikawa Bay is 604 km<sup>2</sup>. It is connected to Ise Bay by the Nakayama and Morozaki channels, which are 9 km wide. Ise Bay water and oceanic water flow into Mikawa Bay from Morozaki channel and flow out through Nakayama channel (Suzuki *et al.* 2002).

Until 1950, eelgrass populations covered most of the shallow water (until DL [datum level] –5 m) in Ise–Mikawa Bay. However, the distribution of the eelgrass populations has decreased significantly as a result of development in the coastal area and water pollution. At present, the main eelgrass population in Aichi prefecture is restricted to the east coast area in Ise Bay and to the

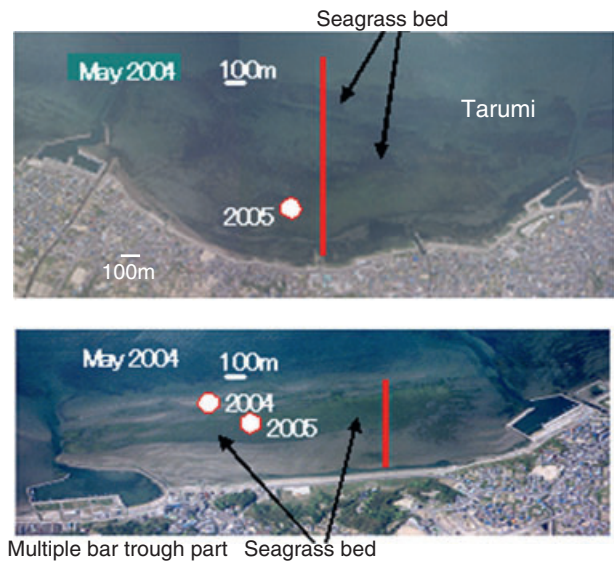
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**Fig. 1** Map of the sites studied in the Ise–Mikawa Bay. The man-made island (shown by the open circle) is constructed to offing 3 km in 2000, and the area of the man-made island is 577 ha.

northern coast area in Mikawa Bay. Tarumi and Kosugaya are typical foreshores of the Ise Bay east coast (Fig. 1). Eelgrass populations at Tarumi are 1000 m wide. Kosugaya beach consists of a multiple bar-trough part and an inshore flat part. Eelgrass populations form a belt-shaped distribution in the hollow of the multiple bar-trough part and an island-like colony in the inshore flat part (Yoshida *et al.* 2006). Isshiki in the northern coast area of Mikawa Bay is located near the mouth of the Yahagi River. Eelgrass populations at this beach are similar to those in Kosugaya. Miya is a semi-enclosed sea area that is interrupted by a breakwater offshore. Eelgrass populations with a width of 300 m are observed in this area.

In the present study, the reproductive features of eelgrass populations were examined by monitoring changes in the eelgrass populations in the east coast areas of Ise Bay and by genetic analysis. Based on the data, we discuss the genetic diversity of multiple eelgrass populations in Ise–Mikawa Bay in relation to changes in the eelgrass populations.



**Fig. 2** An aerial photograph of Kosugaya and Tarumi. The red lines show the positions at which the degree of vegetation cover of eelgrass was examined. The red circles show the positions at which the sampling area was set up for microsatellite analysis.

## Materials and methods

### *Measurement of the distribution of the eelgrass populations*

The degree of vegetation cover of eelgrass (proportion of the area covered by eelgrass in a 50 cm × 50 cm quadrat was quantified into five classes) was estimated by scuba diving from 1993 to May 2005 along a line at the center of each population of eelgrass in Tarumi (34°51'59"N, 136°51'05"E) and Kosugaya (34°50'03"N, 136°51'48"E), located on the east coast of Ise Bay (Fig. 1). The length of the lines from the position of DL ± 0 in an offshore direction was 600 m at Kosugaya and 1200 m at Tarumi (Fig. 2). The position of the sites was determined using a Differential Global Positioning System ([DGPS]; Furuno Electric, Nishinomiya, Japan).

### *Genetic analysis*

Genetic polymorphism of microsatellite markers was analyzed in eelgrass populations of Isshiki (34°46'18"N, 137°01'37"E) and Miya (34°48'15"N, 137°15'24"E) in Mikawa Bay, and in Tarumi and Kosugaya in Ise Bay (Fig. 1).

Leaf blades of eelgrass were harvested during May and June 2005. The sampling area of 10 m × 10 m (100 m<sup>2</sup>) was set up in the center of the eelgrass populations in Tarumi, Kosugaya, Isshiki and Miya. The sampling area was divided into meshes of 2 m with a rope and spindle. Leaf blades of eelgrass were sampled at the center of each

mesh. Twenty-five samples per each population (i.e. a total of 100 samples) were used for the analysis.

The samples were stored at  $-80^{\circ}\text{C}$  until use. DNA was extracted from 0.1 to 0.2 g fresh weight of leaf tissue using the hexadecyltrimethylammonium bromide (CTAB) method (Stacey & Isaac 1994). The DNA samples were genotyped by six microsatellite loci (Reusch *et al.* 1999; Reusch 2000a,b). The thermocycling profile of the polymerase chain reaction (PCR) consisted of a 10 min denaturation at  $95^{\circ}\text{C}$ , followed by 30 cycles of 1 min at  $95^{\circ}\text{C}$ , 1 min at  $57^{\circ}\text{C}$  and 1 min at  $72^{\circ}\text{C}$ , and then an additional 7 min at  $72^{\circ}\text{C}$ . Twenty nanograms of DNA was used as a template for the PCR. The PCR products were diluted to 10% with 12  $\mu\text{L}$  HiDi formamide (+1/40 vol. of Gene Scan -500 Rox size standard) and subjected to electrophoresis (Applied Biosystems). A capillary sequencer, 310 Genetic Analyzer (Applied Biosystems, California, USA), was used. The size (bp) of the microsatellite markers was calculated using an electropherogram with 310 Gene Scan 2.1 using the Gene Scan -500 Rox size standard.

Average heterozygosities ( $H_o$  and  $H_e$ : Nei 1987) and the inbreeding coefficient ( $F_{IS}$ : Wright 1951, 1965) were used to evaluate the genetic diversity of each group. A  $\chi^2$  test was used to test goodness of fit for deviation of genotypic frequencies from Hardy-Weinberg expectation. The genetic differentiation between the populations was determined from the coefficient of pairwise genetic differentiation ( $F_{ST}$ : Nei 1987). A significance test of the  $F_{ST}$  value for each dataset was done by  $10^5$ -fold permutation.

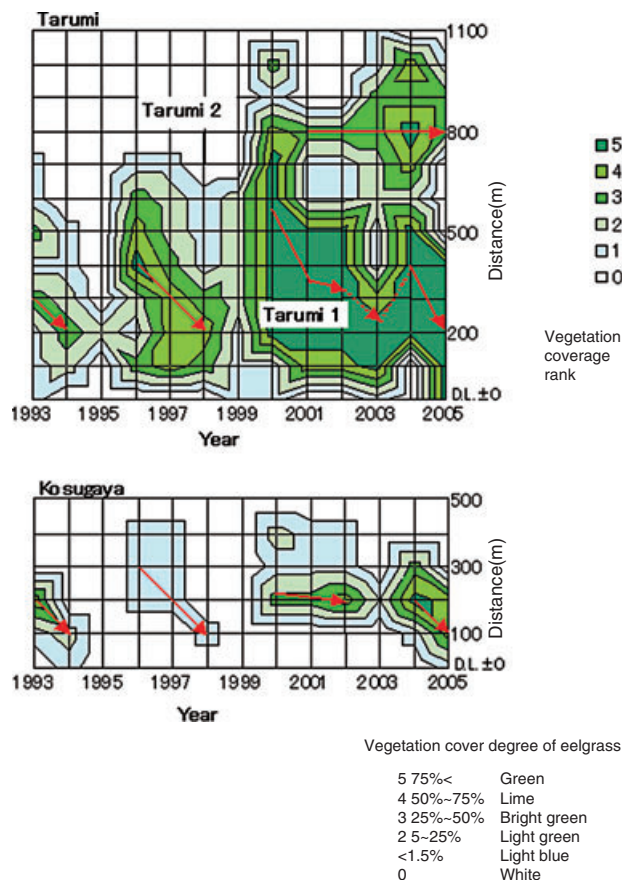
Genetic connectivity was examined using the genetic distance ( $D$ : Nei 1987) between populations using a phylogenetic tree made by the average distance method (UPGMA).  $H_e$ ,  $H_o$ ,  $F_{IS}$ ,  $F_{ST}$  and  $D$  were calculated by microsatellite analyser (Dieringer & Schlotterer 2003). The data of genetic polymorphism at Kosugaya ( $34^{\circ}50'03''\text{N}$ ,  $136^{\circ}51'43''\text{E}$ : Kosugaya2004) in 2004 (Yoshida *et al.* 2006) were also used. The genetic assignment of populations as the origin of individuals was done using GeneClass2 (Piry *et al.* 2004).

## Results

### *Spatial and temporal dynamics of the eelgrass populations*

As Tarumi and Kosugaya are major sites where large meadows of eelgrass have remained, their eelgrass populations were examined from 1993 to 2005 (Fig. 1). The degree of vegetation cover of eelgrass (proportion of the area covered by eelgrass in a  $50\text{ cm} \times 50\text{ cm}$  quadrat was quantified into five classes) was estimated by scuba diving along a line at the center of each population of eelgrass as shown by the red line in Fig. 2.

Figure 3 shows the changes in the distribution of the eelgrass populations on the survey line in Tarumi and



**Fig. 3** Change in the distribution of *Zostera marina* populations on the survey lines in Tarumi and Kosugaya, on the east coast area of Ise Bay, from 1993 to 2005. The changes in the center of gravity of the eelgrass population are shown with the arrows.

Kosugaya. In Tarumi, class 3 or more of vegetation cover was observed in an area 300 m and 500 m from the shoreline in 1993. Interestingly, the distribution of eelgrass changed to an area of 200 m in 1994, 100–610 m in 1996, 80–450 m in 1997, 80–340 m in 1998, 140–850 m and 1000 m in 2000, 80–580 m and 780–810 m in 2001 and 2002, 70–370 m and 760–910 m in 2003, 160–510 m and 630–1040 m in 2004, and 0–460 m and 790–920 m in 2005. It was remarkable that the vegetation cover degree was very low, class 2 or less, over the whole survey line in 1995 and 1999. Similar changes in vegetation cover were also observed at Kosugaya. In Tarumi, the degree of vegetation cover of eelgrass populations on the survey line became uniformly high in 2000. Since 2000 a high degree of vegetation cover in Tarumi has been maintained.

We estimated the center of the eelgrass population from the distribution and vegetation cover degree. Arrows in Fig. 3 show a change in the center of the eelgrass population. It is apparent that periodic change in the distribution of eelgrass populations was observed in Tarumi and

**Table 1** Genetic diversity of *Zostera marina* populations in Ise-Mikawa Bay calculated with allele data at six microsatellite loci

Location	Mean no. alleles	$H_o$	$H_e$	$F_{IS}$
Tarumi	11.3	0.840	0.836	-0.005
Kosugaya	11.0	0.853	0.848	-0.007
Isshiki	11.8	0.773	0.794	0.026
Miya	10.5	0.693	0.696	0.004
Kosugaya2004	12.0	0.842	0.807	-0.043

All  $F_{IS}$  values did not show a significant difference to expectation zero (Hardy-Weinberg equilibrium,  $P > 0.05$ ).  $F_{IS}$ , inbreeding coefficient;  $H_e$ , expected heterozygosity;  $H_o$ , observed heterozygosity.

Kosugaya. The center of the eelgrass populations shifted approximately 100–200 m every year. The degree of vegetation cover of the eelgrass populations decreased with the shift in the center of the populations over 3 years. The closest distance from the shoreline was 400–500 m in Tarumi and 200–300 m in Kosugaya. Once again, eelgrass populations showed regeneration with movement of the center of the populations in the direction of the shore from the next year.

*Genetic diversity*

Table 1 shows the parameters of genetic diversity. The average number of alleles that was detected in these six loci was 10.5–12.0 for the five populations. The average heterozygosity ( $H_o$ ) shows that the genetic diversity was higher than 0.800 for the populations in Tarumi, Kosugaya and Kosugaya2004, 0.773 for the population in Isshiki and 0.693 for the population in Miya. The inbreeding coefficient ( $F_{IS}$ ) did not show a significant difference from an expectation of zero (Hardy-Weinberg equilibrium) in all populations.

*Gene flow*

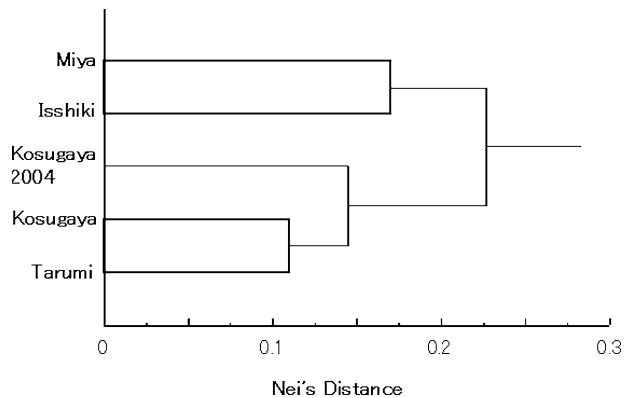
Table 2 shows the coefficient of pairwise genetic differentiation ( $F_{ST}$ ). Tarumi-Kosugaya, Tarumi-Kosugaya2004, Kosugaya-Kosugaya2004 and Isshiki-Miya did not show genetic differentiation. The  $F_{ST}$  of Tarumi-Isshiki, Tarumi-Miya, Kosugaya-Isshiki and Kosugaya-Miya, which corresponds to the  $F_{ST}$  between the site of Ise Bay and the site of Mikawa Bay, showed a value larger than zero ( $P < 0.01$ ). Figure 4 shows Nei's genetic distance ( $D$ ) between the eelgrass populations. The eelgrass populations of Ise-Mikawa Bay could be divided into two groups, Tarumi and Kosugaya, and Isshiki and Miya.

Table 3 shows the numbers of individuals assigned to each population as the most probable origin. Among the

**Table 2** Matrix of pairwise genetic differentiation ( $F_{ST}$ ) among populations of *Zostera marina* in Ise-Mikawa Bay

Location	Tarumi	Kosugaya	Isshiki	Miya	Kosugaya2004
Tarumi	—				
Kosugaya	0.000†	—			
Isshiki	0.045	0.040	—		
Miya	0.086	0.072	0.026	—	
Kosugaya2004	0.004*	0.000*	0.037	0.053	—

†  $F_{ST}$  did not show a significant difference to expectation zero ( $P > 0.01$ ).



**Fig. 4** Neighbor-joining tree based on Nei's genetic distance ( $D$ ).

**Table 3** Numbers of individuals of eelgrass assigned to each population of Tarumi, Kosugaya, Isshiki and Miya as the origin

Location	Location assigned as the origin			
	Tarumi	Kosugaya	Isshiki	Miya
Tarumi	12	7	5	1
Kosugaya	13	12	0	0
Isshiki	6	0	13	6
Miya	0	1	7	17

individuals sampled at Tarumi, seven individuals were assigned to Kosugaya. In addition, 13 individuals sampled at Kosugaya were assigned to Tarumi. Six individuals at Isshiki were assigned to Miya and seven individuals at Miya were assigned to Isshiki. Five individuals at Tarumi were assigned to Isshiki and six individuals at Isshiki were assigned to Tarumi, but individuals at Kosugaya were assigned to neither Isshiki nor Miya.

**Discussion**

*Reproduction modes of the eelgrass populations*

As shown in Fig. 3, we found that eelgrass populations in Tarumi and Kosugaya, in the east coast area of Ise Bay,

showed dynamic regeneration over a period of 4 years with an accompanying shift in the population center to the shore. We call this phenomenon a 'walking meadow'.

The observed shift in the eelgrass population center to shore was approximately 100 m per year (Fig. 3). As the expansion of rhizomes by vegetative propagation is significantly smaller than 100 m per year (Kawabata *et al.* 1993), the transport of seeds by rip currents is a key factor in the shift of the eelgrass population center to the shore. When eelgrass seeds are dispersed, they fall onto the sea bottom near the parents by gravity because they are negatively buoyant (Orth *et al.* 1994). The rip current would carry the eelgrass seeds a short distance and would cause mixing of seeds within eelgrass populations. The rip current in the bay during June and July, which is the period of seed dispersal in Tarumi and Kosugaya, is in the direction from offshore to inshore by a south-west wind (Japan Fisheries Resource Conservation Association (JFRCA) 1997). As a result, seeds are transported by the rip current to shore. Eelgrass seeds of various origins are physically mixed during the transport process by the rip current.

As shown in Fig. 2, a multiple bar trough in Kosugaya is formed by wind waves from the north-west in winter (JFRCA 1997). Seeds transported by the rip current would be trapped more easily in this trough. This transport of eelgrass seeds to shore is repeated every year. A phenomenon similar to that of Kosugaya was also observed in Tarumi before a large man-made island (Fig. 1) was built in 2000.

Table 1 shows that the genetic diversity of the eelgrass populations of Tarumi and Kosugaya was high compared with the diversity on the Atlantic and Californian coasts (Reusch *et al.* 2000; Hammerli & Reusch 2003; Muniz-Salazar *et al.* 2005). Hence, it is believed that the high genetic diversity of eelgrass populations in these areas is achieved by sexual reproduction as well as the physical mixing and transport of seeds.

In Tarumi, the degree of vegetation cover of the eelgrass populations on the survey line became high in 2000, approximately 800 m from the shore (Fig. 3). A high degree of vegetation cover has been maintained since 2000. This could be attributed to a weaker rip current in summer caused by the large man-made island (577 ha; Fig. 1), which was built 3 km offshore in 2000 (JFRCA 1997). The weaker rip current in summer will decrease the frequency of transport of eelgrass seeds to shore, increase the probability of seeds settling in the area, and decrease the loss of seedlings by wind waves in winter. All these factors contribute to an increase in the growth of eelgrass populations in the short term, but reduce the genetic diversity achieved by the physical mixing of the seeds in the long term. Moreover, when the large man-made island blocks the tidal residual current from the inner bay

areas to the bay mouth, and the watercourse is changed, the seed supply from Tarumi, the largest eelgrass population in Ise Bay, to Kosugaya and Mikawa Bay in the downstream would decrease. This would be one reason for the decline in eelgrass populations in Ise–Mikawa Bay.

At present, it is unclear why eelgrass populations in Tarumi and Kosugaya showed dynamic regeneration over a period of 4 years. We conjecture as follows. In summer, the seeds are transported to the trough near the shore by the rip current from offshore to inshore, and the center of the eelgrass populations in Tarumi and Kosugaya moves toward the shore. In the third year, the eelgrass populations reach the marginal areas of the tidal flat. The shift in the population center stops here because the marginal areas of the tidal flat are not suitable for eelgrass growth because they dry up and the water temperature is high (Kawasaki 1988). Over this period of time, the offshore eelgrass populations, whose vegetation cover degree is low because of the inevitable transport of seeds to shore, recover to the original levels based primarily on vegetative propagation. Because it is presumed that 4 years are required to supply a sufficient amount of seeds (Izumi *et al.* 2002), the overall degree of vegetation cover increases every fourth year. It is thought that this 'walking meadow' phenomenon with a cycle of 4 years is attributed to the physical characteristics of the waters (including the rip current to shore in summer and a multiple bar trough formed in winter) as well as to the characteristics of the regeneration process of eelgrass, which grows by both vegetative propagation and sexual reproduction.

#### *Metapopulation structure*

As the pairwise  $F_{ST}$  of the Tarumi and Kosugaya populations is zero, there is no genetic differentiation among these two populations. Therefore, it might be considered that the rate of gene flow between these two populations is high. However, it is plausible that Tarumi and Kosugaya were one large population until recent years so that they maintain high genetic diversity although their populations have been rapidly declined recently. Negligible values of  $F_{ST}$  for Kosugaya2004 with Tarumi or with Kosugaya (Table 2) also support the genetic connectivity of these two populations.

In addition to the short-distance transport of seeds, eelgrass has a system of long-distance transport of seeds by residual tidal current. As spadices containing seeds can float on the sea surface when they are detached from the shoots (Harwell & Orth 2002), seeds can be transported by drift. The long-distance transport of seeds by the drift of spadix of eelgrass can explain the cross-assignment of origin for individuals between Tarumi and Kosugaya populations and between Isshiki and Miya populations. The existence of a residual tidal current in Ise–Mikawa Bay

supports the long-distance transport of seeds by drift of the spadix of eelgrass.

### *Important factors for the sustainability of eelgrass populations*

It has been revealed that the gene flow between the eelgrass populations in Ise–Mikawa Bay can be attributed to the long-distance transport of seeds by the residual tidal current. The rapid reduction in the distribution area of eelgrass populations since 1960 (Takeda 2005) falls over the period in which large-scale reclamation projects in line with harbor development in the inner bay areas and large-scale levee protection works (including high tide breakwater) were undertaken. Thus, it is likely that the rapid decrease in eelgrass populations in Ise–Mikawa Bay since 1960 can be attributed to an extreme reduction in the supply of seeds to downstream, which would result in the disappearance of eelgrass populations, which had been widely distributed in the inner bay areas (i.e. upstream of the residual tidal current). Even today, reduction and fragmentation in the distribution of eelgrass populations are in progress in Ise–Mikawa Bay because of reclamation and other factors. A reduction and fragmentation in the distribution inhibit the transport of seeds to the entire bay and have a quite significant negative impact on the genetic diversity levels that are currently maintained by the eelgrass populations. It is feared that lower eelgrass seed supply to downstream and declining genetic diversity may accelerate a reduction in the eelgrass populations in the entire bay in the future.

Conventionally, efforts to restore eelgrass populations, based primarily on vegetative propagation, have been focused on small-scale eelgrass populations in foreshores as a compensatory measure against reclamation and large-scale development. For this reason, there has been a tendency to neglect the maintenance of a connected structure among local populations and genetic diversity. Targeting eelgrass populations in individual foreshores alone will be ineffective. It is important to have a concept of restoring the entire local populations in a 'self-sustained' manner for the whole bay. To preserve the eelgrass populations, it is essential to take proactive preservation measures, such as securing large-scale parent populations in the upstream waters of the residual tidal current that supplies seeds, selecting settlement areas for the stably supplied seeds based on flow analyses, and improving the habitat environment. These efforts will enable eelgrass populations in different foreshores of Ise–Mikawa Bay to survive as local populations in a self-sustained manner while maintaining connectivity, and will also lead to the preservation of the genetic diversity of eelgrass populations. It is noteworthy that numerical simulations that

have been carried out in developing nurseries for little-neck clams *Ruditapes philippinarum* take into consideration the relationship between the supply of larvae and the water flow of the entire bay (Suzuki *et al.* 2002). Likewise, the preservation of eelgrass populations will require efforts from a macroscopic view that are similar to those made in preserving a tidal flat.

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