

# Evaluation of genetic diversities in *Zostera japonica* for seagrass restoration in brackish water systems : A case study in the Hii River

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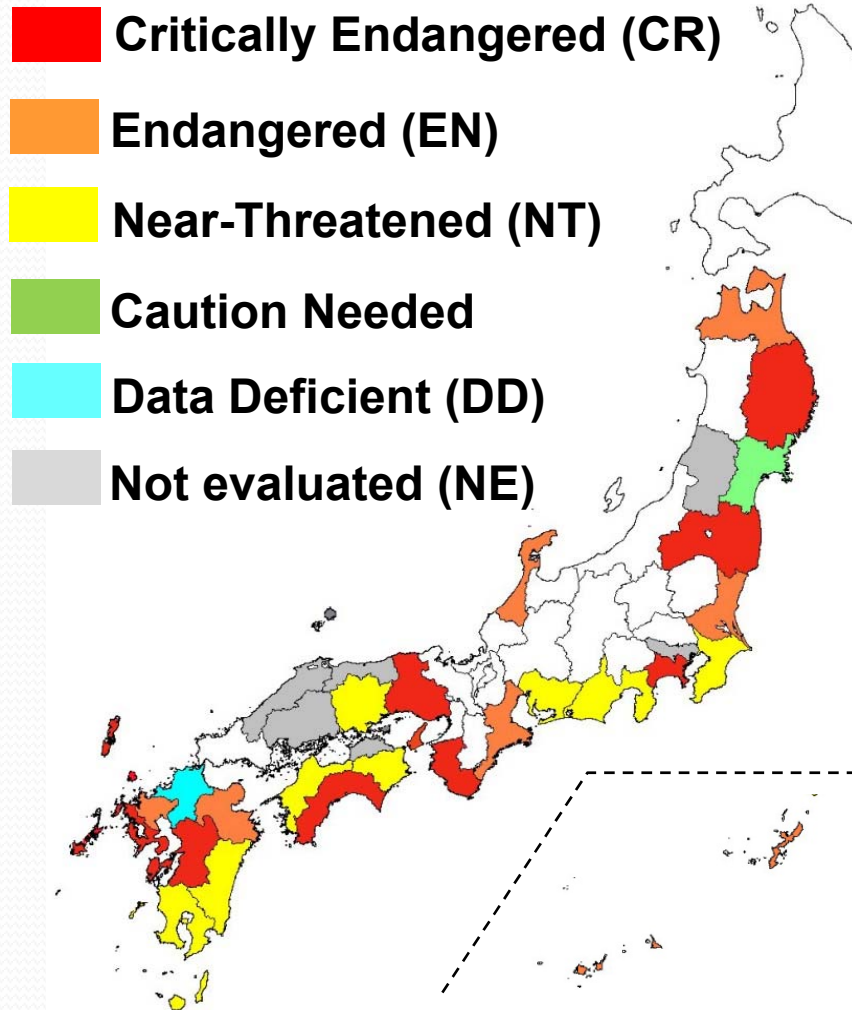
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# What is *Zostera japonica* (ZJ) ?



- Perennial seagrass in brackish water.
- Distributes widely in estuaries and lakes .
- Main component of seagrass bed.
- Reproduction: both sexual (seed) and asexual (clonal) .

# Present status and distribution in Japan



● RDB category in each prefecture:

$Z_j$  is categorized as NT to CR in 26 prefectures out of 32 prefectures.



**Many of local populations are threatened with extinction!**

Fig. RDB category of  $Z_j$  in each prefecture.

# Problems in restoration of seagrass bed

- Recognition of ecological function in seagrass bed.
- A number of seagrass management including restoration and transplantation have been addressed.
- Never disturb genetic diversity of local population and its local endemism (avoidances of genetic pollution and out-breeding depression).

\* Guideline of the Fisheries Agency for seagrass bed restoration (2007)  
“ Careful attentions for donor population selection is needed.  
**‘Fixation Index ( $F_{st}$ ) less than 0.05 is acceptable’**”

To conserve endemic genetic diversity and restore seagrass bed effectively, it is needed to find & preserve donor populations which have enough genetic diversities and gene flow to the restoration site.

# Our objectives

Using DNA (SSR) markers for restoration and monitoring of seagrass bed is important (Procaccini *et al.* 2007 J. Exp. Marine Biol. Ecol. 350-234-259).

- Genetic analysis using SSR markers makes problems clear and gives ideas for effective restoration plan.
- Genetic monitoring should provide an early warning signal that the system is in distress, long before conditions become irreversible and possibly un-restorable.

## From the point of the conservation genetics...

1. The estimation of genetic diversities of local populations in Hii river system (Lake Shinji & Lake Nakaumi)
2. The restoration design in seagrass bed

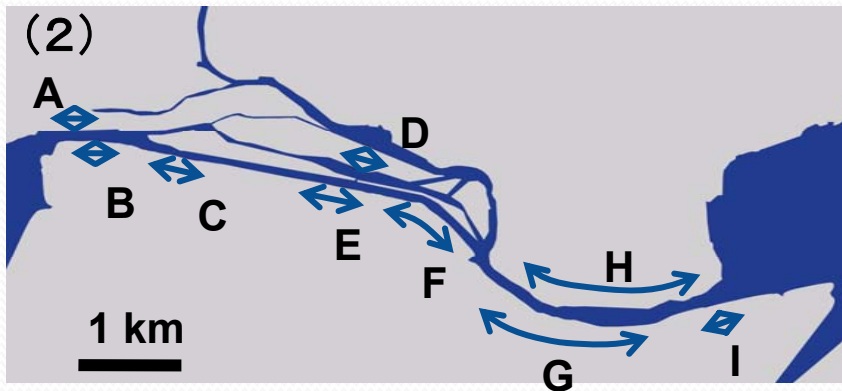
# Study sites: Lower reach of the Hii River



Fig. Distribution of *Z. japonica* populations in the Hii River (modified the Ministry of Land, infrastructure, Transport and Tourism (2008)).

- Located west part of Japan, emptying into the Japan sea.
- In downstream, two large brackish-water lakes (Lake Shinji & Lake Nakaumi: designated wetland under the Ramsar Convention).
- In the past, *ZJ* populations were widely distributed.  
→ Now, the populations are remarkably declined and dispersed due to reclamation, revetment and eutrophication.
- *ZJ* is a important key specie to restore seagrass bed.

# Sampling sites



## ● Sampling sites

L. Nakaumi: 6 points (NA-NF)...1

Ohashi Riv.: 9 points (A-I) ....2

## ● Sampling methods

After examining rough population size, leaves were collected in equal interval (each 1-2m) to 5-60 points.

Fig. Localities and populations sampled.

# Genetic markers and genetic analysis

Table Characteristics of SSR markers.

Locus	Motif	No. of Alleles	Size range (bp)	$H_o / H_E$
Zn B1	(GA) <sub>4</sub> G(GA) <sub>20</sub>	7	108 - 120	0.517 / 0.670
Zn B8	(GA) <sub>14</sub>	20	135 - 191	0.780 / 0.903
Zn D6	(CT) <sub>5</sub> TT(CT) <sub>2</sub> TT(CT) <sub>13</sub> (GTC T) <sub>2</sub> GT(CT) <sub>10</sub>	16	226 - 262	0.675 / 0.892
Zn E7	(GA) <sub>18</sub>	7	149 - 167	0.727 / 0.773
Zn F8	(CT) <sub>17</sub>	19	195 - 249	0.775 / 0.888
Zn H8	(CT) <sub>3</sub> (CA) <sub>2</sub> T (CT) <sub>16</sub>	5	148 - 157	0.110 / 0.104

- The extraction of DNA  
CTAB method (Revised Doyle & Doyle 1987)

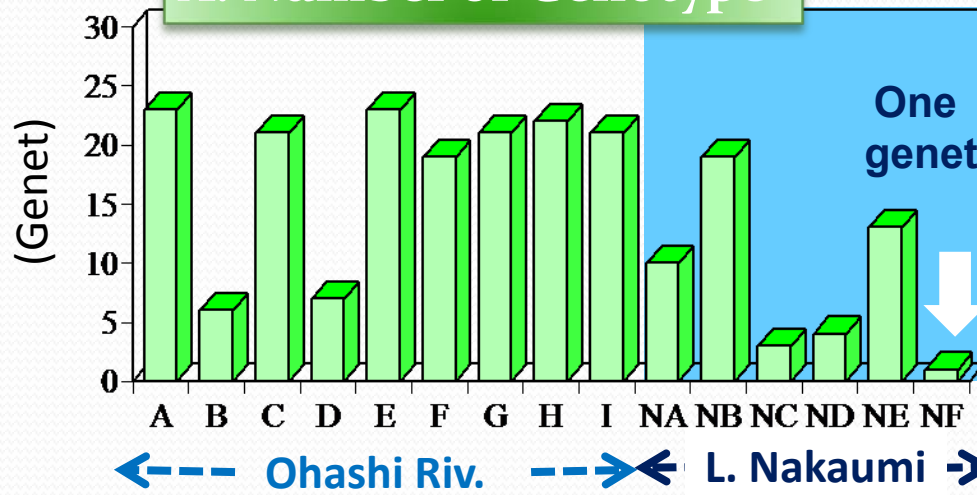
- Microsatellite (SSR) markers  
Using 6 Locus in *Zostera noltii*  
(Coyer et al. 2004)。

- Detection of polymorphism  
and data analyses
  - Fragment analysis using  
ABI310 Genetic Analyzer
  - GenePop ver4.0 (Rousset 2008)

- Number of mean alleles per locus, allele size and mean heterozygosity values show in the present study.

# Result 1: Comparison of Genotypic Diversity

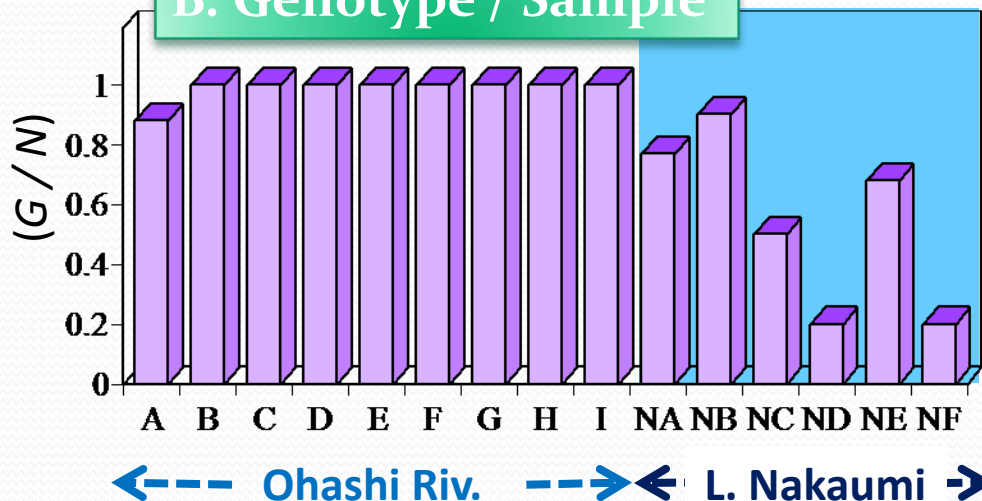
## A. Number of Genotype



● The Ohashi River :  
Almost all samples showed different genotypes (genets)!  
**= High genotypic diversities**

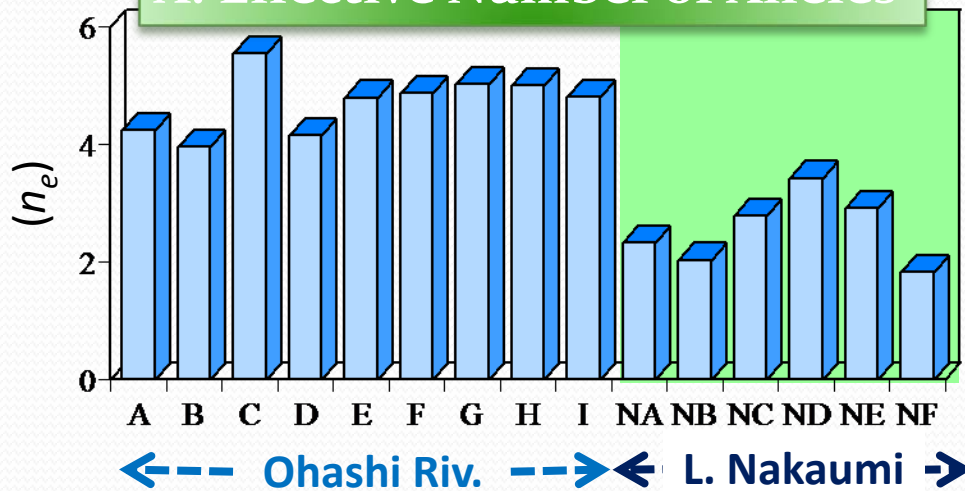
● Lake Nakaumi :  
Low genotype / sample number ratio (the same genotypes were observed repeatedly in different samples)  
**= Low genotypic diversities, clonal reproduction is prominent.**

## B. Genotype / Sample



# Result 2: Comparison of Genetic Diversity

## A. Effective Number of Alleles



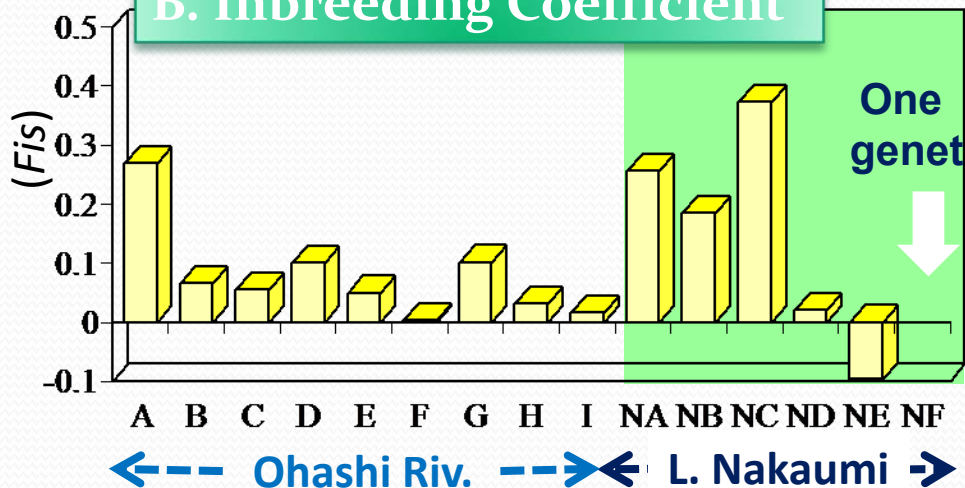
### ● Effective number of alleles

$$n_e = 1 / \sum P_i^2$$

$P_i$  : Freq. of each allele

The Ohashi Riv. > L. Nakaumi

## B. Inbreeding Coefficient



### ● Inbreeding coefficient

$$F_{is} = (H_s - H_i) / H_s$$

The Ohashi Riv. < L. Nakaumi

# Result 3: Gene Flow and Population Connectivity

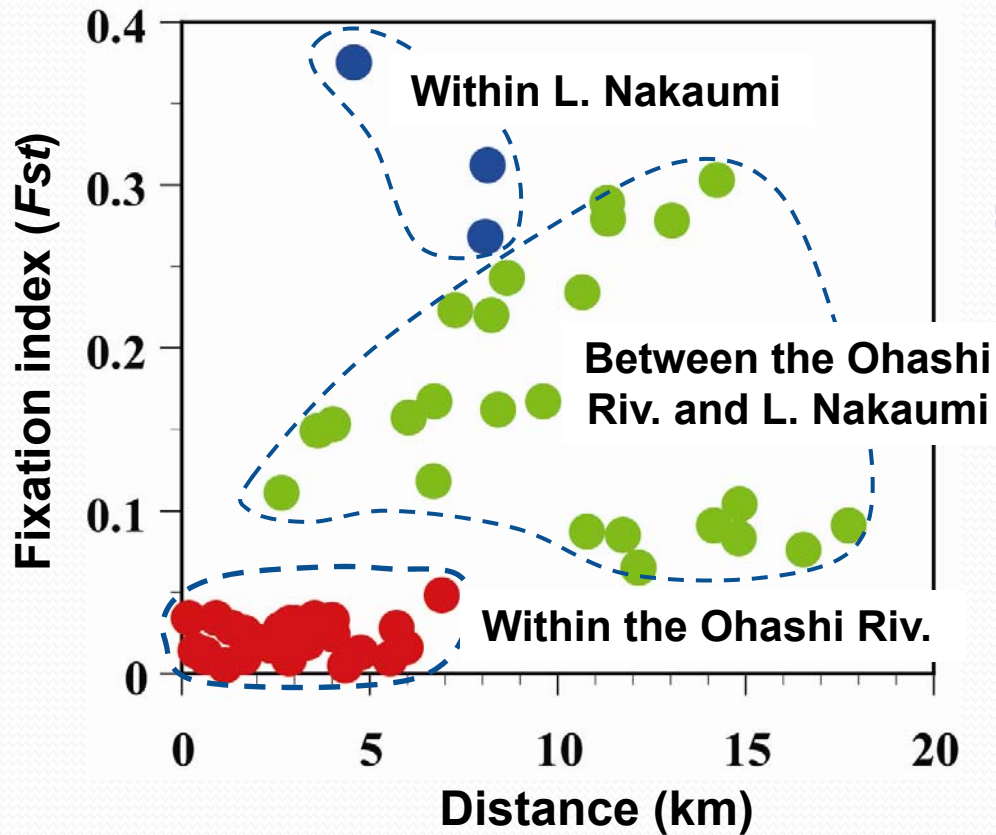


Fig. Pairwise comparisons of genetic and geographic distance among the populations. Omitting Stn. *B*, *NC*, *ND* & *NE* due to few genets.

- **Within the Ohashi Riv.**  
 $F_{st} < 0.05$   
= Frequent gene flow were maintained among the populations.
- **Between the Ohashi Riv. and L. Nakaumi**  
 $F_{st} > 0.07$
- **Within L. Nakaumi**  
 $F_{st} > 0.27$   
= Gene flow frequencies among the populations were low.

# Conclusions & Discussion 1

## Summary of Results: The Ohashi River

- High genetic diversities have been maintained in mid- and down-stream populations.
- Pairwise fixation index among the populations were low ( $F_{st} < 0.05$ ).
- In upstream of the river, one population has shown high inbreeding coefficient.

1. Frequent gene flow (pollen and seed transportation) have been maintained among the populations
2. Connectivity among populations may allow the gene flow.
3. As residual current by tide trend downstream, gene flow in the upstream population may be limited compared with mid- and down-stream ones. Thus frequency of inbred mating may be relatively high in upstream population.

# Conclusions & Discussion 2

## Summary of Results: Lake Nakaumi

- Local populations have low genotypic and genetic diversities
- Pairwise fixation index among the populations were high ( $F_{st} > 0.06$ ).
- High inbreeding coefficient values were observed in some populations.

1. Isolation by distance limits gene flow among the populations, and promotes inbreeding. This may be a cause of low genetic diversities in the populations.
2. To promote gene flow from other populations, connectivity among populations should be restored.
3. Conservation of preserved local populations are also needed to avoid further loss of endemic genetic diversity.