Seagrass-oyster farmers interaction detected by eelgrass DNA analysis in Hinase area of the Seto Inland Sea, Japan

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Eelgrass restoration in Hinase, Okayama, Japan



Location of Hinase and MPA relevant regulations (Based on Kaiyo Daicho of Japan Coast Guard 2015)

Eelgrass restoration in Hinase, Okayama, Japan

- Loss of eelgrass vegetation became significant after the rapid industrialization activities around 1960s .
- Eelgrass bed restoration activity started in 1985 by local fishermen.
- Hinase area was famous for the fishing by coastal pound netting to catch fish and shrimp migrating to eelgrass beds, but gradually the fishing had been replaced by oyster farming with the massive loss of eelgrass. (Tsurita et al. 2016)



(Cited from: http://www.eic.or.jp/library/bio/case/ c3_3.html)



Oyster rafts in Hinase

Eelgrass restoration in Hinase, Okayama, Japan

- The fishers conducted eelgrass bed restoration using a seeding method for several decades even after the oyster farming became the majority, because they already knew eelgrass can maintain a better coastal environment for oyster farming as well as coastal productivity for fishing.
- After 30 years, the eelgrass beds are recovering (Tanaka 2014)
 Year 1940s 1980s 2011

12ha

590ha



Cover



200ha

Fishermen and children, collecting eelgrass to mature the seeds

Seagrass recovery in the Seto Inland Sea around 2010s





Seagrass beds is one of the most important coastal habitat with high productivity and biodiversity providing various ecosystem services.



Rapid recovery of eelgrass vegetation by natural dispersal has been apparent in all regions of the Seto Inland Sea. We demonstrated the contribution of oyster farmers' long-term activities to eelgrass bed restoration in the Hinase area based on a DNA analysis for the eelgrass population-genetic structure.

- Did the recovery of eelgrass bed in Hinase come about by the restoration activity?
- How did the seeding activity by fishermen (oyster farmers) influence the populationgenetic structure of eelgrass meadows in Hinase area?

Study site



<u>9 sites</u>: 2 sites are source of seeds for the seeding activity, and other 7 sites are the restoration area

Methods

- 1. 20-50 eelgrass (*Zostera marina*) shoots were randomly collected as samples in each site in July 2015.
- 2. Genomic DNA was extracted and fragments amplified using QIAGEN PLANT MINI KIT
- 3. All samples were genotyped for 7 microsatellite loci, which were developed by previous studies (Reush et al. 2000, Tanaka et al. 2008, Shimabukuro *et al*. 2012), using GeneMapper
- 4. Genetic diversity of each population and genetic differentiation between populations (F_{st}) were calculated using GenAlEx v6.5
- 5. The relationships between genetic distance, geographic distance and seeding intensity were statistically analyzed

Results: Genetic diversity in each site

Clonal richness was higher in all sites, suggesting that each population would be established by generative reproduction (most of eelgrass shoots raised from seeds) rather than by vegetative reproduction

Katakami		
🔥 Katakar	ni-shita	S. Ofer
2 km	Genjiwan- nishi Genjiw	nago Nishidomari Mahoroba an Kubikiri <u>Kubikiri</u>

Population	No. sample	No. genotype	Clonal richness	No. allele	Allelic richness	Observed heterozygosity (Ho)	Expected heterozygosity (He)	Inbreedin g coefficien t
Otabujima	46	45	0.978	8.857	6.701	0.546	0.562	0.062
Nishidomar i	38	31	0.816	9.571	8.057	0.594	0.599	-0.004
Kubikiri	47	43	0.915	8.714	6.978	0.595	0.582	0.010
Mahoroba	21	20	0.952	6.714	6.714	0.564	0.567	0.006
Katakami	37	36	0.973	10.000	8.326	0.603	0.608	0.018
Katakami- shita	26	22	0.846	8.286	7.992	0.571	0.604	0.022
Genjiwan	51	47	0.922	9.571	7.165	0.596	0.584	-0.039
Genjiwan- nishi	40	31	0.775	8.857	7.619	0.577	0.573	-0.001
Yonago	49	45	0.918	10.143	7.580	0.606	0.596	-0.019
Total	355	320	0.901					

Results: Genetic differentiation between

$F_{\mbox{\scriptsize ST}}$ values between sites and the significance

Otabujima	Nishidoma ri	Kubikiri	Mahoroba	Katakami	Katakami- shita	Genjiwan	Genjiwan- nishi	Yonago	
	0.470	0.084	0.478	0.001	0.024	0.382	0.094	0.219	Otabujima
0.007		0.078	0.383	0.027	0.151	0.527	0.539	0.590	Nishidomari
0.008	0.010		0.319	0.002	0.010	0.026	0.024	0.144	Kubikiri
0.009	0.011	0.010		0.523	0.256	0.300	0.444	0.374	Mahoroba
0.013	0.012	0.013	0.010		0.904	0.009	0.374	0.005	Katakami
0.014	0.013	0.016	0.015	0.007		0.057	0.265	0.060	Katakami- shita
0.006	0.007	0.009	0.010	0.011	0.013		0.560	0.594	Genjiwan
0.010	0.008	0.011	0.011	0.008	0.012	0.006		0.068	Genjiwan- nishi
• Kubikiri • Mahoroba • Yonago • Otabujima • Genjiwan • Senjiwan • Senjiwan • Senjiwan • Nishidomari						C OOS Katakami Kata 2 km	0 010 akami-shita Genjiwa nishi	n- Yonago Ni Genjiwan H Otabuji	Shidomari Mahoroba Kubikiri ma
Coord. 1 PCOA plot									

Results: Seeding intensity

The record of No. seeds sown in each year

Voor	Source area of	No. seeds	Areas seeded			
rear	seeds	$(\times 10^4)$				
1985	大多府島	15	楠戸、現寺湾			
1986	大多府島	175	楠戸、現寺湾、米子湾、他2箇所			
1987	大多府島	252	米子湾、曽島北、鴻島東			
1988	大多府島	204	米子湾			
1989	大多府島	220	楠戸、現寺湾、米子湾、千軒湾*			
1990	大多府島	401	楠戸、現寺湾、米子湾、千軒湾*			
1991	大多府島	342	楠戸、現寺湾、米子湾、千軒湾*			
1992	大多府島	351	現寺湾、米子湾、千軒湾*			
1993	大多府島	251	現寺湾、米子湾、千軒湾*			
1994	大多府島	251	楠戸、現寺湾、米子湾、千軒湾*			
1995	大多府島	229	楠戸、現寺湾、米子湾、千軒湾*			
1996	現寺湾、片上湾	351	現寺湾、米子湾、千軒湾*、他1箇所			
1997	現寺湾、片上湾	430	現寺湾、米子湾、他1箇所			
1998	片上湾	300	米子湾、千軒湾*			
1999	片上湾	300	米子湾、千軒湾*			
2000	久々井湾	300	米子湾、千軒湾*			
2001	片上湾	975	米子湾、千軒湾*			
2002	大多府島	200	米子湾、千軒湾*			
2003	大多府島	200	米子湾、千軒湾*			
2004	大多府島	200	米子湾、千軒湾*			
2005	大多府島	200	米子湾、千軒湾*			
2006	大多府島	200	楠戸			
2007	現寺湾	164	楠戸、森下前			
2008	米子湾、現寺湾	260	楠戸、森下前、千軒湾(五輪)			
2009	大浦、米子、大多府島	185	楠戸、森下前、千軒湾(五輪)			
2010	現寺、米子、大多府島	595	ヨータイ前、千軒湾(五輪)			
2011	桃の木、米子、大多府島	1170	千軒湾(奥泊西・東、西泊、首切)			
2012	米子湾、現寺湾	600	千軒湾(奥泊西・東、西泊、五輪、首切)			
2013	現寺湾	304	千軒湾(奥泊西・東、西泊、まほろば下、首切)			
2014	Drifted flowering shoots	574	千軒湾(奥泊西・東、西泊、まほろば下、首切)			
	Total	10199				



Using this data, we calculated the <u>seeding intensity</u> as the number of seeds totally sowed in each restoration area

(provided from Okayama prefecture)

Seeding intensity as the number of seeds totally sowed from the two source sites (Otabujima or Katakami) by fishermen



Otabujim a	Nishidomari	Mahorob a	Kubikiri	Katakami	Kataka mi-shita	Genjiwan	Genjiwan- nishi	Yonago	
0.000									Otabujima
450.472	0.000								Nishidomar i
320.472	0.000	0.000							Mahoroba
450.472	0.000	0.000	0.000						Kubikiri
0.000	279.214	279.214	279.214	0.000					Katakami
0.000	0.000	0.000	0.000	0.000	0.000				Katakami- shita
603.917	167.714	92.714	167.714	120.343	0.000	0.000			Genjiwan
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		Genjiwan- nishi
1284.417	205.000	0.000	205.000	907.843	0.000	120.343	0.000	0.000	Yonago

Results: Contribution of seeding activity

Natural dispersal was calculated as a geographical distance: the effective distance (m) between sites

All parameters were standardized for the analysis

The data set was analyzed using Mantel's multiple regression matrix with randomization





Results: Contribution of seeding activity



recover the eelgrass.



Discussion & Conclusion

Our DNA analysis suggested that;

Genetic structure of the recovered seagrass meadows in the restoration area would be mainly derived from natural dispersal

The fishermen's seeding activity significantly shortened the genetic distance between the populations with natural dispersal.

Question1: Did the recovery of eelgrass bed in Hinase come about by the restoration activity?

Yes, but the farmers' seeding activity did not disturb the genetic structure by natural eelgrass dispersal

Question2: How did the seeding activity by fishermen influence the population-genetic structure of eelgrass meadows in Hinase area?

They helped eelgrass natural dispersal, suggesting that Their seeding activity did not make artificial eelgrass beds but facilitated natural recovery of eelgrass beds.

The eelgrass-oyster farmer relationship in Hinase would be a good practice as an ideal ecosystem restoration.

Merci de votre attention!

Miyajima world heritage near FRA, Hiroshima