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論 文 内 容 要 旨

Introduction

Carangids of the genus *Seriola* are economically important fish, widely distributed throughout the Pacific and Atlantic oceans. Increasing fishing activity will make a growing pressure to the natural population, in terms of lower effective population size. This will reduce the chance of successful reproduction of fish species, and give a risk to change in genetic variability and population structure. Finally, the species will be brought to `endangered` level and is possibly going to extinction. Therefore the greater awareness and efforts to conserve the genetic resources are needed. Since the genetic variability seems to be an important feature of a population to evaluate short-term fitness individuals and the long-term survival of the population [1], an alternative may be pursuant to the eventual determination of the genetic variability [2]. Recently, molecular genetic markers are widely used for population studies [3]. Of the several genetic markers, microsatellite is being increasingly used due to the highly polymorphism and inheritance in Mendelian way. This study was designed to learn the population genetics on aquaculture fish in genus *Seriola*, by revealing the basic genetic information using genetic markers, for the importance of their risk management and /or conservation.

I. Isolation of microsatellite DNA loci and their polymorphism for carangid of the genus *Seriola*

As part of a program to develop easily assayable and highly polymorphic genetic markers for population studies, microsatellite DNA regions of greater amberjack were cloned and sequenced. Approximately 6.0 x 10^5 bp of greater amberjack were observed, and 2.4 x 10^5 (GT)n loci were estimated in those genome. The average distance between neighboring microsatellites was 25 kbp. Six primer sets have been designed for complement the flanking regions. Four of these primers showed polymorphism in the greater amberjack DNA. The microsatellite loci of greater amberjack were also available to other *Seriola* species, but not for the other genera.

Three microsatellite loci of greater amberjack have been applied to examine the genetic variability of four *Seriola* species i.e. greater amberjack, highfin amberjack, yellowtail and yellowtail kingfish. The highest genetic variability was observed in the kingfish samples, and then followed by greater amberjack, yellowtail and highfin amberjack. Relationship based on the Nei's genetic distance [4] showed greater amberjack was relatively close to the highfin amberjack, while the kingfish was close to yellowtail (Fig. 1). This division agrees with the morphological result.

II. Genetic divergence of kingfish from the natural waters of Japan, Australia and New Zealand

Genetic polymorphism in kingfish, collected from natural waters of Japan, Australia and New Zealand were examined using microsatellite DNA and mtDNA control region markers. Sixteen to 25.7 alleles per locus were observed in 3

microsatellite markers, while the observed (and expected) average heterozygosities were 0.782 (0.918), 0.750 (0.809), and 0.650 (0.888) for Australian, Japanese and New Zealand kingfish respectively (Table 1). Twelve mtDNA haplotypes were detected following digestion of control region sequences with five endonucleases: *Hae*III, *Hinf*I, *Mbo*I, *Rsa*I, and *Taq*I (Table 2). Significant genetic divergence was observed between the kingfish population from Japan and those from Australia-New Zealand. There was no significance differentiation among the Australian and New Zealand population samples. Genetic relationship based on the UPGMA dendrogram of Nei's genetic distance showed that Japanese kingfish was clearly separated from the Australia-New Zealand ones (Fig 2). The Southern Hemisphere of yellowtail kingfish is thought to be one of three morphologically similar, but geographically separate population or subspecies [5]. The Australian and New Zealand species is known as *Seriola lalandi lalandi*, while the Japanese one is *S.lalandi aureovittata.*

III. Genetic variability of greater amberjack used as seed fish in aquaculture-farm of Japan

Four populations of greater amberjack seed fish were collected from aquaculture-farms at Wakayama and Kagoshima prefectures. Two seed populations come from Vietnam while two others were originated from Japan area. Three greater amberjack populations caught from the natural population were used as a control. Genetic polymorphism was assessed using microsatellite DNA and mtDNA control region markers. Moderate to high level of polymorphism was observed among the greater amberjack samples. Nine to 12.7 alleles per locus were observed in three microsatellite loci, while the observed (and expected) average heterozygosities were 0.701 (0.778) and 0.654 (0.738) for aquaculture-farm and natural greater amberjack samples respectively (Table 3). Thirty-three composites of mtDNA control region haplotypes were detected via digestion with five endonucleases, *Hae*III, *Hinf*I, *Mbo*I, *Rsa*I and *Taq*I (Table 4). Significant genetic differentiation was observed among greater amberjack samples. Nei's genetic distances revealed that at least two genetically different sub-populations of greater amberjack were used in the aquaculture-farm of Japan (Fig. 3).

IV. Loss of genetic variability in greater amberjack from the artificial propagated activity

The seed production of greater amberjack was carried out at the Kinki-University and the Prefectural Fisheries Experimental Station of Nagasaki. Twenty to thirty breeders from four to five year's age were used for reproduction. The breeders are distributed into a 200 m^3 concrete pond with proportion of 4:3 (male: female) after injected with gonadrotropin (500 IU/fish) for mass spawning. The offspring were removed to a 20 m^3-concrete pond and reared for six months. DNA was extracted from the offspring to examine the polymorphism using microsatellite DNA

and mtDNA control region markers. The genetic variability of greater amberjack from artificially propagated activity was also compared with those observed in the natural samples. Significant difference in genetic variability was observed among artificially propagated samples. The genetic differentiation was also significant between natural and artificial propagation samples. Lower variability was observed in the artificially propagated samples than those observed in the greater amberjack from natural populations (Table 5 and 6). These genetic change in variability and allele frequency maybe caused by bottleneck effect and random genetic drift.

V. Fluctuation of the genetic variability during spawning time in a hatchery: a reference case from red sea bream.

Genetic monitoring of brood stock in the hatchery is necessary to prevent the erosion of genetic variability and the possibility exposure of deleterious recessive genes. Genetic variability of red sea bream samples from different spawning dates was assayed by the polymorphism of microsatellite DNA and mtDNA control region markers. Fourteen to 18.3 alleles per locus was observed in 3 microsatellite markers, while the observed and expected averages ranged from 0.843 to 0.919 (Table 7). Twenty-three composites of mtDNA haplotypes were detected following digestion of control region sequences with five endonucleases: *Hae*III, *Hinf*I, *Mbo*I, *Rsa*I, and *Taq*I (Table 8). Significant difference of genetic variability was observed among samples from different spawning date. Fluctuation of genetic variability was found during spawning activity. Pooling samples from different dates increased the genetic variability during spawning, and it was comparable with those observed in the red sea bream samples from the natural waters (Fig. 4 and 5). Collecting offspring of different dates may conduct the preservation of genetic variability in hatchery samples.

Conclusion and Suggestion

The development of microsatellite loci has been conducted from greater amberjack genome. Those loci were available also for the other *Seriola* species. Genetic variability of samples collected from natural, aquaculture farm and artificial propagation activity have been examined using microsatellite DNA and mitochondria DNA markers. The present state of genetic variability of species in genus *Seriola* is relatively at level "non-endangered" condition (Table 9). This study showed that high polymorphism was observed in the natural and aquaculture-farm samples, but loss of genetic variability was found in artificially propagated samples. Significant genetic differences were observed between kingfish samples from Japan and Australia-New Zealand. The Southern Hemisphere of yellowtail kingfish is thought to be one of three morphologically similar, but geographically separate populations or subspecies. The Australian and New Zealand species is known as *Seriola lalandi lalandi*, while the Japanese one is *S. lalandi aureovittata.* Significant genetic differentiation was also

observed among greater amberjack used as seed fish in aquaculture-farms. At least two genetically different sub-populations were distributed in the aquaculture-farm of Japan. One of those stocks is come from the Vietnam waters. Further from a reference case in the hatchery experiment, monitoring of genetic variability of red sea bream was conducted during spawning activity, showed that the collecting offspring from different date during spawning may be effective to preserve the genetic variability of hatchery stocks.

Regarding the above results, it is suggested that the Japanese *Seriola* populations should be used for development of aquaculture and preservation of genetic resources of natural populations. This is because the importing the stocks from different subpopulations will rise a risk of undesired effect such as loss of local adaptation, genetic structural change and genetic disturbance. Further, monitoring genetic variability and application of enough effective number of founders should be used in artificial propagated activity to prevent the erosion of genetic variability and the possibility exposure of deleterious recessive genes. It is also possible to maintain the genetic variability in hatchery by collecting the offspring from different dates during spawning time. Finally, using the genetic markers, the additional population samples should be tested to clarify the genetic structure of genus *Seriola* from geographically intermediate areas.

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Table 1. Genetic variability of kingfish collected from Japan (J-1), Australia (A-1 to 3) and New Zealand (N-1) revealed by microsatellite DNA

Sample		Locus			
		Sdn-03	Sdn-06	Sdn-09	Average
J-1	N-samples	58	60	60	
	N-allele	36	13	9	19.3
	Ho	0.7	0.8	0.75	0.75
	He	0.961	0.748	0.723	0.809
	Ho/He	0.728*	1.069	1.037	0.945
A-1	N-samples	80	80	80	
	N-allele	36	20	16	24
	Ho	0.825	0.75	0.75	0.775
	He	0.963	0.905	0.887	0.918
	Ho/He	0.857*	0.829*	0.846*	0.844
A-2	N-samples	69	71	78	
	N-allele	30	21	17	22.7
	Ho	0.884	0.718	0.744	0.782
	He	0.95	0.908	0.754	0.871
	Ho/He	0.931	0.791*	0.986	0.903
A-3	N-samples	76	76	79	
	N-allele	37	23	17	25.7
	Ho	0.763	0.671	0.62	0.685
	He	0.961	0.915	0.891	0.922
	Ho/He	0.794*	0.733*	0.696*	0.741
N-1	N-samples	25	24	24	
	N-allele	21	15	12	16
	Ho	0.72	0.583	0.666	0.656
	He	0.929	0.874	0.863	0.888
	Ho/He	0.775	0.667	0.772	0.738

*)Departure from Hardy-Weinberg Equilibrium at Bonferroni value, P<0.01--> (0.05/5)

Table 2. Genetic divergence of king fish collected from Japan (J-1), Australia (A-1 to 3) and New Zealand (Z-1) revealed by MtDNA analysis

	Haplotype*	J-1	A-1	A-2	A-3	N-1
1	AAEFD	0.182	0	0	0	0
2	OAEFD	0.382	0	0	0	0
3	OAFFD	0.4	0	0	0	0
4	ABEFD	0.018	0	0	0	0
5	OAFFE	0.018	0	0	0	0
6	ABGFE	0	0.013	0.063	0.052	0.04
7	ABGFF	0	0.313	0.313	0.257	0.28
8	ABGFG	0	0.613	0.6	0.597	0.64
9	ABHFF	0	0	0	0	0.04
10	ABHFG	0	0.025	0	0.039	0
11	ACGFF	0	0.038	0.013	0.026	0
12	ACGFG	0	0	0	0.039	0
	No of sample	55	80	80	77	25
	No of haplotype	5	6	4	6	4
	Haplotype Diversity	0.66	0.525	0.538	0.576	0.509

*) Generated by TaqI, HaeIII, MboI, RsaI and HinfI endonucleases

Table 3. Genetic variability of seed fish of greater amberjack collected from aquaculture farm (F-1 to 4) and natural waters (N-1 to 3) revealed by microsatellite DNA

Sample		Locus			Average
		Sdn-06	Sdn-08	Sdn-09	
F-1	N-sample	80	53	80	
	N-allele	9	21	8	12.7
	N-effective allele	4.5	16.7	4.2	8.6
	Ho	0.588	0.622	0.800	0.670
	He	0.777	0.941	0.788	0.835
	Ho/He	0.757*	0.661*	1.015*	0.802
F-2	N-sample	80	70	80	
	N-allele	6	21	5	10.7
	N-effective allele	3.8	10.5	2.9	5.8
	Ho	0.725	0.857	0.738	0.773
	He	0.737	0.905	0.665	0.769
	Ho/He	0.983	0.947*	1.109	1.006
F-3	N-sample	78	43	78	
	N-allele	5	19	5	9.6
	N-effective allele	3.3	12.3	3.2	6.3
	Ho	0.795	0.558	0.794	0.715
	He	0.698	0.919	0.689	0.768
	Ho/He	1.139*	0.607*	1.152*	0.966
F-4	N-sample	77	75	77	
	N-allele	5	22	5	10.6
	N-effective allele	2.9	12.8	2.8	6.1
	Ho	0.714	0.64	0.714	0.689
	He	0.658	0.922	0.649	0.743
	Ho/He	1.089	0.694*	1.100	0.959
N-1	N-sample	79	67	79	
	N-allele	5	22	7	11.3
	N-effective allele	3.2	14	3.6	6.9
	Ho	0.570	0.746	0.700	0.672
	He	0.688	0.929	0.719	0.778
	Ho/He	0.828*	0.803*	0.974*	0.863
N-2	N-sample	80	80	80	
	N-allele	6	22	8	12
	N-effective allele	3	11.9	3.8	6.2
	Ho	0.675	0.65	0.775	0.7
	He	0.667	0.916	0.738	0.774
	Ho/He	1.011	0.709*	1.050	0.924
N-3	N-sample	80	76	80	
	N-allele	5	24	5	11.3
	N-effective allele	2.5	13.8	2.5	6.3
	Ho	0.600	0.579	0.638	0.606
	He	0.602	0.927	0.604	0.711
	Ho/He	0.996	0.625*	1.056	0.893

*)Departure from Hardy-Weinberg expectation at Bonferroni corrected value, P< 0.007----> (0.05/7)

Table 4. Haplotype frequency of greater amberjack from aquaculture farm (F-1 to 4) and natural water (N-1 to 3) generated by five endonucleases

#	Haplotype	F-1	F-2	F-3	F-4	N-1	N-2	N-3
1	AAAAA	0.171	0.463	0.387	0.325	0.5	0.455	0.481
2	BAAAA	0.014	0.06	0.04	0.026	0.014	0.091	0.039
3	CAAAA	0.214	0.06	0.133	0.091	0.097	0.065	0.039
4	DAAAA	0.157	0.313	0.36	0.26	0.278	0.325	0.312
5	EAAAA	0.029	0.015	0.067	0.117	0.014	0.026	0.065
6	FAAAA	-	-	-	-	0.014	-	-
7	FAAAC	-	-	-	-	-	0.013	-
8	FAACC	0.071	-	-	-	-	-	-
9	AAAAB	-	-	-	0.052	-	-	-
10	DAADA	-	-	-	-	0.014	-	-
11	ACAAA	-	-	-	0.013	0.014	-	-
12	DAABA	-	-	-	-	0.014	-	-
13	DABAA	0.014	0.06	-	-	0.028	0.013	-
14	AABAA	0.014	-	-	0.013	0.014	-	0.014
15	AEBAA	0.011	-	-	-	-	-	-
16	BAABA	0.014	0.015	-	-	-	-	-
17	CCAAA	0.014	-	-	-	-	-	-
18	DAACC	0.029	-	-	-	-	-	-
19	DAACA	0.071	-	-	-	-	-	-
20	DAADC	0.014	-	-	-	-	-	-
21	EAABA	-	0.015	-	-	-	0.013	-
22	AAAAC	-	-	-	0.013	-	-	-
23	KAAAA	-	-	-	0.013	-	-	-
24	DCAAA	-	-	-	0.013	-	-	-
25	AACAA	-	-	0.013	-	-	-	-
27	FAACB	0.043	-	-	-	-	-	-
28	FAACA	0.014	-	-	-	-	-	-
29	DAAAB	-	-	-	0.039	-	-	-
30	CAAAB	-	-	-	0.013	-	-	-
32	AADAA	-	-	-	-	-	-	0.013
33	DACAA	-	-	-	-	-	-	0.013
34	AAAAD	-	-	-	0.013	-	-	0.026
35	DAACB	0.1	-	-	-	-	-	
	N-sample	70	67	75	77	72	77	77
	N-haplotype	17	8	6	14	11	8	9
	Haplotype diversity	0.888	0.686	0.706	0.809	0.67	0.683	0.672

Table 5. Genetic variability of seed fish of greater amberjack collected from artificial propagated activity (H-1 to 3) and natural water (N-1 to 3) revealed by microsatellite DNA

Sample		Loci Sdn-06	Sdn-08	Sdn-09	Mean
H-1	N-sample	40	38	40	
Wakayama	N-allele	4	4	3	3.7
	N-effective allele	2.9	2.2	2.9	2.7
	Ho	0.846	0.459	0.821	0.709
	He	0.659	0.554	0.657	0.623
	Ho/He	1.249*	0.829*	1.249*	1.121
H-2	N-sample	40	40	40	
Wakayama	N-allele	3	10	3	5.3
	N-effective allele	2.0	5.8	2.1	3.3
	Ho	0.725	0.417	0.657	0.606
	He	0.504	0.827	0.527	0.619
	Ho/He	1.438*	0.504*	1.281*	1.075
H-3	N-sample	40	40	40	
Nagasaki	N-allele	3	6	3	4.0
	N-effective allele	1.4	2.3	1.4	1.7
	Ho	0.200	0.425	0.225	0.283
	He	0.275	0.567	0.293	0.378
	Ho/He	0.727	0.749*	0.768	0.748
N-1	N-sample	79	67	79	
Oita	N-allele	5	22	7	4.0
	N-effective allele	3.2	14	3.6	11.3
	Ho	0.570	0.701	0.700	0.657
	He	0.688	0.929	0.719	0.778
	Ho/He	0.828*	0.755*	0.974	0.852
N-2	N-sample	80	80	80	
Kochi	N-allele	6	22	8	12
	N-effective allele	3	11.9	3.8	6.2
	Ho	0.675	0.650	0.775	0.700
	He	0.667	0.916	0.738	0.774
	Ho/He	1.011	0.709*	1.050	0.924
N-3	N-sample	80	76	80	
Wakayama	N-allele	5	24	5	11.3
	N-effective allele	2.5	13.8	2.5	6.3
	Ho	0.600	0.579	0.638	0.606
	He	0.602	0.927	0.604	0.711
	Ho/He	0.996	0.625*	1.056	0.893

*)Departure from Hardy-Weinberg equilibrium at Bonferroni corrected level, P<0.008

Table 6. Haplotype frequency of greater amberjack from artificial propagated activity (H-1 to 3) and natural waters (N-1 to 3) generated by five enzymes

#	Haplotype	H-1 Wakayama	H-2 Wakayama	H-3 Nagasaki	N-1 Oita	N-2 Kochi	N-3 Wakayama
1	AAAAA	1.0	0.975	0.025	0.500	0.455	0.481
2	BAAAA	-	-	-	0.014	0.091	0.039
3	CAAAA	-	0.025	0.975	0.097	0.065	0.039
4	DAAAA	-	-	-	0.278	0.325	0.312
5	EAAAA	-	-	-	0.014	0.026	0.065
6	FAAAA	-	-	-	0.014	-	-
7	FAAAC	-	-	-	-	0.013	-
10	DAADA	-	-	-	0.014	-	-
11	ACAAA	-	-	-	0.014	-	-
12	DAABA	-	-	-	0.014	-	-
13	DABAA	-	-	-	0.028	0.013	-
14	AABAA	-	-	-	0.014	-	0.013
21	EAABA	-	-	-	-	0.013	-
32	AADAA	-	-	-	-	-	0.013
33	DACAA	-	-	-			0.013
34	AAAAD	-	-	-	-	-	0.026
	N-sample	38	40	40	72	77	77
	N-haplotype	1	2	2	11	8	9
	Haplotype diversity	0.000	0.049	0.049	0.67	0.683	0.672

Table 7. Genetic variability of red sea bream collected from different spawning date evaluated by microsatellite DNA

Sample	Spawning date (1999)		Locus Pma-01	Pma-03	Pma-05	Mean
I	April 14	N-sample	79	79	79	
		N-allele	14	22	17	17.7
		N-effective allele	3.5	11.2	11.1	8.6
		Ho	0.646	0.734	0.684	0.688
		He	0.716	0.911	0.909	0.843
		Ho/He	0.902**	0.805**	0.752**	0.819
II	April 22	N-sample	77	77	77	
		N-allele	15	21	17	17.7
		N-effective allele	3.3	12.9	11.1	9.1
		Ho	0.701	0.571	0.636	0.636
		He	0.696	0.923	0.909	0.843
		Ho/He	1.007**	0.619**	0.699**	0.775
III	April 30	N-sample	80	80	80	
		N-allele	18	23	18	13.7
		N-effective allele	11.9	13.1	11.9	12.3
		Ho	0.638	0.663	0.65	0.651
		He	0.916	0.924	0.916	0.919
		Ho/He	0.696**	0.717**	0.709**	0.707
IV	May 06	N-sample	77	77	77	
		N-allele	14	23	18	18.3
		N-effective allele	4.1	12.2	8.6	8.3
		Ho	0.714	0.636	0.857	0.736
		He	0.756	0.918	0.887	0.854
		Ho/He	0.944**	0.692**	0.966**	0.867
Pooled		N-sample	313	313	313	
		N-allele	23	26	19	22.7
		N-effective allele	6.2	15.3	12.1	11.2
		Ho	0.674	0.661	0.706	0.681
		He	0.838	0.934	0.917	0.896
		Ho/He	0.804**	0.707**	0.769**	0.761
Wild-Kochi+		N-sample	105	106	105	
		N-allele	24	32	21	25.7
		N-effective allele	3.7	17.8	9.3	10.3
		Ho	0.733	0.906	0.876	0.838
		He	0.731	0.944	0.892	0.856
		Ho/He	1.003	0.96	0.982	0.98

+) Ricardo and Taniguchi (1999)

**)Departure from Hardy-Weinberg equilibrium (P<0.005

Table 8. Genetic variability of red sea bream collected from different spawning date evaluated by MtDNA

	Haplotype	I	II	III	IV	Pooled	Wild-Kochi
1	AABAA	0.200	0.175	0.150	0.075	0.150	0.038
2	BBBBA	0.125	0.050	-	-	0.044	-
3	AABCA	0.150	0.325	0.175	0.250	0.225	0.177
4	AAABA	0.350	0.075	0.175	0.325	0.231	0.215
5	AABBA	0.025	0.025	0.125	0.125	0.075	0.063
6	ABBBA	0.050	-	-	-	0.013	-
7	AACDA	0.050	0.025	-	-	0.019	0.076
8	ABBCA	0.050	-	-	-	0.013	-
9	AAADA	-	0.025	-	-	0.006	0.038
10	AADCA	-	0.025	0.025	0.025	0.019	-
11	AADBA	-	0.125	-	-	0.031	0.038
12	AAACA	-	0.025	0.050	0.125	0.050	0.038
13	AABCB	-	0.025	0.025	-	0.013	-
14	AACBC	-	0.025	-	-	0.006	-
15	AACBA	-	0.025	0.050	-	0.019	0.013
16	AADBC	-	0.025	-	-	0.006	-
17	AABCC	-	0.025	-	-	0.006	0.013
18	AABDA	-	-	0.025	-	0.006	-
19	AAAAA	-	-	0.100	0.025	0.031	0.038
20	CAACA	-	-	0.025	-	0.006	-
21	CABCA	-	-	0.050	-	0.013	-
22	DBADA	-	-	0.025	-	0.006	-
23	ABABA	-	-	-	0.050	0.013	-
24	AACEA	-	-	-	-	-	0.025
25	AADDA	-	-	-	-	-	0.013
26	DAADA	-	-	-	-	-	0.025
27	EAADA	-	-	-	-	-	0.038
28	FABCA	-	-	-	-	-	0.013
29	FAABA	-	-	-	-	-	0.013
30	FABAA	-	-	-	-	-	0.013
31	FCABA	-	-	-	-	-	0.013
32	AAADB	-	-	-	-	-	0.013
33	DACDA	-	-	-	-	-	0.013
34	AAAEA	-	-	-	-	-	0.013
	N-sample	40	40	40	40	160	79
	N-haplotype	8	15	13	8	23	23
		(10.7)*	(19.8)*	(17.6)*	(10.4)*	-	(23.6)*
	Haplotype Diversity	0.801	0.844	0.891	0.801	0.862	0.901

*) Calculation of haplotype number using regression for 160 samples

Table 9. Genetic variability of fish species collected from different ecological revealed by microsatellite DNA markers

Species	NA	NEA	Ho	He	Ne
Thuna*1	12.7	4.5	0.798	0.761	8750
Kingfish	21.7	8.7	0.752	0.809	19250
Greater amberjack	11.7	4.5	0.679	0.778	8750
Red sea bream*2	23.7	6.5	0.83	0.856	13750
Grouper	9.3	2.3	0.504	0.563	3250
Ayu*3	11.6	4.4	0.731	0.784	8500
Endangered population of ayu	2.4	1.67	0.200	0.201	1675
Threespine stickleback	10.8	5.1	0.61	0.853	10250
Common carp*4	3.9	1.7	0.205	0.581	1750
Ornamental-Nishikigo Carp*4	4.4	2.3	0.380	0.462	3500

*1) Takagi et al. (1999)
*2) Ricardo et al. (1999)
*3) Takagi et al. (1999)
*4) Aliah et al. (1999)

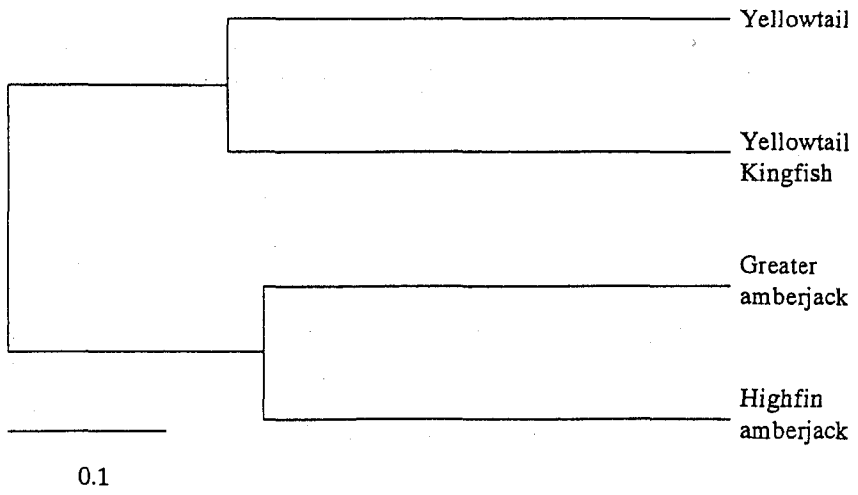

Fig. 1. Genetic relationship among four species of genus *Seriola* based on the UPGMA dendrogram of Nei's genetic distance of allele frequency

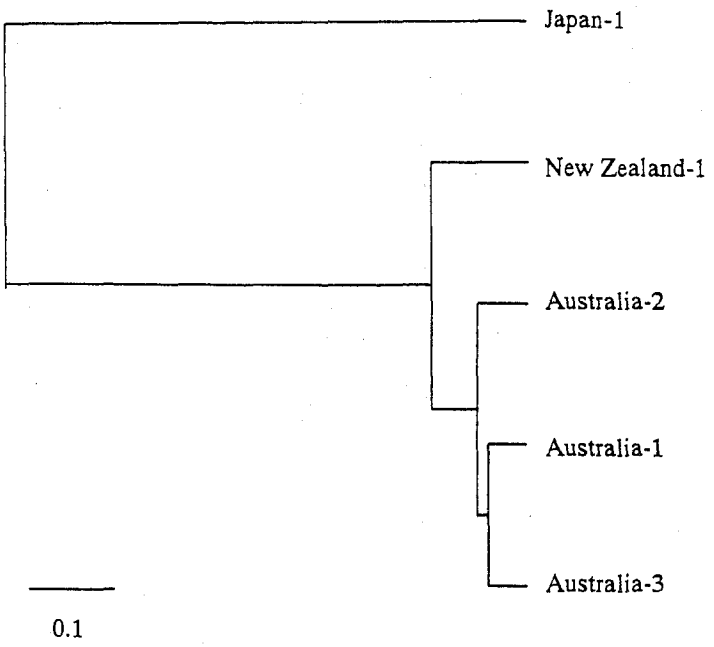

Fig. 2. Genetic relationship among kingfish from Japan, Australia and New Zealand based on the UPGMA dendrogram of Nei's genetic distance of allele frequency

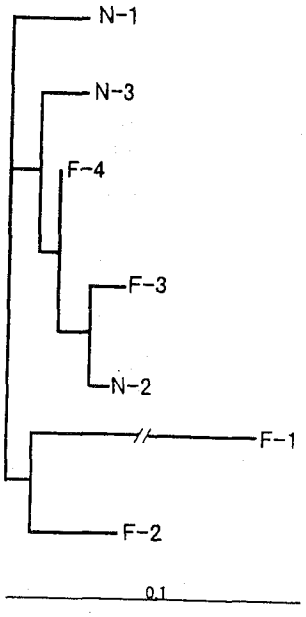

Fig. 3. Genetic relationship among greater amberjack used as seed fish in aquaculture farms of Japan based on the NEIGHBOR JOINING dendrogram of unweighted Nei's genetic disctance of allele frequency

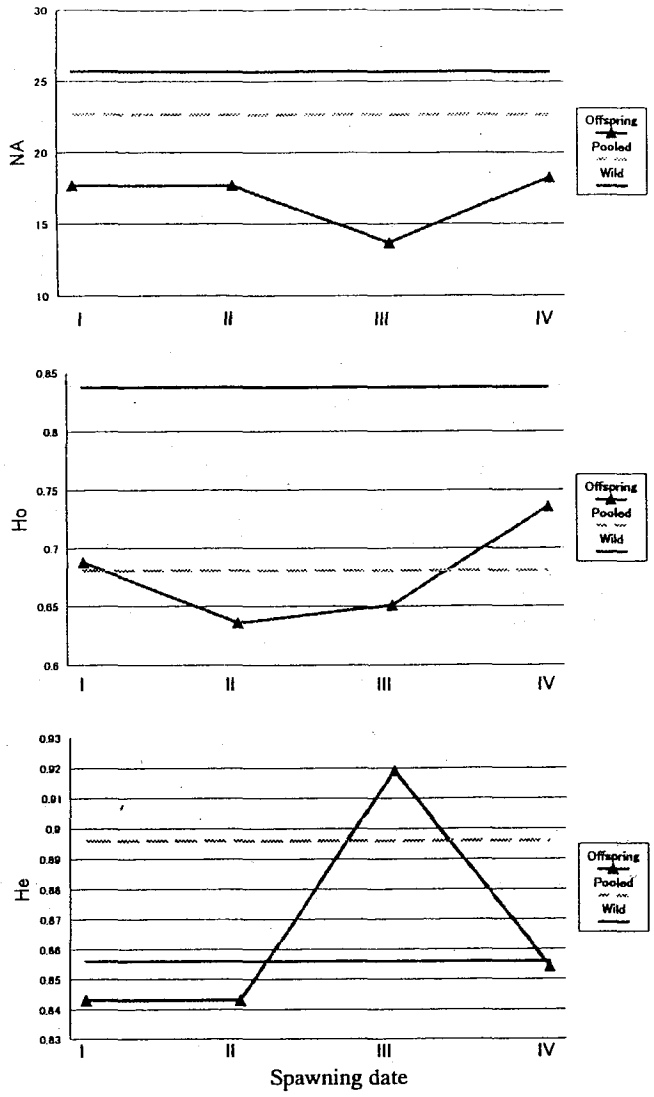

Fig.4. Fluctuation of allele number (NA), observed (Ho) and expected heterozygosities (He) of red sea bream during spawning time

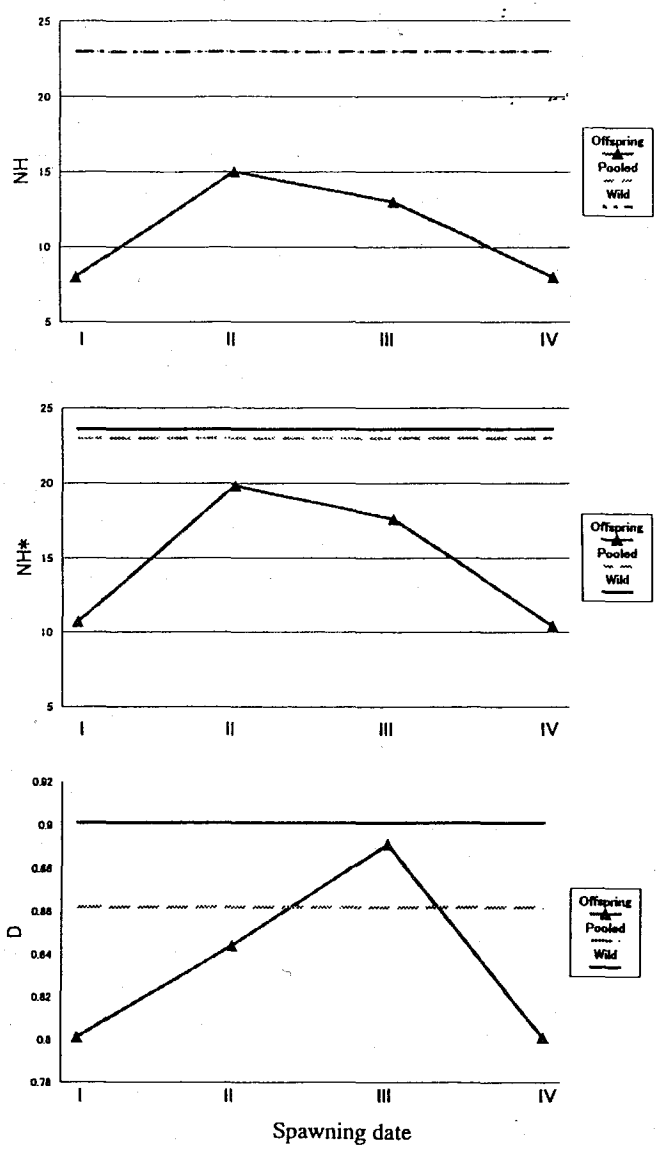

Fig 5. Fluctuation of haplotype number (NH) and haplotype diversity (D) of red sea bream during spawning time. NH* : No of Haplotype calculated by using regression for 160 samples

論文審査結果要旨

近年，日本の海産漁の増養殖において，多品種化がすすめられる中で，ブリ属をはじめとする外国種苗が輸入され，それら導入魚の養殖生け簀からの散逸や遺棄により自然生態系へ広がるケースも認められ，生態的攪乱が懸念されている。本研究は，養殖場周辺海域（生態系）における導入種とそれに対応する在来種の集団遺伝学的特性を査定し，外来種導入の潜在的な利点および有害性を評価するとともに管理手法を開発する事を目的としている。

第1章では，マイクロサテライトDNA多型マーカーを検出するためのプライマーシークエンスの開発を試みている。カンパチを用いて開発したプライマーシークエンスにより当該種及びその近縁魚類（ブリ，ヒラマサ，ヒレナガカンパチ）のマーカーを検出したところ，遺伝的多様性はヒラマサが最も高く，カンパチ，ブリ，ヒレナガカンパチの順に低くなっていること，遺伝的類似性については，ブリとヒラマサの2種，カンパチとヒレナガカンパチの2種がそれぞれ相対的に近縁であることを解明した。

ヒラマサは南北太平洋に広く分布する回遊魚である。第2章では，日本近海およびオセアニア海域で採取したヒラマサのマイクロサテライトDNAマーカーを検出し，それらの集団構造（繁殖の単位）に関する解析を行っている。遺伝的多様性は，日本およびニュージーランド集団においてやや低く，オーストラリア集団では高かった。日本近海集団とオセアニア海域の2標本群間に遺伝的異質性が確認されたことから，両海域にはそれぞれ独自の繁殖集団が存在することが強く示唆された。

カンパチは，養殖において使用するため外国（ベトナム）から大量の天然種苗が導入されている。第3章では，ベトナム産カンパチと日本産カンパチのゲノム遺伝子の構成と集団間の遺伝的異質性の検定を試みたところ，両海域間で明らかな差異が認められ，両海域の集団がそれぞれ独自の産卵場に由来することが示唆された。ベトナム産カンパチの養殖利用は，遺伝的異質集団の導入のケースに当たるため，養殖対象として利用するに際して，その潜在的利点と有害性について総合的な評価が求められるとした。

第4章では，カンパチ養殖用人工種苗の遺伝的多様性や遺伝子構成の変化を，マイクロサテライトDNA多型およびmtDNA制限酵素切断片長多型マーカーにより評価する試みを行っている。人工種苗における遺伝的多様性は野生集団に比べて著しく低下しており，産卵に加わった雌親魚は事実上数個体以内であり，集団の有効なサイズ（Ne）が限界サイズを大きく下回っていることを解明した。

第5章では，人工種苗生産において集団の有効なサイズを限界サイズ以上に維持するための手法開発調査を試みている。産卵期間に複数回採卵したところ，それぞれの採卵日の仔魚の遺伝的多様性は低下しているが，これらをプールすると親の集団と同じレベルに維持できることを明らかにした。

本研究では，外来種苗の利用および人工種苗の遺伝的管理手法に関する指針を提案している。また，ブリ類の集団構造に関して科学的新知見が多く含まれており，野生集団の遺伝的保全に関して現時点において説得力のある指針を示したことの保全生物学的意義は大きい。よって，審査員一同は本論文の著者を博士（農学）の学位を授与するに値するものと判定した。