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論 文 内 容 要 旨

Chapter 1. General introduction

The Mekong River is the longest river in Southeast Asia. It is an inland water system with the highest degree of bio-diversity in the world including about 1,200 fish species. The Pangasiidae family is one of the most important indigenous fish of the Mekong Basin and some species member of this family i.e., Mekong giant catfish, *Pangasianodon gigas* and Giant pangasius, *Pangasius sanitwongsei* are considered as a flagship species in the relation with the conservation issue. The Mekong giant catfish and Giant pangasius have been classified as critically endangered and rare on the IUCN Redlist since 2001 and 1994, respectively. The pangasiid fishes distribute from North to South in the main steam of the River and reservoirs, except Mekong giant catfish which endemics in the Mekong River. The pangasiid fishes migrate long distance from pool habitats in dry-season within river channels, to feeding habitats of the floodplains in rainy-season. Important sizes of fish differences of the pangasiid fishes are reported. Several species are great economic importance and some are use in aquaculture.

At present, genetic structure of pangasiid fish in the Mekong River is not clearly understand. It is not clear that the fish that occur in various parts of the Mekong River are genetically same or not. The objectives of this thesis are to develop and characterize micorsatellite DNA markers, 1) to develop technique, 2) to identify fish belonging to the Pangasiidae family by molecular DNA markers, 3) to assess populations structures of common species in Upper Mekong River, 4) to assess population structure of wild and hatchery populations of Mekong giant catfish, and 5) to develop a management plan for conservation of Mekong giant catfish.

Chapter 2. Development and characterization of microsatellite DNA markers developed for Mekong Giant Catfish

Microsatellite DNA (msDNA) markers for the Mekong giant catfish were

developed from the captive stock samples using $(GT)_{15}$ probe. Ninety-six colonies were positive colonies. Nine of 11 primers set were polymorphic. The number of alleles per locus (N_A) was ranged from 1 to 4. The expected (H_E) and observed (H_O) heterozygosities were ranged from 0.000 to 0.574 and 0.000 to 0.800 (Table 1). In addition, these primers were successfully amplified in the four closely related species, *P. bocourti*, *P. conchophilus*, *P. larnaudii* and *P. sanitwongsei*. These primers have proven to be useful for the population genetic structure and taxonomic study in these species of the genus.

Chapter 3. Identification and relationship of the species of Pangasiidae by DNA markers

Ten pangasiid fish species including four genera; *Pangasius*, *Pangasianodon*, *Pteropangasius* and *Helicophagus* were identified based on msDNA and mtDNA markers. Both msDNA and mtDNA showed concordant results in classifying these pangasiid fishes. The phylogenetic relationship of Pangasiidae studied on morphological characters were supported by our results which showed genetic grouping by molecular markers being parallel with morphological taxa in the Pangasiidae family (Figure 1). The *Pangasianodon* clade includes two species; *P. gigas* and *P. hypophthalmus* which were closely related with each other. Basen on both the msDNA and mtDNA markers . The *Pangasius* clade includes six species; *P. bocourti*, *P. conchophilus*, *P. kremfi*, *P. larnaudii*, *P. macronema* and *P. sanitwongsei* based on both the msDNA and mtDNA markes. In the other hand, *Pteropaangasius* and *Heilicophagus* include only one species; *P. pleurotaenia* and *H. waandersii* that placed on different cluster with *Pangasius* and *Pangasianodon* (Figure 1).

The unidenfied larvae of juvenile were used as blank samples for testing the efficiency of the markers for species identification. By comparativde analysis of the

DNA markers of the blank wsaples with the known species, it is verified that these molecular markers were highly effective to identify the unknown fish samples. Blank samples 1, 2 and 3 were closely related with *P. larnaudii*, *P. hypophthalmus* and *P. pleurotaenia*, respectively (Table 2, Figure 1).

Chapter 4. Comparison of genetic diversity of the 6 Pangasiidae species collected from the Upper Mekong River based on DNA markers

Genetic diversity of six pangasiid fishes was investigated using 9 msDNA markers and sequencing analysis of mtDNA control region. Six species including *P. bocourti*, *P. conchophilus*, *P. hypophthalmus*, *P. macronema*, *P. sanitwongsei* and *H. waandersii* were collected from the Upper Mekong River. Both the msDNA and mtDNA showed concordant results in demonstrating similar genetic diversity and population structuring of pangasiid fishes (Table 3 and 4). High genetic variability was found in *P. macronema* while lower genetic variability was found in *P. sanitwongsei* and *P. hypophthamus* when observed in term of both msDNA (N_A, H_E) and mtDNA haplotypes. In migratory fishes such as *P. bocourti*, *P. conchophilus*, *P. macronema* and *H. waandersii*, genetic divergence among populations were not large. Population structure of pangasiid fishes were also observed which no evidence for isolation by distance. For conservation of pangasiid fishes of the Upper Mekong River, stock management of each populations should be separately performed by upper and lower reaches, and control of harvest through fisheries should be established to conserve each species.

Chapter 5. Evaluation of genetic divergence of the endangered Mekong giant catfish collected from natural water and hatcheries based on DNA markers

Genetic variability of the Mekong giant catfish collected from wild and hatchery populations was investigated using 10 msDNA markers and sequencing analysis of

mtDNA control region. Both msDNA and mtDNA showed concordant results in demonstrating similar genetic diversity and population structure of the Mekong giant catfish (Table 5 and 6). Marked reductions of genetic variability in the hatchery populations compared with the wild populations were observed in the genetic indices of msDNA (N_A, H_E, H_O) and mtDNA haplotypes. Genetic variability of the Mekong giant catfish was low being concordant with other endangered fish species reported to date. F_{ST} and Φ_{ST} also suggested that the magnitude of the genetic divergence within and among hatchery populations were significant but were not significant in the wild, between populations from upper and lower reaches (Table 7). Actually, haplotype network showed shared haplotypes among Thailand, Cambodia and hatchery populations (Figure 2). The smaller genetic diversity observed in the hatchery populations may be caused by bottleneck effect due to the limited number of effective parents when each population was founded.

Effective population size (Ne) of the wild Mekong giant catfish was 2,245 being similar with the Ne value of endangered vertebrates that reported including the cape fear shiner, *Notropis mekistocholas* (Ne=7-4463) (Saillant et al. 2004) and black rhinoceros, *Diceros bicornis* (Ne=1472-5173) (Harley et al. 2005). It is recommend to enhance genetic variability, to eliminate the accumulation of inbreeding effects in hatchery populations by mean of increasing population size and to prevent inbreeding using selective breeding base on the principle of minimal kinship broodstock management.

Chapter 6. Simulation of broodstock management by minimal kinship selective breeding in hatchery populations of the Mekong giant catfish

The effectiveness of the minimal kinship (*MK-Pair*), average minimal kinship (*MK-Ave*) and random breeding on preservation of genetic diversity of the hatchery populations of the Mekong giant catfish were compared based on the indices of genetic

variabilities by DNA markers. The genetic variability in descendant which was reproduced by computer by the method of minimal kinship selective breeding (MKSB) was maintained in higher values as shown in the simulation experiment. The MKSB average value based selection (*MK-Ave*) and the pairwise value based selection groups (*MK-Pair*) were effective to minimize loss of genetic variation. H_E and N_A when these are compared with non-selected group (Table 8). For, H_E, the differences of the values of MKSB with that of the non selective group approaches were prominent when the selection intensity (Nc/Na) being the ratio of contributing parent (Nc) and reproduced fish in each generation (Na) was more than 0.20 was kept. The *MK-Ave* approach could increase about 49.9% of H_E within 20^{th} generations under the condition of selection intensity 0.20 (at least 100 parents were used and 500 offspring) were kept in each generation (Figure 3). On the other hand, the *MK-Ave* selective breeding showed a better result to prevent the loss of N_A than *MK-Pair* in all examined selection intensities when the number of selection intensity was more than 0.04.. *MK-Ave* selective breeding approach could conserve about 98% of N_A for 20 generation when at least selection intensity of 0.20 were used as breeders in each generation (Figure 4). Furthermore, if the selection intensity was set at 0.04, inbreeding will not occur in the *MK-Ave* selective breeding approach. For preserving genetic diversity of Mekong giant catfish, it is recommended to use average minimal kinship breeding approach under the selection intensity of 0.20, which mean to use 100 parents, and to keep 500 offspring in each generation.

Chapter 7. Recommendation to broodstock management of the Mekong Giant catfish and pangasiid fish based on DNA markers and computer simulation

From the results, the recommendations to broodstock management of pangasiid fish are as follow:

1) Mitochondrial DNA marker is more effective in identifying of pangasiid fish larvae than msDNA markers. Management policy for pangasiid fishes such as setting optimal sustainable harvesting limit must be established. Overfishing in vulnerable area, especially at the spawning sites will lead to a reduction in effective population size and yield, and ultimately the loss of genetic diversity and population viability.

2) The Mekong giant catfish exhibits the genetic divergence according to each year of hatchery populations, each population could be considered as "Management Units" because wild fish are impossible to catch enough number of spawner from natural for seed production. All populations should be directed towards preserving the genetic integrity of each group.

3) Captive broodstock used for restocking program should be founded by collecting fish from the wild populations, and the offspring should be released back into natural habitat from which the founders were derived.

4) Breeding of the Mekong giant catfish should be done by using minimal kinship selective breeding approach in order to retain genetic diversity of the captive stock. Genetic diversity of both wild and hatchery populations should be monitored regularly in order to assess the success of its management plan (Figure 5).

Table 1. Characterization of msDNA primers in the Mekong giant catfish

Locus	Repeat motif	T_a (°C)	EP(bp)	SL (bp)	N_A	H_O	H_E	Accession no.
Pdgi-8	$(TG)_{14}$	60	126	188-192	2	0.733	0.481	DQ097706
Pdgi-9	$(TG)_{15}$	48	155	153-155	2	0.267	0.405	DQ097705
Pdgi-11	$(CA)_{12}$	60	211	202-210	2	0.200	0.508	DQ097707
Pdgi-13	$(CA)_{10}$	55	144	142-144	2	0.067	0.067	DQ097708
Pdgi-16	$(TG)_{12}$	55	156	153-157	3	0.800	0.574	-
Pdgi-18	$(GA)_{14}$	55	145	144-160	2	0.300	0.521	DQ097709
Pdgi-19	$(TG)_{15}$	55	170	170	1	0.000	0.000	DQ097710
Pdgi-20	$(CA)_5CG(CA)_{11}$	55	168	157-171	4	0.467	0.568	DQ097711
Pdgi-21	$(TG)_4TT(TG)_{13}$	52	147	143-147	2	0.600	0.481	DQ097712
Pdgi-22	$(GA)_7GC\ (GA)_{14}$	53	212	203	1	0.000	0.000	DQ097713
Pdgi-24	$(CA)_{16}$	55	175	174-184	4	0.267	0.251	DQ097714

Abbreviations follow; T_a = temperature annealing, EP= expected product length, SL= size product length, N_A=number of alleles per locus, H_E=expected heterozygosity, H_O=observed heterozygosity, Accession no. = gene bank Accession no

Table 2. Unbiased genetic distance between each pangsiid species; below diagonal based on msDNA, above diagonal base on mtDNA

	Boc	Con	Gig	Hyp	Kre	Lar	Mac	Ple	San	Hwa	Un1	Un2	Un3
Boc	**	0.169	0.276	0.234	0.115	0.161	0.118	0.278	0.161	0.369	0.158	0.233	0.295
Con	1.319	**	0.323	0.269	0.186	0.223	0.192	0.312	0.222	0.386	0.226	0.275	0.311
Gig	5.845	3.011	**	0.193	0.288	0.269	0.290	0.281	0.268	0.385	0.267	0.186	0.298
Hyp	3.242	2.465	2.453	**	0.25	0.272	0.240	0.293	0.242	0.357	0.269	0.02	0.299
Kre	1.780	0.871	2.809	3.190	**	0.158	0.105	0.229	0.126	0.339	0.158	0.244	0.243
Lar	0.813	1.243	2.695	2.016	1.501	**	0.160	0.289	0.147	0.331	0.006	0.264	0.290
Mac	1.448	2.630	3.532	3.148	3.075	1.272	**	0.262	0.15	0.353	0.162	0.235	0.267
Ple	0.992	1.637	2.559	3.023	1.297	0.867	1.016	**	0.255	0.307	0.290	0.287	0.039
San	1.113	1.604	1.582	3.882	1.877	1.070	1.533	1.208	**	0.355	0.149	0.232	0.250
Hwa	1.601	1.468	3.440	5.373	1.836	1.397	2.774	1.623	1.754	**	0.326	0.355	0.316
Un1	0.874	1.419	2.085	2.773	1.527	0.232	1.467	1.003	1.256	1.641	**	0.261	0.291
Un2	4.215	2.939	1.904	0.261	2.637	2.102	3.806	4.442	1.000	1.000	1.879	**	0.289
Un3	1.056	1.811	4.802	2.365	2.328	0.948	1.062	0.415	1.213	1.313	1.120	1.000	**

Abbreviations follow; Boc = *P. bocourti*, Con= *P. conchophilus*, Gig= *P. gigas*, Hyp= *P. hypophthalmus*, Kre= *P. kremfi*, Lar= *P. larnaudii*, Mac= *P. macronema*, Ple= *P. pleurotaenia*, San= *P. sanitwongsei*, Hwa= *H. waandersii*, Un1= unknown1, Un2= unknown2 and Un3= unknown3.

Table 3. Genetic variability of each pangsiid species based on msDNA markers

Allelic	*P. bocourti*			*P. conchophilus*			*P. hypophthalmus*		*P. macronema*			*P. sanitwongsei*				*H. waandersii*	
variation	NP	NK	LK	NP	NK	MK	NK	UB	NP	NK	UB	CR1	CR2	CR3	UB	NP	NK
N	30	20	27	22	35.5	15.5	24	12	10.5	22	36	34.5	31	20	9	58	16
N_A	4.89	4.56	5	8.0	8.56	7	3.00	2.11	7.78	11.40	14.11	2.33	1.44	1.33	1.78	5.22	3.56
R_A	17.45	12.28	16	15.00	22.03	11.25	13.50	7.06	9.14	16.70	25.06	18.42	16.22	10.67	5.39	31.6	9.78
H_E	0.463	0.449	0.458	0.597	0.547	0.516	0.324	0.245	0.684	0.749	0.747	0.220	0.127	0.084	0.133	0.364	0.288
H_O	0.463	0.443	0.457	0.462	0.453	0.406	0.389	0.250	0.636	0.651	0.643	0.194	0.151	0.072	0.130	0.382	0.254
HWE	0.300	0.857	**0.000**	**0.000**	**0.000**	**0.0001**	**0.000**	**0.002**	0.006	**0.000**	**0.000**	**0.0007**	0.673	0.331	0.973	0.021	0.026

Abbreviations follow; N= number of individual, N_A=number of alleles per locus, R_A=alleles richness, H_E=expected heterozygosity, H_O=observed heterozygosity, HWE= probability of conformity to Hardy-Weinberg equilibrium; bold mark is deviate from HWE (p<0.006), NP= Nakorn Phanom Province, NK= Nongkhai Province, MK= Mukdahan Province, UB= Ubonrachathani Province and CR= Chiang Rai Province.

Table 4. Genetic diversity of each pangsiid species based on mtDNA markers.

	P. bocourti			*P. conchophilus*			*P. hypophthalmus*		*P. macronema*			*P. sanitwongsei*				*H. waandersii*	
	NP	NK	LK	NP	NK	MK	NK	UB	NP	NK	UB	CR1	CR2	CR3	UB	NP	NK
Sample size	15	20	22	10	20	12	22	12	8	22	30	21	27	12	4	45	5
Polymorphic sites	21	18	23	36	44	39	2	0	21	48	52	2	8	13	17	22	12
No. of substitutions																	
Transitions	17	15	19	32	38	34	1	0	18	32	39	1	4	10	8	15	11
Transversions	3	1	3	1	2	1	1	0	3	12	12	1	6	3	9]	3	0
Indels	1	2	1	4	5	5	0	0	0	6	5	0	0	0	0	0	1
No. of haplotype	12	14	15	10	19	9	3	1	8	18	29	3	11	9	4	20	4
h	0.971	0.958	0.952	1.00	0.995	0.955	0.41	0.00	1.00	0.980	0.998	0.55	0.613	0.94	1.00	0.865	0.900
π	0.015	0.012	0.011	0.028	0.025	0.028	0.001	0.00	0.019	0.020	0.021	0.00	0.004	0.01	0.024	0.007	0.016

Other abbreviations are as follow: Nucleotide diversity (π), Haplotype diversity (h) NP= Nakorn Phanom Province, LK= Laksila district, Nakorn Phanom Province NK= Nongkhai Province, MK= Mukdahan Province, UB= Ubonrachathani Province and CR= Chiang Rai Province.

Table 5. Genetic diversity of wild and hatchery populations of Mekong giant catfish based on msDNA markers

Allelic variation	Populations									
	WTH	WCB	H84	H91	H92	H93	H99	H05	All wild	All hatchery
N	11	9	43	63	17	14	20	30	20	187
N_A	2.8	3	2.9	3.5	3.2	2.9	2.3	1.7	3.2	3.6
R_A	2.74	3	2.49	2.75	2.86	2.77	2.00	1.70	3.2	3.14
H_E	0.483	0.467	0.407	0.469	0.427	0.469	0.342	0.291	0.472	0.449
H_O	0.418	0.433	0.451	0.456	0.465	0.464	0.445	0.393	0.425	0.445
HWE	0.255	0.878	0.036	**0.000**	0.716	0.171	0.023	0.002	0.185	**0.000**

Abbreviations follow; N= number of individual, N_A=number of alleles per locus, R_A=alleles richness, H_E=expected heterozygosity, H_O=observed heterozygosity, HWE= probability of conformity to Hardy-Weinberg equilibrium; bold mark is deviate from HWE (p<0.005), WTH= wild from Thailand, WCB= wild from Cambodia, H= hatchery population each years (1984, 1991, 1992, 1993, 1999 and 2005).

Table 6. Genetic diversity in Mekong giant catfish based on mtDNA control region

	Populations									
	WTH	WCB	H84	H91	H92	H93	H99	H05	All wild	All hatchery
No. of samples	10	9	42	61	16	14	20	29	19	182
No. variable site	8	10	7	8	6	5	5	0	8	12
No. substitutions										
Transitions	8	9	7	8	6	5	5	0	8	12
Transversions	0	1	0	0	0	0	0	0		
No. haplotypes	6	8	5	6	5	4	2	1	6	11
h	0.89	0.79	0.60	0.74	0.68	0.75	0.10	0.00	0.89	0.87
π	0.009	0.008	0.006	0.005	0.004	0.006	0.001	0.000	0.009	0.007

Other abbreviations are as follow: π=nucleotide diversity, h= haplotype diversity, WTH= wild from Thailand, WCB= wild from Cambodia, H= hatchery population each year (1984, 1991, 1992, 1993, 1999 and 2005).

Table 7. Estimates of F_{ST} and Φ_{ST} based on msDNA and mtDNA sequences

	msDNA		mtDNA	
	F_{ST}	*P*	Φ_{ST}	*P*
Global (no subdivision)	0.031	0.0000	0.415	0.0000
Among wild populations	-0.008	0.7459	0.046	0.1355
Among hatchery populations	0.110	0.0000	0.466	0.0000
Wild vs Hatchery	0.034	0.0000	-0.006	0.5862
Thailand vs Hatchery	0.031	0.0039	0.016	0.2333
Cambodia vs Hatchery	0.034	0.0009	0.007	0.3441

P=Significant level base on random allelic permutation testing.

Table 8. Genetic diversity in term of H_E and N_A of the combined hatchery populations of the Mekong giant catfish

	Selection Intensity	*MK-Pair*				*MK-Ave*				Rd			
		G0	F10	F20	% loss	G0	F10	F20	% loss	G0	F10	F20	% loss
H_E	0.04	0.449±0.00	0.566±0.01	0.551±0.02	-22.8	0.449±0.00	0.578±0.06	0.568±0.05	-26.5	0.449±0.00	0.404±0.04	0.364±0.06	18.9
	0.10	0.449±0.00	0.616±0.01	0.591±0.01	-31.5	0.449±0.00	0.665±0.01	0.665±0.01	-48.2	0.449±0.00	0.427±0.03	0.407±0.03	9.4
	0.20	0.449±0.00	0.638±0.01	0.664±0.01	-47.9	0.449±0.00	0.661±0.00	0.673±0.00	-49.9	0.449±0.00	0.434±0.02	0.424±0.03	5.6
N_A	0.04	36.00±0.00	28.6±1.62	26.12±1.64	27.4	36.00±0.00	31.14±2.44	29.68±2.89	17.6	36.00±0.00	26.76±1.85	23.4±2.12	35.0
	0.10	36.00±0.00	33.58±0.92	32.7±1.13	9.2	36.00±0.00	35.3±0.64	35.3±0.65	1.9	36.00±0.00	30.84±1.46	28.00±1.92	22.2
	0.20	36.00±0.00	35.00±0.70	34.88±0.75	3.1	36.00±0.00	35.4±0.67	35.38±0.67	1.7	36.00±0.00	33.16±1.15	30.98±1.52	13.9

G0=founder generation; F10 and 20 = generation 10 and 20.

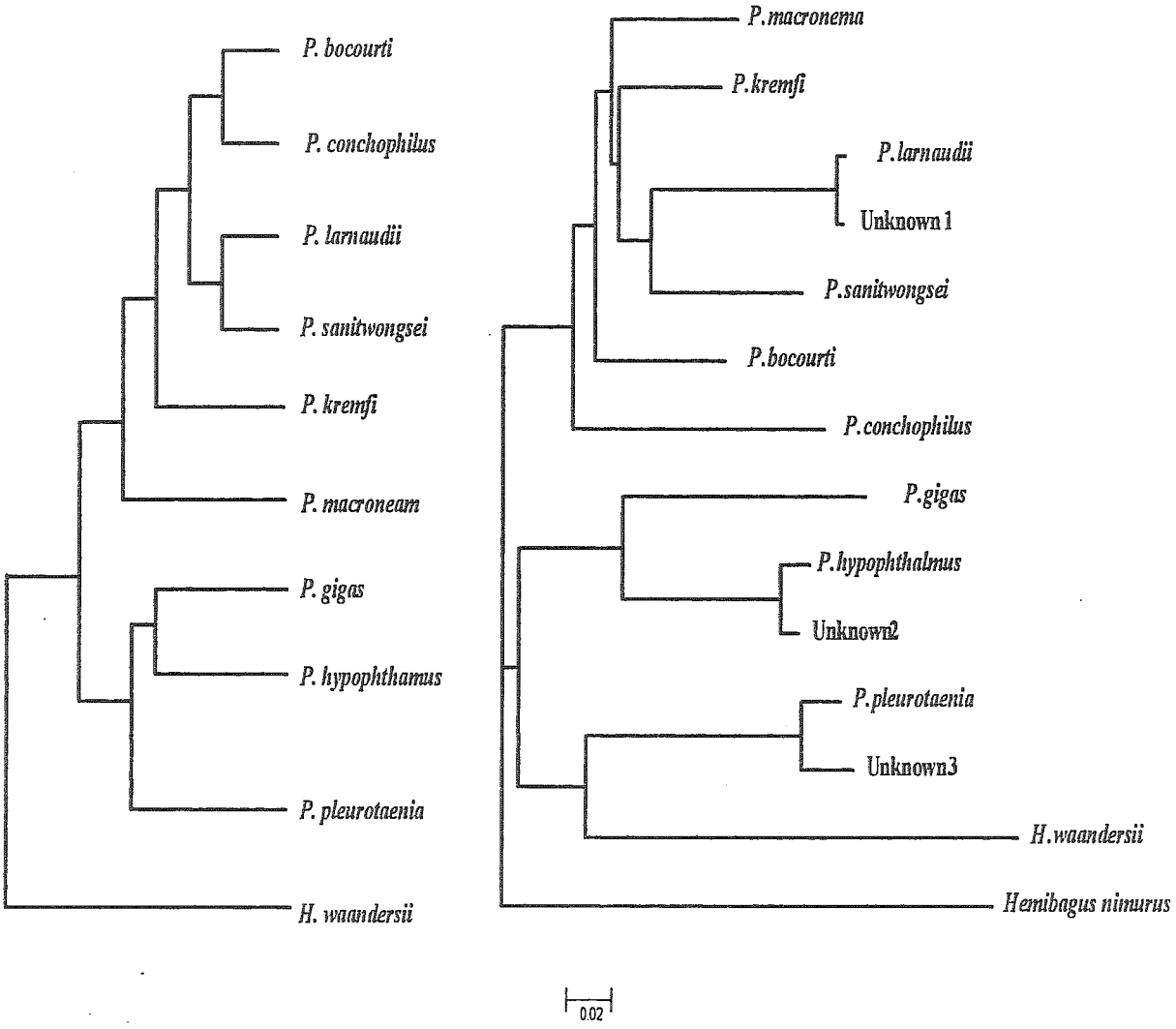

Figure 1. Phylogenetic relationship among several species on the Pangasiidae family, generated from morphological character data (left) and mtDNA marker (right).

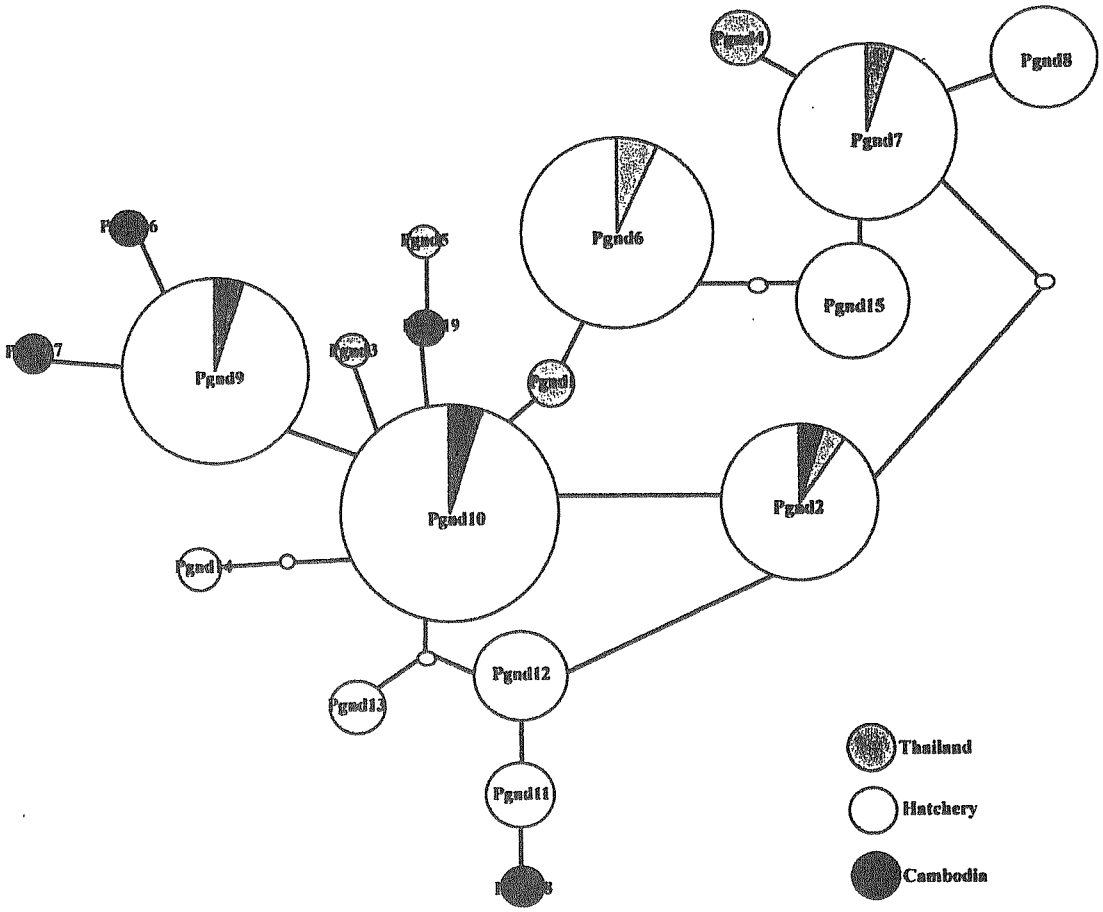

Figure 2. Statistical parsimony network of Mekong giant catfish mtDNA control region haplotypes. Each circle represents a haplotype and frequencies are indicated by each circle size. Blue circles indicate the haplotypes only present in wild from Thailand, yellow circles indicate the haplotypes from hatchery and red circles indicate the haplotypes from wild Cambodia.

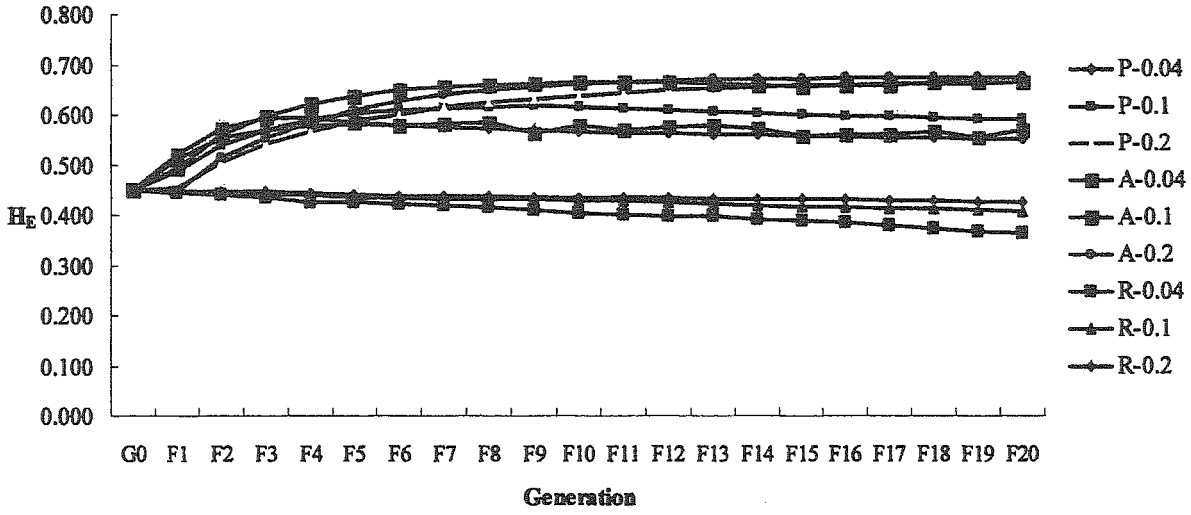

Figure 3. Average changes in heteryzygosity (H_E) in total hatchery from 50 repeats simulations for 20 generations under (P) minimal kinship selective breeding, (A) Average minimal kinship selective breeding and (R) random selective breeding using selection intensity 0.04, 0.1 and 0.2 per generation.

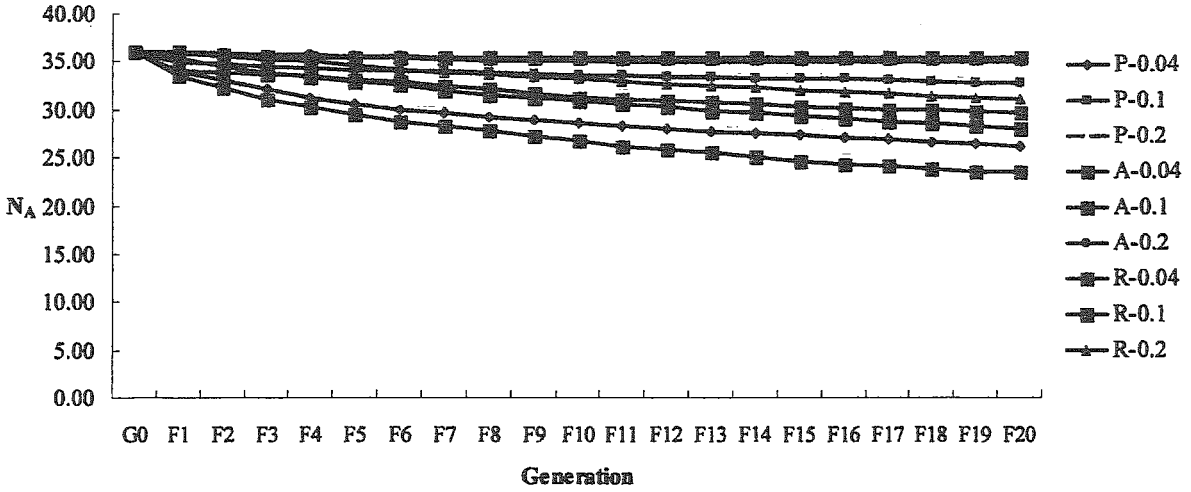

Figure 4. Average change number of alleles (N_A) in total hatchery from 50 repeats simulations for 20 generations under (P) minimal kinship selective breeding (A) Average minimal kinship selective breeding and (R) random selective breeding selection intensity 0.04, 0.1 and 0.2 per generation.

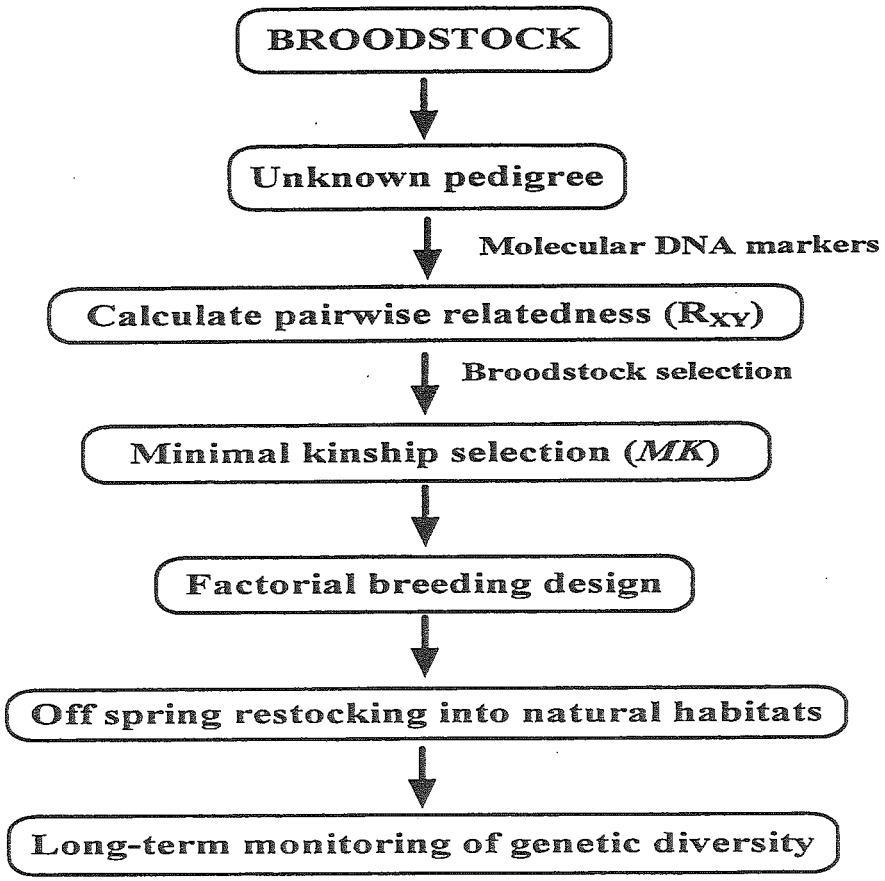

Figure 5. Diagram of the proposal for broodstock management to minimize loss of genetic variability in the stock enhancement program.

論文審査結果要旨

メコン川は東南アジア最長の河川で，淡水魚の種多様性の中心的存在である。ナマズ類のPangasidae科は在来種の中でも最大のグループで，とりわけメコンオオナマズ *Pangasianodon gigas* と *Pangaisus sanitwongsei* は遺伝資源の保全における象徴的重要魚種であり，絶滅危惧種に指定されている。これらの魚種の遺伝的集団構造はほとんど未解明の状態にあり，メコン川内の分集団の遺伝的分化も不明の状態である。本研究は，本種の絶滅リスクの査定・評価を視野におき，集団の遺伝的管理手法の開発を目指したものである。

最初に，集団遺伝学的分析に必要な高感度マーカーであるマイクロサテライトDNAマーカーの開発を試み，多数のプライマーセットを作製し，多型性を備える11つのプライマーセットの開発に成功し，それらのマーカー座の特性評価を行っている。次に，ナマズ目4属の遺伝的類縁関係をマイクロサテライトDNAマーカーおよびミトコンドリアDNAマーカーにより分析し，両者が良く対応していることを解明した。これらのマーカーは，メコン川のナマズ類の魚種鑑定において成魚期だけでなく仔稚魚期においても有効であることを示し，今後，種々の資源生物・生態学においてその効果を発揮することが期待できる。また，メコン川のナマズ類6種について遺伝的集団構造の解析を試み，河川内分集団間で距離による隔離のないことを解明し，メコン川の沿岸国間での協調的資源管理の必要性を指摘している。

さらに，絶滅危惧種であるメコンオオナマズについては，放流用種苗生産のための親魚が保存されており，その多様性保全とリスク評価・管理が必要となる。このため，遺伝マーカーによる多様性低下防止手法として，Minimal Kinship選択交配法（MK法）が有効と考え，遠縁の個体を選び交配を行う継代シミュレーションを30世代にわたって実行し，遺伝的多様性レベル維持に関する効果を評価・検討している。本シミュレーション法が遺伝的多様性レベル維持のための情報を得る上で効果的であることを明らかにしたが，対象集団の特性や，親魚の数によっては逆効果を引き起こす危険性も示唆され，今後，種苗生産の現場においてMK法を実施する際には，継代シミュレーションに基づくリスク予測を実施し，リスク防止条件を解明した後に，種苗生産とその放流を実施すべきとする管理マニュアルを提案した。

以上のように，本研究は現存のメコン川のナマズ集団の多様性をDNAマーカーによって評価し，これらのデーターを用いたシミュレーションにより，野生集団の遺伝的保全に配慮した放流事業のあり方について価値ある提案を行っている。よって，審査委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。