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Utility of Isozyme as a Genetic Marker for Estimating the Effects of Release and Stock Enhancement of Fleshy Prawn *Fenneropenaeus chinensis*

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We evaluated the utility of applying isozyme analysis and two tagging methods, visible implant fluorescent elastomer (VIE) injection and uropod-cutting, to monitor the effects of releasing nursery-reared fleshy prawn (*Fenneropenaeus chinensis*) into natural habitat in Korea. One hundred thousand farmed prawns (70 mm long) were tagged by clipping off the outer left uropod and injecting them with VIE. This marked seed population was released at Muchangpo, Korea, on 11 and 19 July 2002. Two months later, total catch and catch per unit effort (CPUE) at three locations (Hongwon, Muchangpo, and Anmyundo) were determined. Total catch and CPUE increased nearly 18% over the previous year in Hongwon and Muchangpo. The mixing rate, estimated by uropod regeneration pattern, was 0.33% at Hongwon, 0.53% at Muchangpo, and 0.21% at Anmyundo. The recapture rate was about 3.5%. Isozyme analysis confirmed that the mixing rate was highest at Muchangpo. Moreover, fleshy prawns from Muchangpo were genetically most related to the seed population, indicating that the released prawns had largely remained near the released site. We also confirmed that isozyme genes are valuable as genetic markers for qualitative analyses of a released seed population.

Key words: Fleshy prawn, Genetic marker, Isozyme, Recapture rate, Release effect, *Fenneropenaeus chinensis*

Introduction

The fleshy prawn *Fenneropenaeus chinensis* belongs to the Family Penaeidae and is found primarily in Korea and China, but has recently been reported near the coast of Kyushu, Japan. This species is of considerable commercial importance, primarily due to its large size; it is farmed in the West Sea, East China Sea, and Korean Bight, where wild stocks are also fished via trawling (Holthuis, 1980).

National hatcheries and local governments in Korea have released more than 100 million fleshy prawns on the west coast each year since 1988 to enhance stocks. However, the effects of the release on stock enhancement have been uncertained. Thus, a proper investigation of the influence seeds on the wild fleshy prawn population was essential.

Many tagging methods, including fin-clipping, hole-punching, branding, and tattooing, have been applied. Because the crustaceans molt, these markers are lost after molting. For the crustaceans, marking methods for shrimp were reviewed by Farmer (1981). In kuruma prawn ranching projects in Japan, stainless steel and gold wire tags were described by Ariyama et al. (1994). Toyota et al. (1997) have developed a marking method for the kuruma prawn reducing the pigment area of regenerated uropods after cutting.

Genetic markers have also been studied for tagging of released seeds (Seki and Taniguchi, 1988; Taniguchi and Takahashi, 1989; Seki et al., 1994), and the utility of the method has been demonstrated in rainbow trout (Campton and Johnston, 1985) and Atlantic salmon (Birt et al., 1991). For organisms that

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shed their outer layer, such as crustaceans, a genetic marker, which will not be lost during the animal's lifetime, is critical.

To facilitate the development of a low-cost marking method for mass monitoring of fleshy prawn in the short term, we describe the effects of a mass prawn release, including an assessment of mixing rates, and the procedural requirements for enhancing natural stocks. We also evaluate isozyme genes as a genetic markers, and describe genetic diversity and isozyme variability among fleshy prawn populations to develop the genetic resources of this species.

Materials and Methods

Seed production and release of fleshy prawn

One hundred mature female *F. chinensis* were collected from the coast of Beopseongpo, Jeollanam-do, on 13 May 2002, and transported to Boryung National Hatchery, National Fisheries Research & Development Institute (NFRDI). The collected females were maintained in 7×7 m concrete tanks containing filtered seawater. Active females were moved to circular tanks of 100 m^2 in surface area, and the water temperature was raised to between 16 and 18°C to induce spawning. After the prawns laid eggs, the tanks were weakly aerated to scatter the eggs, which were then maintained in standing water until the hatching. The average body length was 22.1 ± 1.4 cm and the average body weight of the mature females was 72.0 ± 12.8 g.

During the nauplius stages, food was not provided. Zoea stages were fed with microalgae (*Skeletonema costatum*, *Chaetoceros* sp., and *Nitzschia* sp.). When the larvae molted to the protozoan stage, rotifers and artificial feed for larvae were provided. At the mysis stages, newly hatched *Artemia nauplii* were provided. Other methods for larvae culture followed Kim and You (1989). At least 13.5 million shrimp larvae (postlarva stages 10-12) were produced and released into the sea near Muchangpo, Boryung, and the coast of Biin, Chungcheongnam-do, on 4 and 5 June 2002.

Nursery rearing and marking for release

Juveniles of PL stages 10-12 were transferred to the shrimp pond in Hwaseong City on 4 June 2002, and cultured for 34 days. After reaching approximately 72 mm in body length (body weight 2.3 g), about 50,000 shrimp were collected and marked by injecting 0.01mL of visible implant fluorescent elastomer (VIE) into the sixth abdominal segment or cutting off the outer left uropod completely. Also, 1 million shrimp larvae were transferred to the shrimp pond in Taean-gun on 4 June and cultured for 42 days. When the juveniles had reached a body length of 76.7 mm (body weight 3.1 g), about 50,000 shrimp were collected and marked, using the same techniques.

The 100,000 marked shrimp were released near Seokdaedo, 1 mile southwest of Muchangpo, Boryung-si, Chungcheongnam-do, in twice waves: 50,000 for 11 July, and 50,000 on 19 July (Fig. 1).

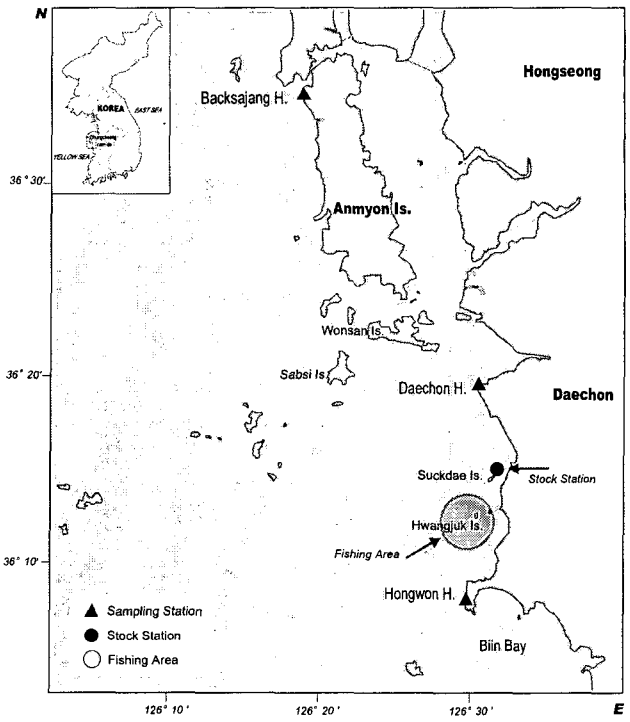

Fig. 1. Release and subsequent observation sites of fleshy prawns off the west coast of Korea.

Amount of catch and the mixing rate

To evaluate the total catch, catch per unit effort (CPUE), and mixing rate of the seed population, we focused on the ports of Anmyundo, Muchangpo, and Hongwon in Chungcheongnam-do, where the consignment sale corners of the National Federation of Fisheries Cooperatives (NFFC) are located. The sites were visited three times during September and October during fishing season. The total catch and the NFFC log of boats arriving at those three ports were collected and reviewed. Marked shrimp were determined by direct observation of the regenerated uropod shape and any traces of visible fluorescent material on the abdomen of shrimp. The number of recaptured released fleshy prawns was estimated using the follow formula: (total catch / mean body weight) × mixing rate (%).

Genetic analysis using isozymes

Isozymes were analyzed to assess the genetics and variability of fleshy prawns in Korea, and elucidate the genetic differences of the artificially raised seeds. Horizontal starch gel electrophoresis was used for isozyme analysis (Taniguchi et al., 1983a), and ten isozymes (G3PDH, GPI, IDHP, LDH, MDH, MEP, MPI, PGDH, PGM, and PROTEIN) from muscle tissue were analyzed. Wild shrimp were collected at Narodo in Goheung, Heuksando, Youngkwang, Seochun, Muchangpo, and Anmyundo.

Results and Discussion

Regeneration and identification standards for the uropods

In 2002, 24 marine shrimp hatcheries in Korea produced about 935 million fleshy prawn seeds. Nearly 750 million postlarval shrimp were stocked in shrimp ponds, and about 110 million postlarva were released on the west coast in Chungcheongnam-do and Jeollabuk-do.

Fleshy prawns whose uropods had been cut off were reared in indoor tanks for 4 months, and the regeneration status of the uropods was continually observed. The uropods regenerated during the rearing period, and less than 10% mortality occured. While the uropods eventually completely regenerated (Fig. 2), their shape, color, and pigment patterns were unique.

Cut uropods were regenerated with a shorter and/or the density and distribution of pigments, different from those of normal ones, which were mainly concentrated on the lower part. The length of the regenerated uropods was sometimes shorter and similar to that of the inner uropods. Thus the length of uropod and the density and distribution of pigments could be used as identification standards, and the method of cutting uropods was confirmed as a useful approach for marking fleshy prawns.

The uropod-cutting method has been used as a marker for the investigation of release effects for the kuruma prawn. The uropod-cutting marker of kuruma prawn has basically the cutting uropods pigmentation regeneration. The uropod cutting marker of Kuruma prawn has basically the cutting uropods pigmentation regeneration rate as indicator. As the result of releasing the kuruma prawn by cutting its uropod, the cutting uropods length is almost regenerated after about 1 month, but the uropod's central melanophore did not regenerate (Toyota et al., 1998). Because pigmentation regeneration rate depends on the size of the shrimp at the time the uropod is cut off, it can be easily identified in seeds larger than 50 mm, but is not so obvious to the unaided eye below 50 mm.

Catch rate and CPUE by fishing region

The fleshy prawn catch was investigated from sales records of three NFFCs in the September and October fishing seasons of 2002 (Table 1). A total of 6,470 kg of fleshy prawn was caught by 791 boats at Hongwon port in 30 days of fishing during September and October; however, fishing activity was discontinued earlier than normal due to a significant decrease in the catch. During the same period, a total of 4,290 kg was caught by 345 boats at Muchangpo port in 41 days, and at Anmyundo, a total of 9,094 kg was caught by 654 boats in 42 days of fishing. In 2001, the catch was evaluated at the same ports during same months. Total catch at Hongwon in 2002 increased 20% over the previous year, and that at Muchangpo in 2002 increased 16%. The total catch at Anmyundo decreased compared to the previous year. The CPUE was the lowest at Hongwon at 9.7, the highest at Anmyundo with 24.6 while at Muchangpo it was in the middle with 13.4. At Hongwon and Muchangpo, the fishing boats were small (2-5 tons) and remained near the coast; the fishing period showed decreased

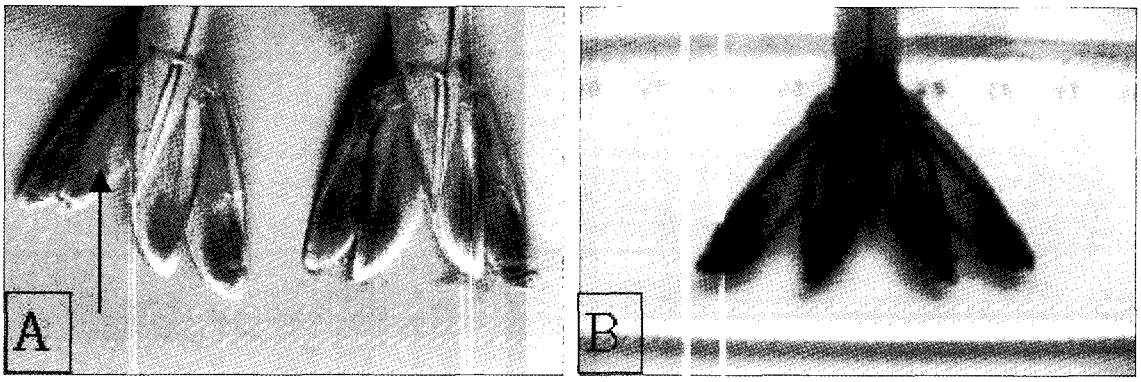

Fig. 2. Regeneration of uropod: A, short uropod (↑); B, normal uropod.

Table 1. Total catche (kg) and catch per unit effort (CPUE) of fleshy prawn at each site

	Month	Hongwon (Seochun)	Muchangpo (Boryung)	Anmyundo (Taean)
Fishing days	Apr.	-	-	-
	Aug.	4	0	5
	Sept.	23	26	27
	Oct.	7	15	15
	Total	34	41	47
Number of Fishing boat	Apr.	-	-	-
	Aug.	63	0	45
	Sept.	523	300	475
	Oct.	268	45	179
	Total	854	345	699
Total catch (kg)	Apr.	-	-	-
	Aug.	920	0	2,243
	Sept.	5,299	3,632.5	7,668.2
	Oct.	1,171	657	1,426.9
	Total	7,390	4,289.5	11,338
CPUE (kg/no.)	Apr.	-	-	-
	Aug.	14.6	0	49.8
	Sept.	10.1	12.1	16.1
	Oct.	4.37	14.6	7.97
	Total	9.7	13.4	24.6

catch and increased fishing activity. When the CPUE was investigated at the three ports in 2001, the CPUE of Hongwon port, Muchangpo port and Anmyundo were 8.7, 10.8 and 18.6 respectively. CPUEs of three ports were all increased than the 2001.

Mixing and recapture rates of released fleshy prawns

Three attempts were made at each site to locate released shrimp in September and October 2002. Table 2 shows the size of shrimp according to site. In September, when fall fishing activity began, the average length of shrimp at

Hongwon and Muchangpo were 167.8 and 165.6 mm, respectively, both larger than the 160.1 mm at Anmyundo. The observation of uropods of recaptured shrimp were assessed directly at each site (Table 3). Of a total of 1,640 shrimp observed at Hongwon, 5 were recaptured shrimp with a regenerated uropod (0.33%). At Muchangpo, out of a total of 1,655 shrimp examined six shrimp were identified as released shrimp with a cut-off uropod, and the mixing rate was highest (0.53%) among the three sites.

When total catch is multiplied by one shrimp's weight, total number of caught shrimps can be known. It is estimated that 153,600 shrimps in September, and 23,420 shrimps in October were caught at Hongwon. 115,300 and 13,000 shrimp were caught at Muchangpo in September and October, respectively. An estimated grand total of 614,920 shrimps were caught at the three sites collectively over the study period. Based on calculations of the incidental catch rate at each site, we estimate that 663 released shrimps were recaptured at Hongwon and 1.332 were recaptured at Muchangpo. An estimated 2,788 of 100,000 shrimps released in 2002 for the were recaptured in during the study period. Accordingly, when the survival rate of released seed's is 80%, the recapture rate is estimated at approx. 3.5%. But, it is actually estimated at the rate lower the above rate. The recapture rate of released seeds largely depends on the natural mortality or initial loss in wild environments.

When this recapture rate from the result of marked released shrimp is applied to the released seeds from Boryung Hatchery, a catch of more than 500,000 shrimp would be expected, but the actual catch at Hongwon and Muchangpo in 2002 was only 330,000 shrimp. The body length of individuals in this seed population was around 10 mm, whereas the marked, nursery-reared seeds released in this study were 75 mm in body length. Thus a much lower survival rate would be expected for the former. Assuming a survival rate of 10%, the estimated recaptured population would be around 50,000, but the actual recapture would be still lower. Therefore, it is important to consider the release size of prawns to improve the effect of releasing because the initial loss is greater when seeds of 10 mm body length are released.

Toung and Zhang (1998) investigated fleshy prawn releases and recapture rates in China over a 10-year period (1984-1993). They reported that when 10- mm long seeds were released, the recapture rate was 0.2-0.3%; when 52-mm long seeds through nursery culture were released, the recapture rate was 1.16%; and when 63-mm long seeds were released, the recapture rate was 2.07%. Thus, the bigger shrimp at release, the higher the recapture rate will be.

The recapture of kuruma prawns investigated at

Table 2. Body length and body weight of wild fleshy prawn captured at each site (mean ± SD)

Month		Hongwon	Muchangpo	Anmyundo
September	Body length (mm)	167.8±11.58	165.6±12.27	160.1±10.57
	Body weight (g)	30.2±5.88	26.9±5.89	26.9±5.99
October	Body length (mm)	182.6±14.28	179.7±16.29	161.8±16.49
	Body weight (g)	38.6±8.97	36.0±9.35	28.5±8.8

Table 3. Number of fleshy prawn with an abnormal uropod (i.e. released shrimp) and total shrimp observed at each site

Observed month	Hongwon		Muchangpo		Anmyundo	
	Released shrimp/ Observed number	Mixing rate (%)	Released shrimp/ Observed number	Mixing rate (%)	Released shrimp/ Observed number	Mixing rate (%)
Sept.	2/520	0.39	4/455	0.88	2/748	0.27
Oct.	3/1,120	0.27	2/1,200	0.17	1/704	0.14
Mean	-	0.33	-	0.53	-	0.21

Fukuoka, Japan in 1987 was estimated at 3.2%, and Morikawa et al. (1996) reported that 3.1% out of 200,000 released prawns were recaptured.

As shown in Fig. 1, released shrimp in the present study were mostly caught by fishing boats that worked near Hwangjukdo, between the ports at Muchangpo and Hongwon, suggesting that released fleshy prawns did not move to the open sea during the growth period and settled near the release site. Thus, fleshy prawns may be sedentary, a behavior that could contribute greatly to enhancing coastal shrimp resources if the shrimp are released systematically.

Genetic diversity and variability in isozymes

Fourteen loci were detected using 10 isozymes. The electrophoresis patterns of MDH, PGM, and MPI are shown in Fig. 3. Two loci were detected in MDH; MDH-1*, which showed no genetic variation, and MDH-2*, which was estimated to have three alleles, 120, 100, and 90. Two loci were detected in PGM: PGM-1* and PGM-2*, which had four alleles (115, 100, 70, and 40).

Four alleles (115, 100, 90, and 70) were also detected in MPI*. A total of 25 alleles was estimated from the 14 detected loci, and the average number of alleles was 1.8, which is common in marine

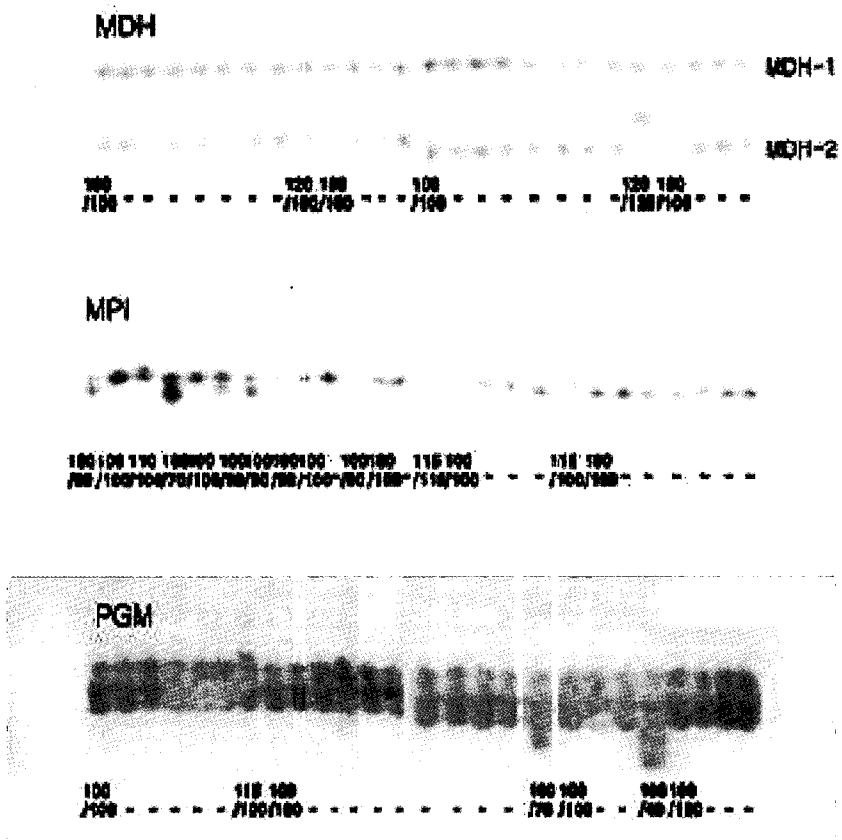

Fig. 3. Electrophoresis patterns of MDH, MPI, and PGM in fleshy prawn.

Table 4. Allele frequencies at 14 loci in seven samples of fleshy prawn from Korea

Locus	Allele	Narodo (P)	Heuksando (P)	Youngkwang (P)	Hongwon (O)	Muchangpo (O)	Anmyundo (O)	Artificial (A)	Artificial (B)
G3PDH*	*100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
LDH*	*100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
MDH-1*	*100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
MDH-2*	*120	0.00	0.033	0.00	0.03	0.03	0.00	0.05	0.038
	*100	1.00	0.956	1.00	0.96	0.96	1.00	0.95	0.962
	*90	0.00	0.011	0.00	0.01	0.01	0.00	0.00	0.00
IDHP-1*	*120	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00
	*100	1.00	1.00	1.00	0.99	0.99	1.00	1.00	1.00
GPI*	*140	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00
	*100	0.90	0.933	0.95	0.93	0.88	0.89	0.825	0.837
	*60	0.10	0.067	0.05	0.06	0.12	0.10	0.175	0.163
MEP	*100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
MPI*	*115	0.03	0.011	0.00	0.03	0.02	0.03	0.038	0.025
	*100	0.90	0.90	0.95	0.92	0.87	0.92	0.962	0.887
	*90	0.07	0.089	0.05	0.05	0.11	0.05	0.00	0.088
	*70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PGDH*	*100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
PGM-1*	*100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
PGM-2*	*115	0.08	0.122	0.033	0.09	0.02	0.09	0.05	0.00
	*100	0.92	0.867	0.95	0.91	0.97	0.91	0.95	0.962
	*70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	*40	0.00	0.011	0.017	0.00	0.01	0.00	0.00	0.038
PROT-1*	*100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
PROT-2*	*100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sample number		50	45	35	50	50	50	40	40
Mean of allele	1.79	1.308	1.538	1.308	1.615	1.615	1.385	1.308	1.385
Ho	0.0414	0.037	0.046	0.018	0.042	0.046	0.043	0.048	0.052
He	0.0401	0.039	0.048	0.022	0.042	0.046	0.039	0.042	0.048
Ho/He	1.032	0.949	0.958	0.818	1.0	1.0	1.103	1.143	1.083

*P, parental; O, captured shrimp; A, first release; B, second release.

crustaceans and reflects low variability (Hedgecock et al., 1982).

The frequency and distribution of alleles estimated from each group are shown in Table 4. Polymorphic loci (P) with more than 95% of the main allele ranged from 0.231 to 0.385 and the average was 0.2926. Polymorphic loci (P*) with less than 95% of the main allele ranged from 0.154 to 0.308, and the average was 0.2156, showing very low variability. The observed value for heterozygosity (Ho) in each group in the natural population was lowest at Youngkwang (0.018) and highest at Heuksando (0.046) and Muchangpo (0.046). The heterozygosity of released seeds was 0.05, similar to that at Muchangpo. The overall average heterozygosity for wild population was 0.414. The ratio of observed value of heterozygosity to expected value of heterozygosity (Ho/He) was close to 1.0 in most groups, indicating relatively balanced genetic properties. The value for released seeds was 1.113, somewhat higher than that for wild populations, while it was considered that effective population size was not used rather than inbreeding because used parents were not domestic parents, but not greatly different from wild populations in genetic diversity and variability. This result suggests that the dispersal of planktonic larvae and the migration ability of adult prawns lead to sufficient genetic exchange among populations to preclude substantial differentiation. Mixing at wintering grounds may also lead to a homogeneous genetic structure. Because of weak geographic partitioning and strong vagility, it is more common for marine species to have lower intraspecific diversity than terrestrial species.

Based on mtDNA analyses, fleshy prawn populations in the Yellow Sea appear to come from a single homogenous population with little variation. Therefore, it may be reasonable to treat this species as one unit stock in the Yellow Sea (Hwang et al., 1997).

Distinct genetic markers that can discriminate wild populations from released seeds were not identified in this study. However, when the genetic relationships among groups were compared using the distribution and frequency of alleles, released seeds were most closely related to shrimp captured at Muchangpo (Fig.

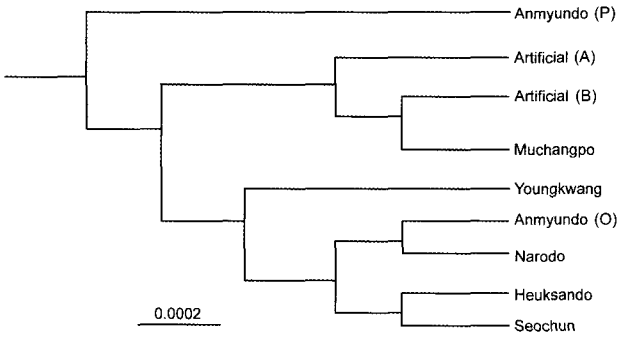

Fig. 4. Dendrogram of fleshy prawn populations based on UPGMA clustering of genetic distances.

4).

Fig. 4 shows the genetic relationships based on isozyme analysis. Among wild populations, very little genetic differentiation was observed between the parent group (P) captured in the spring and the relevant year's group (O) captured in the fall, despite the generational gap and geographical distance between them. Thus, no independent group of fleshy prawn in Korea has differentiated via reproduction or geographic isolation, i.e., all groups are genetically similar. While it is assumed that more than 10 million seeds released each year have contributed slightly to the stock enhancement of the area, since artificial seed A and B, which were the same seed as those produced at Boryung Hatchery and released at the coast of Muchangpo and then provided nursery culture in different ponds, were shown to be the genetically closest to Muchangpo group. However, it was relatively different from other groups genetically, but not statistically significant. Also, the parental group captured at Anmyundo in the spring had the greatest genetic distance from all other groups examined, because egg-laying parental shrimps are not usually captured in Anmyundo but fleshy prawn from the outer sea are mostly captured.

If isozymes are to be used for determining the effectiveness of fleshy prawn releases, it is necessary to repeatedly analyze the isozymes of seeds each year over several years to determine the standard loci with unchanged allele frequencies. If such standard alleles are identified, it may be possible to quantitatively determine the percentage of released seeds (Y_L) at each station using the equation (Sato et al., 1982):

$$Y_L = (P_O - P_M) / (P_L - P_M) \times 100\ (\%),$$

where P_O represents the standard allele frequencies observed at each station, and P_L and P_M are the values of standard allele frequencies for released and wild seeds, respectively.

Isozymes have been used for group analyses of marine systems, geographical differentiation of species, and interspecies and intraspecies genetic characteristics, and many practical theories have been developed and applied to other fields, such as breeding, ecology, and resource sciences. Group analyses, however, are limited by short time periods after reproductive isolation or by the bottleneck effect because isozymes are mostly monomorphic loci, and even polymorphic loci show low variability. Thus, it is necessary to identify a highly variable genetic marker that is individual-specific and can detect slight variation among groups and determine genetic character.

Recently, microsatellite DNA analysis was developed as a highly variable genetic marker with excellent reproducibility. The strategy has been applied widely to various fields, such as pedigree analyses, gene mapping, quantitative trait loci studies, and group analyses. In addition, this technique was used to study the effects of releasing red seabream and black seabream, and yielded very promising results. Therefore, we conclude that more precise data on release effects, and therefore improve stock management, can be achieved by using microsatellite DNA analysis.

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