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Genetic analysis of additional samples from the 'J' stock and implications for the estimation of mixing proportion of 'J' and 'O' stocks in sub-area 11

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ABSTRACT

In the context of a co-operative research project on cetacean between Korea and Japan, the stock identity of minke whales distributed in the Sea of Japan (East Sea) is being investigated since 1998. A total of 53 by-catch samples has been collected, 37 in the Korean coast between 1998 and 2000 and 16 in the Japanese coast between 1993 and 2000. Genetic diversity in the recent by-catch samples was higher than that in a past Korean commercial samples taken in 1982. Results of homogeneity tests showed a statistically significant difference between the recent by-catch and past commercial samples. No significant difference was found between the by-catch samples of Korea and Japan. The mixing proportion of 'J' and 'O' stocks in sub-area 11 was estimated based on five combinations of the 'baseline' sample of the 'J' stock (using both past commercial and recent by-catch samples). The 'J' stock contribution in the mixed assemblage of sub-area 11 was higher when the recent by-catch samples were used as 'baseline' sample. This result urge for a better definition of the sample representing the 'J' stock as recommended by Pastene *et al.* (1998). Further studies within the Sea of Japan (East Sea) using additional available samples is need for such purpose.

INTRODUCTION

Studies based on genetics and on other biological parameters indicated that at least two biological stocks occur in the western North Pacific: one distributed around Korean Peninsula and west of Japan and the other distributed in the Okhotsk Sea and Pacific side of Japan (see review by Pastene *et al.*, 2000). The IWC refers to these stocks as 'J' and 'O' stocks, respectively.

Based on samples from past coastal whaling conducted in Korea and Japan, Wada (1991) showed a statistically significant allozyme heterogeneity in the April sample from the southern part of Okhotsk Sea (sub-area 11). Goto and Pastene (1997) reported also a significant mtDNA heterogeneity in the same sub-area and month using restriction fragment length polymorphism (RFLP) analysis of the control region. These authors interpreted such heterogeneity as the temporal mixing of 'J' and 'O' stocks in that locality.

The mixing proportion of 'J' and 'O' stocks in sub-area 11 was estimated using RFLP haplotype frequency data and a maximum likelihood method (Pastene *et al.*, 1998). The proportion of 'J' stock female animals in the April sample in sub-area 11 was 0.4075 (SE=0.0806) and that of male animals in August was 0.3147 (SE=0.1160).

Pastene *et al.* (1998) made several suggestions for future works in this field as follows:

1) MtDNA control region sequencing and nuclear DNA (microsatellite) data, which could be considered more powerful

molecular techniques than RFLP analysis, should be used to evaluate the mixing proportion.

2) Investigation on the possibility of more than two stocks in the area.
3) Clarify whether the sample set from sub-area 6 used in the genetic analyses is representative of 'J' stock.
4) Analysis of samples from sub-area 12

As regard to the item 1), Goto *et al.* (2000) estimated the mixing proportion of the 'J' stock in sub-area 11, by month and sex, using three genetic markers: maternal inherited mtDNA RFLP and sequencing of the control region, and biparental inherited nuclear DNA (microsatellite) for five loci.

Regarding to items 2) and 3), additional samples from sub-area 6 became available since 1998 under the context of a co-operative research project on cetacean between Japan and Korea. Until 1993, the only samples available for genetic analysis were from a past commercial whaling operation in September/October 1982 around the Korean Peninsula (n=28). The co-operative genetic study has involved the collection of samples from by-catches in both countries so that the period and area of sampling in the Sea of Japan (East Sea) can be expanded.

We presented here preliminary results of a mtDNA sequencing analysis on the by-catch samples from Korea and Japan. The analysis was made with the objective to understand further the genetic composition of the 'J' stock. In addition, we estimate the proportion of the 'J' stock in sub-area 11, by month and sex, using mtDNA sequencing haplotype and several combinations for the sample as representative of the 'J' stock.

MATERIALS AND METHODS

Samples and mtDNA sequencing analysis

By-catch sample

The co-operative genetic study has involved the collection of samples from by-catches in Korean and Japan. Sampling localities in the Korean Peninsula (both historical commercial and recent by-catches) are shown in Figs. 1 and 2, respectively. Sampling localities of by-catches in the western side of Japan are shown in Fig. 3. A total of 37 samples was collected in the Korean coast between 1998 and 2000 (Table 1) while a total of 16 was collected in the Japanese coast between 1993 and 2000 (Table 2). Samples from a past commercial whaling operation in September/October in 1982 around the Korean Peninsula (n=28) were used for comparison. All these samples were sequenced for a portion of the mtDNA control region using the same methods used by Goto and Pastene (2000).

Data analysis

The degree of mtDNA diversities was estimated using the index of nucleotide diversity (Nei, 1987 pp. 256). Homogeneity tests among groups were conducted using the chi-square test (Rice, 1989) and Hudson *et al.* (1992)'s sequence (Kst*) and haplotype (Hst) statistics. The degree of divergence was inferred as being larger than zero, if an equal or more extreme value of Kst*/Hst was observed in less than 5% of 10,000 Monte Carlo simulations.

Analysis of mixing proportion

Samples

In addition to the by-catch and past Korean commercial samples from the Sea of Japan (East Sea), samples available for this study were from past coastal whaling operation in Japan in sub-area 7 (n=138) and 11 (n=147), JARPN surveys in sub-areas 7 (n=139), 9 (n=188) and 11 (n=80) (See detail in Goto *et al.*, 2000). The data from commercial operations and JARPN are pooled to increase the power to identify mixing proportions.

Mathematical model for estimation of mixing proportion

We estimated the proportion of the 'J' stock in the mixed assemblage of sub-area 11 by the maximum likelihood method. Variances of these estimates consist of the variance from the sampling from mixed stock (sub-area 11) and the sampling the baseline stocks ('O' and 'J' stocks) (Kishino *et al.*, 1994). This method had already been applied by Pastene *et al.* (1998) for estimation of mixing proportion in sub-area 11 using mtDNA RFLP haplotype frequency data.

Baseline stocks

'J' stock

Using the available samples from the Sea of Japan (East Sea), we defined five combinations to be used as the baseline sample of the 'J' stock:

Case 1: Korean past commercial samples only (n=28)
Case 2: Korean by-catch samples (n=37)
Case 3: Korean commercial and by-catch samples (n=65)
Case 4: Korean and Japanese by-catch samples (n=53)
Case 5: Korean and Japanese by-catch samples and Korean past commercial samples (n=81)

'O' stock

Whales taken in commercial operations in the past and by the JARPN surveys in sub-area 7 (n=277), were considered as the representative sample of the 'O' stock. In order to ensure that no substantial number of 'J' stock animals are present in this sub-area, we conducted an estimation of mixing proportion in sub-area 7 having sub-area 6 as baseline of the 'J' stock (see the cases above) and sub-area 9 (samples from JARPN, n=188) as baseline of the 'O' stock. In general results for the five baseline cases are similar. For both female and males, the estimations for most of the cases are zero. In some cases they are large as 0.092, but these estimations have large standard errors, 0.053 (data not shown). These results indicates that no substantial proportion of 'J' stock animals occur in sub-area 7 in each month This assumption is safe with 95% confidential intervals, when it is assumed that estimated mixing proportions are normally distributed. On the other hand, Goto *et al.* (this meeting) showed that no significant deviation from Hardy-Weinberg equilibrium was detected in sub-area 7 using microsatellite data. Therefore, for the analyses that follow, it is assumed that sub-area 7 contains predominantly individuals from the 'O' stock.

RESULTS

Samples

Most of the samples from by-catches in Korea were collected in the eastern coast of the Korean Peninsula (n = 30) as shown in Fig. 2, while five and one samples were collected in the southern and western coast, respectively. These samples were collected all through the year and there was no monthly bias in the number of samples collected. Body length ranged from 3.8 to 8.0 meters, with a mean body length of 5.1 meters, and it is estimated from body length that most of the samples were immature animals.

On the samples taken off the western coasts of Japan, only one animal was taken from sub-area 10 and the rest was collected in sub-area 6 (Fig. 3). As with the samples from by-catches in Korea, the collected animals were evenly distributed among the twelve months. Body length was from 3.2 to 6.0 meters, with a mean length of 4.5 meters, and it is estimated that all of the samples were immature animals.

On the other hand, the samples from historical commercial whaling in Korea used in this study were restricted to the southeastern area (off Korea) and to the month of September and October, with the exception of one individual, which was collected in the western side of the Korean Peninsula.

Haplotype frequencies

Table 3 shows the haplotype frequencies in samples from past Korean commercial whaling and those from by-catches in Japan and Korea. Samples taken in sub-areas 7, 8, and 9 from JARPN surveys are also shown for comparison. Five haplotypes were detected in the 28 samples from past Korean commercial whaling. Of these, haplotype 1 was predominant, accounting for 64%. As for the samples from by-catches, thirteen haplotypes were detected in the Korean samples and nine in Japanese ones, and all together, a total of twelve new haplotypes were recognized. Haplotype 1 was also predominant in the samples from by-catches, but it accounted for only 24% of Korean and 25% of Japanese samples. These frequencies were considerably lower than in samples from commercial whaling, implying that there is higher diversity in the by-catch samples. On the other hand, the specific haplotypes which were not detected in the samples from the Sea of Japan (East Sea) (commercial whaling as well as by-catches in Japan and Korea) accounted for 85.6% of the JARPN samples in sub-areas 7, 8 and 9 (headed under 'others' in the table).

Genetic diversity

Table 4 shows the results of haplotype and nucleotide diversities. Samples from past commercial whaling rated low for both values, but those from by-catches showed higher values for both haplotype and nucleotide diversities, close to the results for the samples collected from the JARPN surveys.

Comparison between past commercial and by-catch samples

Table 5 shows the results of the homogeneity tests using three statistical methods among samples from past commercial whaling, by-catches in Korea, those in Japan and JARPN. No significant difference was found between the samples from by-catches in Korea and Japan, but there was a statistically significant difference between the two and the samples from the 1982 Korean commercial whaling. On the other hand, significant differences were found between samples from the Sea of Japan (East Sea) and these from JARPN (Pacific side of Japan).

Mixing proportions in sub-area 11

To estimate the mixing proportion in sub-area 11, we defined five combinations for the baseline stocks for sub-area 6: the past Korean commercial samples, Korean by-catches only, Korean commercial and by-catch samples, Korean and Japanese by-catch samples and Korean and Japanese by-catch samples + Korean past commercial samples. Table 6 shows estimates (and their standard errors) of the mixing proportions for the 'J' stock in sub-area 11 by month, sex and each baseline cases in sub-area 6. These mixing proportions were determined by assuming that the samples of sub-area 6 (five cases) are representative of the 'J' stock while those of sub-area 7 are representative of the 'O' stock. The mixing proportions of the 'J' stock in sub-area 11 that used combinations including by-catches (Cases 2, 3, 4 and 5) were similar among them. However, the mixing proportion estimated for these cases was relatively higher than in the case using only commercial samples, with the exception of female in August and of male in May and June. Consequently, a significant mixture of ' J' stock was observed only for females in April and males in August when only commercial samples were used as a baseline stock. There was a significant mixture of females in July as well when the by-catch samples are added. Although the estimates for females in June and males in July are relatively high, these estimations showed a large standard error.

DISCUSSION

Under the co-operative genetic studies between Korea and Japan, efforts have been made to collect samples from by-catches in recent years, making it possible to conduct genetic analyses on Korean and Japanese by-catch samples. In this study, we have conducted mtDNA analyses on the samples collected so far. It was found that genetic diversity is higher for the recent by-catch samples than the past commercial samples in 1982. It was confirmed that there is a heterogeneity in haplotype composition between past and recent samples. Also, we estimated the mixing proportions of the 'J' stock in sub-area 11 based on five combinations of commercial and by-catch samples. The estimated mixing rate based on four cases that included by-catch samples show relatively higher values than the case of using only commercial samples as the baseline stocks in sub-area 6.

Possible assumptions for the differences between past commercial and recent by-catches are as follows:

1) Commercial samples in 1982 could have been collected from a restricted area and time, so that such sample is not representative of all genetic diversity of the 'J' stock.
2) Monthly changes in haplotype distribution
3) Possibility of migration of the ' O' stock animals to sub-area 6, and
4) The ' J' stock is composed from more than one stocks

Regarding to assumption 1) and 2), past Korean commercial samples were collected from restricted season and area, while by-catch samples were collected from extended season and area as mentioned above. The larger genetic diversity detected in by-catch samples than in historical ones could be expected due to extended season and area. As regard to 3) and 4), Abe *et al.* (2000) showed that no significant deviation from Hardy-Weinberg equilibrium has been observed in the Japanese by-catches (n=10). This means that probably there is no a mixing assemblage in this area. It should be noted, however, that the samples in the Sea of Japan (East Sea) in their analysis are few and therefore we need to analyze new samples especially those to western side of Korean Peninsula from where few samples were available at this stage.

In order to make the mixing proportion estimates between O and J stocks in sub-area 11 useful for management purpose, we need to verify first the above assumptions. A better definition of the J stock will contribute to make these estimates more robust. For that purpose we are still collecting samples from by-catches under the co-operative genetic study between Korea and Japan. We believe that the analyses of these samples should contribute to a deeper understanding of the stock structure.

In addition, this paper deals with only the mtDNA analyses results, but we intend to conduct analyses on microsatellite DNA as well. Analyses using maternal and bi-parental genetic markers should contribute to clarifying the stock structure and to estimating the mixing proportion.

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Table 1. List of samples collected from by-catchs of minke whales in the Korean Peninsula between 1998 and 2000. See Fig. 2 for sampling localities.

Individual No.	Month	Body length(m)	Sex	Area
98KB10	981121	5.8	F	KB
98KB06	990131	5.0	F	KB
98KB04	990305	4.9	M	KB
98KB03	990311	4.6	F	KB
98KB01	990326	3.9	F	KB
98KB08	981210	4.7	M	KN
98KB05	990222	5.3	F	KW
98KB02	990223	5.1	F	KW
99KB27	990418	4.4		KB
99KB28	990429	4.6	F	KB
99KB19	990501	4.5		KB
99KB21	990501	4.5		KW
99KB22	990508	5.5	F	KN
99KB24	990520		F	KB
99KB26	990603	4.2	F	KB
99KB23	990610	8.0		JJ
99KB20	990811	5.8	F	KN
99KB25	990820			KB
99KB11	991008			KB
99KB13	991021			KB
99KB09	991022	4.8		KB
99KB01	991028	5.3		KB
99KB12	991120			KB
99KB08	991129	5.1		KB
99KB14	991201	5.1	M	KB
99KB02	991204	7.0	F	KW
99KB03	991207			KB
99KB06	991208			KB
99KB05	991211			KW
99KB04	991212			KB
99KB07	991218	3.8		KN
99KB18	000211	4.9		KB
99KB16	000302			CN
99KB17	000309			KB
99KB15	000318			KB
99KB29	000324	4.4	F	KB
99KB10	000328			

KB : KyeongSangBukDo
KW : KangWonDo
KN : KyungSangNamDo
CN : ChungCheongNamDo
JJ : JeJuDo

Table 2. List of samples collected from by-catch of minke whales in the Japanese coast between 1993 and 2000. See Fig. 3 for sampling localities.

Individual No.	Month	Body length(m)	Sex	Pref.
JBM095	961024	4.5	F	Hokkaido
JBM146	990103	5.4	M	Niigata
JBM149	990127	5.6	F	Niigata
JBM173	991220	6.0	M	Niigata
JBM165	990604	3.2	F	Niigata
JBM147	990104	4.5	M	Ishikawa
JBM071	950908	4.5	M	Ishikawa
JBM182	000404	4.6	F	Toyama
JBM184	000411	4.3	F	Toyama
JBM188	000508	3.2		Toyama
JBM189	000510	4.8		Toyama
JBM129	980123	5.2	M	Shimane
JBM185	000420			Shimane
JBM118	970724	3.2	F	Yamaguchi
JBM037	930606	4.3	M	Fukuoka
JBM158	990228	4.1	F	Nagasaki

Table 3. Frequency of mtDNA haplotypes in the samples from past commercial samples from Korea, recent bycatch (Korea and Japan) and JARPN.

	Past Comm.	Bycatch		JARPN
	Korea	Korea	Japan	SA
Haplotype	1982	1998-2000	1993-2000	7,8,9
1	18	9	4	9
2	2	0	0	0
3	6	1	2	0
4	1	3	1	1
5	1	0	0	0
6	0	0	2	20
7	0	1	0	27
8	0	4	1	1
9	0	2	1	1
10	0	1	0	1
11	0	2	1	0
12	0	7	3	0
13	0	3	0	0
14	0	1	0	0
15	0	2	0	0
16	0	1	0	0
17	0	0	1	0
Other	0	0	0	358
Sum	28	37	16	418

Table 4.Haplotype diversity (*H*) and nucleotide diversity (π) in samples of minke whales from the Sea of Japan. NP: western North Pacific minke whale, SH: Antarctic minke whale.

	Origin	Sampling period	Sample size	*H*	π
Sea of Japan	Commercial	1982	28	0.5529	0.0046
	By-catch(Korea	1998-2000	37	0.8919	0.0068
	By-catch(Japan	1993-2000	16	0.9015	0.0073
NP	JARPN	1994-1999	418	0.9522	0.0079
SH	JARPA	1989, 1996	119	0.9882	0.0147

Table 5. Comparison among commercial samples in Korea, by-catch samples in Korea and by-catch samples in Japan using χ^2(upper), Hst(middle) and Kst*(lower). P values are shown in parenthesis.

	Korea by-catch (n=37)		Japan by-catch (n=16)		JARPN SA7, 8, 9 (n=418)	
Korea commercial (n=28)	-	(0.0000)	-	(0.0008)	-	(0.0000)
	0.0699	(0.0000)	0.0524	(0.0108)	0.0288	(0.0000)
	0.0761	(0.0001)	0.0605	(0.0177)	0.0606	(0.0000)
Korea by-catch (n=37)			-	(0.5982)	-	(0.0000)
			-0.0089	(0.8724)	0.0101	(0.0000)
			-0.0106	(0.8882)	0.0402	(0.0000)
Japan by-catch (n=16)					-	(0.0000)
					0.0043	(0.0004)
					0.0150	(0.0000)

Table 6: Results of mixing proportions for the J stock in sub-area 11 from mtDNA sequencing data, by month, sex and each baseline case in sub-area 6.

(a) Females

Month	Sample size	Case 1 Korean Commercial (n=28)		Case 2 Korean by-catch only (n=37)		Case 3 Korean comm. and by-catch (n=65)		Case 4 By-catch (Korea and Japan) (n=53)		Case 5 All samples (n=81)	
		Sequence		Sequence		Sequence		Sequence		Sequence	
		Est	SE	Est	SE	Est	SE	Est	SE	Est	SE
April	55	0.350	(0.063)	0.555	(0.065)	0.594	(0.065)	0.567	(0.065)	0.605	(0.065)
May	41	0.035	(0.032)	0.051	(0.039)	0.051	(0.040)	0.056	(0.042)	0.054	(0.042)
June	17	0.015	(0.060)	0.142	(0.094)	0.142	(0.095)	0.142	(0.094)	0.143	(0.095)
July	23	0.121	(0.070)	0.380	(0.101)	0.379	(0.101)	0.380	(0.101)	0.380	(0.101)
August	16	0.013	(0.064)	0.000	(0.133)	0.000	(0.081)	0.000	(0.132)	0.000	(0.088)

(b) Males

Month	Sample size	Case 1		Case 2		Case 3		Case 4		Case 5	
		Sequence		Sequence		Sequence		Sequence		Sequence	
		Est	SE	Est	SE	Est	SE	Est	SE	Est	SE
May	9	0.000	(0.314)	0.000	(0.331)	0.000	(0.325)	0.000	(0.327)	0.000	(0.323)
June	8	0.000	(0.354)	0.000	(0.372)	0.000	(0.366)	0.000	(0.368)	0.000	(0.363)
July	28	0.036	(0.035)	0.133	(0.066)	0.127	(0.066)	0.157	(0.076)	0.144	(0.073)
August	22	0.254	(0.094)	0.292	(0.103)	0.351	(0.104)	0.289	(0.103)	0.445	(0.109)

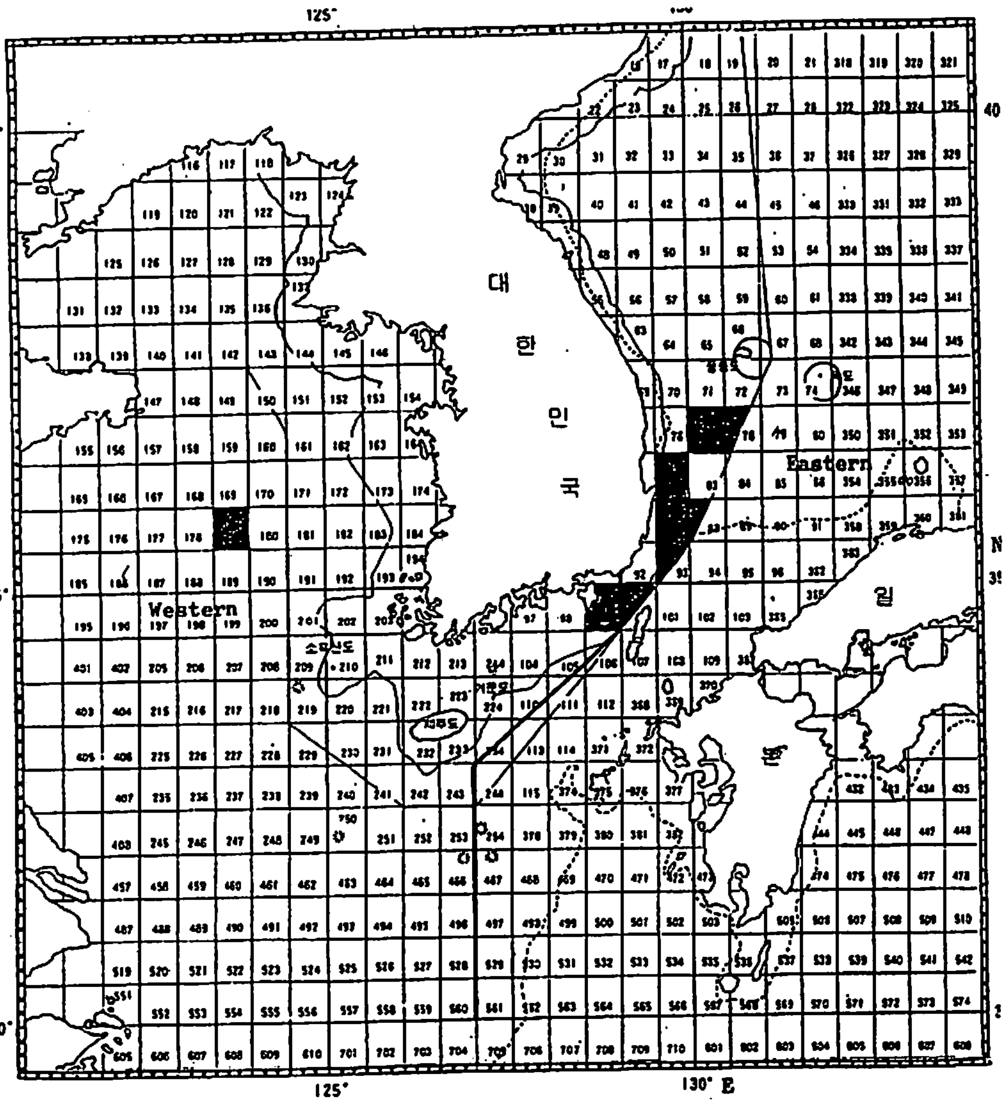

Fig. 1: Geographical location of samples of minke whales obtained in 1982 from a Korean commercial whaling operation.

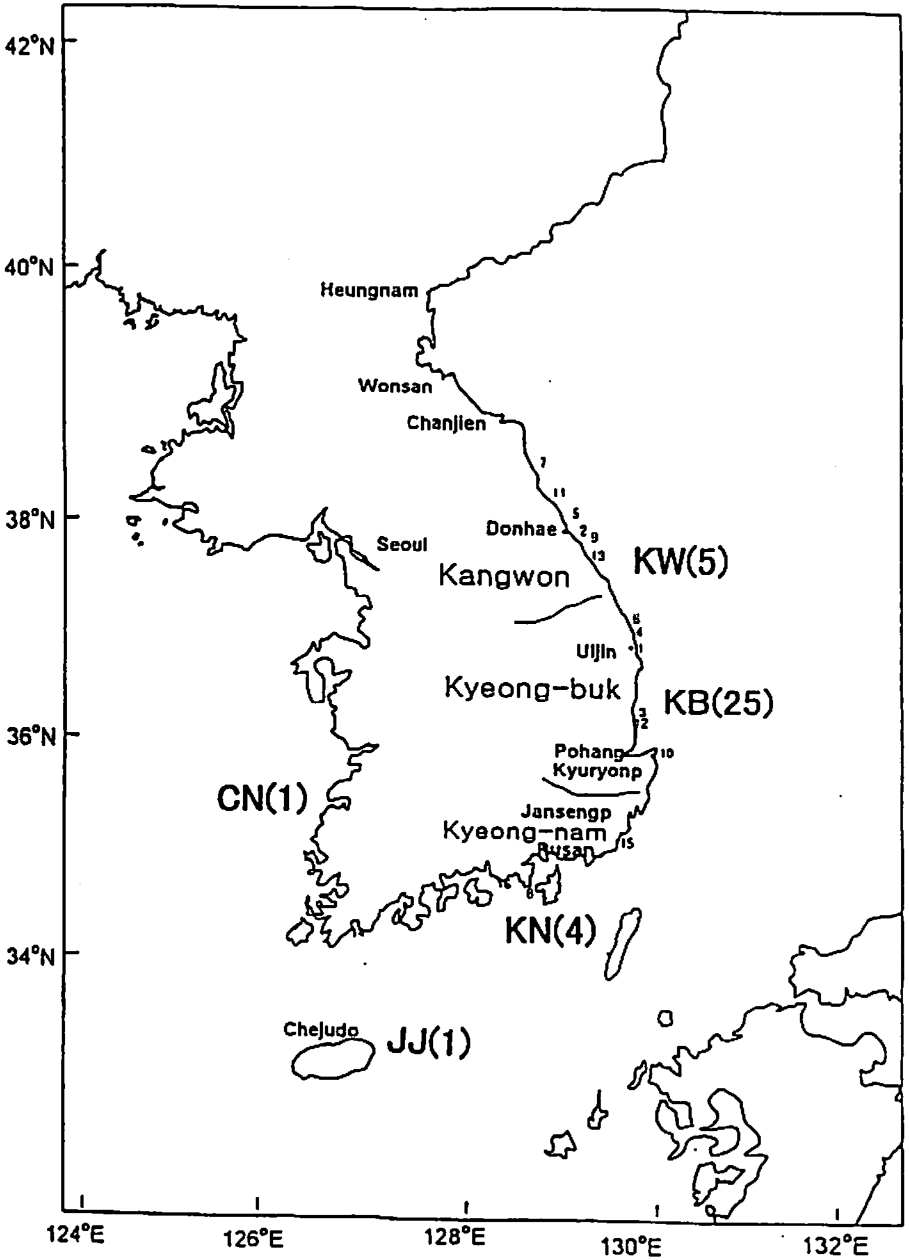

Fig. 2: Sampling localities (by-catches) in the Korean Peninsula.

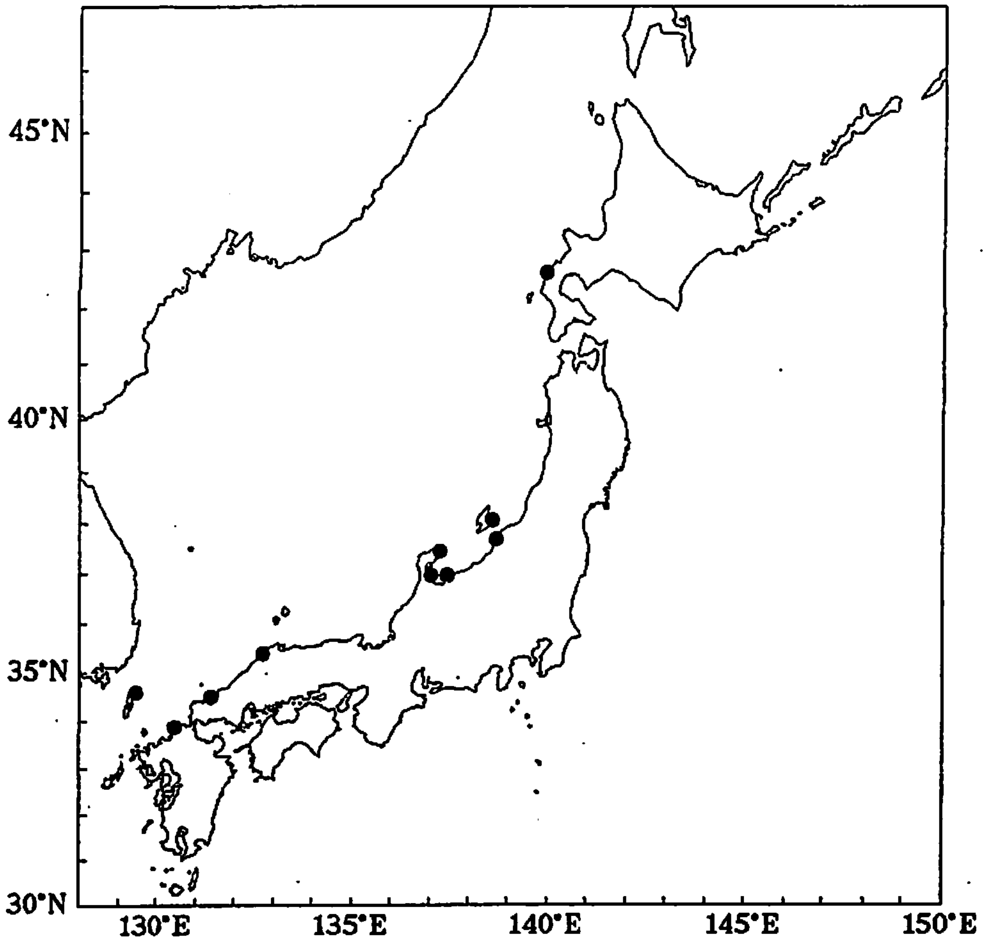

Fig. 3: Sampling localities (by-catches) in the western side of Japanese coast.