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Population Structure in Minke Whale from the North Pacific Examined by the Persistent Organic Pollutants as Chemical Tracers

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ABSTRACT

The population structure of minke whales (*Balaenoptera acutorostorata*) in the North Pacific was investigated from the views of environmental sciences. Persistent organic pollutants (POPs) such as PCBs and organochlorine pesticides were analysed for 76 minke whales in the North Pacific collected during 1996 and 1999, and the data was applied to principal component analysis. Two groups were constructed, and one of the groups (including 4 individuals) collected from the Sea of Okhotsk in 1996 showed higher concentrations of DDTs and HCHs. Considering that the background levels of these contaminants are greater in the Sea of Japan than those in the western North Pacific, and genetic studies suggested the presence of at least two stocks of minke whales in the Sea of Okhotsk, it seems reasonable to conclude that these four individuals were derived from the Sea of Japan and mixed with the North Pacific populations. Little evidences for new population structure in the North Pacific has been found. This study suggested the availability of the persistent environmental pollutants as the chemical tracers to evaluate the ecological aspects in the North Pacific minke whales.

Keyword: Population structure, Minke whale, Persistent organic pollutants (POPs), Chemical tracers

INTRODUCTION

In order to manage and protect the fishery resources, it is important to understand the ecological information such as population, distribution and migration pattern of marine organisms. The abundance estimation of minke whales in the Sea of Okhotsk and the northwest Pacific was 25,000 (IWC, 1992). The minke whale population in the Sea of Japan was estimated at, at least 1,600 (IWC, 1997). The population structure has been mainly examined by the methods used genetics and morphology in minke whales around Japan. Wada and Numachi (1991) and Goto and Pastene (1997a) found the significant differences of allozymes and RFLP analysis of mtDNA between Japanese and Korean coastal minke whales.

These suggest the occurrence of two distinct populations, which were J stock (the Sea of Japan, Yellow Sea and East China Sea) and O stock (the Pacific coast of Japan and the Sea of Okhotsk). On the other hands, International Whaling Commission (IWC) had proposed the hypotheses, which is the existence of other offshore stock structures of minke whales in the western North Pacific (IWC 1994). Thus, several studies have been conducted to check this hypothesis from the point of genetics, morphology, stable isotope analysis, parasites, and the environmental sciences, such as the accumulation of persistent organic and inorganic compounds.

Persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and organochlorine pesticides are less degradable and bioaccumulative through the aquatic food chain. Using these characters of POPs, several attempts have been made to evaluate some ecological and physiological parameters in marine mammals so far. Subramanian *et al.* (1988) and Prudente *et al.* (1997) reported the different patterns and the concentrations of PCBs congeners and isomers in Dall's porpoises from the Bering Sea, the North Pacific, the Okhotsk Sea and the Sea of Japan, and suggested the availability of POPs to estimate the distribution and migration patterns of animals. Muir *et al.* (1996) reported the significant difference of POPs accumulation patterns among sampling locations by the principal component analysis (PCA) based on the concentrations of 88 individual organochlorines in beluga whales. Similar attempts had been applied to the accumulation of trace metals in marine organisms (Courtney *et al.*, 1994).

In this context, the main objectives of this study is to examine the population structure of minke whales around Japan by using the residue levels of persistent organochlorines such as PCBs, DDTs, HCHs, HCB and chlordane compounds (CHLs). The samples were derived from western North Pacific and the Sea of Okhotsk during 1996–1999. Statistical analysis was applied to several organochlorine contaminants in minke whales. The obtained data will support to evaluate the population structure and their distribution pattern in the minke whales around Japan.

MATERIALS AND METHODS

Samples

Minke whale samples used in this study were collected from the western North Pacific and the Sea of Okhotsk in the 1996 and 1999 JARPN surveys. Sampling locations and the number were shown in Fig. 1 and Table 1. Blubber samples were frozen shipped to the laboratory and stored –20℃ until chemical analysis.

Chemical Analysis

Persistent organochlorines were analyzed following the method described by Tanabe *et al.* (1994). Samples were homogenized with anhydrous sodium sulfate and extracted by Soxhlet apparatus with mixed solvents of diethyl ether and hexane (3:1). After Kuderna-Danish concentration, the extract was passed through 20g of Florisil (Wako Pure Chemical Co. Ltd.) packed dry column to remove fat. Organochlorines were eluted with 150ml of 20% hexane-washed water in acetonitrile and were collected in a separatory funnel containing 100ml of hexane and 600ml of hexane-washed water. After partitioning, the hexane layer was concentrated, cleaned up with sulfuric acid, and passed through a florisil column for separation of PCBs from organochlorine pesticides. The first fraction eluted with hexane contained PCBs, *p,p'*-DDE, *trans*-nonachlor and HCB. The second fraction eluted with 20%

dichloromethane in hexane contained HCHs, *p,p'*-DDD, *p,p'*-DDT and CHLs. The quantification of organochlorine residues was made on a gas chromatograph (Hewlett Packard: 5890 Series II) equipped with ECD (electron capture detector) and a moving needle-type injection port (splitless and solvent cut mode, Shimadzu, Co. Ltd., Japan). The GC column used was fused silica capillary (0.25 μm i.d. $\times$ 30 m length) coated with DB-1 (J&W Scientific Co. Ltd., 100% dimethyl polysiloxane, 0.25 μm film thickness). The oven temperature was programmed from 60℃ to 160℃ at a rate of 20℃ / min with a hold of 10 min and from 160℃ to 260℃ at a rate of 2℃ / min with a final hold of 20 min. Injector and detector temperatures were maintained at 250 and 300℃, respectively. Helium and nitrogen were the carrier (20-30 cm/s) and make-up (60 mL/min) gases, respectively. The concentration of organochlorines was quantified from the peak area of the sample to that of the corresponding external standard. An equivalent mixture of Kanechlor 300, 400, 500 and 600 was used as a standard for PCBs determination. Total PCB concentrations were calculated by adding the concentrations of individually resolved peaks. The percentage recoveries of organochlorines in this method were 100±5% for pesticides and 97±11% for PCBs. Concentrations of organochlorines were not corrected for recovery efficiencies.

Principal component analysis (PCA) was applied to five variables of organochlorine compounds concentrations with the software of Excell Statistics ver. 3.0 for Windows. Significant differences of the concentrations were analysed by Mann-Whitney *U*-test by the same software.

RESULTS AND DISCUSSION

Concentrations and patterns

Persistent organochlorine pollutants were detected from all minke whales analysed. The lipid percentage of blubber and the average±standard deviation of the POPs concentrations were shown in Table 1. The residue levels were significantly higher in males than those in females in 1996 samples ($p<0.01$). This is in accordance with the results of other cetaceans such as fin whale (Aguilar *et al.*, 1988) and minke whale in Southern Hemisphere (Aono *et al.*, 1997), suggesting the lactational transfer of contaminants from mother to their calf during the reproductive process. DDTs and PCBs showed higher concentrations (5,500±4,900 ng/g lipid wt., 4,800±2,600 ng/g, respectively) among the POPs in 1996 male samples, whereas PCBs levels were the highest in samples collected from 1997, 98 and 99. When comparing the concentrations of OCs in males among the sampling years, DDTs and HCHs were significantly higher in 1996 males than the others ($p<0.05$). In contrast, PCBs and CHLs showed relatively elevated concentrations in 97 and 98 samples rather than those of 96 and 99 samples. The lipids were about 80 % in 1996 samples, while lower and variable values observed in 97, 98 and 99 samples. Although it is unclear about the reasons exactly, sampling locations and growth stage of animals might be related to the different pattern and the concentrations of POPs and lipid percentage in minke whales.

As for the composition of organochlorine pesticides, *p,p'*-DDE, β-HCH and *trans*-nonachlor were dominant among their total concentrations, respectively. In case of DDT compounds, *p,p'*-DDT occupied 11 % to the total DDTs in 1996 males, which were relatively higher than the 97 (4.6%), 98 (9%) and 99 (6%) samples. The compositions of HCHs and CHLs were less variable among the year of collecting samples.

Population structure

PCA was applied to the concentrations of PCBs, *p,p'*-DDE, β-HCH, HCB and *trans*-nonachlor in all minke whales analysed. The cumulative contribution rates and loadings of component I and II and the score plot were shown in Table 2 and Fig. 2, respectively. The positive and dominant loadings of the component I were PCBs (0.932) and *trans*-nonachlor (0.922), and β-HCH (0.851) and *p,p'*-DDE (0.443) showed the positive loadings in the component II. These facts imply that the horizontal axis (component 1: 62.9 % of the total information) and the vertical axis (component II: 24.4 % of the total information) indicate to the increasing concentrations of PCBs and *trans*-nonachlor toward the right, and p,p'-DDE and β-HCH toward to above, respectively (Fig. 2). Consequently, two groups were constructed by the PCA. Group II included four male animals from the Sea of Okhotsk in 1996. The average of DDTs and HCHs concentrations in Group II were 12,000 and 2,300 ng/g lipid wt., which levels were greater than the average concentrations of other samples in this region (DDT: 3,400 ng/g, HCHs: 480 ng/g). Maricar *et al.* (1997) presented the residue levels of organochlorines in Dall's porpoises collected from the Sea of Japan and the northwestern North Pacific. The elevated concentrations of DDTs and HCHs were found in Dall's porpoises from the Sea of Japan than those in the North Pacific. The major source of these contaminants is thought to be the atmospheric transport from Asian countries because the usage of HCHs had continued in China even in 1990s (Li *et al.*, 1996). In additions, the Sea of Japan have a semi-closed systems and the water exchange is very limited (Kannan *et al.*, 1998), resulting in the long-term contamination by POPs in this region. As described previously, there are two different minke whale populations, which are J and O stocks around Japan. In this context, it seems reasonable to conclude that minke whales of Group II were derived from the Sea of Japan (J stock) and mixed with O stock in the Sea of Okhotsk. Pastene *et al.* (1998) also suggested the presence of at least two stocks of minke whales in the sub-area 11 in 1996 samples by the genetic studies of mtDNA.

The rest of the sample plots could be comprised as Group I, which would represent the 'O stock' population in the North Pacific. No significant genetic and morphological differences of minke whales were detected among the sampling sub-area, 7, 8 and 9 in the North Pacific (Goto and Pastene, 1997b, 1998; Fujise and Kato, 1996), which were in accordance with the results of this study. These observations suggest that the possibility of the occurrence of new population stock (e.g. W stock proposed by IWC) in the North Pacific is likely to be small.

This study suggested the availability of the persistent environmental pollutants as the chemical tracers to evaluate the ecological aspects in the North Pacific minke whales. Although a careful interpretation is needed because of the small sample size, the results obtained various methods (genetics, morphology and other approaches) would allow the comprehensive understanding of more detail stock identity in the North Pacific minke whales.

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Table 1 Concentrations of organochlorine compounds (ng/g lipid wt.) in the blubber of minke whales collected from the Sea of Okhotsk and western North Pacific in the 1996 and 1999 JARPN surveys.

Year	Location (area no.*)	Sex**	Number	Lipid	PCBs	DDTs	HCHs	HCB	CHLs
1996	Sea of Okhotsk (11)	M	19	80±5	4800±2600	5500±4900	910±880	150±35	820±430
1996	Sea of Okhotsk (11)	F	11	81±6	1700±1000	1300±1000	220±120	180±150	500±410
1997	W.N. Pacific (8, 9)	M	10	66±13	6400±3900	3300±2800	320±130	210±120	1100±580
1998	W.N. Pacific (7E, 8)	M	10	63±12	6100±2300	3000±1400	450±80	270±75	1200±550
1999	Sea of Okhotsk (11)	M	26	55±12	3200±1800	2900±2000	450±260	210±82	790±440

*: Corresponding to Fig. 1

**: M; male, F; female

Table 2 Cumulative proportion (CP) and loadings of component I and II examined by PCA in minke whales

	CP	PCBs	*p,p'*-DDE	*β*-HCH	HCB	*trans*-nonachlor
component 1	0.629	0.932	0.821	0.419	0.760	0.922
component 2	0.873	-0.118	0.443	0.851	-0.440	-0.300

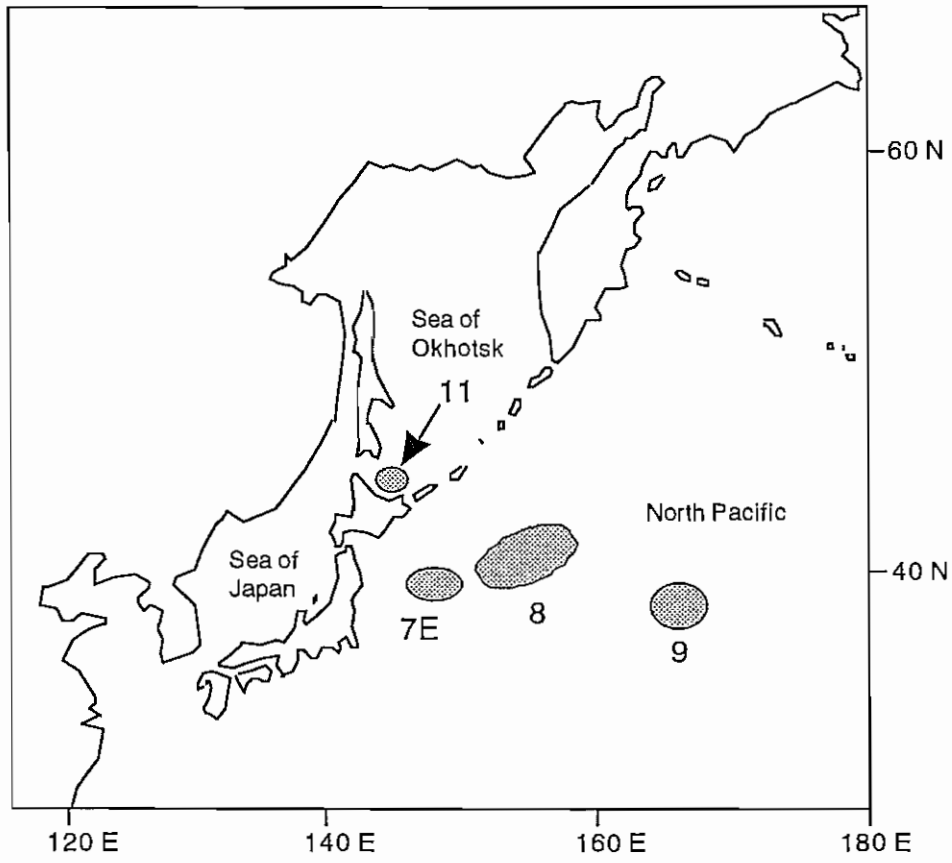

Fig. 1 Sampling locations of minke whales in the Sea of Okhotsk and the western North Pacific.

Number indicate the sampling locations (See Table 1)

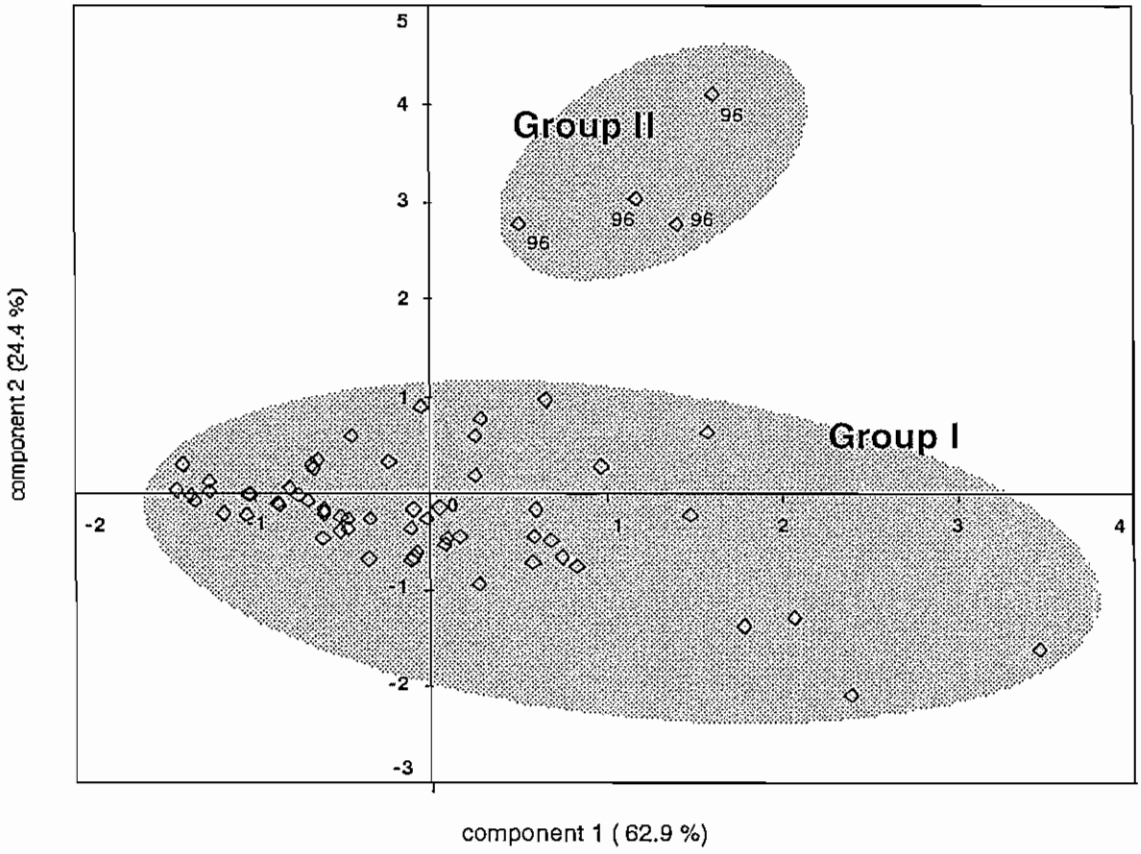

Fig. 2 Score plot of PCA applied to organochlorine concentrations (fat wt.) in minke whales from the Sea of Okhotsk and western North Pacific during 1996 and 1999

The number of plots in Group II indicates the year of collecting samples