An Attempt to Identify Stocks in the Western North Pacific Minke Whale (*Balaenoptera acutorostrata*) Using the Accumulation Levels of Heavy Metals and Organochlorines as Ecological Tracers

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ABSTRACT

We attempt to identify stocks in the western North Pacific minke whales by using ecological tracers based on the accumulation levels of iron (Fe) mercury (Hg) and cadmium (Cd) in liver, and of polychlorinated biphenyls (PCBs), DDTs, chlordane compounds (CHLs), hexachloro-cyclohexanes (HCHs) and hexachlorobenzene (HCB) in blubber of minke whale samples, collected by the JARPN surveys in 1994-1999. In the southern part of the Okhotsk Sea (sub-area 11), some animals presented higher concentration levels of DDTs, especially of p,p'-DDT, and PCBs. These whales also showed a relative lower concentration of Hg. It is suggested that these whales could belong to the Sea of Japan-Yellow Sea- East China Sea stock (J-stock). A reverse pattern was found in the rest of the whales in that sub-area, in regard the concentration levels in Hg and p,p'-DDT. It is further suggested that these other individuals could belong to the Okhotsk Sea-Western Pacific stock (O stock). A discriminant analysis revealed the no significant differences were observed among Pacific sub-areas (sub-areas 7, 8 and 9) and then we found no evidence to support the occurrence of hypothetical W stock. In this paper the usefulness of heavy metals and organochlorines accumulation as ecological tracer to identify stocks is examined.

INTRODUCTION

In the western North Pacific two stocks of minke whales have been proposed on the basis of genetics and non-genetics studies. One is the Okhotsk Sea-West Pacific stock (namely O-stock) and the other was the Sea of Japan-Yellow Sea-East China Sea stock (namely J stock) (Omura and Sakiura, 1956; Ohsumi, 1983; Kato, 1992; Best and Kato, 1992; IWC, 1992) . Furthermore the hypotheses of the occurrence of a third stock (W stock) in offshore areas of the western North Pacific and the occurrence of sub-stocks had been established by the Scientific Committee of the IWC (IWC, 1994).

The Japanese Whale Research Program under Special Permit in the North Pacific (JARPN) was started to verify these hypotheses (Government of Japan, 1994; 1995). As the stock identity issue should be treated under a multi-approaches perspective (see Donovan, 1991), the research program combine studies on genetics, morphology/morphometric, ecological markers and differences in the level of chemical pollutants, among others.

In general organochlorines and heavy metals such as Hg are amplified through the food chain. The accumulation level of these chemicals reflects mainly the feeding habit because they are stable and persistent. Recently considerable discussion has been arising with regard the use of pollutants as indicator of stock. Several studies have attempted to examine the biological and ecological characteristics of cetaceans using chemical tracers such as organochlorine contaminants (PCBs and DDTs) and heavy metals (Hg and Cd) (Honda, 1985; Honda *et al.*, 1987; Fujise, 1987; Subramanian *et al.*, 1987, 1988; Tanabe *et al.*, 1987; Tatsukawa *et al.*, 1990).

Organochlorines are man-made chemicals and are considered to be reflecting the long-term pollution. The use of organochlorines such as PCBs and DDTs has been limited to closed system. Thus, it has been reported that the accumulation levels in the blubber of northern fur seals have decreased (Tanabe *et al.*, 1994). On the other hand, it has been reported that the accumulation of these organochlorines in blubber of marine mammals is a function of the age. As an example, the accumulation pattern with age in the case of southern minke whales, was examined by Aono *et al.* (1997).

Regarding to heavy metals, Cd is considered to have a shorter residence time in the body of marine mammals rather than organochlorines. Then, the accumulation level of Cd reflects the latest Cd intake through the food. In contrast, Hg is considered to have a longer residence time than Cd. Furthermore it has been reported that the accumulation levels of Hg increase with the age. As an example, age- and length-related changes in these metals in the liver of the southern minke whales were investigated by Fujise *et al.* (1997).

In this paper we examine the stock structure of the western North Pacific minke whales using the level of accumulation of organochlorines such as PCBs and DDTs and heavy metals such as Hg, Fe and Cd, as ecological tracers. Especially we attempt to study the stock structure in the southern part of Okhotsk Sea (sub-area 11), where it is known that two different stocks mix to each other on a spatial and temporal scale.

MATERIALS AND METHODS

Samples

A total of 203 animals was used in this study. These samples were collected during the six JARPN surveys (1994-1999). A summary of these samples is shown in Table 1. In the field, blubber, liver and kidney samples were collected for organochlorines and heavy metals (Hg, Fe, and Cd) analyses. These tissues were stored at –20°C until analysis.

Chemical analyses

Organochlorines analyses

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. × 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°C to 160°C at a rate of 20°C / min with a hold of 10 min and from 160°C to 260°C at a rate of 2°C / min with a final hold of 20 min. Injector and detector temperatures were maintained at 250°C and 300°C, 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.

Heavy metals analyses

Liver sample was used for the analyses of heavy metals such as cadmium (Cd), iron (Fe), and mercury (Hg). Homogenized samples of tissue and prey species (5-10g wet wt) were digested to a transparent solution with a mixture of nitric, perchloric and sulphuric acids. The resultant solutions were then diluted to a known volume with deionized water and transferred to acid-washed test tubes with teflon screw-caps The concentration of iron, manganese and zinc were determined in a flame atomic absorption spectrophotometer. Copper and cadmium were measured after diethyl dithiocarbamate-methyl isobutyl ketone (DDTC-MIBK) treatment. by flame atomic absorption spectrophotometry (Honda *et al.,* 1983; Fujise *et al.,* 1988). For mercury, homogenized samples were digested with mixture of nitric and sulphuric acids in a flask equipped with a Liebig condenser and followed by $KMnO4$ digestion. Excess $KMnO4$ was reduced with 20% hydroxylamine hydrochloride solution and the mercury was converted to Hg0 with tin(II)chloride. Determination was made with a spectrophotometer (Honda *et al.,* 1983). Recoveries ranged 90.0 to 99.9%. The precision of this analysis method was examined by analysis of Hg in dolphin muscle, the coefficients of variation (CV%) were below 7.0%.

The principal interpretation for the use of chemical tracer as stock indicators

In this study Hg level in liver was considered as a base, which reflects the intake for long-term through the food. Concentration levels of other chemicals were compared between areas under this base. If the different concentration levels of individuals was observed in the same area, it should be considered that these animals feed on the food (prey) that have a different concentration level of the chemicals (i.e. different prey species).

Although the analysis was conducted on a total of 130 individuals (Table 1), our analysis on stock identity was limited to only mature male individuals because the age-related accumulation characteristics of pollutant and because excretion in mature females via lactation.

Statistical analysis
In order to examine the geographical variation and stock variation of concentrations of the heavy metals and organochlorines, we used the principal component analysis (PCA), canonical discriminant analysis (DA) in SPSS 10.0J for Windows (SPSS Japan, Inc.)

RESULTS AND DISCUSSIONS

Results of the analyses of organochlorines and heavy metals are shown in Tables 2 and 3 for Okhotsk Sea and Pacific side of Japan, respectively. The body length and blubber thickness of each individual is also showed in both tables. In addition, the allocation of stock in each individual, according to mtDNA RFLP and sequencing analyses (Goto *et al.,* 2000) and the occurrence of scars on the skin of the individuals examined in this study, is given. A summary of the pollutant information, by sub-area, sex and reproductive status, is shown in Table 4.

Length –related accumulations of organochlorines and heavy metals
Because the age data for the whale is not available for all samples, we examined the relationships between body length and concentration of heavy metals (Hg, Cd and Fe) in liver and organochlorines (p,p'-DDT, ΣDDTs, β-HCH, ΣHCHs, PCBs, HCB and ΣCHLs) in blubber. As mentioned earlier, in this examination, only mature males are used because females and immature individuals seems to be affected by intake or excretion of organochlorines through pregnancy and lactation. Those correlations are shown in Fig. 1, by geographical locality (Okhotsk Sea = sub-area 11 in 1996 and 1999, Pacific = Pacific side of Japan or sub-areas 7, 8 and 9 during 1994 and 1998).

From these figures most of chemicals are well correlated to their body length, except for HCB and CHLs. This means that the concentration levels increase with body length. This result is similar to those found for other marine mammals.

Geographical differences in the concentration of organochlorines and heavy metals
Fig. 1 also shows comparisons in the accumulation patterns of these chemicals, between the southern Okhotsk Sea (sub-area 11) and the Pacific sub-areas (sub-areas 7, 8 and 9). Concentrations of PCBs, DDTs and HCHs in sub-area 11 were higher than those in the Pacific sub-areas. In contrast, levels of hepatic Hg and hepatic Cd in sub-area 11 were lower than those in the Pacific sub-areas.

In order to examine further the geographical differences in the accumulation levels of these chemicals, a principal component analysis (PCA) and a canonical discriminant analysis (DA) were applied.

Table 5 shows the result of the PCA using analytical data for seven chemicals (L-Hg, L-Cd, PCBs, ΣDDTs, ΣHCHs, HCB, and ΣCHLs) of males collected in sub-areas 7, 8, 9 and 11 during 1994 and 1999. A total of seven principal components was determined. PC1 is mostly correlated to PCBs and CHLs. It was also correlated to DDTs partly. PC2 is correlated to HCHs and DDTs. PC3, PC4 and PC5 are correlated to HCB, hepatic mercury (L-Hg) and hepatic cadmium (L-Cd), respectively. The cumulative contribution rate for PC1-PC5 covered for 95.6 % of all variations.

Table 6 shows the result of canonical discriminant analysis using the first five components scores (PC1-PC5). Following the discriminant function, the following formulae was obtained from the DA analysis.

$$Z = -0.680*PC2+0.490*PC3+0.530*PC4+0.893*PC5-0.087 \quad (1),$$
$$\text{Wilks's } \Lambda = 0.492 \ (p<0.001).$$

This function revealed that the correct discrimination rate was 93.8%.

As mentioned above, out of these chemicals four chemicals are selected: L-Hg, L-Cd, p,p'-DDT, β-HCH. The results of PCA and DA using these four chemicals are shown in Tables 7 and 8, respectively. The discriminant function and Wilks's Λ is as follows:

$$Z = -0.517*PC1+0.877*PC2+0.539*PC3-0.054 \quad (2),$$
$$\text{Wilk's } \Lambda = 0.633 \ (p<0.001).$$

In this case the correct discrimination rate was 84.0%.

An examination of the possibility of the existence of hypothetical W stock

Heterogeneity in the accumulation levels of these chemicals in the Pacific side of Japan (sub-areas 7, 8 and 9) is investigated using PCA and DA. Results of the PCA analysis is shown in Table 9, and those of the DA analysis is shown in Table 10. From these analyses, the derived discriminant functions were not significant statistically (Wilks' Λ = 0.916; p=0.531).

No significant differences were observed among the Pacific sub-areas (sub-areas 7, 8 and 9) and then we found no evidence from the analysis of pollutants, supporting the existence of the hypothetical W-stock in offshore areas.

Identification of O-J stocks

Fig.2 shows the relationship between Hg and other chemicals (PCBs, DDTs, HCHs, HCB, CHLs, p,p'-DDT, Cd and Fe) concentrations. Only mature males were used here.

In the DDTs figures, especially p,p'-DDT, two or three different groups are identified: one group revealed a high hepatic Hg and lower p,p'-DDTs in blubber; the second group revealed a lower hepatic Hg and higher p,p'-DDTs; the third group presented a low concentration in both hepatic Hg and p,p'-DDTs in blubber.

Considering their sampling area, the first group is composed of animals collected in the Pacific side (sub-areas 7, 8 and 9), the main distribution area of the O stock. The second group contains individuals collected in the southern Okhotsk Sea (sub-area 11), where O and J stocks mix to each other. The third group contains individuals sampled in both sub-areas (Fig. 3).

It is known that the main body of the J stock is distributed in the Sea of Japan and the main body of the O stock in the Pacific side of Japan and Okhotsk Sea. Geographic differences in the concentration levels of PCBs and DDTs were reported for blubbers of Dall's porpoise (Subramanian *et al.,* 1986). DDTs and PCBs were higher in the Sea of Japan than those in the Pacific side. For Hg, it was reported that lower concentration of this metal in liver of Dall's porpoise was observed in the Sea of Japan than those of individuals collected in the western North Pacific (Fujise, 1987).

Tamura and Fujise (2000) reported the major prey species of minke whales in the western North Pacific, and they found differences between the southern Okhotsk Sea and Pacific side of Japan. The Okhotsk Sea (sub-area 11) minke whales feed on krill while minke whales in the Pacific sub-areas (7, 8 and 9) feed on the pelagic fishes such as Pacific saury and Japanese anchovy. Thus minke whales in the Pacific side of Japan (i.e. O-stock) mainly consumed pelagic fishes that are located in a high trophic level. It was expected that these animals show the higher Hg accumulation levels. In contrast, minke whales distributed in the southern Okhotsk Sea, which mainly consumed krill (low trophic level), were expected to have lower levels of Hg than those of the Pacific group.

Considering the biological half-life (BHL) of Hg (c.a. two or three hundred days), if a different accumulation level of Hg was observed between two groups, this suggests that they ate the different prey species and/or their feeding area are different.

The occurrence of the three groups in Fig. 3, which have different pattern of accumulations, could be explained as follows: the first group, which inhabit the Pacific area, showed lower DDTs levels and relative higher Hg intake because of their food habitat (piscivorous). This group could represent the O-stock minke whales. The second group inhabiting the Sea of Japan, where relative large amount of pesticides such as DDTs maybe discharged from the Asian continent to the sea, high concentrations of DDTs were observed in their blubber. These whales feed mainly on low trophic level of preys such as krill. Then, this maybe represent the J-stock minke whales.

Considering the above criteria, of 19 males collected in sub-area 11, 6 individuals are considered as of the 'J-stock' (Table 2). These animals are compared with the results of mtDNA analysis (Table 2). MtDNA RFLP haplotype '1' was observed frequently in whales of the O-stock and haplotypes '3' and '5' were observed frequently in 'J-stock' whales (Goto and Pastene, 1997). However, these authors also mentioned that these markers are not absolute.

Most of the whales identified as of the J stock by our pollutant analysis, have haplotypes '3' and '5'. However one whale had haplotype '1'.

Table 2 also compare our results with the records for occurrence of scar in the surface skin of the whale during the cruise (Fujise *et al.,* 2000). In the 1999 JARPN, 12 pregnant females were collected in the sub-area 11. Five of 12 pregnant whales have the larger foetuses that are indicating the 'J-stock' breeding cycle. Other 7 pregnant females have the foetuses that are collected normally in the Pacific sub-areas in the JARPN and these animals showed to have the 'O-stock' breeding season. Skin surfaces of these animals were also different. The pregnant females having larger foetuses show smooth skin. But the pregnant whale having normal size of foetuses showed to have a sandy skin with a number of scars in their surface. These observations suggested that the whale having a smooth skin indicate to 'J-stock', but the whale with a rough skin having scars indicate to 'O-stock'.

Thus the results of our pollutant analysis complement well with the observations of scars and with the results of the genetic analysis. Then the use of chemical tracer is useful to separate J and O stocks individuals.

Still pendent the question on the third group of animals in Fig. 3. A possible explanation could be that these animals are younger than those of the first and second groups. Another possibility is that some groups having different food preferences exist within the O-stock. In the JARPN surveys, several prey species were observed in the sub-areas 7, 8 and 9, although the major prey species was recorded as Pacific saury and Japanese anchovy. The list of prey species is wide from krill to salmon and Japanese pomflet. However, no detailed geographical studies are available for these prey species.

Further analyses involving adequate samples from wider areas covering from the coastal to offshore and Pacific and Okhotsk Sea are necessary. Also there is a need to incorporate age information in our analysis.

Examinations of stock estimation for individual base derived from the mtDNA analyses
Goto *et al.* (2000) examined the possibility of identifying individual J stock animals from a mixed assemblage. That study was based on mtDNA RFLP and sequencing analyses. Using the mtDNA criteria we examined differences in chemical accumulations between O and J stocks. Only animals from sub-area 11 are used. Tables 11 and 12 show the results of PCA and DA, respectively. The discriminant factors obtained from the DA are shown below:

$$DF1 = -0.800*PC1+0.824*PC2 \quad (3),$$
$$DF2 = 0.782*PC1+0.759*PC2 \quad (4),$$
$$\text{Wilks's } \Lambda = 0.591 \ (p<0.001).$$

Using this function, the correction rates are 51.9 % in total. Fig. 4 shows the plot of DF1 and DF2 values in each individual. This plot suggests that three O-stock groups (96, 99 and other years) have almost similar location in this figure. These groups are different from J-stock groups (96 and 99). Furthermore it seems that some differences occur between J stock groups in 1996 and 1999. Concentration levels of the J stock group in 1999 are different from the J stock in 1996 and are similar to those for O-stock animals. These differences between J groups are not unknown at this stage. Further multidisciplinary examination is required using additional J stock samples.

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Table 1. Summary of samples used in this study

Year	Sub-area	Number of samples analysed		
		Male	Female	Combined
1994	9	18	3	21
1995	9	15	8	23
1996	7	1	1	2
	8	8		8
	11	19	11	30
1997	8	5	0	5
	9	5	0	5
1998	7	5	0	5
	8	5	0	5
1999	11	26	0	26
Total		107	23	130

Table 2. Body length (BL), Blubber thischness (BT), and concentrations of heavy metals (Fe, Hg and Cd) in liver and organochlorines (PCBs, DDTs, p,p'-DDTs, HCHs, HCB, CHLs) in blubber of minke whales colleceted in the southern Okhotsk Sea (sub-area 11). This table also shows stock estimates from the mtDNA RFLP hyplotypes (M. Goto, pers. comm.) and records of occuerence of the scar on the skin surface.

Specimen. No.	Sub-Area	BL (m)	Sex	Status*	BT (cm)	O or J**	Scar***	Heavy metals in liver (ppm) L-Fe	L-Hg	L-Cd	Organochlorines concentrations (ppb) PCBs	p,p'-DDT	DDTs	β-HCH	HCHs	HCB	CHLs
96NP-025	11	5.1	M	12	2.8	2	3	60	0.27	0.41	1200	61	700	120	190	160	380
96NP-036	11	6.2	M	12	2.9	1	1	447	0.57	0.12	2100	390	2300	450	530	120	280
96NP-042	11	7.5	M	14	2.5	1	0	891	0.17	0.69	5200	330	6400	1700	1700	170	480
96NP-045	11	7.3	M	14	3.0	1	1	866	0.17	0.92	5600	1100	10000	1700	1700	140	760
96NP-046	11	7.4	M	14	2.9	1	1	567	0.08	0.93	5800	890	4800	950	1000	140	930
96NP-020	11	7.5	M	14	2.8	1	1	1684	0.16	0.56	2800	100	1300	380	430	93	470
96NP-041	11	7.5	M	14	3.4	1	1	3889	0.27	0.94	5600	350	3200	850	900	110	930
96NP-033	11	7.6	M	14	2.8	1	1	2116	0.09	0.29	4700	1700	10000	1600	1600	170	950
96NP-034	11	7.6	M	14	3.4	1	2	1764	0.17	0.29	5100	1300	11000	2200	2300	140	920
96NP-028	11	6.8	M	14	4.6	2	3	185	0.52	1.23	1400	73	590	100	150	68	300
96NP-029	11	6.8	M	14	4.1	2	3	284	0.22	0.75	1400	65	800	140	190	78	210
96NP-040	11	6.8	M	14	4.4	2	3	381	0.31	0.37	1600	140	770	100	160	110	370
96NP-023	11	7.1	M	14	4.5	2	3	158	0.27	1.00	1700	82	1100	190	250	120	320
96NP-031	11	7.2	M	14	4.4	2	3	65	0.17	1.17	2100	93	820	160	210	91	350
96NP-035	11	7.2	M	14	5.0	2	3	206	1.14	1.47	2200	180	1300	190	250	120	460
96NP-026	11	7.4	M	14	4.7	2	3	212	0.90	1.62	5700	750	8700	230	270	120	1000
96NP-027	11	7.4	M	14	2.8	2	3	401	0.41	0.86	2400	73	1100	260	300	96	540
96NP-019	11	7.6	M	14	3.9	2	3	501	0.22	1.37	4400	420	4800	240	290	112	850
96NP-024	11	7.7	M	14	4.3	2	3	175	0.28	1.04	6900	490	5600	370	440	120	950
99NP-093	11	7.4	M	14	3	1	0	91	0.04	0.28	1700	120	1600	510	550	120	320
99NP-055	11	6.8	M	14	2.9	1	1	254	0.41	0.17	1500	200	1900	330	370	120	410
99NP-057	11	7.0	M	14	4	1	1	35	0.03	0.52	1100	52	1200	400	440	130	270
99NP-095	11	7.6	M	14	2.6	1	1	440	0.30	0.63	3300	89	2200	560	600	120	600
99NP-082	11	7.7	M	14	3	1	1	589	0.23	0.42	2500	140	2400	640	670	120	500
99NP-081	11	7.7	M	14	3.1	2	1	347	0.29	0.42	1700	72	1400	300	320	94	250
99NP-056	11	6.9	M	13	4.1	2	3	137	0.30	2.98	560	36	310	73	110	73	170
99NP-091	11	7.0	M	14	3.5	2	3	160	0.71	1.18	780	45	330	59	85	58	110
99NP-084	11	7.2	M	14	3.3	2	3	11	0.11	2.58	1000	41	830	150	180	90	260
99NP-094	11	7.2	M	14	2.9	2	3	345	0.74	5.31	1000	49	580	63	84	68	310
99NP-077	11	7.2	M	14	4	2	3	108	0.90	0.88	620	160	870	160	190	58	190
99NP-078	11	7.3	M	14	2.9	2	3	107	0.35	3.11	1600	81	1100	150	180	97	400
99NP-070	11	7.3	M	14	3.9	2	3	116	0.25	3.96	400	21	260	48	64	42	140
99NP-074	11	7.3	M	14	3.6	2	3	262	0.16	1.76	1000	66	850	230	290	120	320
99NP-086	11	7.3	M	14	4.6	2	3	524	0.39	1.88	1800	34	1100	140	160	79	410
99NP-089	11	7.4	M	14	2.8	2	3	496	0.58	1.40	2000	82	2300	78	88	69	420
99NP-079	11	7.4	M	14	4.9	2	3	149	0.42	2.67	1700	64	1200	130	160	100	390
99NP-090	11	7.4	M	14	4.3	2	3	137	2.87	1.97	2900	190	2500	200	250	250	750
99NP-083	11	7.5	M	14	2.8	2	3	223	0.28	2.69	1800	29	1200	150	160	93	320
99NP-097	11	7.5	M	14	3.5	2	3	371	0.17	2.66	2200	210	3000	180	210	160	630
99NP-100	11	7.5	M	13	3.9	2	3	23	0.20	2.17	1200	34	260	90	130	91	180
99NP-080	11	7.5	M	14	3.1	2	3	289	0.71	0.98	2400	120	1600	160	190	190	630
99NP-087	11	7.7	M	14	3.7	2	3	35	1.33	2.96	1500	100	2100	150	180	100	490
99NP-085	11	7.7	M	14	3.1	2	3	220	0.59	1.27	4300	190	3600	240	290	230	1000
99NP-088	11	7.9	M	14	2.5	2	3	179	0.89	5.12	2300	92	2400	370	410	150	810
99NP-096	11	8.0	M	14	3.7	2	3	19	0.30	1.48	2200	110	1700	200	240	140	680
96NP-030	11	7.6	F	25	3.7	1	1	134	0.14	0.84	440	78	520	130	180	51	100
96NP-021	11	4.7	F	22	2.9	2	1	54	0.04	0.32	2300	190	1900	150	250	500	1100
96NP-039	11	5.6	F	22	3.8	2	2	293	0.18	0.15	3400	320	2000	140	200	230	890
96NP-018	11	5.9	F	22	2.9	2	3	97	0.27	1.06	1500	80	670	86	140	140	400
96NP-047	11	6.4	F	22	3.8	2	3	444	0.16	1.51	630	92	650	98	140	88	300
96NP-044	11	7.1	F	24	4.7	2	3	428	0.29	1.08	970	62	520	110	170	93	210
96NP-043	11	7.9	F	25	4.4	2	3	108	0.12	1.39	1500	77	710	110	170	90	260
96NP-032	11	8.0	F	25	4.0	2	3	141	0.17	1.89	620	27	170	31	76	37	71
96NP-038	11	8.0	F	25	4.0	2	3	428	0.18	0.47	930	75	920	130	160	110	250
96NP-037	11	8.1	F	25	5.0	2	3	682	0.21	0.78	1200	48	410	36	69	49	140
96NP-022	11	8.3	F	24	3.3	2	3	425	0.29	2.15	1700	300	2700	350	400	240	690

*: Status , 12 = Immature male, 14 = mature male, 22 = immature female, 24-25: mature female

**: Estimation for J-stock (1) or O-stock (2), which were based on the haplotypes derived from the mtDNA RFLP and sequence analyses

***: Occurrence of scars in the skin surface: 0=absent of scar, 1=few scars are exist, 2=frelatively scars, 3=large number of scars

Table 3. Body length (BL), blubber thischness (BT), and concentrations of heavy metals (Fe, Hg and Cd) in liver and organochlorines (PCBs, DDTs, p,p'-DDTs, HCHs, HCB, CHLs) in blubber of minke whales colleceted in the pscific side of Japan (sub-areas 7, 8 and 9). This table also shows stock estimates from the mtDNA RFLP hyplotypes (M. Goto, pers. comm.) and records of occuerence of the scar on the skin surface.

Specimen.	Sub-	BL	Sex	Status*	BT	O or J**	Scar***	Heavy metals in liver (ppm)			Organochlorines concentrations (ppb)						
No.	Area	(m)			(cm)			L-Fe	L-Hg	L-Cd	PCBs	p,p'-DDT	DDTs	β-HCH	HCHs	HCB	CHLs
98NP-023	7E	6.9	M	14	3.3	2	3	265	0.96	1.34	7100	400	3500	230	300	300	1600
98NP-028	7E	7.8	M	14	3.0	2	3	602	0.35	3.10	4000	140	2200	150	160	99	680
98NP-030	7E	7.3	M	14	2.2	2	3	208	0.34	3.56	2700	71	960	160	270	180	500
98NP-031	7E	7.2	M	14	2.6	2	3	475	0.37	3.35	4500	140	2100	370	440	230	920
98NP-032	7E	7.6	M	14	2.5	2	3	311	0.64	2.32	4000	230	2300	170	230	120	860
96NP-057	7W	7.9	M	14	3.2	2	3	233	0.46	0.92	1600	120	1300	240	320	150	380
96NP-006	8	7.8	M	12	2.7	2	3	152	0.96	1.74	4000	170	1800	240	290	130	590
96NP-016	8	4.7	M	14	3.5	2	3	81	0.84	2.34	3400	180	1800	130	170	150	730
96NP-011	8	5.9	M	14	2.5	2	3	203	0.47	2.24	4300	62	1000	170	200	76	510
97NP-074	8	7.0	M	14	3.2	2	3	326	0.72	1.78	4100	130	1900	210	240	130	760
98NP-075	8	7.2	M	14	2.6	2	3	606	1.00	2.91	2400	130	1100	170	220	160	510
97NP-070	8	7.2	M	14	3.8	2	3	83	0.98	3.83	3600	120	1500	150	180	110	670
98NP-074	8	7.3	M	14	2.6	2	3	285	0.82	6.35	2400	130	870	210	240	150	490
98NP-079	8	7.3	M	14	2.5	2	3	56	0.47	2.84	3000	140	1500	190	230	120	610
97NP-079	8	7.3	M	14	2.8	2	3	265	0.68	2.65	1900	44	660	100	130	78	310
96NP-003	8	7.4	M	14	2.4	2	3	119	0.81	2.77	4400	110	1300	350	460	160	730
96NP-014	8	7.5	M	14	3.0	2	3	704	0.49	1.49	7100	430	6200	270	300	200	1100
96NP-009	8	7.5	M	14	3.0	2	3	120	1.83	3.84	5400	270	3200	280	330	200	710
97NP-084	8	7.6	M	14	3.8	2	3	160	0.81	3.14	2500	97	1000	150	180	90	510
96NP-013	8	7.6	M	14	1.9	2	3	83	0.88	4.22	5400	61	2100	230	280	160	940
97NP-076	8	7.6	M	14	4.6	2	3	140	0.79	3.19	4100	170	2200	160	180	96	630
98NP-076	8	7.6	M	14	2.1	2	3	461	1.10	4.31	3200	140	1800	240	270	150	620
96NP-010	8	7.7	M	14	2.5	2	3	222	0.78	1.71	3800	58	1500	160	180	88	600
98NP-063	8	7.8	M	14	3.0	2	3	161	0.46	1.32	4000	140	2000	290	350	200	800
95NP-081	9	4.5	M	12	3.1	2	2	36	0.13	0.37	2300	86	1300	110	240	220	550
95NP-009	9	5.9	M	12	2.8	2	3	127	0.39	0.50	460	49	400	76	100	92	140
94NP-014	9	6.1	M	12	3.9	2	3	152	0.27	1.40	490	31	220	68	140	66	97
95NP-019	9	6.6	M	14	3.1	2	3	40	0.55	2.77	1700	160	1900	300	360	100	370
95NP-005	9	6.6	M	14	3.0	2	3	44	0.66	0.83	2500	340	1900	150	220	160	460
95NP-070	9	6.8	M	14	3.4	2	3	453	0.50	4.38	900	38	540	130	170	63	220
95NP-014	9	6.9	M	14	3.3	2	3	252	0.93	1.39	1500	91	1000	130	170	110	410
94NP-019	9	7.0	M	14	3.7	2	3	74	0.77	2.87	1600	270	1900	330	650	320	500
94NP-015	9	7.1	M	14	2.4	2	3	157	0.82	1.93	1700	100	910	110	180	87	250
95NP-096	9	7.2	M	14	4.0	2	3	158	0.87	6.85	1000	64	830	95	120	49	210
94NP-003	9	7.2	M	14	2.8	2	3	157	0.57	1.99	1800	230	1700	140	200	160	580
94NP-008	9	7.2	M	14	2.9	2	3	69	0.72	2.20	1500	160	1700	190	240	140	540
94NP-012	9	7.3	M	14	3.9	2	3	68	1.05	3.13	2100	130	1000	110	170	76	260
94NP-011	9	7.3	M	14	4.3	2	3	121	0.91	2.77	2900	170	1900	230	280	140	380
94NP-006	9	7.3	M	14	3.1	2	3	60	2.45	5.84	1300	250	1800	220	250	100	380
94NP-002	9	7.4	M	14	2.7	2	3	206	0.55	2.58	1800	300	2300	270	360	210	610
94NP-009	9	7.4	M	14	3.0	2	3	983	1.37	3.62	1500	200	1600	190	280	130	440
94NP-017	9	7.4	M	14	3.6	2	3	272	0.77	4.68	2400	120	2600	280	350	130	470
95NP-083	9	7.4	M	14	3.5	2	3	55	1.36	8.15	2400	73	1600	210	230	74	260
95NP-095	9	7.4	M	14	3.2	2	3	69	0.57	3.91	2300	160	1900	180	210	93	350
95NP-090	9	7.4	M	14	3.0	2	3	230	1.55	5.39	2600	150	2300	250	300	130	410
94NP-016	9	7.5	M	14	2.8	1	3	739	1.50	4.00	2200	250	2500	390	470	130	480
95NP-063	9	7.5	M	14	3.0	2	3	453	1.09	3.64	2000	76	1300	350	390	130	390
95NP-073	9	7.5	M	14	4.1	2	3	454	0.77	3.84	590	66	930	140	170	75	260
95NP-007	9	7.6	M	14	2.9	2	3	97	0.47	2.26	3500	420	6000	610	750	300	870
97NP-006	9	7.6	M	14	2.2	2	3	145	3.36	2.65	11000	25	7200	240	270	290	1800
94NP-010	9	7.6	M	14	3.8	2	3	298	0.91	2.35	1600	100	970	100	130	89	260
97NP-010	9	7.6	M	14	2.8	2	3	163	0.48	3.75	2900	120	1300	150	180	100	580
94NP-004	9	7.7	M	14	4.8	2	3	188	0.88	7.22	2300	200	1500	370	480	130	570
94NP-005	9	7.7	M	14	4.7	2	3	551	2.54	1.44	2800	270	1900	380	550	150	630
97NP-017	9	7.7	M	14	3.5	2	3	146	0.70	6.29	3700	170	1900	160	200	170	730
94NP-020	9	7.9	M	14	3.4	2	3	177	0.32	1.89	2000	170	1400	180	270	130	450
95NP-040	9	7.9	M	14	2.9	2	3	98	0.50	2.40	3200	150	2500	420	460	140	610
94NP-018	9	8.0	M	14	4.2	2	3	158	3.27	1.56	2900	230	1800	320	420	130	580
97NP-016	9	8.0	M	14	2.6	2	3	295	0.61	3.46	3000	37	1400	190	200	100	500
94NP-021	9	8.1	M	14	3.5	2	3	262	0.78	3.98	2700	180	1800	270	390	210	370
97NP-011	9	8.1	M	14	3.0	2	3	533	1.36	3.50	5600	49	2000	290	350	230	840
95NP-017	9	8.4	M	14	3.5	2	3	386	0.70	3.59	3900	120	2500	330	370	110	630

*: Status , 12 = Immature male, 14 = mature male, 22 = immature female, 24-25: mature female

**: Estimation for J-stock (1) or O-stock (2), which were based on the haplotypes derived from the mtDNA RFLP and sequence analyses

***: Occurrence of scars in the skin surface: 0=absent of scar, 1=few scars are exist, 2=frelatively scars, 3=large number of scars

Table 4. Body length (BL), blubber thickness (BT) and concentration of heavy metals in liver and kidney, and concentration of organochlorines in blubber of North Pacific minke whales in each sub-area and sex (L-Fe: hepatic iron, L-Hg: hepatic mercury, L-Cd: hepatic cadmium, K-Cd: renal cadmium)

Sex	Sub area	Year			BL m	BT cm	L-Fe ppm	L-Hg ppm	L-Cd ppm	K-Cd ppm	PCBs ppb	p,p'-DDT ppb	DDTs ppb	β-HCH ppb	HCHs ppb	HCB ppb	CHLs ppb
Male	11	1996	Imm.	Mean	5.7	2.9	253	0.15	36.6	0.61	1650	226	1500	285	360	140	330
				S.D.	0.8	0.1	274	0.03	19.6	0.11	636	233	1131	233	240	28	71
				Min	5.1	2.8	60	0.10	9.2	0.44	1200	61	700	120	190	120	280
				Max	6.2	2.9	447	0.22	74.8	0.90	2100	390	2300	450	530	160	380
				n	2	2	2	23	23	23	2	2	2	2	2	2	2
		1996	Mat.	Mean	7.3	3.7	844	0.33	0.9	3.61	3800	479	4252	668	714	118	635
				S.D.	0.3	0.8	1004	0.29	0.4	1.31	1899	499	3759	699	691	28	283
				Min	6.8	2.5	65	0.08	0.3	1.67	1400	65	590	100	150	68	210
				Max	7.7	5.0	3889	1.14	1.6	6.57	6900	1700	11000	2200	2300	170	1000
				n	17	17	17	17	17	17	17	17	17	17	17	17	17
		1999	Mat.	Mean	7.4	3.5	218	0.52	2.0	6.30	1733	93	1492	222	254	114	422
				S.D.	0.3	0.6	165	0.57	1.4	3.98	895	58	885	159	164	50	225
				Min	6.8	2.5	11	0.03	0.2	1.30	400	21	260	48	64	42	110
				Max	8.0	4.9	589	2.87	5.3	17.00	4300	210	3600	640	670	250	1000
				n	26	26	26	26	26	26	26	26	26	26	26	26	26
	7	1996-1998	Mat.	Mean	7.4	2.8	349	0.52	2.4	5.07	3983	184	2060	220	287	180	823
				S.D.	0.4	0.4	156	0.24	1.1	3.06	1861	118	888	82	94	75	433
				Min	6.9	2.2	208	0.34	0.9	2.13	1600	71	960	150	160	99	380
				Max	7.9	3.3	602	0.96	3.6	10.30	7100	400	3500	370	440	300	1600
				n	6	6	6	6	6	6	6	6	6	6	6	6	6
	8	1996	Imm.		7.8	7.8	152	0.9607	1.744	4.7122	4000	170	1800	240	290	130	590
				n	1	1	1	1	1	1	1	1	1	1	1	1	1
		1996-98	Mat.	Mean	7.2	2.9	240	0.82	3.0	6.29	3824	142	1861	204	244	136	661
				S.D.	0.8	0.7	189	0.32	1.3	3.97	1305	92	1275	66	82	42	184
				Min	4.7	1.9	56	0.46	1.3	2.40	1900	44	660	100	130	76	310
				Max	7.8	4.6	704	1.83	6.3	16.78	7100	430	6200	350	460	200	1100
				n	17	17	17	17	17	17	17	17	17	17	17	17	17
	9	1994-1995	Imm.	Mean	5.5	3.3	105	0.26	0.8	4.73	1083	55	640	85	160	126	262
				S.D.	0.9	0.6	61	0.13	0.6	4.81	1054	28	579	22	72	82	250
				Min	4.5	2.8	36	0.13	0.4	1.47	460	31	220	68	100	66	97
				Max	6.1	3.9	152	0.39	1.4	10.25	2300	86	1300	110	240	220	550
				n	3	3	3	3	3	3	3	3	3	3	3	3	3
		1994-97	Mat.	Mean	7.5	3.3	246	1.06	3.5	8.86	2554	161	1951	240	308	140	504
				S.D.	0.4	0.6	212	0.75	1.7	4.67	1759	92	1281	112	147	65	280
				Min	6.6	2.2	40	0.32	0.8	3.17	590	25	540	95	120	49	210
				Max	8.4	4.8	983	3.36	8.1	27.05	11000	420	7200	610	750	320	1800
				n	35	35	35	35	35	35	35	35	35	35	35	35	35
Female	11	1996	Imm	Mean	5.7	3.4	222	0.16	0.8	2.64	1958	171	1305	119	183	240	673
				S.D.	0.7	0.5	181	0.09	0.6	1.90	1179	111	746	31	53	183	384
				Min	4.7	2.9	54	0.04	0.2	0.40	630	80	650	86	140	88	300
				Max	6.4	3.8	444	0.27	1.5	4.72	3400	320	2000	150	250	500	1100
				n	4	4	4	4	4	4	4	4	4	4	4	4	4
		1996	Mat.	Mean	7.9	4.2	335	0.20	1.2	3.94	1051	95	850	128	175	96	246
				S.D.	0.4	0.6	214	0.07	0.6	1.24	452	92	849	106	110	69	209
				Min	7.1	3.3	108	0.12	0.5	1.45	440	27	170	31	69	37	71
				Max	8.3	5.0	682	0.29	2.1	5.57	1700	300	2700	350	400	240	690
				n	7	7	7	7	7	7	7	7	7	7	7	7	7
	7E	1996	Mat.		7.1	2.9	941	0.82	2.8	3.95	2700	100	1200	300	380	155	530
				n	1	1	1	1	1	1	1	1	1	1	1	1	1
	9	1994-95	Mat.	Mean	7.2	3.5	752	0.77	3.9	8.37	1416	127	1314	134	193	85	246
				S.D.	1.1	0.6	1037	0.48	2.5	4.14	1171	123	1102	94	112	40	115
				Min	4.8	2.5	29	0.04	1.5	3.20	230	9	130	45	71	32	81
				Max	8.2	4.3	3427	1.50	8.0	14.8	4000	430	3600	340	410	160	480
				n	11	11	11	11	11	11	11	11	11	11	11	11	11

Table 5. Result of the PCA using data for seven chemicals (L-Hg, L-Cd, PCBs, Σ DDTs, Σ HCHs, HCB and Σ CHLs) of male minke whales collected in sub-areas 7, 8, 9 and 11 during 1994-1998.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
L-Hg	0.129	-0.079	0.111	0.967	0.173	0.004	0.002
L-Cd	-0.039	-0.132	-0.031	0.167	0.976	-0.018	-0.001
PCBs	0.934	0.238	0.175	0.107	-0.029	0.032	-0.166
Σ DDTs	0.525	0.695	0.116	0.008	-0.100	0.467	0.010
Σ HCHs	0.159	0.969	0.102	-0.094	-0.127	-0.029	-0.001
HCB	0.334	0.126	0.925	0.123	-0.034	0.026	0.001
CHLs	0.878	0.153	0.366	0.115	-0.029	0.075	0.227
Cumulative contribution rate	29.6	51.6	66.7	81.2	95.6	98.9	100.0

Table 6. Result of the DA for geographical location of sample using five pricipal component scores (PC1-PC5) by the PCA in Table 5.

$Z = -0.680*PC2+0.490*PC3+0.530*PC4+0.893*PC5-0.087$

Wilks' Lambda = 0.492 ($p<0.001$)

	Result of discrimination					
	Okhotsk Sea		Pacific side		Total	
Sampling location						
Okhotsk Sea	17	(89.5)	2	(10.5)	19	(100)
Pacific side	4	(6.5)	58	(93.5)	62	(100)
				% of correct = 93.8 %		

Table 7. Result of the PCA using data for four chemicals (L-Hg, L-Cd, p,p'-DDT, and β -HCHs) of male minke whales collected in sub-areas 7, 8, 9 and 11 during 1994-1998.

	PC1	PC2	PC3	PC4
L-Hg	-0.079	0.168	0.980	-0.065
L-Cd	-0.108	0.971	0.174	-0.123
p,p'-DDT	0.469	-0.156	-0.081	0.866
β -HCHs	0.897	-0.122	-0.095	0.415
Cumulative contribution rate	26.0	51.3	76.5	100.0

Table 8. Result of the DA for geographical location of sample using three pricipal component scores (PC1-PC3) by the PCA in Table 7.

$Z = -0.517*PC1+0.877*PC2+0.539*PC3-0.054$

Wilks' Lambda = 0.633 ($p<0.001$)

	Result of discrimination					
	Okhotsk Sea		Pacific side		Total	
Sampling location						
Okhotsk Sea	17	(89.5)	2	(10.5)	19	(100)
Pacific side	11	(17.7)	51	(82.3)	62	(100)
				% of correct = 84.0 %		

Table 9. Result of the PCA using data for four chemicals (L-Hg, L-Cd, p,p'-DDT, and β -HCHs) of male minke whales collected in sub-areas 7, 8 and 9 during 1994-1998.

	PC1	PC2	PC3	PC4
L-Hg	0.082	0.990	0.043	0.103
L-Cd	0.992	0.081	-0.087	0.043
p,p'-DDT	-0.095	0.045	0.964	0.243
β -HCHs	0.049	0.112	0.245	0.962
Cumulative contribution rate	25.1	50.1	75.1	100.0

Table 10. Result of the DA for stock estimated by mtDNA, using four pricipal component scores (PC1-PC4) by the PCA in Table 9.
DC1= 0.522*PC1+0.823*PC2-0.314*PC3, DC2= 0.399*PC1+0.089*PC2+0.898*PC3
Wilks' Lambda = 0.916 (p=0.531)

	Result of discrimination							
	Sub-area 7		Sub-area 8		Sub-area 9		Total	
Sampling location								
Sub-area 7	4	(66.7)	2	(33.3)	0	(0)	6	(100)
Sub-area 8	4	(22.2)	7	(38.9)	7	(38.9)	18	(100)
Sub-area 9	10	(26.3)	11	(28.9)	17	(44.7)	38	(100)

% of correct = 45.2 %

Table 11. Result of the PCA using data for four chemicals (L-Hg, L-Cd, p,p'-DDT, and β -HCHs) of male minke whales collected in sub-areas 7, 8, 9 and 11 during 1994-1999.

	PC1	PC2	PC3	PC4
L-Hg	-0.034	0.158	0.985	-0.067
L-Cd	-0.107	0.975	0.163	-0.104
p,p'-DDT	0.901	-0.124	-0.035	0.415
β -HCHs	0.451	-0.127	-0.088	0.879
Cumulative contribution rate	25.7	50.9	76.0	100.0

Table 12. Result of the DA for stock estimated by mtDNA, using three pricipal component scores (PC1-PC3) by the PCA in Table 11.
DC1= -0.800*PC1+0.824*PC2, DC2= 0.782*PC1+0.759*PC2
Wilks' Lambda = 0.591 (p<0.001)

	Result of discrimination											
	0 (94-98)		J (96)		O (96)		J (99)		O (99)		Total	
Estimate by mtDNA												
O stock (94-98)	31	(59.6)	2	(3.8)	11	(21.2)	3	(5.8)	5	(9.6)	52	(100)
J stock (96)	0	(0)	5	(62.5)	1	(12.5)	2	(25.0)	0	(0)	8	(100)
O stock (96)	2	(10.0)	4	(20.0)	6	(30.0)	5	(25.0)	3	(15.0)	20	(100)
J stock (99)	0	(0)	0	(0)	0	(0)	5	(100)	0	(0)	5	(100)
O stock (99)	6	(28.6)	0	(0)	5	(23.8)	2	(9.5)	8	(38.1)	21	(100)

% of correct = 51.9 %

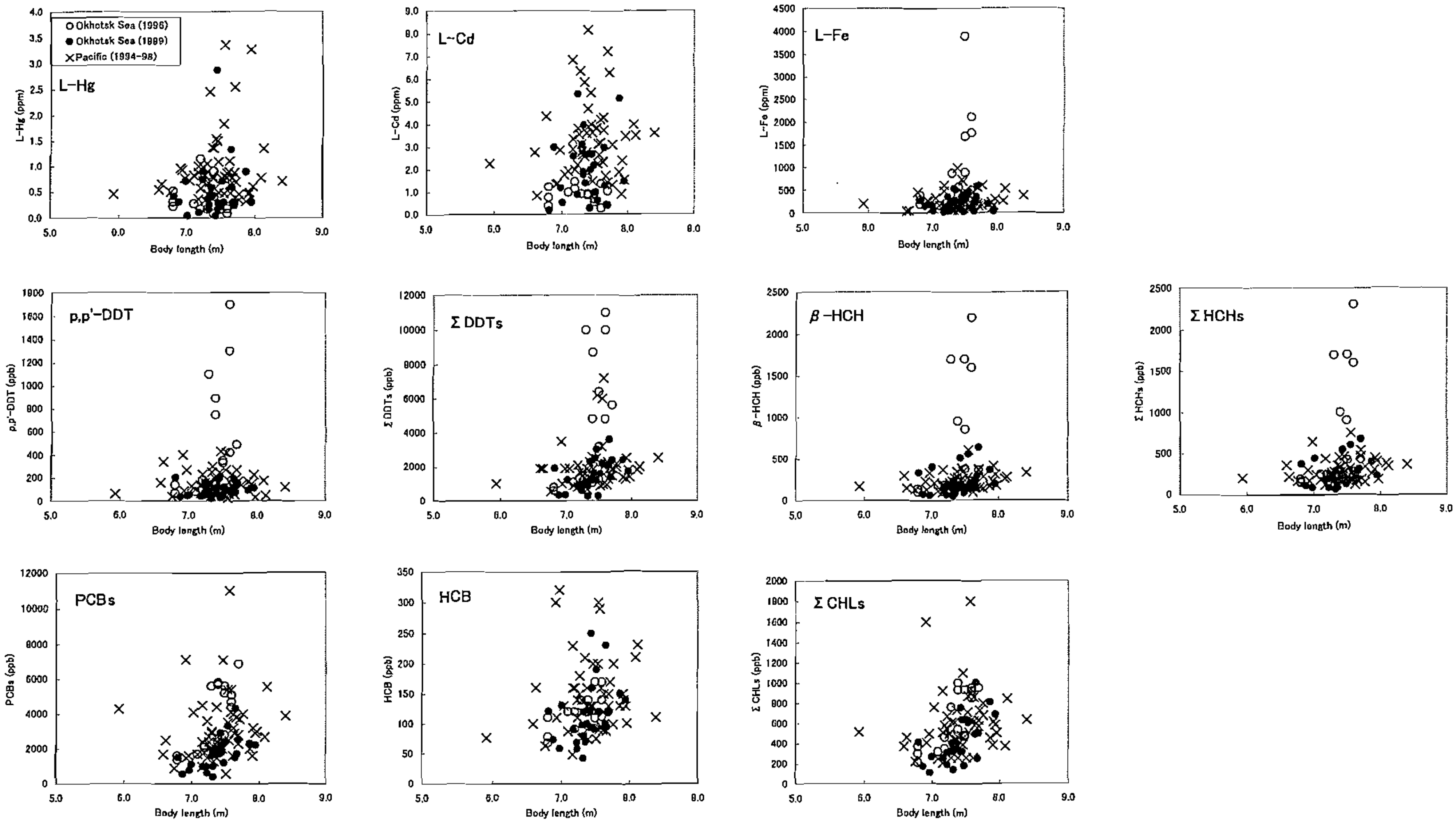

Fig. 1. Lengh-trends of concentrations of heavy metals (Fe, Hg and Cd) in liver and organochlorines (p,p'-DDT, Σ DDTs, β-HCH, Σ HCHs, PCBs, HCB and Σ CHLs) in blubber of mature male North Pacific minke whales, respectively. Figure shows the geographical locations (Okhotsk Sea = sub-area 11, and Pacific = Pacific side of Japan, sub-areas 7, 8 and 9) and sampling year.

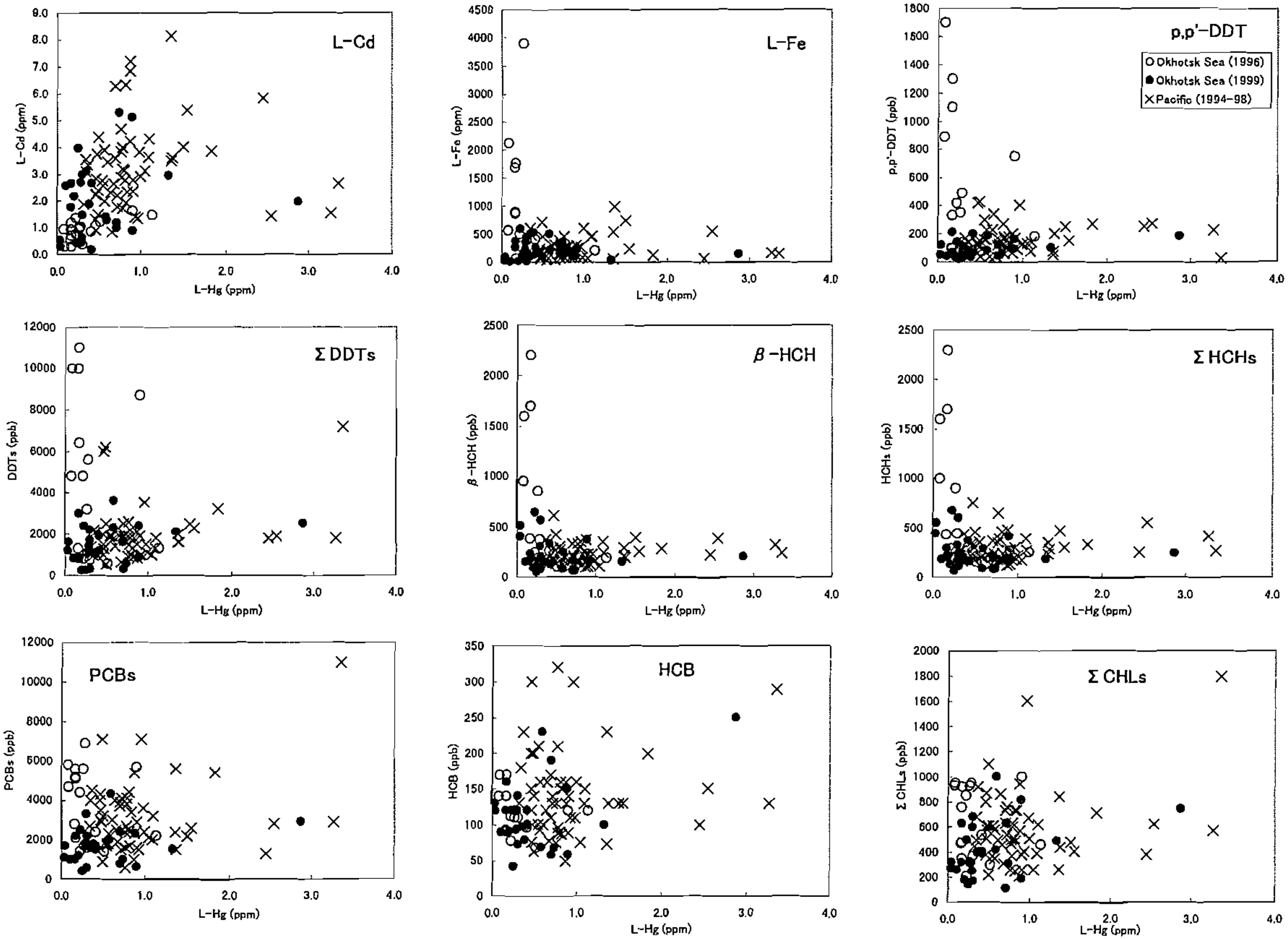

Fig. 2. Relationships of hepatic Hg concentrations to the concentrations of heavy metals (*Fe* and Cd) in liver and organochlorines (p,p'-DDT, DDTs, β-HCH, HCHs, PCBs, HCB and Σ CHLs) in blubber of mature male North Pacific minke whales, respectively. *Figure* shows the geographical locations (Okhotsk Sea = sub-area 11, and Pacific = Pacific side of Japan, sub-areas 7, 8 and 9) and sampling year.

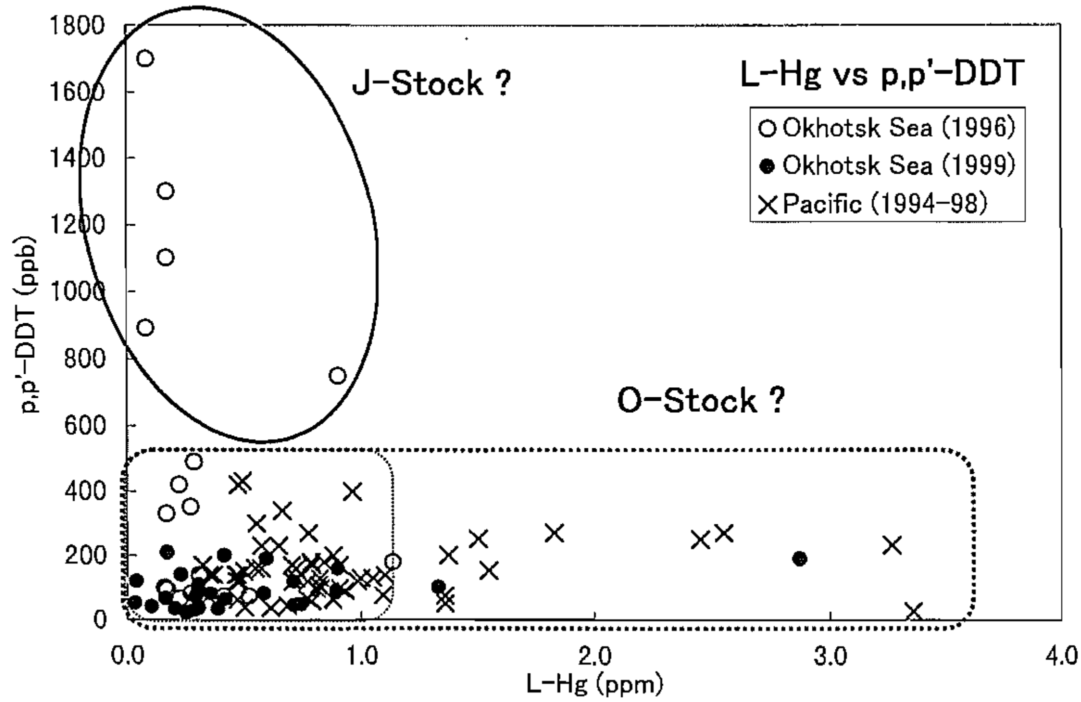

Fig. 3. Schematic diagram of Juggement of stock using relationship between concentrations Hg in liver and p, p'-DDT in blubber

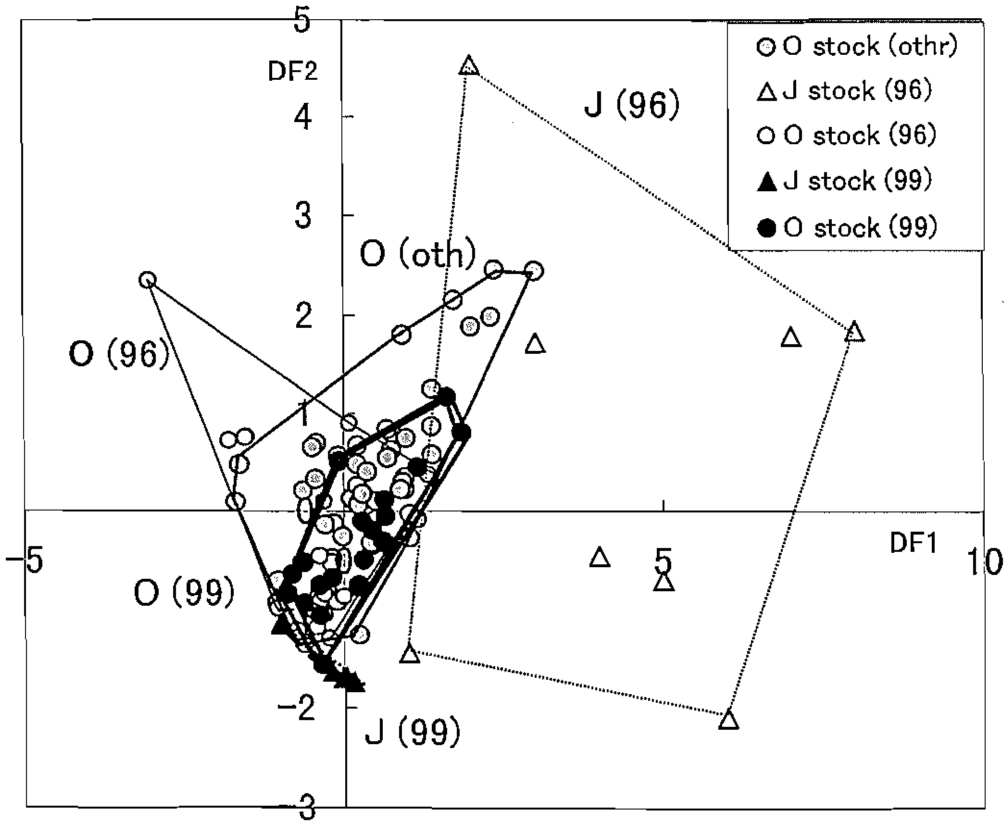

Fig. 4. Plot of DF1 and DF2 for minke whale individuals derived from the DA in Table 12.