Utility of non-genetic information for stock identification - The case of the western North Pacific minke whale -

Yoshihiro Fujise, Ryoko Zenitani and Mutsuo Goto

The Institute of Cetacean Research, 4-18 Toyomi-cho, Chuo-ku, Tokyo 104-0055, Japan

ABSTRACT

Individuals identified as belonging to the "J" and "O" stocks of minke whale in the western North Pacific by mtDNA analysis are examined by three non-genetic markers: occurrence of scar as one of the external characters, foetal length reflecting the conception date and accumulations of pollutants. Striking differences were found by these three markers between "J" and "O" stocks, and genetic and non-genetics markers matched almost perfectly. This means that these non-genetic markers sufficiently identify the breeding season and habitat environment of the animal stocks in question. Utility of these non-genetic markers is discussed.

KEYWORD: NORTH PACIFIC MINKE WHALE, STOCK IDENTIFICATION, NON-GENETICS

INTRODUCTION

It has been reported that there are two different stocks of minke whales in the waters around Japan, the Sea of Japan-Yellow Sea-East China Sea stock (the "J" stock) and the Okhotsk Sea-Western Pacific stock (the "O" stock) (Omura and Sakiura, 1956; Ohsumi, 1983; Kato *et al.*, 1992; Wada, 1983; 1984). However, at a meeting of the working group for the RMP implementation simulation trials for minke whales, it was suggested that the available information on the stocks was inconclusive and that there may be a sub-stock within the stocks and/or another stock (the "W" stock).

The JARPN (JARPN II) surveys in the western North Pacific are conducted on the stock structure of minke whales in the area in terms of genetics, biology, environmental chemistry, and parasites with the view of verifying the above mentioned hypothesis (Fujise *et al.*, 2000a). The surveys also attempt stock identification of individual animals (Goto *et al.*, 2000; Fujise *et al.*, 2000b). The JARPN survey results have been discussed in detail at the JARPN review meeting held in February 2000 (IWC, 2001).

The Working Group on Stock Definition of the Scientific Committee was established in 1998 to develop stock definitions for the purpose of effective management of whales (IWC, 1999). One of the tasks assigned by the Working Group is to review the utility of non-genetic approaches, as well as genetics, in developing definition s of stocks.

This paper discusses the utility of non-genetic information for stock identification, and whether or not it is possible to identify the stock of the individual animal based on external morphological scars, conception date determined by foetus length, and accumulation level of pollutants and other environmental chemistry information in addition to genetic data, which are part of the JARPN surveys on stock structure.

MATERIALS AND METHODS

We used the data in the documents SC/F2K/J18 (Fujise *et al.*, 2000b) and SC/F2K/J28 (Goto *et al.*, 2000) presented at the 2000 JARPN review meeting (Table 1). The stock of individual animals was identified using the following information:

Genetic

A phylogenetic approach was used to estimate the "J" stock animals in JARPN samples (Goto *et al.*, 2000). Haplotypes contained in a same cluster and sharing RFLP haplotypes 3 and 5 (which are the main RFLP haplotypes of the "J" stock) or being sampled in the Sea of Japan, were considered to be from the "J" stocks

(Goto *et al.*, 2000)

External morphology
There have been frequent sightings of minke whales with numerous white scars on the skin (Fig.1). They are considered to be scars of bite marks by *Isistitus* sp., and since they have been inflicted when minke whales migrate to tropical and subtropical waters where *Isistitus* sp. live. Older animals tend to have more scars than the younger ones (Fig. 2). In fact, some of the large mature animals are covered with white scars so that the original body color is almost completely obscured.

Two mature pregnant animals, one with no scars at all and the other with scars, were collected in sub-area 11 during a survey conducted in July 1997. The scarred animal was carrying a foetus of about 50 cm, and the unmarred one, a large foetus of about 140 cm. It seemed unlikely that they shared the same breeding ground, and the natural assumption would be that they were from different ones. In view of the above foetal length, we inferred that the unmarred animal was of "J" stock and the scarred one from the "O" stock, and conducted the following analysis. However, although white scars are an indication of the stock the animal belongs to, in the case of immature animals, it is difficult to determine whether the animal in question is too young to have been scarred or that it lives in waters where there are no *Isistitus* sp., since the morphological marks are inflicted after birth. We have, therefore, excluded immature animals from the analysis (Fig. 2).

Conception date determined by foetus length
The breeding season differs between the "J" and the "O" stocks. The former is thought to breed in winter and the latter in autumn (Kato, 1992). Therefore, we identified the "O" stock and the "J" stock from the relationship between foetal length and sampling date (Fig. 3).

Accumulation of pollutants
Organochlorines including DDT and PCBs are man-made chemicals, and the accumulation levels are known to vary depending on the area and the sex and age of the animal. It is also known that the heavy metals such as Hg and Cd are varied in the internal organs such as liver and kidney of the animals depending on the food habitat and the their sex and age. In cetaceans, Dall's porpoises in the Sea of Japan and those in the Pacific Ocean show different levels of DDT accumulation, and the figures are considered useful in learning their ecology, especially in determining their habitats (Subramanian *et al.*, 1986). Fujise (1987) has estimated the habitat of Dall's porpoises, based on the relation between their Hg and Cd accumulation levels.

In the North Pacific minke whales, Hg and DDT levels were examined as a preliminary comparison with other stock estimation results (Fujise *et al.*, 2000b). It was found that in the Pacific Ocean (sub-areas 7, 8 and 9), they consume higher trophic level prey such as Pacific saury and Japanese anchovy, while those in the Sea of Japan (sub-area 11) mainly consume krill, which is of low trophic level. Therefore, the minke whales belong to Pacific side ("O" stock) shows higher Hg levels, and lower (p,p'-) DDT levels in comparison with the whales belong to "J" stock.

In this paper, we used these results of estimation for stock using the pollutant accumulations. Since the accumulations of organochlorines and heavy metals are varied depending to age and sex, the adoption of this estimation is limited for the mature male and some of immature and mature females. Then, in this study, we used the result to grouping to two groups: Option A is that all whales analysed are used (regardless of their sex and age), and Option B is used only the data for individuals (mainly mature males) shows typical accumulation level for the stock belonged.

RESULTS

Table 2 shows the comparison results by different estimation methods. The estimated stock identity has been compared with the results derived from genetic data and other methods.

Scars
The results of stock estimation using scars matched 97% of the "O" stock and 95.5% of the "J" stock determined by genetic data. Further, they corresponded 100% with the results determined by foetal length and the accumulation level of pollutants.

Foetal length
The stock identity of 39 pregnant females was determined using foetal length. The results matched 91.2% of the

"O" stock and 100% of the "J" stock determined by genetic data. They also corresponded 100% with the results determined by scar length and the accumulation level of pollutants.

Accumulation of pollutants
Identification of the "J" and "O" stocks estimated by the levels of organochlorines and heavy metals matched 90.8 to 95.0% of the "O" stock and 80.0% of the "J" stock determined by genetic data. They also corresponded 100% with the results determined by scar and foetal length.

DISCUSSION

Stock identification is mainly determined using genetic methods. For western North Pacific minke whales, haplotypes based on mtDNA control region sequence is used to determine the stock identity of individual animals. In this research, to determine stock identity, we added breeding season and other biological parameters as well as pollutant levels and scars that reflect the habitat conditions to the results of stock estimation by genetic methods. We found that the estimation results using the aforementioned parameters were more or less consistent with the results determined by genetic data. It has been made clear that it is possible to use the parameters for determining stock identity.

Results of stock estimates by scar, foetal length and the accumulation level of pollutants show a perfect match, which means that they sufficiently reflect the breeding season and habitat environment of the animals in question. It strongly supports the idea that they are useful in determining stocks that are separated by different habitat environment.

However, though small in number, there were animals for which the results from the parameters were not consistent with the results determined by genetic data. This may be due to a) the fact that absolute markers of the sequencing haplotypes for the "J" stocks are not established at this stage, since animals identified as having the "J" stock haplotype occur in the Pacific side of Japan in lower frequencies, and b) that there is a possibility of migration between the "J" and "O" stocks. Further detailed discussions on these points are necessary.

One disadvantage of the estimation methods employed in this research is that they cannot be applied to all animals. For instance, foetal information only applies to pregnant females and cannot be used for non-pregnant animals. The scars from *Isistitus* sp. bite marks are effective for mature animals. Since they are inflicted after birth, we cannot say an immature animal is of "J" stock merely because it is not marked (however, if it is scarred, it is not of "J" stock even if the animal is immature). Further, a considerable amount of the accumulated pollutants such as DDT and other organochlorines are transferred from the mother to the child during pregnancy and lactation, so that a female with low accumulation level does not necessary mean it is from a habitat that is not highly contaminated (i.e., the Pacific Ocean).

Therefore, we need to understand and recognize both the advantages and disadvantages of the different estimation methods, and determine stock identity by combining the information with genetic data, so that it will be possible to identify stock with more accuracy.

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Table 1. Summary of samples used in this study

	Number of samples estimated		
	Male	Female	Combined
Genetics	419	79	498
Scars	416	75	491
Foetal length	0	39	39
Pollutants	107	23	130

Table 2. Comparison of J and O stock estimation by individual base for North Pacific minke v in diferent estimation method. Options A and B is explained in the text page 2.

		Genetics			Scars			Foetus length		
		Match	Miss	%	Match	Miss	%	Match	Miss	%
Genetics (n=498)	O									
	J									
	Total									
Scars (n=491)	O	455	14	97.0						
	J	21	1	95.5						
	Total	476	15	96.9						
Foetus length (n=39)	O	31	3	91.2	31	0	100			
	J	5	0	100	5	0	100			
	Total	36	3	92.3	36	0	100			
OC (Option A) (n=103)	O	89	9	90.8	88	0	100	3	0	100
	J	4	1	80.0	3	0	100	0	0	
	Total	93	10	90.3	91	0	100	3	0	100
OC (Option B) (n=25)	O	19	1	95.0	20	0	100	3	0	100
	J	4	1	80.0	3	0	100	0	0	
	Total	23	2	92.0	23	0	100	3	0	100

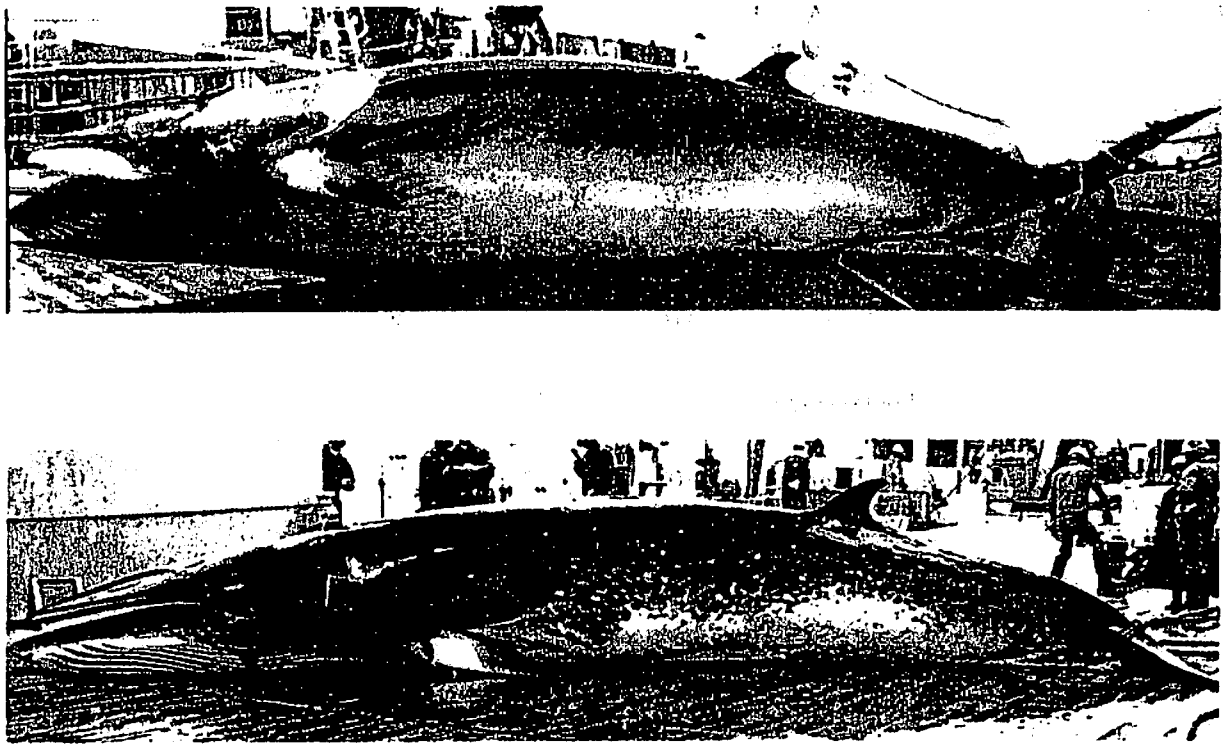


Fig. 1. Comparison of occurrence of scars in the body surface of mature minke whale.
Upper: type of few scars are exist (Specimen No.098 in the 1999 JARPN).
Lower: type of large number of scars are existed (Specimen No. 085 in the 1999 JARPN)

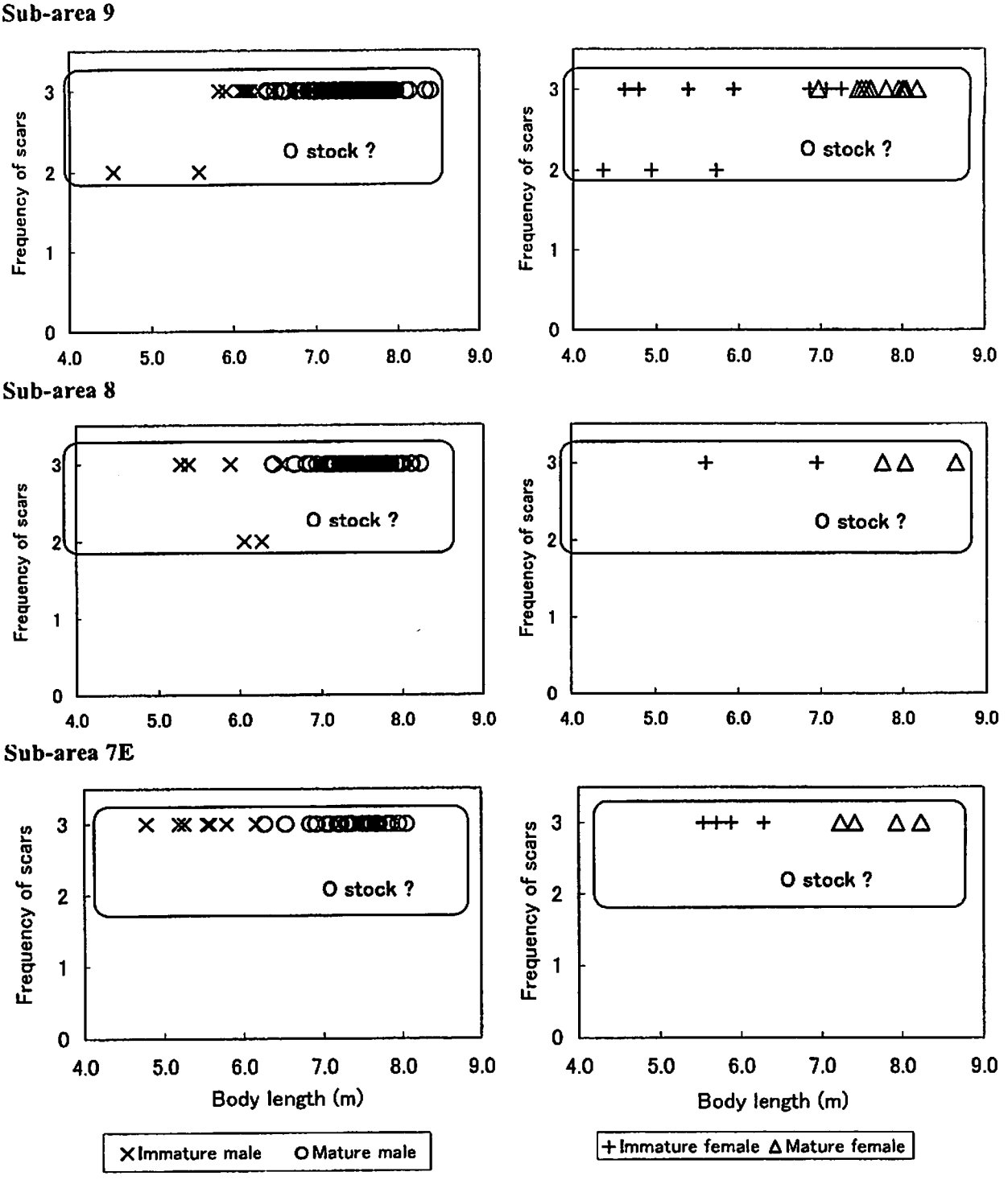
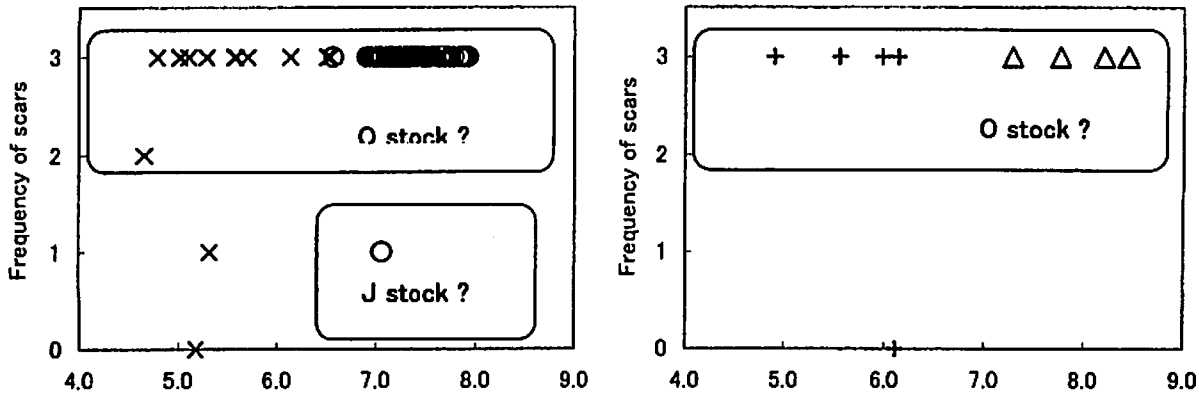


Fig. 2. Relationship between boldy length and frequency of scars of the body surface of minke whales by sex and mature in each sub-area. Criteria of the J and O stock identification used in this study are also shows.

Sub-area 7W



Sub-area 11

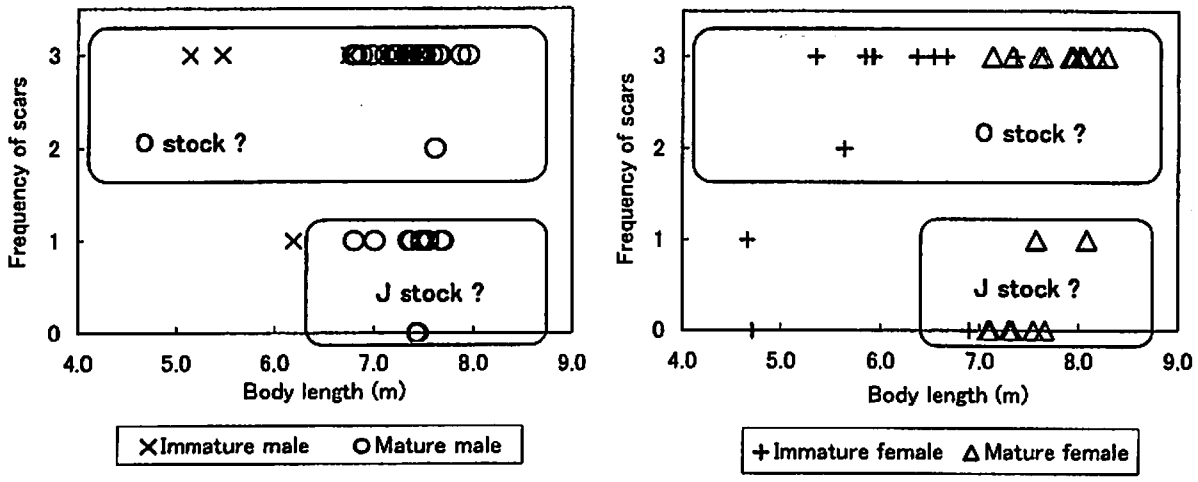


Fig. 2. (Continued).

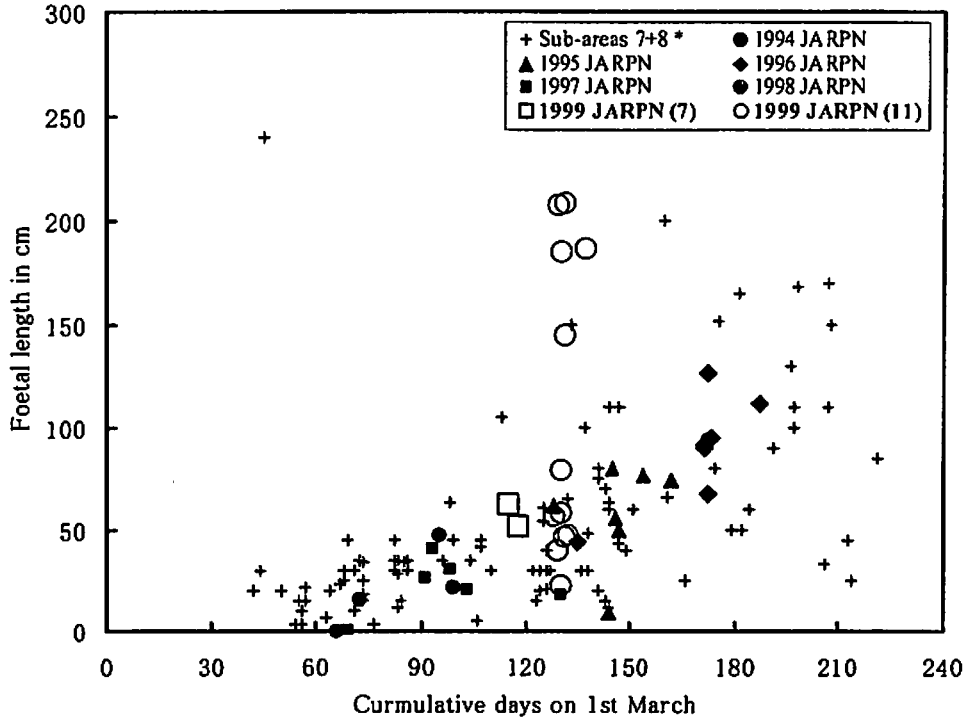


Fig. 3. Relationship between foetal length and sampling date.

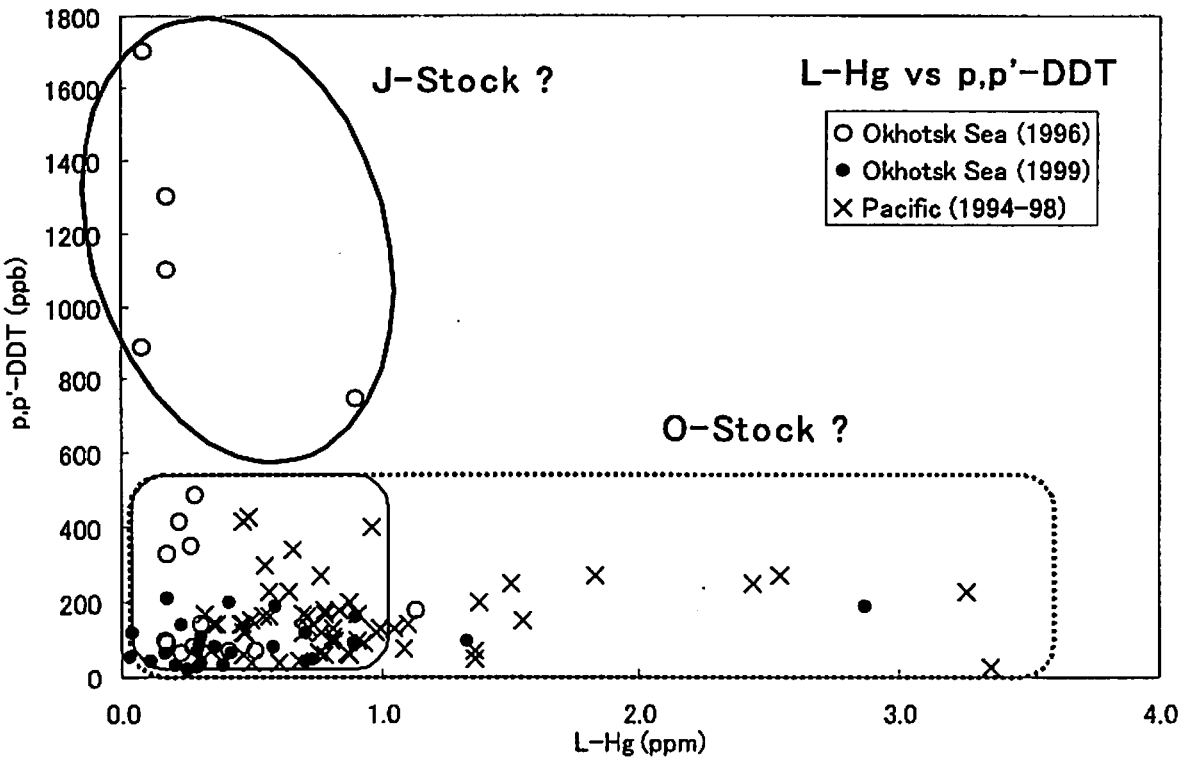


Fig. 4. Schematic diagram of Judgment of stock using relationship between concentrations of Hg in liver and p, p'-DDT in blubber