

Technical Report (not peer reviewed)

Review of biological information of the J stock common minke whale based on genetically identified individuals

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ABSTRACT

The findings from a review of the biological and ecological information of the J stock common minke whale held at the Institute of Cetacean Research are summarized in this paper. Information reviewed included spatial/temporal distribution, morphology, morphometrics, reproductive characteristics, feeding ecology, environmental pollutants, and ecological markers, based on the individuals of this stock identified by genetic markers. Results of this review confirm biological and ecological differentiation of the J stock within the North Pacific common minke whale, and corroborate the view that this stock should be managed independently.

INTRODUCTION

In the western North Pacific, at least two biological stocks of common minke whales *Balaenoptera acutorostrata* are known to exist: the Okhotsk Sea-West Pacific (O stock) and the Sea of Japan-Yellow Sea-East China Sea (J stock) (Omura and Sakiura, 1956; Ohsumi, 1977; 1983). The two stocks are differentiated in morphological and reproductive characteristics (Omura and Sakiura, 1956; Ohsumi, 1977; Kato, 1992), as well in genetics (Wada and Numachi, 1991 for allozymes; Goto and Pastene, 1997 for mtDNA; and Kanda *et al.*, 2009a; b for microsatellites), suggesting their reproductive isolation. The International Whaling Commission (IWC) had proposed some boundaries for these stocks (Donovan, 1991).

Previous genetic studies showed that both stocks mix with each other spatially and temporally in the southern part of the Okhotsk Sea (northern Hokkaido) (Wada, 1991; Pastene *et al.*, 1998). Since then, a substantial number of genetic samples of western North Pacific common minke whales became available, and modern and more powerful genetic markers have been applied to those samples in recent years. The application of such markers to the new samples enabled finer studies on stock structure of this species in this ocean basin (Pastene *et al.*, 2016a; b).

In recent years microsatellite DNA data from a substantial number of genetic samples of common minke whales in the western North Pacific have been used in genetic analyses for individual assignment, for example, by using the program STRUCTURE (Pritchard *et al.*, 2000). Genetic individual identification has allowed the study of several biological and eco-

logical aspects separately for both J and O stocks common minke whales. The objective of this document was to review the biological and ecological information accumulated on the J stock based on these approaches.

INDIVIDUAL IDENTIFICATION BASED ON MICRO-SATELLITE DNA ANALYSES

Microsatellite DNA data and the program STRUCTURE version 2.0 (Pritchard *et al.*, 2000) were used to determine the most likely number of genetically distinct stocks present in the samples of common minke whales in the western North Pacific. The program is a model-based clustering method for inferring stock structure (K , the number of stocks in the model) using multilocus genotype data with and without information on sampling locations. Individual assignment was conducted for the most plausible K using the estimated individual proportion of membership probability. The ancestry model used for the simulation was the admixture model, which assumes individuals may have mixed ancestry. The allele frequency model used was the correlated allele frequencies model, which assumes frequencies in the different stocks are likely to be similar due to migration or shared ancestry.

Further details of the genetic analyses on individual identification of common minke whales can be found in Goto *et al.* (2017).

BIOLOGICAL AND ECOLOGICAL CHARACTERIZATION OF THE J STOCK

Several biological and ecological studies have been conducted based on genetically-identified J stock individuals.

Table 1

Summary of different biological and ecological studies of the J stock common minke whales including focal points, samples used and key references. JARP: Japanese Whale Research Program under Special Permit in the western North Pacific; JARPNI: JARP Phase II; NEWREP-NP: New Scientific Whale Research Program in the western North Pacific.

Focal points	Samples/Years/Sources	References
<u>Distribution and movement</u>		
Spatial distribution	<i>n</i> =4,275 (1994 to 2014), JARP/JARPNI; bycatch	Goto <i>et al.</i> (2017)
Temporal distribution	<i>n</i> =2,522 (2001 to 2014), JARPNI; bycatch	Goto <i>et al.</i> (2017)
Distance from the coastal line	<i>n</i> =986 (1994 to 2007), JARP/JARPNI; bycatch	Kanda <i>et al.</i> (2010)
<u>Morphology and morphometry</u>		
Flipper color pattern	<i>n</i> =220 (2012 and 2013), JARPNI	Nakamura <i>et al.</i> (2016)
Fluke color pattern	<i>n</i> =164 (2007), JARPNI	Nagatsuka (2008; 2010)
Morphometry	<i>n</i> =500 (2000 to 2007), JARP/JARPNI	Hakamada and Bando (2009)
<u>Reproduction</u>		
Conception date	<i>n</i> =107 (1994 to 2007), JARP/JARPNI	Bando <i>et al.</i> (2010a)
<u>Feeding ecology</u>		
Stomach contents	<i>n</i> =742 (1996 to 2018), JARP/JARPNI; NEWREP-NP	Present study
<u>Ecological markers</u>		
Total Hg levels	<i>n</i> =59 (2012 and 2013), JARPNI	Yasunaga and Fujise (2016)
Cookie cutter shark scar	<i>n</i> =1,037 (2002 to 2007), JARP/JARPNI	Bando <i>et al.</i> (2010b)

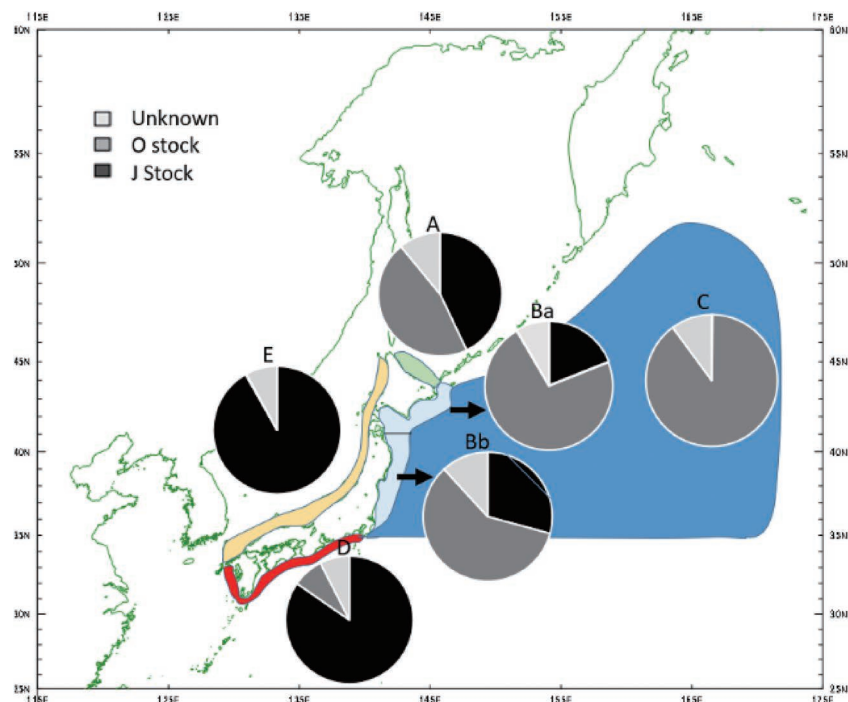


Figure 1. Spatial occurrence of O and J stocks common minke whales in waters around Japan based on genetic individual identification (modified after Goto *et al.*, 2017).

Description of the results of the main focal points are presented in this section (Table 1).

Distribution and movement

Spatial distribution along the Japanese coast

As shown in Figure 1, almost all of the individuals collected from the Sea of Japan side belong to the J stock,

whereas almost all of the individuals from the offshore North Pacific belong to the O stock. The southern part of the Pacific side of Japan was mainly occupied by the J stock. Northern Hokkaido and the northern part of the Pacific side of Japan represent areas where both stocks overlap geographically.

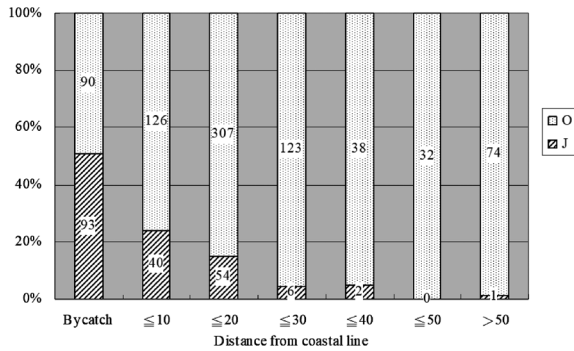


Figure 2. Proportion of the minke whales assigned to the J and O stocks collected from sub-area 7W by the distance from the Japanese coastal line (after Kanda *et al.*, 2010).

Temporal distribution along the Pacific coast of Japan

In the southern part of the Pacific side of Japan, the J stock was predominant throughout the year (around 80% in proportion). In the Northern part of the Pacific side of Japan, the proportion of the J stock increased in autumn/winter and decreased in spring/summer. Conversely, the proportion of O stock decreased in autumn/winter and increased in spring/summer. The fact that the J stock is distributed in the southern part of the Pacific side of Japan throughout the year suggests that the Kuroshio Current—one of the strongest west-boundary currents of the subtropical gyre—is serving as the stock boundary between O and J stocks (Goto *et al.*, 2017).

Distance from the coastal line

In terms of the distance from the Japanese coastal line in the North Pacific side e.g., sub-area 7W (sub-areas Ba and Bb in Figure 1), the proportion of the common minke whales was different between the J and O stocks (Figure 2). The proportion of the J stock whales decreased from coastal areas towards offshore areas. Such a clinal distribution supports the mixing of the two stocks in sub-area 7W.

Migratory routes

The migratory routes to feeding areas of adult and juvenile J stock animals are schematically shown in Figure 3. Migratory routes to breeding areas are assumed to be the reverse in the case of adults. Adult animals were assumed to migrate northward and southward for feeding and breeding, respectively, through the central corridor of the Sea of Japan. The northward migration limit was not clear at this stage because there were no genetic samples available from the central and northern parts of the Okhotsk Sea. In the case of juveniles, it was assumed that they were making short northward and southward

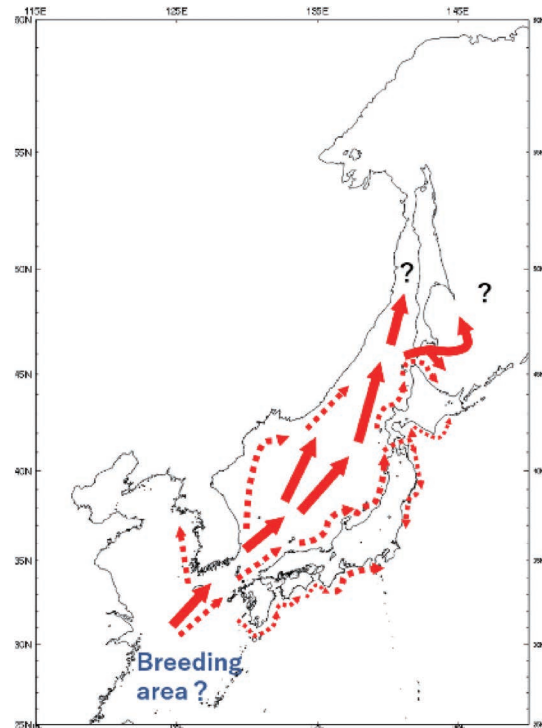


Figure 3. Assumed feeding migration route of J stock animals (modified after Hatanaka and Miyashita, 1997 and Goto *et al.*, 2010). Solid line: mature, dotted line: immature.

migrations along the coastal area for feeding because bycatch juvenile animals were observed throughout the year on the Japanese coast.

Morphology and morphometry

Morphology

Nakamura *et al.* (2016) studied the unique white patch on the flipper of whales sampled by JARPNII during 2012 and 2013 to elucidate stock differences. They focused on the morphological differences in the size and pattern of the white patch on the flipper of each whale. The length of the white patch along the anterior (ventral) margin of the flipper tends to be proportionally smaller in the J stock. The pattern of the boundary area of the white patch named as the ‘Grayish Accessory Layer (GAL)’ was remarkably different between the two stocks (Figure 4). Among animals with ‘no GAL’ type, 94% were J stock. Conversely, 96% of the animals with GAL expanding over the half the flipper width were O stock.

Fluke color pattern

Nagatsuka (2008) found that common minke whales had different black and white patterns on the underside of their flukes. She then separated the sampled whales into three different fluke color types (Figure 5). Differences

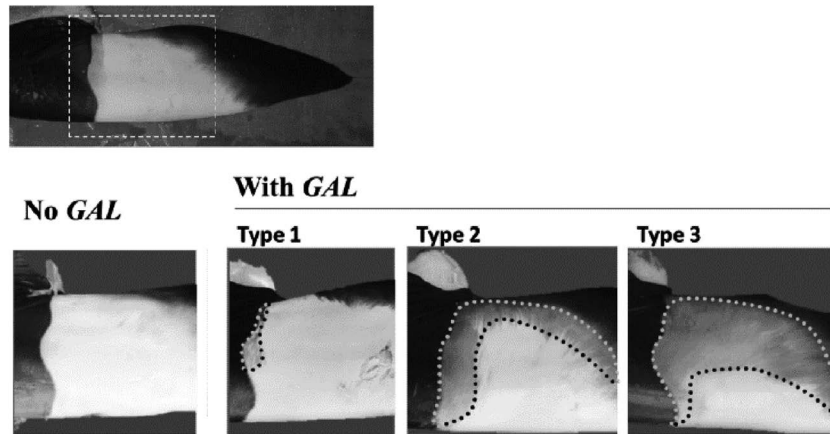


Figure 4. Characteristics of the white patch along the ventral part of the flipper in common minke whales from the western North Pacific. The pictures show the basis for the classification based on the GAL types (GAL: surrounded by dotted line) (after Nakamura *et al.*, 2016).

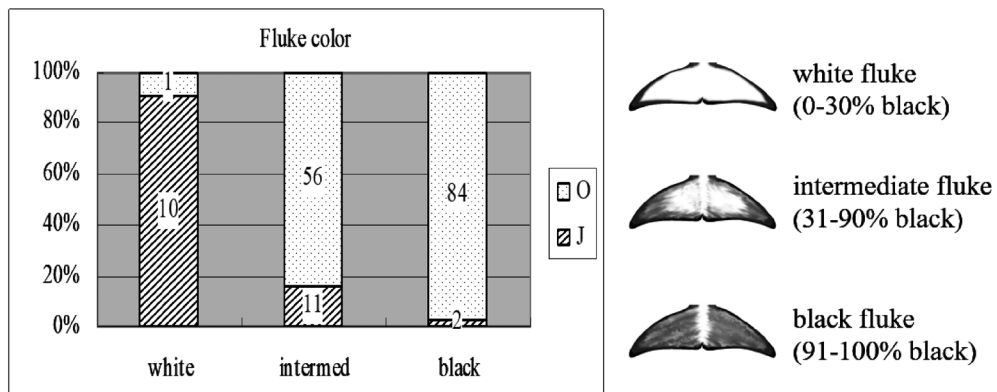


Figure 5. Proportion of the minke whales assigned to J and O stock collected from sub-area 7W by the fluke color pattern, and diagram of the fluke color pattern (after Kanda *et al.*, 2010).

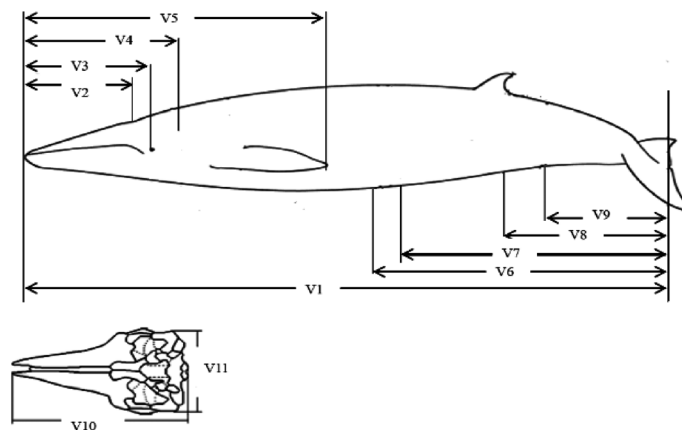


Figure 6. External measurements of western North Pacific common minke whales examined for comparative stock analyses (after Hakamada and Bando, 2009). Measurements V3, V4, V7, V10 and V11 showed significant differences in morphometrics between J and O stocks.

in the frequencies of these three types were observed between the individuals assigned genetically as J and O stocks sampled during the 2007 JARPNII coastal and off-shore surveys (Nagatsuka, 2010). The frequencies of the

three types were recalculated for the samples collected only from sub-area 7W (Figure 5). The frequencies were different between the individuals assigned to J and O stocks ($p < 0.001$).

Morphometry

Hakamada and Fujise (2000) examined the difference between the J and O stocks, using the external measurement data obtained during the 1994–1999 JARPN surveys. Significant differences were observed between the two stocks, especially that J animals exhibited shorter lower body than O stock animals. Hakamada and Bando (2009) examined morphometric data of common minke whales sampled by JARPNII to investigate the differences between J and O stocks (Figure 6). Analysis of covariance (ANCOVA) using body length as a covariate was used to test if there were significant differences in morphometric measurements among the groups compared. Measure-

ments V3, V4, V7, V10 and V11 showed significant differences in morphometrics between J and O stocks.

Reproduction

Conception date

Bando *et al.* (2010a) examined the distribution of conception dates of J and O stock animals collected from the coastal area of western North Pacific (sub-area 7W) and the Okhotsk Sea (sub-area 11) during 1994–2007 JARPN/JARPNII surveys (Figure 7). In sub-area 7W the conception date of the J stock was in August ($n=1$) and January ($n=2$); in sub-area 11 they were distributed between October and March ($n=8$). These results suggested that the

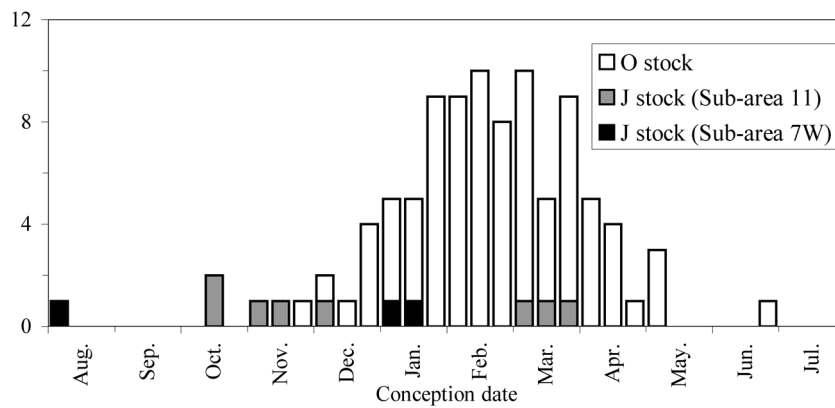
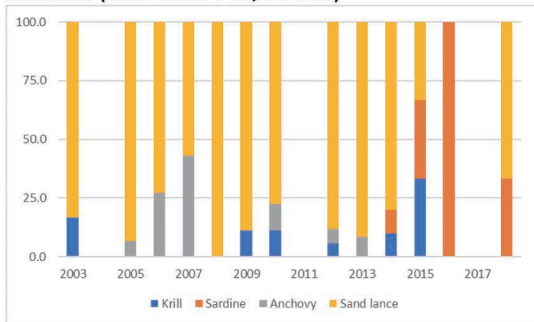
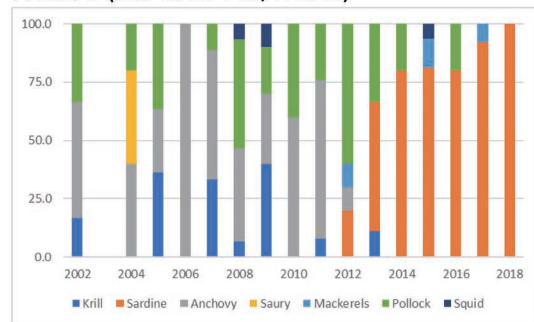


Figure 7. Seasonal distribution of conception dates of the J and O stock common minke whales in ten-day periods (after Bando *et al.*, 2010a).

Sanriku (sub-area 7W, South)



Kushiro (sub-area 7W, North)



Abashiri (sub-area 11)

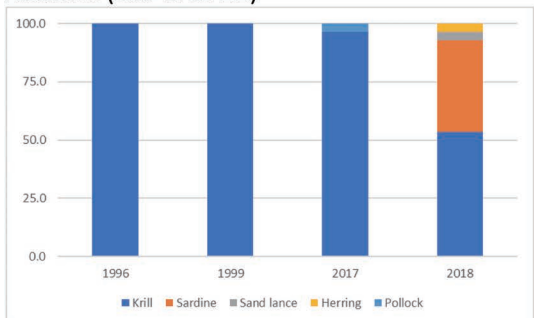


Figure 8. Yearly and geographical change of main prey species of J stock common minke whales.

conception period of the J stock extends from autumn to winter. However, the sample sizes are too small to reach a definitive conclusion.

Feeding ecology

The feeding ecology of common minke whales around Japan has been examined in previous published papers (Kasamatsu and Tanaka, 1992; Tamura and Fujise, 2002; Konishi *et al.*, 2009). However, there have been few published papers specifically on the feeding habits of J stock common minke whales. Results of the analyses of genetically identified J stock individuals showed that these whales fed on various prey species such as Pacific krill (*Euphausia pacifica*), Japanese sand lance (*Ammodytes personatus*), Japanese anchovy (*Engraulis japonicus*), Pacific saury (*Cololabis saira*), walleye pollock (*Gadus chalcogrammus*), and Japanese common squid (*Todarodes pacificus*), and that the main prey species changed both yearly and geographically (Figure 8). These results suggested that J stock common minke whales appear to

be an opportunistic feeder, changing their prey species in response to prey availability.

Environmental pollutant (total Hg levels)

Yasunaga and Fujise (2016) compared the accumulation patterns of total Hg concentrations in muscle and liver between J and O stocks of common minke whales. The analyses were based on 35 O stock and 24 J stock immature animals taken from sub-area 7W in the 2012 and 2013 JARPNII surveys (Figure 9). Multiple linear regression analyses in total Hg concentrations of the whales adjusted for confounders (age index, sex, blubber thickness and year) showed no discernible effect between the two stocks. Results suggested that there is no stock-dependent difference of total Hg and that there is no exposure risk among the common minke whales from the coastal waters of Japan.

Ecological markers

Cookie cutter shark scar

Bando *et al.* (2010b) investigated cookie cutter shark-

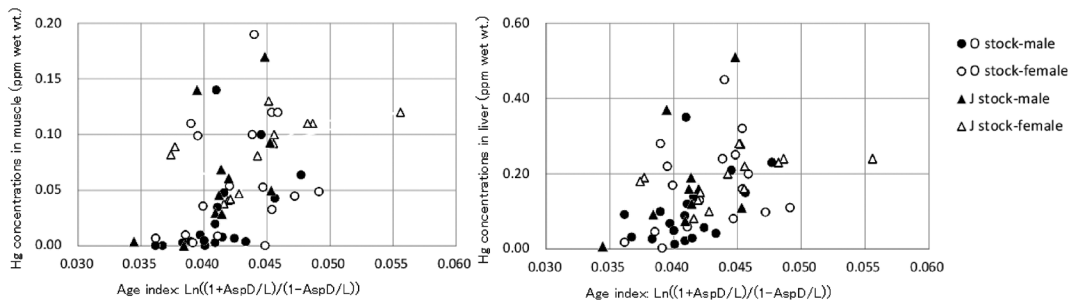


Figure 9. Relationship between Hg concentrations (ppm wet wt.) in muscle in relation to age (left) and Hg concentration in liver in relationship to age (right) in common minke whales from sub-area 7W (after Yasunaga and Fujise, 2016).

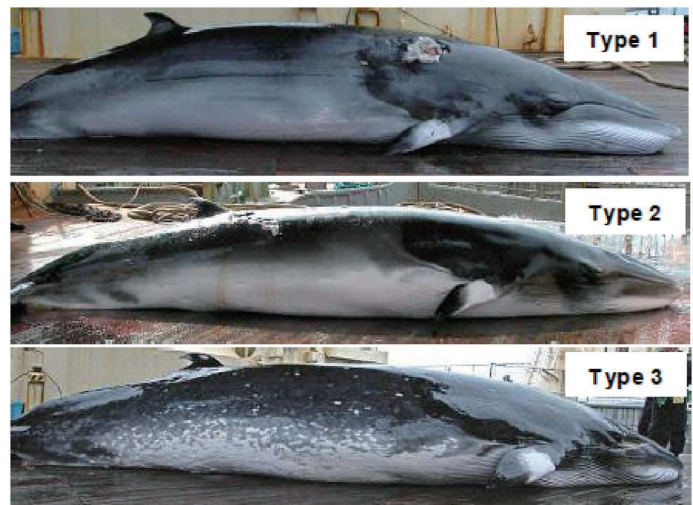


Figure 10. Three types of cookie cutter shark-induced scars in common minke whales. J stock animals had fewer scars than O stock animals.

Table 2

Types of cookie cutter shark-induced scars in common minke whales in the coastal areas of western North Pacific off sub-area 7W (North: off Hokkaido, South: off Sanriku) and Pacific offshore area (sub-areas 8 and 9) (modified from Bando *et al.*, 2010b).

Sub-area	Type of scar	Microsatellite DNA		
		O	?	J
7W North	Type 1 None	0	2	17
	Type 2 1–20 scars	14	5	31
	Type 3 more than 20 scars	291	31	5
	Total	305	38	53
7W South	Type 1 None	0	3	19
	Type 2 1–20 scars	10	5	22
	Type 3 more than 20 scars	213	25	2
	Total	223	33	43
8	Type 1 None	0	0	0
	Type 2 1–20 scars	3	1	0
	Type 3 more than 20 scars	95	11	0
	Total	98	12	0
9	Type 1 None	0	0	0
	Type 2 1–20 scars	4	1	0
	Type 3 more than 20 scars	203	23	1
	Total	207	24	1

induced scars as an ecological marker to determine stock structure in western North Pacific common minke whales collected by JARPNII surveys during 2002–2007. Three types of common minke whales were identified from the density of scars (Figure 10). Prevalence of scars differed clearly between J and O stock animals. However, this ecological marker cannot be considered as an absolute marker to differentiate the two stocks as evidenced in Table 2. J stock animals had fewer scars than O stock animals. In both stocks, prevalence increased with body length, and almost all animals of more than 7 m in body length had scars. Nevertheless, these results were consistent with the occurrence of the two stocks J and O, the former with fewer scars distributing in coastal areas while the latter with more scars distributing in both coastal and offshore areas.

SUMMARY

Table 3 shows a summary of the biological and ecological characteristics of J stock individuals. The individual identification by the genetic markers has been very useful in looking for stock characterization and differences in several traits, such as distribution and movement, morphology and morphometry, reproduction, feeding ecology and environmental pollutant, as presented in this paper. Except for feeding ecology and environmental pollutant (total Hg levels), these traits indicate that J stock individuals are biologically and ecologically distinguished from O

Table 3

Summary of the studies on biological and ecological characteristics of the genetically identified individuals of J stock common minke whales reviewed in this study.

Focal point	Characteristic
Spatial distribution	Whales occupy the Sea of Japan side and the southern part of the Pacific side of Japan; they overlapped geographically with O stock in northern Hokkaido and the northern part of the Pacific side of Japan.
Temporal distribution	Whales are predominant throughout the year in the southern part of the Pacific side of Japan. Their proportion increases in autumn/winter and decreases in spring/summer in the Northern part of the Pacific side of Japan.
Distance from the coastal line	Their proportion decreases from coastal areas towards offshore.
Flipper color pattern	Characterized for almost no GLA in their flippers in comparison with the O stock (Figure 4).
Fluke color pattern	Characterized by a higher proportion of white color in flukes in comparison with the O stock (Figure 5).
Morphometry	Characterized by short lower body in comparison with the O stock.
Conception date	Most likely extends from autumn to winter.
Feeding ecology	Feed on various prey species such as krill, schooling pelagic fishes, and Japanese common squid. They appear to be an opportunistic feeder changing their prey species in response to prey availability in their feeding ground.
Environmental pollutant (total Hg levels)	Levels are similar to those of the O stock animals in the coastal waters of Japan. Levels suggest that the health risk is low.
Cookie cutter shark scar	Fewer scars in comparison with the O stock (Figure 10).

stock individuals. The J stock can be defined as a group of individuals sharing a common gene pool maintained by random mating and should therefore be managed independently.

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