

## Cruise report of the sighting and biopsy sampling survey in the Okhotsk Sea, summer 2010, including individual stock identification of common minke whales

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### ABSTRACT

From 13 July to 26 August in 2010, the common minke whale biopsy sampling survey was conducted in the Okhotsk Sea, using the research vessel *Shonan-maru No. 2*, where the restricted closing mode searching was carried out. The research area was set in the Okhotsk Sea including the Russian EEZ. During the searching of track lines of 1,327.7 n.miles, all the 285 cetacean schools (1,259 individuals) were encountered. These include 38 schools (42 animals) of common minke whales, 59 (93) of fin whales, 3 (4) of North Pacific right whales, and one animal each of sei and sperm whales. Common minke whales were detected mainly at shallow and coastal waters along 200 m isobath. The 24 schools (28 animals) of common minke whales were targeted for biopsy sampling and 12 samples were collected from eight individual whales with two Larsen guns. Stock identification of the animals was tried onboard with the RFLP analysis of mitochondrial DNA control region for the biopsy samples, using two restriction enzymes. From the analysis, seven animals were assigned to O stock and one individual was assigned to J stock. Sex identification was also conducted from amplification of the SRY gene located on Y-chromosome with PCR, which indicated that six of eight animals were male. After the genetic analysis, biopsy samples, extracted DNAs, and PCR products were left at the Russian waters.

KEYWORDS: COMMON MINKE WHALE, BIOPSY SAMPLING, SIGHTING SURVEY, ONBOARD GENETIC ANALYSIS, STOCK IDENTIFICATION, OKHOTSK SEA

### INTRODUCTION

For stock assessment of the Sea of Japan-Yellow Sea-East China Sea stock (J-stock) of common minke whales, a series of sighting surveys has been conducted in the related waters including the Okhotsk Sea (An et al., 2006, 2008, 2009; Iwasaki *et al.*, 1995, 2000; Kim et al., 1999, 2000, 2001, 2002, 2003, 2004, 2005; Miyashita, 2004, 2005, 2006, 2007, 2008; Miyashita and Yoshida, 2003; Sohn et al. 2001). In the Okhotsk Sea, it is known that animals not only of J-stock but also of O-stock (Okhotsk Sea-Western Pacific stock) migrate for feeding in spring and summer (e.g., Goto

and Pastene, 1997). For RMP implementations, abundance estimates in stock by stock basis are requested, and therefore the mixing ratio of two stocks is needed.

Studies on stock identification at individual level have been conducted mainly from genetic analysis (e.g., Goto et al., 2000; Kanda et al., 2009). These studies give us information on mixing ratio of common minke whales in the Okhotsk Sea. In summer 2009, a sighting survey for common minke whales was conducted in the waters, in which biopsy sampling was carried out for genetic analysis (Yoshida, et al., 2010a). Unfortunately, however, all the samples could not be brought out from the Russian waters, because of discrepancies in domestic legal status of common minke whale related to CITES as well as in domestic legal systems regarding international trade between Russia and Japan.

In summer 2010, the common minke whale biopsy sampling survey was conducted in the Okhotsk Sea again, where the restricted closing mode searching was carried out. At the survey, genetic analysis for individual stock identification and additional sex identification was tried on board within the Russian waters. The present report is summary of the cruise. Results of the onboard genetic analysis of biopsy samples are also shown. After the analysis, biopsy samples, extracted DNAs, and PCR products were left at the Russian waters.

## **SURVEY DESIGN AND METHODS**

Research area was set north of 46°N in the Okhotsk Sea, where open sea and the Russian EEZ was included. The following areas were excluded due to no permission: north of 57°N, east of 152°E, and the Russian territorial waters (Fig. 1). The research area was same with it set for the last 2009 survey (Yoshida et al. 2010a). In the research area, three survey blocks were placed and track lines were designed to cover the blocks widely under the zigzag form: all the 15 lines totaling 2371.5 nautical miles were predetermined.

The research vessel, *Shonan-maru No. 2* with a top barrel elevated 20 m above the sea surface was engaged in the survey. The restricted closing mode survey was carried out, in which closing was made only for common minke whales, though opportunistic closing was sometimes carried out for other species. Survey was conducted when the weather conditions were acceptable for searching (Beaufort wind scale  $\leq 4$  and visibility  $\geq 2$  nmi). The daily survey began at 0600 (or 30 min after sunrise) and finished at 1800 (or 30 min before sunset). The vessel followed the predetermined line at 11.5 kts. Two topmen in the barrel of the vessel searched sea surface for cetaceans by naked eyes. Species identification and school counting was conducted using binocular. At the upper-bridge, crews and scientists were also engaged in searching. When common minke whales were encountered, biopsy sampling was carried out, with two Larsen guns.

Genetic analysis for individual stock identification was tried onboard with the RFLP analysis of mitochondrial DNA control region for the biopsy samples, using two restriction enzymes (*Psh* B I and *Hae* III)(see, Yoshida et al. 2010b). The restriction fragment patterns were visualized and then haplotypes were defined. Frequencies of the mtDNA haplotypes from the two restriction enzymes were calculated for 2,300 common minke whales collected in 2001-2007, which were assigned to the either stocks (O or J) from the microsatellite analysis (Kanda *et al.* 2009). Stock identification of individual common minke whales was made from the haplotype frequencies by stock. Additional sex identification was conducted from amplification of the SRY gene located on Y-chromosome with PCR.

## **CRUISE SUMMARY**

### Scientists and observer onboard

The following scientists and observer were onboard:

Scientists:	Shigeru Noji (Chief scientist, NRIFS)
	Aoi Nozawa (Scientist, NRIFS)
Russian observer:	Ivan Istomin (VNIRO)

### Narrative

13 July : The vessel left Shiogama port, Miyagi Prefecture.

14 July : The vessel started the passing mode survey under transit cruise to the Russian check point (E-5).

15 July : The vessel entered the Russian EEZ at 14:00 and passed the check point (E-5) at 20:40.

16 July : The vessel continued the passing mode survey under transit cruise to the way point No. 1a.

17 July : The vessel continued the passing mode survey, but strong wind prevented the vessel from searching.

18 July : The vessel arrived at the way point of No. 1a, then drifted throughout the day from low visibility.

19 July : The vessel started the restricted closing mode survey, but often drifted from bad weather condition.

20 July : Low visibility from heavy rain sometimes prevented the vessel from searching. A biopsy sample was collected.

21 July : The vessel passed the way point of No. 2b. A dense fog sometimes disturbed the vessel, but weather was generally good.

22 July : The vessel passed the way points of No. 3a and 3b. The vessel often drifted from low visibility.

23 July : A dense fog sometimes prevented the vessel from searching, but weather was generally good.

24 July : The vessel passed the way point of No. 4. The vessel often sailed under top down from bad weather conditions.

25 July : Weather conditions were not so good throughout the day. The vessel often sailed under top down.

26 July : The vessel passed the way points of No. 5a and 5b. The vessel could conduct searching throughout the day.

- 27 July : The vessel could conduct searching throughout the day, under good weather conditions. The vessel passed the way point of No. 6. Two biopsy samples were collected.
- 28 July : The vessel could conduct searching throughout the day. The vessel arrived at the way point of No. 7 at 13:34, then started transit cruise to the way point of No. 13 in Block SW. Three biopsy samples were obtained.
- 29 July : The vessel arrived at the way point of No. 13 at 09:24, then started the restricted closing mode survey in Block SW. The vessel passed the way points of No. 14, then drifted from rough sea. Two biopsy samples were collected.
- 30 July : The vessel could not conduct the survey throughout the day, from stormy weather.
- 31 July: From a dense fog, the vessel often sailed under top down, then drifted throughout the day. The first half of the survey was finished. The vessel started the transit cruise to Abashiri at 18:00.
- 1 August : On the way to Abashiri, the vessel sailed without searching from rough sea.
- 2 August : The vessel continued transit cruise to Abashiri. All the 9 biopsy samples collected were discarded to the sea in the Russian EEZ, under the observation of the captain, Russian observer, and scientists. Then, the vessel passed the Russian check point (E-5) and went out of the Russian EEZ at 19:43.
- 3 August : The vessel entered the Abashiri port at 08:00.
- 4 August : Refueling at the Abashiri port.
- 5 August : The vessel left the Abashiri port at 08:40, then conducted transit cruise. The vessel entered the Russian EEZ at 16:00, passed the check point (E-5) at 21:00, and arrived at the way point of No. 18.
- 6 August : The vessel drifted throughout the day, from rough sea.
- 7 August : In the morning, sea was rough. So, the vessel started top down cruise to the way point of No. 17a. After the weather conditions recovered, the vessel started the survey to the way point of No. 16.
- 8 August : Throughout the day, the visibility was low. The vessel sailed under top down to the way point of No. 16, then drifted.
- 9 August : The vessel conducted the survey, but often sailed under top down from low visibility.
- 10 August : In the morning, the vessel drifted from low visibility, then started the survey after the weather recovered.
- 11 August : The vessel drifted throughout the day from rain.
- 12 August : The vessel drifted throughout the day from rough sea.
- 13 August : The vessel conducted the survey, but often drifted from rough sea.
- 14 August : The vessel conducted the survey, under good weather. The vessel passed the way point of No. 15. Two biopsy samples were collected.
- 15 August : The wind was a bit strong. But, the vessel could conducted the survey. At 14:42, the vessel arrive at the point, at which the first half of the survey was finished. Then, the vessel started transit cruise to the way point of No. 8 in Block SE.

- 16 August : The vessel started the survey from the way point of No. 8 at 06:00, but soon later stopped searching from rough sea.
- 17-21 August : Dense fog and strong wind often prevented the vessel from searching. The vessel passed the way points of No. 9 on 17th, No. 10 on 19th, and No. 11 on 21st.
- 22 August : Dense fog often prevented the vessel from searching. The vessel arrived at the way point of No. 12 at 13:50, then started the transit cruise to Shiogama.
- 23 August : On the transit cruise to Shiogama, the vessel conducted the closing mode survey on a track line between way points of 17 and 18, where searching was not conducted from bad weather. A biopsy samples was collected there. After the genetic analysis, all the three biopsy samples collected during the second half of the survey were discarded to the sea in the Russian EEZ, under the observation of the captain, Russian observer, and scientists. Then, the vessel passed the Russian check point (E-5) and went out of the Russian EEZ at 18:37.
- 24 August : The vessel conducted passing mode survey on the transit cruise to Shiogama.
- 25 August : The vessel sailed to Shiogama, without searching effort.
- 26 August : The vessel arrived at Shiogama port at 08:15.

### Survey results

All the 1,327.7 n. miles of track lines was searched under the primary searching mode, which was 56.0% of the predetermined lines. Unfortunately, most of predetermined track lines set in offshore block SE was not searched from bad weather conditions and a dense fog (see, Fig. 2).

During the searching including additional transit cruise, all the 285 cetacean schools (1,259 individuals) were encountered. These include 38 schools (42 animals) of common minke whales, 59 (93) of fin whales, 3 (4) of North Pacific right whales, and one animal each of sei and sperm whales (Table 1). Common minke whales were sighted mainly at shallow and coastal waters of around 200 m depth (Fig. 2). Fin whales were detected at deeper and offshore waters than common minke whales (Fig. 3). North Pacific right whales were encountered on three occasions (Fig. 4). In the last 2009 cruise, most of 17 sightings of this species were recorded in offshore waters. In the present cruise, bad weather conditions and a dense fog prevented the vessel from searching in offshore waters, which may have resulted in only three sightings of North Pacific right whales. Three animals of two right whale schools were targeted for photo-id research. Examination of digital images of head (callosities and lip patches) of the animals indicated no re-sightings.

The 25 common minke whale schools (29 individuals) were targeted for biopsy sampling. Two Larsen guns were used. The 30 darts were launched at 11 whales and 12 biopsy samples were collected from eight individuals (see, Table 2, Fig. 5). The biopsy samples collected were analyzed onboard. Seven animals were assigned to O stock with probability of 58.2-98.4% and one individual was assigned to J stock with 92% (Table 2; see, Yoshida et al. 2010b). The animal assigned to the J stock was detected at southern part of the Okhotsk Sea on August 23 (Table 2, Fig. 5). From additional sex identification, six of the eight animals were identified as male. After the analyses, biopsy samples, extracted DNAs, and PCR products were left at the Russian waters.

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We express our sincere thanks to the captain and crew of *Shonan-maru No. 2* for their hard work and providing an excellent survey platform during the cruise. We especially thank Shigeru Noji and Aoi Nozawa for his excellent work. We also express our sincere appreciation to the Russian Government for issuing the permission for the present survey in the Russian waters. We gratefully thank to Toshinori Uoya and Hiroko Furukawa of the Fisheries Agency of Ministry of Agriculture, Forestry and Fisheries, Japan for their help and arrangement for the cruise. We acknowledge Luis A. Pastene, and Mutsuo Goto of the Institute of Cetacean Research, and Toshie Wakabayashi and Takashi Yanagimoto of the National Research Institute of Far Seas Fisheries, for their valuable advises and suggestions for genetic analysis.

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Table 1. Sightings by *Shonan-maru No. 2* in the common minke whale biopsy sampling survey in the Okhotsk Sea, summer 2010.

Species	Research area				Transit				Total			
	Primary		Secondary		Primary		Secondary		Primary		Secondary	
	Sch.	Animal	Sch.	Animal	Sch.	Animal	Sch.	Animal	Sch.	Animal	Sch.	Animal
Common minke whale	20	21	4	5	10	10	4	6	30	31	8	11
Fin whale	41	62	5	8	4	5	9	18	45	67	14	26
Sei whale	0	0	0	0	1	1	0	0	1	1	0	0
North Pacific right whale	2	3	1	1	0	0	0	0	2	3	1	1
Sperm whale	1	1	0	0	0	0	0	0	1	1	0	0



Table 2. Results of stock identification of eight common minke whales, from mtDNA restriction fragment patterns. ID numbers are attached to each of individual whales, from which biopsy samples were taken. Stock identification was made from haplotype frequencies calculated for 2,300 common minke whales collected in 2001-2007, which were assigned to the either stocks (O or J) by microsatellite analysis (Kanda *et al.* 2009). Results of additional sex identification and estimated body length (m) are also shown.

ID number	Sampling date	Sex	Stock identification			Estimated body length
			<i>Psh</i> B I	<i>Hae</i> III	<i>Psh</i> B I & <i>Hae</i> III	
1	2010.7.20	F	J (61.8)	O (82.0)	O (58.2)	7.3
2	2010.7.27	M	O (98.1)	O (82.0)	O (98.4)	8.1
3	2010.7.27	M	O (98.1)	O (82.0)	O (98.4)	7.6
4	2010.7.28	M	O (98.1)	O (82.0)	O (98.4)	7.6
5	2010.7.28	M	O (98.1)	O (82.0)	O (98.4)	8.0
6	2010.7.29	M	J (61.8)	O (82.0)	O (58.2)	6.8
7	2010.8.14	F	O (98.1)	O (82.0)	O (98.4)	7.6
8	2010.8.23	M	J (61.8)	J (87.4)	J (92.0)	6.8

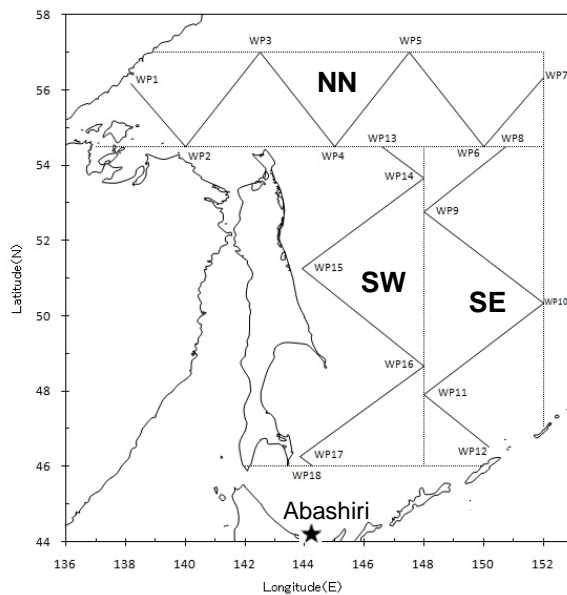


Figure 1. Survey blocks and predetermined track lines set in research area, for the 2010 common minke whale biopsy sampling survey.

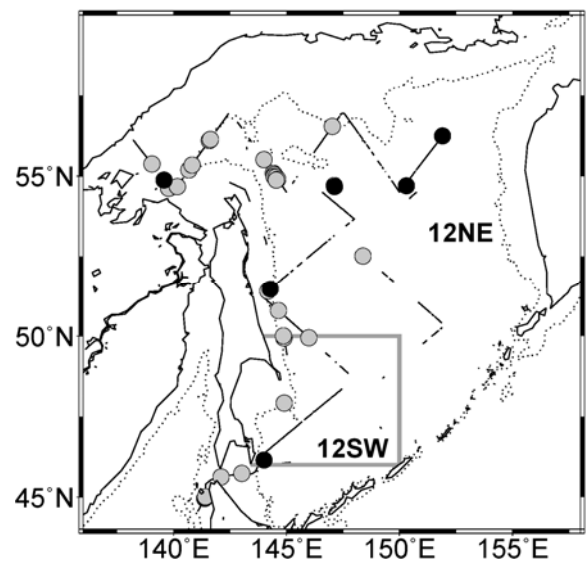


Figure 2. Survey tracks and sighting positions of common minke whale schools made during the survey (gray circle: primary sighting; black circle: secondary sighting). Isobath is at 200 m depth.

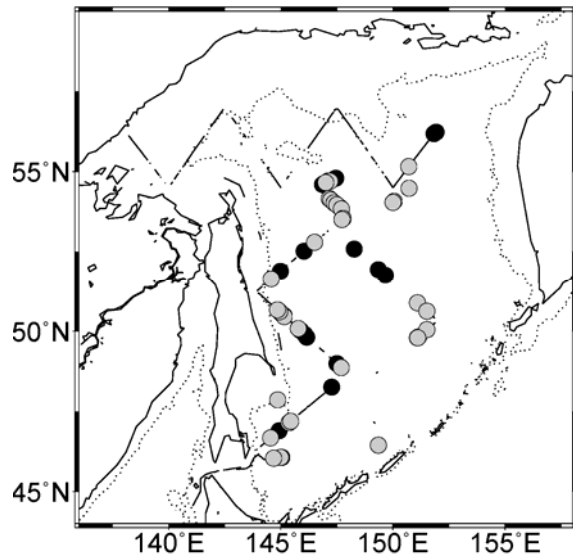


Figure 3. Survey tracks and sighting positions of fin whale schools made during the survey (gray circle: primary sighting; black circle: secondary sighting). Isobath is at 200 m depth.

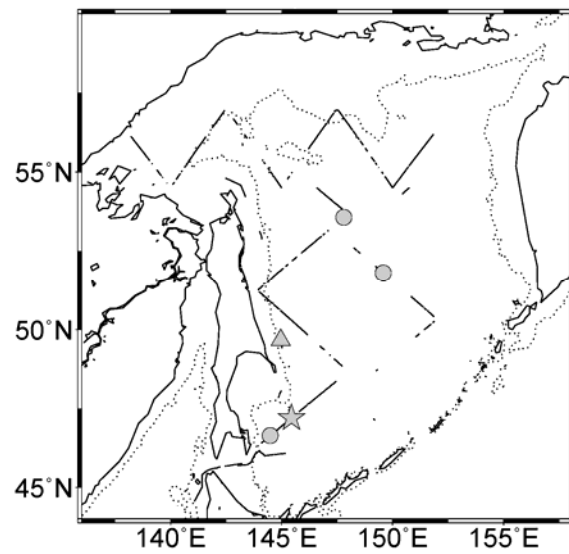


Figure 4. Survey tracks and sighting positions of schools of North Pacific right whale (gray circle), sperm whale (gray star), and sei whale (gray triangle). Isobath is at 200 m depth.

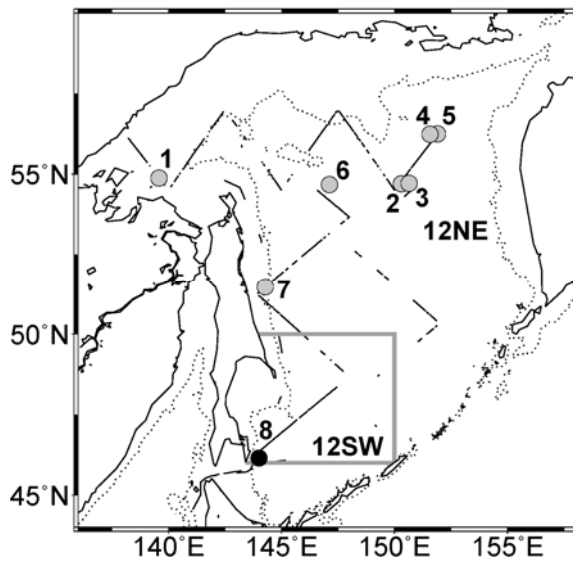


Figure 5. Sighting positions of eight common minke whales, from which biopsy samples were taken. Gray and black circles indicate animals assigned to O and J stocks from genetic analysis, respectively. For ID number attached to each of animals, see Table 2.