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INTERNATIONAL WHALING COMMISSION

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ABSTRACT

This paper reports the results of biological sampling of the Antarctic minke whale during the second New Scientific Whale Research Program in the Antarctic Ocean (NEWREP-A) survey conducted in Area III-E (45°E-70°E) and IV (70°E-130°E), south of 60°S during the 2016/17 austral summer season. The paper also presents the results of the sighting surveys and photo-ID and biopsy sampling of large whales by the sighting sampling vessels (SSVs). Three SSVs and one research base vessel were engaged in the survey for 83 days. A total of 311 primary sightings (involving 526 individuals) of Antarctic minke whale were made during 3,274 n.miles of searching distance. A total of 333 Antarctic minke whales (178 females and 155 males) were sampled, and a number of biological samples and data required for the two main objectives of NEWREP-A were obtained from each whale taken. Earplugs for age determination were collected from all whales. A total of 24 whales were photo-identified: humpback whale (20) and killer whale (4) in the research area. A total of four biopsy samples of humpback whale were collected from photo-identified individuals. The samples and data collected in this survey are available for interested national and international scientists under the guidelines for research collaboration posted at the home page of the Institute of Cetacean Research (ICR): http://www.icrwhale.org/NEWREP-AProtocol.html.

KEYWORDS: SCIENTIFIC PERMITS; ANTARCTIC; FEEDING GROUNDS; ANTARCTIC MINKE WHALE; BIOPSY SAMPLING; PHOTO-ID

INTRODUCTION

The survey of the New Scientific Whale Research Program in the Antarctic Ocean (NEWREP-A) was started in the 2015/16 austral summer season, after the review of the research plan was completed by the International Whaling Commission Scientific Committee (IWC SC) following the guidelines in Annex P (IWC, 2015a; see GOJ, 2016 and Matsuoka *et al*., 2016).

Research under NEWREP-A requires the collection of various types of samples and data which are important for addressing the main objectives I and II of the program. For example, under objective I (ii) samples and data for obtaining the age, sexual maturity and body length of the whales are required. Under objective I (iii) data and samples for studying morphometric, morphological and genetic differences among whales are required. All that information together with other obtained by non-lethal means (e.g. abundance under objective I (i)) is necessary for the specifications of RMP *ISTs* for Antarctic minke whales (Objective I (iv)).

Under objective II (iii), stomach contents of the whales are required to estimate prey composition and consumption by Antarctic minke whales; and blubber thickness, fat weight and girth data are required to study the nutritional condition of the whales. All that information together with other obtained by non-lethal means (e.g. whale abundance under objective II (ii) and krill biomass and oceanographic information under objective II (i)) is necessary for ecosystem modelling work (Objective II (iv)).

Age data at the annual scale is required by the Statistical Catch-at-Age Analysis (SCAA) under objective I (ii). Age information can be obtained only from internal earplugs and therefore only through lethal sampling methods. The NEWREP-A review workshop agreed that at present, the technique commonly used for the determination of the biological parameters used in the SCAA model require earplug for age determination (IWC, 2015b).

Calculation of sample size of Antarctic minke whale in NEWREP-A was based on the biological parameter Age at Sexual Maturity (ASM). ASM is of great importance not only for contributing information on the proportion of matured animals in the SCAA (related to the main objective I) but also as an important indicator of changes in the nutritional condition of the whale population (related to main objective II). The age-at-50% sexual maturity (ASM 50) was used to set the annual sample size of 333 Antarctic minke whales (see GOJ, 2015 for details).

The second multidiscipline survey of the NEWREP-A was conducted in Areas III-E (45°E-70°E), IV and V-W during the austral summer season 2016/17. The report of the dedicated sighting survey is presented by Isoda *et al*. (2017) and that of the krill and oceanographic survey is presented by Wada *et al.* (2017).

The objective of this paper is to present the results of the biological survey of 333 Antarctic minke whales sampled during the field survey of NEWREP-A in Area III-E and IV. Sighting data obtained by the Sighting Sampling Vessels (SSVs) are also presented in this paper.

SURVEY DESIGN

Research area

The sampling survey was set south of 60°S in Area IV. The area was divided into the East and West sector at 100°E. Each sector was divided into the South and North strata. The boundary between the South and North strata in the sector was defined by a line 45 n.miles from the ice-edge. The southern boundary of the West-south stratum between 70°E and 80°E was fixed at 66°S and the Prydz Bay was defined as the southern area of this boundary (Figure 1). Estimated pack-ice line (ice-edge) was obtained from direct observation from the vessels, from the Defense Meteorological Satellite Program (DMSP; Maslanik and Stroeve, 1999) and from the Advanced Microwave Scanning Radiometer 2 (AMSR2; JAXA, 2016).

Research vessels

Three research vessels *Yushin-Maru* (*YS1*), *Yushin-Maru No.2* (*YS2*) and *Yushin-Maru No.3* (*YS3*) were engaged in sighting and sampling (SSVs). They were equipped with a top barrel platform (TOP), upper bridge platform (UBP), and a whaling cannon. Biological research of the whales sampled was carried out on board the research base vessel, *Nisshin-Maru* (*NM*). One researcher was on board each *YS1*, *YS2* and *YS3*. A total of eighteen researchers, included the cruise leader and vice cruise leader, were on board the *NM*. Specifications of the research vessels were shown in Table 1 and Figure 2.

Backup plan for contingencies

As research activities could be disrupted by both natural and human factors including dangerous sabotage activities by an extreme anti-whaling NGO, the research has a contingency backup plan (GOJ, 2015). The backup plan would address three aspects; (i) adjustments of research protocols at the scene of disruption, (ii) adjustment of research plans including research period, sample size, and research areas after the year of disruption, and (iii) consideration of analysis methods to compensate the effects of disruptions. This plan was in response to the recommendation made at the Expert Workshop to Review the Japanese JARPA II Special Permit Research Programme (IWC, 2014).

Cruise track-line

Survey courses were established in offshore and ice edge waters of the research area by the line transect method. Two SSVs advanced along parallel track-lines 7 n.miles apart (main course and sub-courses). Each of the SSVs changed the track-line order every day to avoid possible sighting bias by fixed position. Starting point of the day was set at the position where one of the vessels ended the surveys on the previous day in the most advanced position. The other vessel moved to the starting position of the next day after the end of the daily survey.

The predetermined track-line of the sampling survey was shown in Figure 3a. Track-line for each vessel consisted of two legs in the northern stratum at 3°20' longitudinal degree intervals, and six legs in the southern stratum at 1°40' longitudinal degree intervals in a 10° longitudinal band in the research area (Nishiwaki *et al*., 2014) except for the Prydz Bay. A latitudinal zig-zag line was set for the Prydz Bay. Track-lines were decided based on the original longitude line, which was selected at random in the western and eastern part of the stratum, respectively. The interval of legs and number of legs in each stratum could be changed in consideration of delay caused by bad weather conditions and other factors. Survey course angle and distances were calculated using a Tamaya Navigator NC-2100G (Ver.1.2.1; Tamaya Instrument Co., Japan).

Sighting protocols

Survey mode

Survey mode of sampling survey for Antarctic minke whale was conducted using Normal Closing mode (NSC). Sighting activities of the survey mode were classified into two types according to 'On-effort' or 'Off-effort'. Both survey activities followed the protocol endorsed for the IWC/SOWER surveys (e.g. Matsuoka *et al*., 2003).

Sighting protocol

Sighting protocols were the same as those in IDCR/SOWER. Research effort began 60 minutes after sunrise and ended 60 minutes before sunset, with a maximum 12 hours per day (approximately 06:00–18:00). Time-zone change was recorded at 30 minute intervals, effective from 01:00h.

Time was adhered to local 'ship' time ranging between +3.0 and +9.0 GMT. Data collected throughout the survey and all associated reporting were in accord with the local 'vessel' time. Searching activity was conducted when the weather conditions were suitable for observations: minke whale visibility better than 1.5 n.miles and the wind speed less than 21 knots (northern strata) or 26 knots (southern strata). Vessel speed during the sighting survey was 11.5 knots with slight adjustment to avoid vibration of the vessels. Sighting effort was conducted by the boatswain and top-men from the TOP (there were always two primary observers in the TOP) and the upper bridge where the helmsman, captain or officer-on-watch, researchers, and the chief engineer (or second engineer) were also present.

Experiment

Preliminary sighting distance and angle experiment was examined in each SSV for evaluating the accuracy of sighting distance and angle given by observers. Observers on each vessel were required to assess eight sets of angles and distance estimation from two platforms (TOP and UBP). The experiment was conducted using a buoy with a radar reflecting transponder, using angle board and scope (7x) equipped with estimate scales on the eye lens. All trials were conducted under good sighting conditions.

Whale sampling

The target sample size for Antarctic minke whales was 333 animals (GOJ, 2015). Whales were sampled using a random sampling method (Kato *et al*., 1989). One or two whales were sampled randomly from each primary sighted school using harpoon with 30g penthrite grenade. Sampled whales were immediately transported to the research base vessel, where biological measurements and biological sampling were carried out.

Biological measurements and sampling

Morphometric and body weight

After photographing the lateral side of each whale, a series of standard measurements was taken, including body length to the nearest 1cm and body proportion at seven different points (to the nearest 1cm). Skull measurements (length and greatest breadth to the nearest 0.5cm were taken for most whales using a large pair of vernier calipers. Measurements of blubber thickness were taken at two points on the lateral side of the body. Girth dimensions were taken from all animal sampled. Body weights of each whale were measured using an electronic track scale (EDP-1801 and EDI-801, Yamato Scale Co., Ltd., Japan) on board the *NM* to the nearest 0.01tons. Body parts and organs weight of six whales were weighted for studying energy storage, using an electronic hanging scale (Kubota) and a marine scale (M1100 and S-182, Marel, Iceland).

Total fat weight

Blubber weight of 35whales was measured for assessment of nutritional condition using an electronic hanging scale and a marine scale.

Definition of sexual maturity

The maturity of the females was determined by the presence of at least one corpus luteum or corpus albicans in either ovary. In the case where no corpus luteum or corpus albicans was observed, the female was categorized as immature. The definition of male sexual maturity was defined preliminary based on the weight of one testis. If the testis was over 0.4kg, the whale was determined as sexually mature (Kato, 1986). Reproductive status of female

whales was classified into four categories (ovulating, pregnant, resting and pregnant and lactating), based upon observation of the ovary, uterus, and mammary gland. Pregnancy of the animal was defined based on conceptus with placental development in the uterus. Body length and weight of foetus was measured in the same way as in adult whales. Sex of the foetus was classified into three categories (female, male or sex unidentified).

Earplug sampling

Left and right earplugs were collected for age determination by the routine procedure (Omura, 1963). After removing the mandibles, the proximal part of the earplug was exposed along the surrounding the external part of the ear canal from the tympanic bulla using a knife, for subsequent incision. The external part of the ear canal was carefully cut open so as not to incise the earplug, and then the earplug was collected with glove-finger using a scalpel. Earplugs were fixed and stored in 10% formalin solution. As a supplement for age determination studies, the largest baleen plates were collected from whales of either sex smaller than 7.0m long. Skin tissue samples were collected from 7 different points of body surface and stored at -80°C for an age-determining study based on DNA methylation.

Stomach contents

Conventional stomach content records were obtained from all sampled whales. Prey species from the fore- and main-stomach contents are weighed for each whale sampled (319 whales) and stored at −20°C or in 10% formalin solution (51 whales).

Other biological samples

Ocular lenses of each adult whale and foetuses with body length less than 10cm were collected, and stored at −80°C for age estimation purposes. Blubber samples were collected at seven different points from six specimen for feasibility study of the age determination method based on DNA methylation. Ovary, mammary gland and endometrium from female animals were dissected from the uterus and stored at −20°C for reproductive study. Tissue samples of testis from male animals for the histological observation were collected and fixed using 10% formalin solution. After measurements of blubber thickness (two points), blubber samples were taken from all specimen for the study of feeding ecology. Muscle and liver samples were sampled and stored at −20°C for pollutant studies. Skin samples were collected and fixed in ethanol solution (70%) for genetic studies.

RESULTS AND DISCUSSION

Narrative of the cruise

YS1 and *YS2* departed Shimonoseki (Japan) on 18 November 2016. *YS3* departed Shiogama (Japan) on 17 November 2016 for dedicated sighting survey. *NM* departed from Innoshima (Japan). During transit from Japan to the research area, the sighting surveys were conducted from 2 December 2016 to 11 December 2016 in the area between 20°S and 60°S outside of national EEZs. As for sampling survey, three SSVs and one research base vessel were engaged in the survey for 83 days. A total of 311 primary sightings (involving 526 individuals) of Antarctic minke whale were made during 3,274 n.miles of searching distance. A total of 333 Antarctic minke whales (178 females and 155 males) were sampled, and a number of biological samples and data required for the two main objectives of NEWREP-A were obtained from each whale taken. A contingency backup plan has been applied for this research cruise as a result of a countermeasure against dangerous sabotage activities by an anti-whaling NGO. As a backup plan, a third SSVs, YS3, conducted biological sampling survey from 24 January to 7 March in 2017, and the research area was reconfigured for Area III-E (45°E-70°E) and IV (70°E-130°E), south of 60°S. The total research period of the sampling survey was 83 days from 15 December 2016 to 7 March 2017. SSVs and *NM* arrived at Shimonoseki on 31 March 2017.

Area coverage

Figure 3b shows the main cruise tracks of the SSVs. The searching distance of the SSVs in each stratum is shown in the Appendix. The total searching distance during the 83-days research period was 8,603 n.miles. The percentage of actual coverage in the whole research area was 22.4% (main course). The actual coverage was less than half compared with predetermined track-line due to bad weather, especially in the northern part of the research

area and anti-whaling NGO in Area IV. The survey in the sector 90°E to 100°E and almost all Prydz Bay area of Area IV was cancelled to avoid meeting vessels of anti-whaling NGO.

Geographical distribution of sampled whales

A total of 333 Antarctic minke whales were caught from a total of 311 primary sightings (involving 525 individuals). All whales were sampled in a random manner. Geographical distribution of sighting and sampling were shown in Figure 3c and 3d, respectively. Of the total, 178 whales were females and 155 males (male sex ratio was 0.47). Table 2 show the mean body length and weight of the sampled whales, by sex, reproductive status and stratum.

Sampling efficiency

A total of 333 individuals were sampled from 301 targeted school (340 individuals). The technical sampling efficiency (number of whales sampled per number of individuals targeted) was 0.98.The main reason for missing a targeted whales (7 cases) was the quick movement of the whale (4 cases out of 7). Two struck and lost cases occurred.

Biological measurements and sampling

External measurements

Table 3 summarizes the information on external body proportions of the sampled whales, and provides the percentage of each measurement in relation to body length. The female and male external body measurements had nearly identical percentages. Figure 5 shows the body length distribution of the sampled whales, by sex. The maximum body length of males and females was 9.41m and 10.27m, respectively. The range for males was 4.72–9.41m and for females 5.12–10.27m.

Body parts weight of sampled whale

Six whales of body parts and organ weights (kg) was measured. A total of 35 whales of total fat weight were weighted. Table 4 shows the number of whales total fat weight measured from the sampled whales by sex.

Sexual maturity of sampled whale

Table 2 shows that mature animals were dominant in the East-South stratum of Area III-E, which is similar to results of previous surveys. Immature animals were more frequent (69.8%) in the North strata. Maturity rate of both sexes were high (74.2 % in female and 74.8 % in male) in the South strata. Two lactating females with pregnancy were sampled in the East-North (17 and 18 December 2016).

The occurrence of lactating females was recorded in the early period of the survey, as in previous surveys. Secretion was observed in the mammary glands of the early-pregnant animals. These observations suggested that pregnant females migrated to the feeding ground after parturition. The animal with maximum testicular weight was also observed in the early period (December). These results reflect seasonal changes in the reproductive activity of the adult Antarctic minke whales.

Stomach contents

A total of 319 stomachs were examined (in 14 cases the both fore- and main stomach were broken). Table 5 shows the frequency of appearance of dominant prey species in the forestomach, by strata. Antarctic krill (*Euphausia superba*) was the dominant prey species (51.4% of the whales examined) in Area III-E and IV. In the southern part of Area III-E, south of 76°S, the dominant prey species was ice krill (*Euphausia crystallorophias*). *Thysanoessa*. *Sp*. was found in mainly northern part in Area IV.

Other sampling

Age information is important for the main objective 1 of NEWREP-A. During the survey earplugs were collected from all animals sampled. Eye lenses were also collected for the purpose of age determination based on the ratio of aspartic acid enantiomers in the lens nucleus. To conduct a feasibility study of the age determination method based on DNA methylation, a total of six tissue samples were collected from the sampled whales.

Some other samples collected such as baleen plate, prey species from stomach contents, blood, muscle, and biopsy skin would be useful for analysis of stable isotopes analyses to estimate the duration of Antarctic minke whale in the feeding grounds.

Data and samples obtained from this survey will be validated and stored at the Institute of Cetacean Research (ICR), Japan, and they are available to national (Japan) and international scientists under established guidelines (see http://www.icrwhale.org/NEWREP-AProtocol.html). Catch data record will be submitted to the IWC secretary, as in the previous surveys.

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Table 1
Specifications of the research vessels.

	Yushin-Maru	*Yushin-Maru No.2*	*Yushin-Maru No.3*	*Nisshin-Maru*
Call sign	JLZS	JPPV	7JCH	JJCJ
Length overall [m]	69.61	69.61	69.61	129.58
Molded breadth [m]	10.4	10.8	10.8	19.4
Gross tonnage [GT]	724	747	742	8,145
Barrel height [m]	19.5	19.5	19.5	–
Barrel height (IO) [m]	13.5	13.5	13.5	–
Upper bridge height [m]	11.5	11.5	11.5	–
Bow height [m]	6.5	6.5	6.5	–
Engine power [PS/ kW]	5,280/ 3,900	5,280/ 3,900	5,280/ 3,900	7,320/ 5,383
Captain	Nobuo Abe	Chikamasa Ohkoshi	Hidenori Kasai	Hiroshi Eguchi

Table 2
Mean (±SD) body length and weight of Antarctic minke whale samples obtained in the NEWREP-A 2016/17.

	Stratum	Sex	Sexual Maturity	Reproductive status	N	Body length (Mean±SD)	Body weight (Mean±SD)
Area III	East-South	Male	Immature		8	6.97±1.57	4.42±2.54
			Mature		57	8.57±0.35	7.35±0.87
		Female	Immature		8	7.01±1.24	4.27±1.90
			Mature	Maturing	0	–	–
				Resting	13	8.80±0.35	8.10±1.14
				Pregnant	79	9.07±0.36	9.22±0.99
				Pregnant & Lactation	0	–	–
Area IV	West-South	Male	Immature		4	6.30±0.75	3.29±0.98
			Mature		6	8.28±0.49	6.63±0.77
		Female	Immature		8	6.38±1.05	3.48±1.44
			Mature	Maturing	0	–	–
				Resting	1	8.53	6.91
				Pregnant	2	8.82±0.93	7.88±1.71
				Pregnant & Lactation	0	–	–
	East-South	Male	Immature		16	6.68±0.88	3.53±1.15
			Mature		18	8.29±0.28	6.79±0.83
		Female	Immature		13	6.61±0.67	3.62±1.06
			Mature	Maturing	1	8.98	7.80
				Resting	1	8.71	8.30
				Pregnant	5	8.97±0.26	7.89±1.08
				Pregnant & Lactation	0	–	–
	West-North	Male	Immature		3	6.14±1.41	2.99±1.89
			Mature		5	8.36±0.58	7.12±1.30
		Female	Immature		1	7.16	4.24
			Mature	Maturing	0	–	–
				Resting	0	–	–
				Pregnant	2	9.23±0.43	8.59±1.33
				Pregnant & Lactation	0	–	–
	East-North	Male	Immature		15	6.27±0.60	2.98±0.85
			Mature		1	7.66	5.37
		Female	Immature		11	6.03±0.73	2.78±0.89
			Mature	Maturing	0	–	–
				Resting	0	–	–
				Pregnant	3	9.00±0.40	7.67±1.26
				Pregnant & Lactation	2	8.92±0.48	7.51±1.50
	Prydz Bay	Male	Immature		5	6.35±0.85	3.12±1.16
			Mature		17	8.54±0.33	7.44±1.03
		Female	Immature		12	6.77±1.02	3.91±1.54
			Mature	Maturing	0	–	–
				Resting	1	9.29	8.81
				Pregnant	15	9.09±0.49	8.56±1.42
				Pregnant & Lactation	0	–	–
Total					333	8.08±1.23	6.66±2.53

Table 3
External body proportions of Antarctic minke whales in the NEWREP-A 2016/17.

Measurement Point	Female			Male		
	No of Measured Samples	Mean (cm)	%BL	No of Measured Samples	Mean (cm)	%BL
Total length (body length)	178	829	100.0	155	783	100.0
Tip of snout to centre of eye	177	159	19.2	153	146	18.6
Tip of snout to tip of flipper	173	373	45.0	149	346	44.2
Tip of snout to end of ventral grooves	178	417	50.3	154	389	49.7
Tip of snout to umbilicus	178	449	54.2	153	420	53.6
Tip of snout to anus	178	613	73.9	154	578	73.8
Girth of chest, half	168	229	27.6	144	212	27.1
Girth of abdominal, half	174	212	25.6	150	198	25.3
Skull length, condyle to tip of premaxilla	173	198.2	23.9	153	182.3	23.3
Skull, greatest width	165	108.5	13.1	152	99.8	12.7

Table 4a
Body parts and organ weight (kg) of Antarctic minke whales in NEWREP-A 2016/17.

Catch date	Sex	Body length (m)	Total parts	Blubber	Meat	Bone	Viscera and others
26 Dec 2016	M	8.81	7,320	1,403	4,028	984	923
1 Jan 2017	M	7.05	3,929	789	2,179	518	455
13 Jan 2017	M	7.75	4,862	1,051	2,551	670	607
21 Jan 2017	F	8.93	7,302	1,619	3,832	1,003	867
30 Jan 2017	F	5.55	1,994	490	981	253	276
14 Feb 2017	M	6.97	3,732	787	2,062	475	418

Table 4b
Number of whale total fat weight measured in the NEWREP-A 2016/17.

Sex	No. of individuals measured
Female	15
Male	20
Total	35

Table 5
The frequency of the appearance of dominant prey species found in forestomach contents of Antarctic minke whales sampled in the NEWREP-A 2016/17.

Stratum	Antarctic krill		Ice krill		*Thysanoessa.sp*		Empty		Broken	
	N	%	N	%	N	%	N	%	N	%
Area III										
East-South	80	24.0	8	2.4	0	0.0	63	18.9	14	4.2
West-South	12	3.6	0	0.0	0	0.0	6	1.8	3	0.9
Area IV										
East-South	30	9.0	0	0.0	0	0.0	19	5.7	5	1.5
West-North	6	1.8	0	0.0	2	0.6	3	0.9	0	0.0
East-North	19	5.7	0	0.0	1	0.3	10	3.0	2	0.6
Prydz Bay	24	7.2	0	0.0	0	0.0	20	6.0	6	1.8
Total	171	51.4	8	2.4	3	0.9	121	36.3	30	9.0

N: number of whales examined; Broken: animals with broken stomach by harpoon.

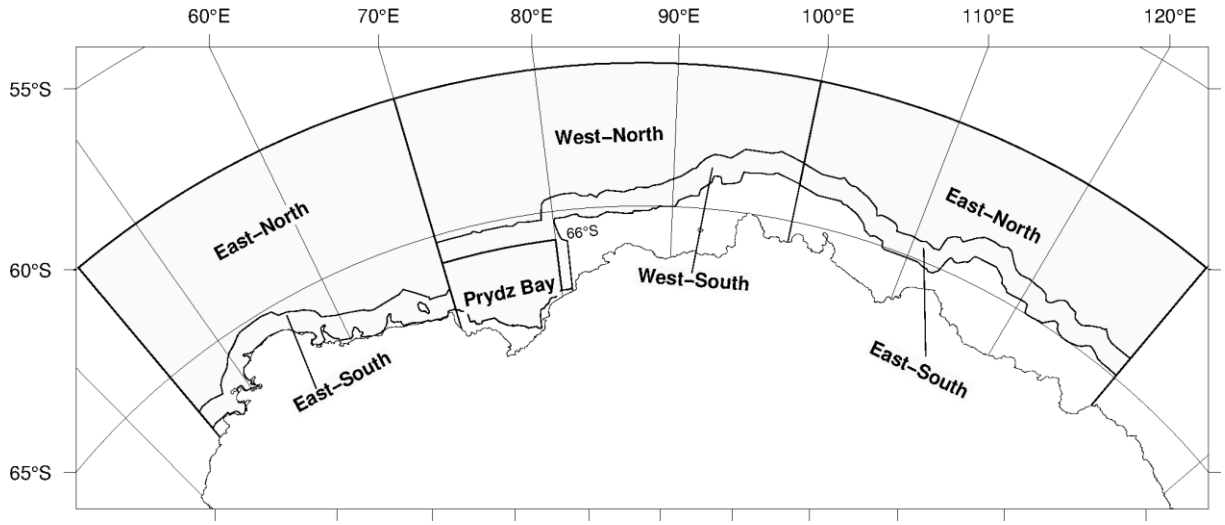

Figure 1. Stratification of sampling survey for Antarctic minke whales in the NEWREP-A 2016/17 research area. Gray shade; research area of the NEWREP-A 2016/17. The boundary between North and South strata of each research area was the 45n.mile lines from estimated ice-edge line except for the Prydz Bay. The boundary between South and Prydz Bay boundary of west strata of Area IV (from 70°E to 80°E, in longitude) was 66°S in latitude. The map using Lambert conic conformal projection.

Figure 2. Research vessels which were used in the NEWREP-A biological sampling survey in 2016/17. Upper left: *Yushin-Maru*; upper right: *Yushin-Maru No.2*; lower left: *Yushin-Maru No.2*; lower right: *Nisshin-Maru*.

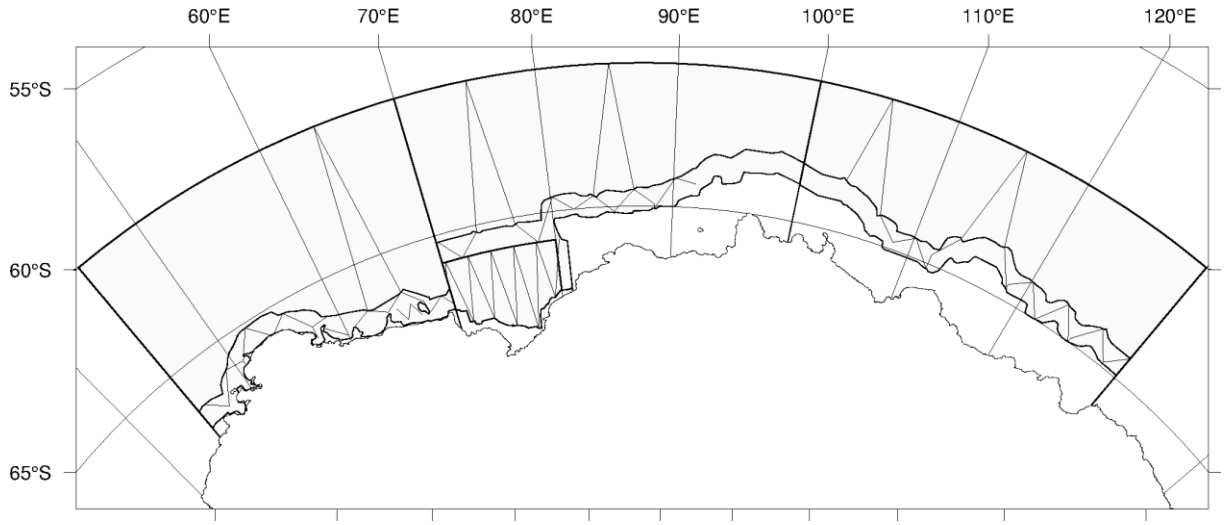

Figure 3a. Predetermined track-line (black lines: main course) of sampling survey for Antarctic minke whales in the NEWREP-A 2016/17. The map using Lambert conic conformal projection.

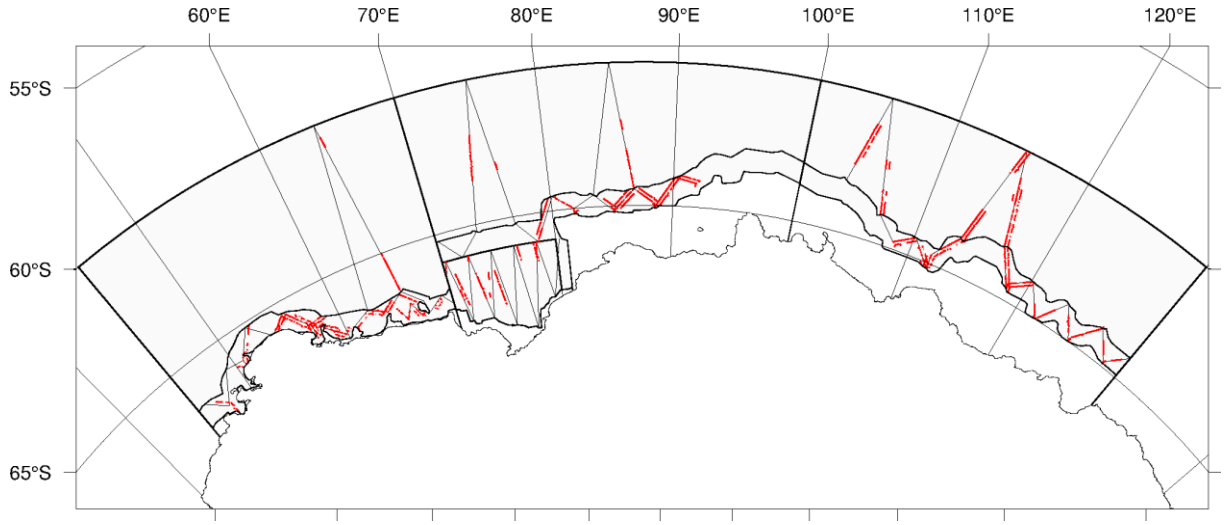

Figure 3b. The portion of the vessel's track-line (red line) that was surveyed on the effort of sampling survey for Antarctic minke whales in the NEWREP-A 2016/17. Two or three parallel lines show the main- and sub-courses.

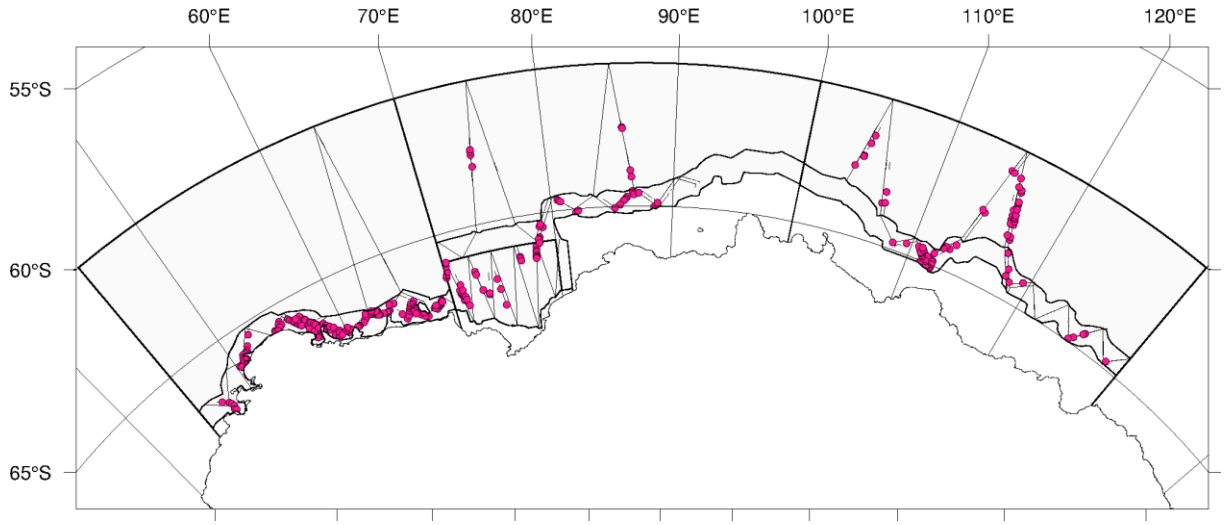

Figure 3c. Geographical distribution of primary sighting position of Antarctic minke whales (pink closed circle) sighted in the NEWREP-A 2016/17 sampling survey. Black bold line of the map was the boundary line for a stratum of the research area. Gray lines were the predetermined track-line. The black line was the track-line of the searching effort. The map using Lambert conic conformal projection.

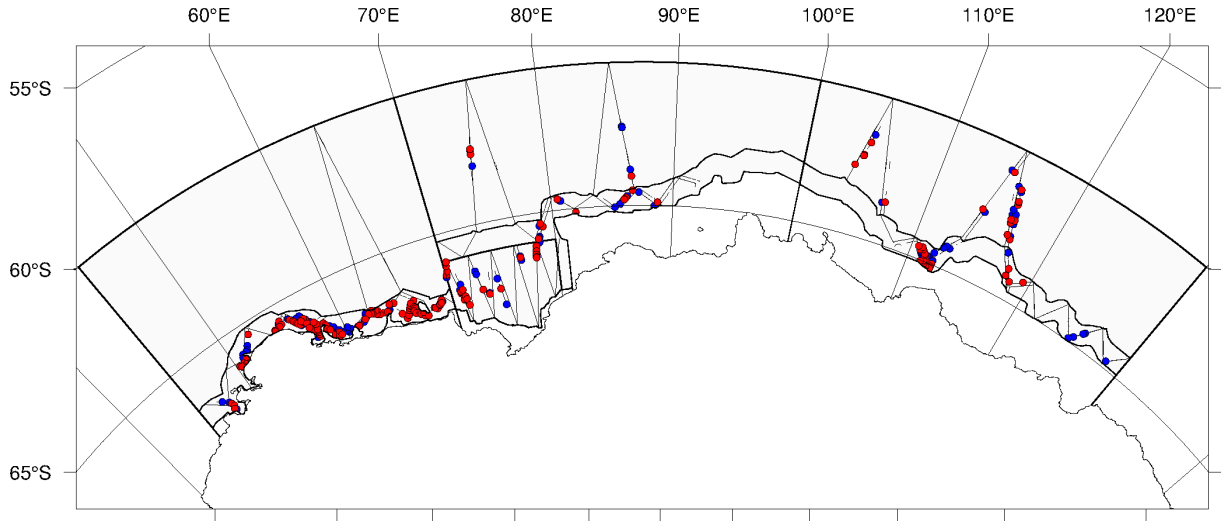

Figure 3d. Geographical distribution of sighting position of Antarctic minke whale sampled in the NEWREP-A 2016/17. One plot showed the sighting position of the one school of the primary sighting. In cases where the school consists of multiple individuals, some samples were sampled less than two individuals/school. Red closed circle: female; blue closed circle: male. Black bold line of each map was the boundary line for a stratum of the each research area. Gray lines were the predetermined track-line. The black line was the track-line of the searching effort. The map using Lambert conic conformal projection.

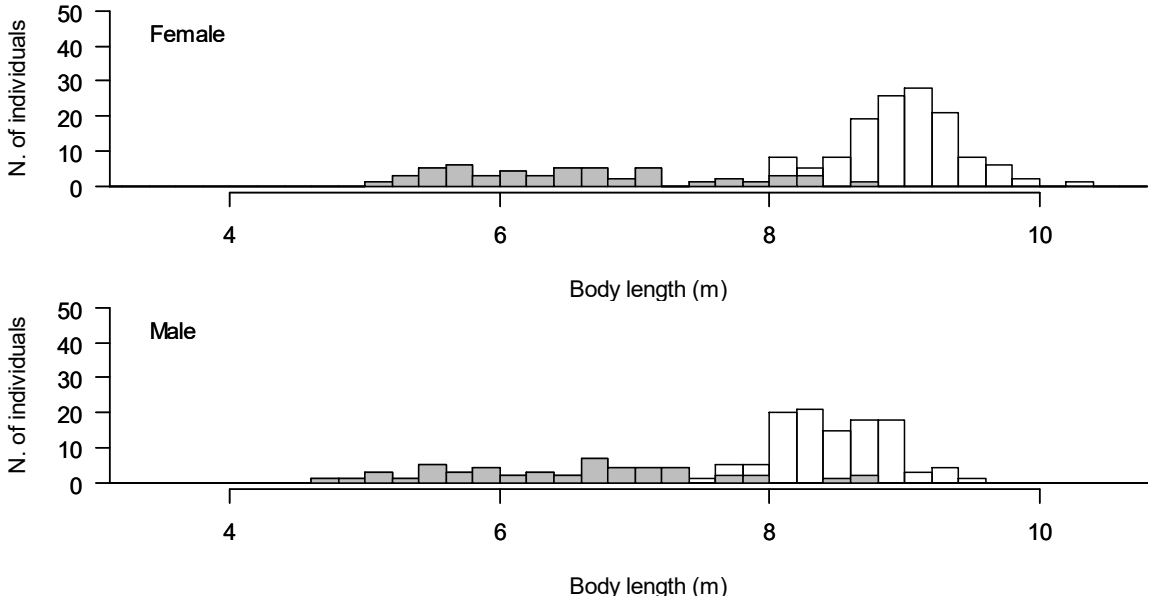

Figure 4. Histogram of the Antarctic minke whales body length data values obtained in the NEWREP-A 2016/17. Upper: female; lower: male. Two vectors colours mean the value of the stature in maturity. White: mature individuals; gray: immature individuals. A vector giving the breakpoints between histogram cells were 0.2m intervals.

APPENDIX

Sighting results by the sighting and sampling vessels during NEWREP-A 2016/17 in Area III-E (45°E-70°E) and IV

IDENTIFICATION OF SPECIES

Guidelines for species identification were based on the IWC-SOWER methods for classification of identification (IWC, 2008): 'Positive identification of species was based on multiple cues and usually required clear observation of the whale's body. Occasionally, repeated observations of the shape of the blow, surfacing, and other behavioural patterns were sufficient; this judgement was made only by the Cruise leader or other designated researcher. Identification of species was recorded as 'probable' based on multiple cues, which were nevertheless insufficient to be absolutely confident of identification. This usually occurred when blows and surfacing patterns could be confirmed, but the whale's body could not be clearly seen. Details of recording procedures during sightings can be found in 'Information for Researchers''. From initial observations of morphologic characteristics, killer whale data were divided into three ecotypes (Pitman and Ensor, 2003).

DETERMINATION OF GROUP SIZE

Following guidelines were used in determining group size (IWC, 2008): 'Schools where the number of animals or an accurately estimated range of the number of animals was determined, were classified as confirmed schools. Data from the confirmed schools can be used to determine a mean school size. Therefore, it is critical that the confirmed schools accurately represent the size of schools in the survey area. Normally, schools believed to be confirmed for school size are approached to within 1 n. mile for large whales and to within 0.3 n. miles for minke whales. Allowing for context-specific differences (i.e. environmental conditions and animal behaviour), every effort was made to be consistent with regard to the maximum time spent on the identification of species and confirmation of numbers. Normally, if the sighting was thought to be minke whales, no more than 20 minutes (after the closure has been completed) should be spent on confirmation. This reduces the potential for confusion with other sightings in the vicinity. Counts of individuals provided by the sighting summary represent best estimates of school sizes in the research area, except when indicated otherwise'.

OTHER RESEARCH ACTIVITIES

Photo-ID

Photo-ID data of individual whales was collected for stock structure analyses as well as examining mixing and movements during the research time. Data were captured by digital photograph using Digital camera (300mm lens, Canon Co., Ltd., Japan). Target species for photo-ID were blue whale (*B. musculus*), humpback whale (*Megaptera novaeangliae*), southern right whale (*Eubalaena australis*), and killer whale.

Biopsy sampling

Samples of skin biopsy were collected by biopsy from the target whales. The target species were blue whale, fin whale (*B. physalus*), sei whale (*B. borealis*), southern right whale, sperm whale (*Physeter macrocehpalus*), killer whale, pygmy right whale (*Caperea marginata*), and southern bottlenose whale (*Hyperoodon planifrons*) sighted in the research time. The system for biopsy sampling was a Larsen gun (Larsen, 1998). The open sight was replaced with an electronic aiming device (red-dot-sight), which allows faster aiming and thus faster shooting. The biopsy darts consisted of a carbon fibre shaft, which is high-pressure moulded to a polyethylene float that also functions as a stop to limit penetration into the tissue. In the float end of the dart, a threaded insert is used for attaching the screw-on biopsy-sampling tip. The biopsy tip is a stainless steel cylinder with a 9mm outer diameter, an internal diameter of 7mm and three internal barbs for sample retention. All collected samples were stored at −80°C.

Feasibility study on satellite tracking of Antarctic minke whale

Feasibility study on satellite tracking of Antarctic minke whale was carried out in both the sampling and the dedicated sighting survey. Results of the feasibility study are presented by Isoda *et al*., (2017).

RESULT

Sightings

The searching effort and experiment times are summarised in Table 1. The WP of the SSV's track-line of sampling survey is shown in Table 2. Total searching distance in the research area was 736.6 n.miles in Area III-E (45°E-70°E) and 2,537.2 n.miles in Area IV (main and sub course). Sighting records are not suitable for design-based abundance estimation as the sighting records of the sampling and survey vessels were made by NSC mode using TOP and UBP except for examination of the distribution of specified species using model-based estimation.

The sightings recorded in the sampling survey are shown in Table 3a–b. Figures 1a–b shows the geographical distribution of baleen whales sighted in sampling survey (Figure 1a: blue and fin whales; Figure 1b: humpback whales). Figure 2 shows the geographical distribution of toothed whales (sperm whale, Southern bottlenose whale, Arnoux's beaked whale, and killer whales) sighted in sampling survey.

In Area IV, Humpback whale was the most abundant species followed by Antarctic minke whale. Distribution of Antarctic minke whale was narrower than that of humpback whales, widely found in III-E and IV. In Area III-E Antarctic minke whale was concentrated at the ice edge.

Effort was made to identify killer whales into three ecotypes (types A, B and C). A total of 13 schools (101 individuals) were sighted and the most sighted schools (12 schools) were 'not identified ecotypes (undetermined)' due to the difficulty of approaching the whales, and recorded as 'killer whales (undetermined)'. Photo-ID data suggested that the one school of killer whales might have been type C. Further observation, experiments, and analysis of this species will be continued during the next cruise.

Photo ID and biopsy

A total of 24 photo-ID data was obtained from humpback and killer whales. The number of photo-ID and biopsy samples are summarised in Tables 4a–4b. Distribution of the sighting position of photo-ID data and biopsy sampling are shown in Figure 3a and 3b, respectively. Table 5 summarises the details of the photo-ID and biopsy sampling. The records will provide useful data for understanding the stock structure, mixing and movements of these species. These data will be submitted to the IWC secretary and will be analysed comparing previous IWC and other relevant catalogues (e.g. Olson, 2012, Matsuoka and Pastene, 2009).

In terms of biopsy skin sampling, a total of 4 individuals were sampled from humpback whales. These biopsy samples obtained during surveys of the NEWREP-A 2016/17 will be used for microsatellite DNA loci analysis for studies of stock structure in the Antarctic feeding ground. Biopsy samples may also be used for other research (e.g. chemical markers as body condition indicator, stable isotope, or hormones).

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Table 1
Summary of research effort of the sighting and sampling vessels during NEWREP-A 2016/17.

	Strata	Date		Days	NSC		NSP		Photo-ID Biopsy
		Start	End		Time	Dist	Time	Dist	Time
Area IV	West-North	12-Jan 14:30	6-Feb 18:00	7	30:16	343.1	0:00	0.0	0:00
	East-North	16-Dec 10:22	22-Dec 17:26	10	60:43	696.4	0:00	0.0	0:00
	West-South	13-Jan 15:32	18-Jan 18:00	7	42:10	466.4	0:00	0.0	0:00
	East-South	15-Dec 6:00	8-Jan 18:00	16	65:47	740.4	0:00	0.0	1:01
	Prydz Bay	20-Jan 6:00	15-Feb 18:00	9	25:35	290.9	0:00	0.0	0:00
Area III	East-North	3-Feb 10:00	13-Feb 18:00	2	9:03	106.0	0:00	0.0	0:00
	East-South	30-Jan 13:39	7-Mar 15:11	21	56:08	630.6	0:00	0.0	0:10
Total		15-Dec 6:00	7-Mar 15:11	72	365:53	4174.4	0:00	0.0	1:11

Table 2
Predetermined waypoints (WP) and the actually covered ratio in Area III and IV.

WP No.	Leg. No.	Latitude	Longitude	Course	Plan	Effort	Covered (%)
East-South in Area III Area code: 32, Mode: NSC							
301	301	67°29'S	070°00'E	319°	31.6	0.2	0.5
302	302	67°05'S	069°07'E	227°	52.1	4.7	8.9
303’	303	67°40'S	067°27'E	000°	35.0	0.1	0.3
303	303	67°05'S	067°27'E	311°	52.8	0.0	0.0
304	304	66°30'S	065°47'E	232°	49.7	15.7	31.5
305	305	67°00'S	064°07'E	296°	44.2	29.6	66.8
306	306	66°40'S	062°27'E	243°	44.2	3.4	7.8
307	307	67°00'S	060°47'E	243°	44.2	0.0	0.0
308	308	66°15'S	059°07'E	262°	60.1	22.6	37.6
308’	308	66°20'S	057°27'E	180°	28.0	16.5	59.0
309	–	66°48'S	057°27'E	–	–	–	–
308’	309	66°20'S	057°27'E	323°	68.7	35.7	52.0
310	310	65°25'S	055°47'E	233°	51.1	31.0	60.6
311	311	65°55'S	054°07'E	317°	61.3	0.0	0.0
312	312	65°10'S	052°27'E	216°	68.9	6.6	9.5
312’	312	66°05'S	050°47'E	180°	11.0	0.0	0.0
313	313	66°16'S	050°47'E	291°	43.6	0.0	0.0
314	314	66°00'S	049°07'E	211°	76.4	0.0	0.0
315	315	67°05'S	047°27'E	311°	52.8	0.0	0.0
316	–	66°30'S	045°47'E	–	–	–	–
801	801	67°00'S	064°55'E	152°	33.8	16.3	48.1
802	802	67°30'S	065°35'E	027°	33.8	17.3	51.1
803	803	67°00'S	066°15'E	152°	33.8	11.1	32.7
803’	803	67°30'S	066°55'E	180°	10.0	0.1	1.1
804	–	67°40'S	066°55'E	–	–	–	–
East-North in Area III Area code: 34, Mode: NSC							
404	404	66°30'S	065°47'E	353°	393.1	106.0	27.0
405	405	60°00'S	064°07'E	186°	403.0	0.0	0.0
406	406	66°40'S	062°27'E	–	–	–	–

Table 2
Continued.

WP No.	Leg. No.	Latitude	Longitude	Course	Plan	Effort	Covered (%)
East-South in Area IV Area code: 42, Mode: NSC							
501	501	64°09'S	130°00'E	224°	56.1	26.4	47.0
501’	901	64°49'S	128°29'E	180°	4.0	3.8	95.5
502	502	64°53'S	128°29'E	323°	72.5	29.6	40.9
503	503	63°55'S	126°49'E	218°	70.1	62.2	88.7
503’	903	64°50'S	125°09'E	180°	10.0	8.4	83.5
504	504	65°00'S	125°09'E	326°	78.1	34.2	43.8
505	505	63°55'S	123°29'E	213°	78.1	42.9	54.9
506	506	65°00'S	121°49'E	326°	78.1	28.1	36.0
507	507	63°55'S	120°09'E	235°	53.0	39.7	74.9
507’	907	64°25'S	118°29'E	180°	10.0	8.7	86.7
508	508	64°35'S	118°29'E	329°	87.1	58.0	66.6
509	–	63°20'S	116°49'E	–	–	–	–
511	511	63°45'S	113°29'E	218°	70.3	45.9	65.3
511’	911	64°40'S	111°49'E	180°	31.0	20.3	65.5
512	512	65°11'S	111°49'E	320°	66.6	19.5	29.3
513	513	64°20'S	110°09'E	239°	49.8	43.7	87.6
513’	913	64°45'S	108°29'E	180°	20.0	0.2	1.2
514	514	65°05'S	108°29'E	326°	78.1	0.0	0.0
515	–	64°00'S	106°49'E	–	–	–	–
West-South in Area IV Area code: 41, Mode: NSC							
556	556	64°10'S	091°49'E	282°	45.0	0.0	0.0
557	557	64°00'S	090°09'E	213°	78.1	74.6	95.5
558	558	65°05'S	088°29'E	000°	10.0	1.9	18.8
558’	957	64°55'S	088°29'E	305°	52.4	37.0	70.7
559	559	64°25'S	086°49'E	223°	62.1	46.8	75.3
559’	959	65°10'S	085°09'E	180°	2.0	2.0	100.0
560	560	65°12'S	085°09'E	311°	56.4	8.4	14.9
561	561	64°35'S	083°29'E	311°	56.4	9.8	17.4
562	562	65°00'S	081°49'E	239°	49.5	40.9	82.7
563	563	64°30'S	080°09'E	305°	52.3	21.3	40.8
564	564	66°00'S	078°29'E	204°	99.4	0.0	0.0
565	–	65°15'S	076°49'E	–	–	–	–
567	567	65°15'S	073°29'E	222°	61.2	0.0	0.0
568	568	66°00'S	071°49'E	317°	61.2	0.0	0.0
569	569	65°15'S	070°09'E	222°	5.6	0.0	0.0
570	–	65°19'S	070°00'E	–	–	–	–
Prydz Bay Area code: 45, Mode: NSC							
701	701	66°32'S	070°00'E	013°	33.3	9.2	27.6
702	702	66°00'S	070°19'E	170°	137.3	13.3	9.7
703	703	68°15'S	071°19'E	009°	137.3	12.3	8.9
704	704	66°00'S	072°19'E	170°	142.2	46.3	32.6
705	705	68°20'S	073°19'E	009°	142.2	0.0	0.0
706	706	66°00'S	074°19'E	172°	166.9	0.0	0.0
707	707	68°45'S	075°19'E	007°	166.9	0.0	0.0
708	708	66°00'S	076°19'E	172°	181.8	0.0	0.0
709	709	69°00'S	077°19'E	007°	181.8	21.3	11.7
710	710	66°00'S	078°19'E	168°	122.5	0.0	0.0
711	711	68°00'S	079°19'E	011°	122.5	0.0	0.0
712	712	66°00'S	080°19'E	166°	102.9	0.0	0.0
East-North in Area IV Area code: 44, Mode: NSC							
603	603	63°55'S	126°49'E	348°	239.9	4.4	1.8
604	604	60°00'S	125°09'E	191°	239.9	0.0	0.0
605	–	63°55'S	123°29'E	–	–	–	–
609	609	63°20'S	116°49'E	346°	205.8	110.9	53.9
610	610	60°00'S	115°09'E	191°	230.1	122.6	53.3
611	–	63°45'S	113°29'E	–	–	–	–
615	615	64°00'S	106°49'E	348°	244.8	60.6	24.7
616	616	60°00'S	105°09'E	194°	196.1	88.8	45.3
617	–	63°10'S	103°29'E	–	–	–	–
West-North in Area IV Area code: 43, Mode: NSC							
659	659	64°25'S	086°49'E	350°	269.4	78.9	29.3
660	660	60°00'S	085°09'E	189°	279.2	0.0	0.0
661	–	64°35'S	083°29'E	–	–	–	–
665	665	65°15'S	076°49'E	351°	318.7	15.8	5.0
666	666	60°00'S	075°09'E	188°	318.7	82.0	25.7
667	–	65°15'S	073°29'E	–	–	–	–

Table 3a
Number of the great whales sighted in Area III-E and Area IV.

Area III-E (45°E-70°E)

Species	Area III-E								Subtotal			
	East-South				East-North							
	Prim.		Second.		Prim.		Second.		Prim.		Second.	
	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.
Blue whale	0	0	0	0	0	0	0	0	0	0	0	0
Fin whale	10	22	5	14	1	2	0	0	11	24	5	14
Sei whale	0	0	0	0	0	0	0	0	0	0	0	0
Antarctic minke whale	155	254	30	74	0	0	1	1	155	254	31	75
Like Antarctic minke whale	0	0	0	0	0	0	0	0	0	0	0	0
Humpback whale	52	83	15	28	19	33	0	0	71	116	15	28
Southern right whale	0	0	0	0	0	0	0	0	0	0	0	0
Sperm whale	1	1	0	0	0	0	0	0	1	1	0	0
Baleen whale	2	2	0	0	0	0	0	0	2	2	0	0

Area IV

Species	Area IV																Subtotal			
	West-South				East-South				West-North				East-North				Prydz bay							
	Prim.		Second.		Prim.		Second.		Prim.		Second.		Prim.		Second.		Prim.		Second.		Prim.		Second.	
	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.
Blue whale	2	4	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	2	4	2	2
Fin whale	34	94	0	0	2	6	0	0	11	28	3	8	2	3	1	2	28	104	0	0	77	235	4	10
Sei whale	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Antarctic minke whale	21	29	0	0	51	108	14	33	10	11	6	8	33	38	1	1	41	86	3	7	156	272	24	49
Like Antarctic minke whale	0	0	0	0	1	2	0	0	0	0	0	0	2	2	0	0	0	0	0	0	3	4	0	0
Humpback whale	74	156	0	0	155	284	15	35	41	85	0	0	125	237	2	8	35	64	1	4	430	826	18	47
Southern right whale	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Sperm whale	11	11	0	0	4	4	1	1	1	1	0	0	0	0	0	0	0	0	0	0	16	16	1	1
Baleen whale	2	10	0	0	1	3	0	0	0	0	0	0	3	7	0	0	0	0	0	0	6	20	0	0

Table 3b
Number of the other toothed whales sighted in Area III-E and Area IV.

Area III-E (45°E-70°E)

Species	Area III-E								Subtotal			
	East-South				East-North							
	Prim.		Second.		Prim.		Second.		Prim.		Second.	
	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.
Southern bottlenosed whale	0	0	0	0	0	0	1	1	0	0	1	1
Arnoux's beaked whale	1	18	1	30	0	0	0	0	1	18	1	30
Unidentified Beaked whale	1	2	1	1	0	0	0	0	1	2	1	1
Killer whale*	23	217	6	64	0	0	0	0	23	217	6	64

*Small cetaceans are outside the competence of the IWC.

Area IV

Species	Area IV																Subtotal			
	West-South				East-South				West-North				East-North				Prydz bay							
	Prim.		Second.		Prim.		Second.		Prim.		Second.		Prim.		Second.		Prim.		Second.		Prim.		Second.	
	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.	Sch	Ind.
Southern bottlenosed whale	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0
Arnoux's beaked whale	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unidentified Beaked whale	10	19	0	0	6	7	0	0	2	5	0	0	2	4	0	0	0	0	0	0	20	35	0	0
Killer whale*	5	35	0	0	2	13	0	0	2	16	0	0	3	10	0	0	4	43	1	25	16	117	1	25

*Small cetaceans are outside the competence of the IWC.

Table 4a
Number of individuals photo-identified in Area III-E and Area IV.

Species	# individuals photographed
Blue whale	0
Humpback whale	20
Killer whale	4
Total	24

Table 4b
Number of biopsy samples collected in the sampling survey.

Species	# individuals collected
Blue whale	0
Humpback whale	4
Killer whale	0
Total	4

Table 5
Details of the encounter duration in photo-ID and biopsy sampling.

Date	Sight No.	Species	Sighting position Lat.	Long.	School size	Est. body length of target ind. [m]	# marked Individual	# of shoot	Opportunity of shoot	Position of shoot	Biopsy sample No.	Notes.
YS1												
30 Jan 2017	1	Humpback	67°3.16'S	66°37.20'E	1	9	1	4	G	RD,FL,LD	–	
30 Jan 2017	2	Humpback	67°4.19'S	66°42.04'E	1	13.4	1	2	G	FL,LD	–	
YS2												
21 Dec 2016	1	Humpback	62°28.38'S	113°59.10'E	4	12.7,12.8,13.2,13.4,	4	12	P	HD,LD,RD,OT,	–	
29 Dec 2016	1	Humpback	64°18.37'S	130°5.93'E	2	12.7,13.0	2	6	P	RD,OT,	–	
31 Dec 2016	1	Humpback	64°20.29'S	124°59.36'E	2	13.1,13.3	2	9	P	RD,HD,OT,	–	
3 Jan 2017	5	Humpback	65°8.90'S	111°54.10'E	2	12.5,12.8	2	5	P	LD,RD,	J16YS2H001	
3 Jan 2017	8	Humpback	65°12.74'S	112°1.23'E	1	11.8	1	2	G	LD,	J16YS2H002	
3 Jan 2017	10	Humpback	65°13.13'S	112°12.30'E	2	12.8,13.5	2	8	P	LD, RD,OT,	J16YS2H003 J16YS2H004	
3 Jan 2017	11	Humpback	65°11.01'S	112°24.91'E	3	10.7,12.3,12.8	3	6	P	FL,LD,RD	–	
20 Feb 2017	1	Humpback	64°3.43'S	67°30.43'E	2	13.2,13.6	2	11	G	FL, LD,OT	–	
YS3												
6 Mar 2017		Killer	67°26.46'S	67°19.40'E	10	7.7,7.5,7.0,4.0	4	8	G	RL	–	

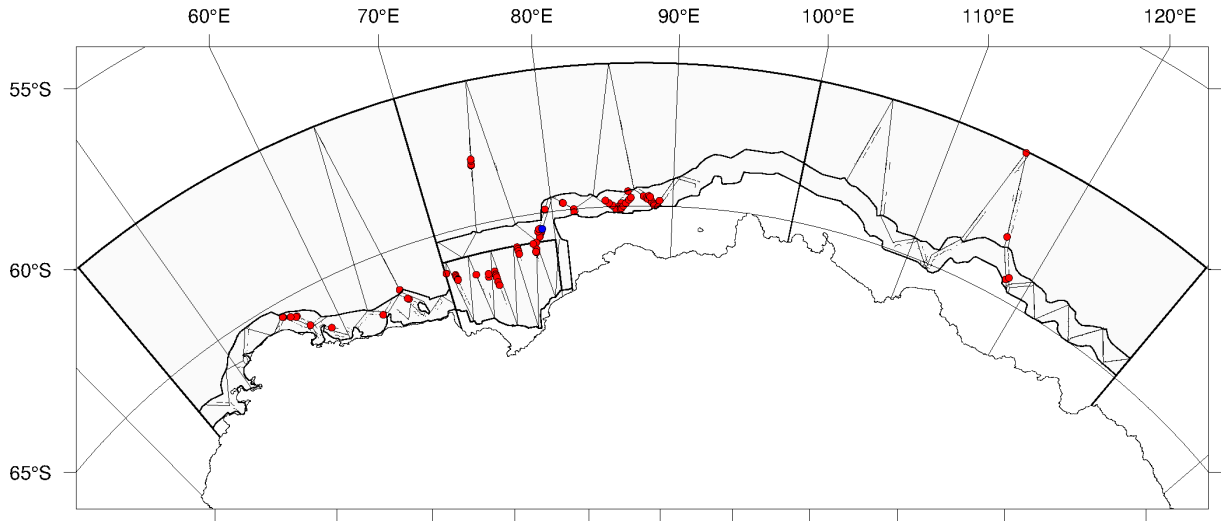

Figure 1a. Geographical distribution of primary sighting position of blue (blue closed circle) and fin (red closed circle) whales sighted in the NEWREP-A 2016/17 sampling survey. Gray line of the map shows the predetermined track-line. The black line was the track-line of the searching effort. The map using Lambert conic conformal projection.

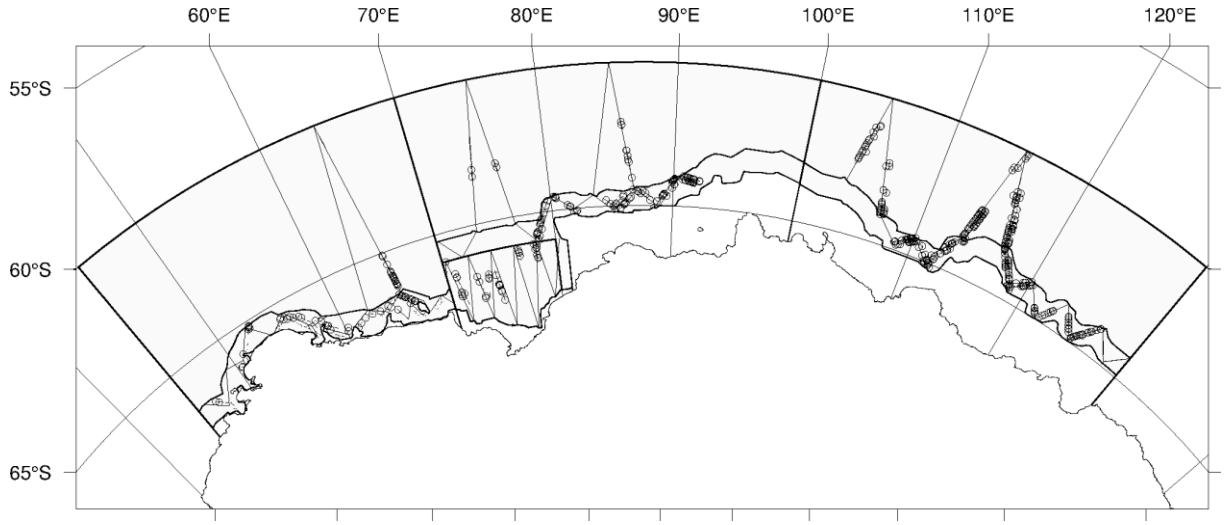

Figure 1b. Geographical distribution of primary sighting position of humpback whales (black open circle) sighted in the NEWREP-A 2016/17 sampling survey. Black bold line of the map was the boundary line for a stratum of the research area. Gray line was the predetermined track-line. The black line was the track-line of the searching effort. The map using Lambert conic conformal projection.

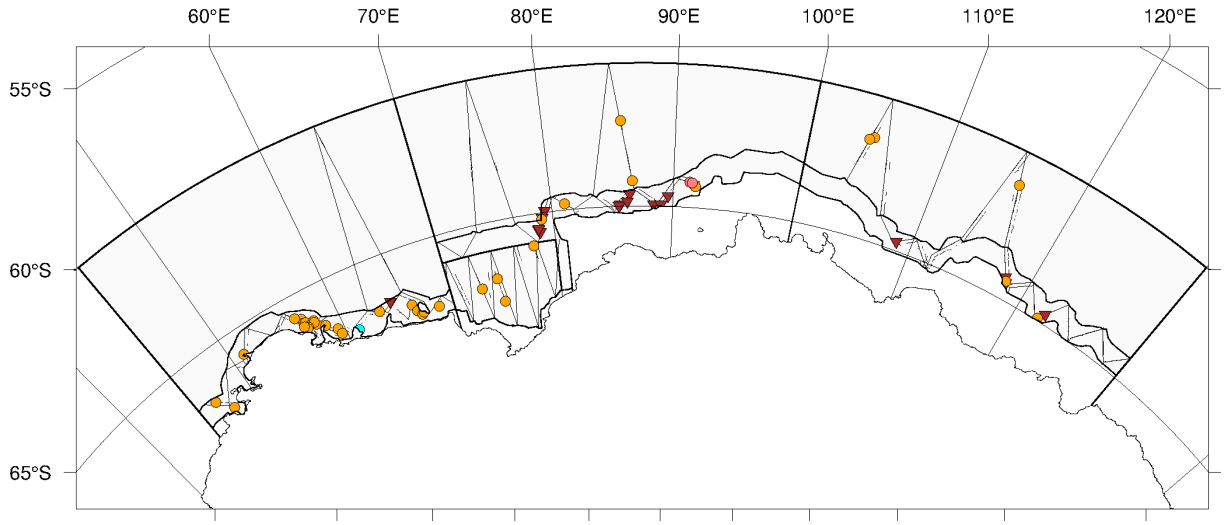

Figure 2. Geographical distribution of primary sighting position of sperm (brown closed reverse triangle), Southern bottlenose (light red closed circle), Arnoux's Beaked (cyan closed circle) and killer (orange closed circle) whales sighted in the NEWREP-A 2016/17 sampling survey. Black bold line of the map was the boundary line for a stratum of the research area. Gray lines were the predetermined track-line. The black line was the track-line of the searching effort. The map using Lambert conic conformal projection.

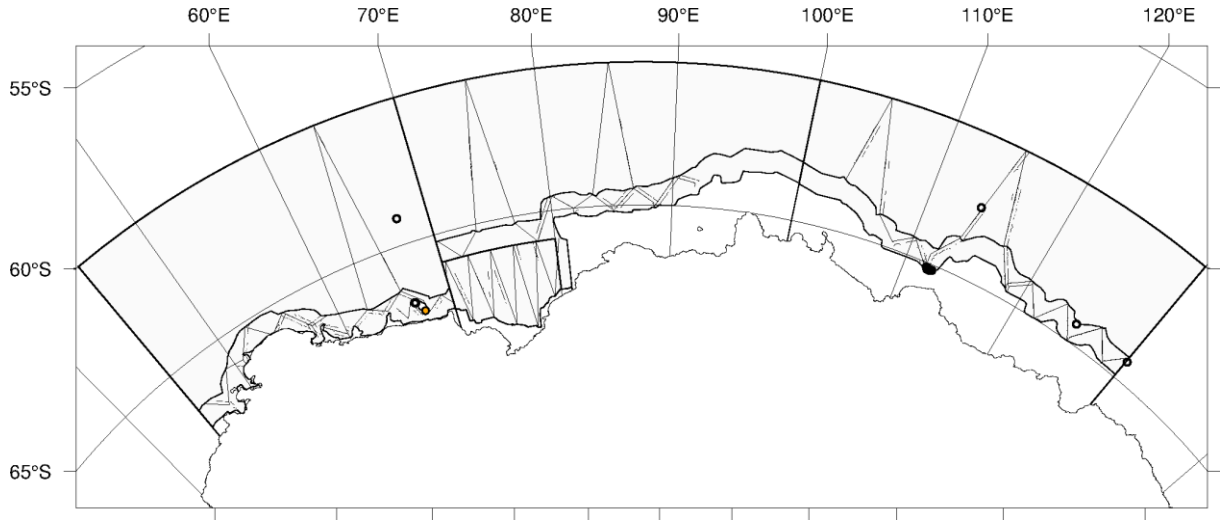

Figure 3a. Geographical distribution of photo-ID data from humpback (black open circle) and killer (orange closed circle) whales collected during the NEWREP-A 2016/17 sampling survey. A single symbol could represent more than one photograph data. Black bold line of the map was the boundary line for a stratum of the research area. Gray lines were the predetermined track-line. The black line was the track-line of the searching effort. The map using Lambert conic conformal projection.

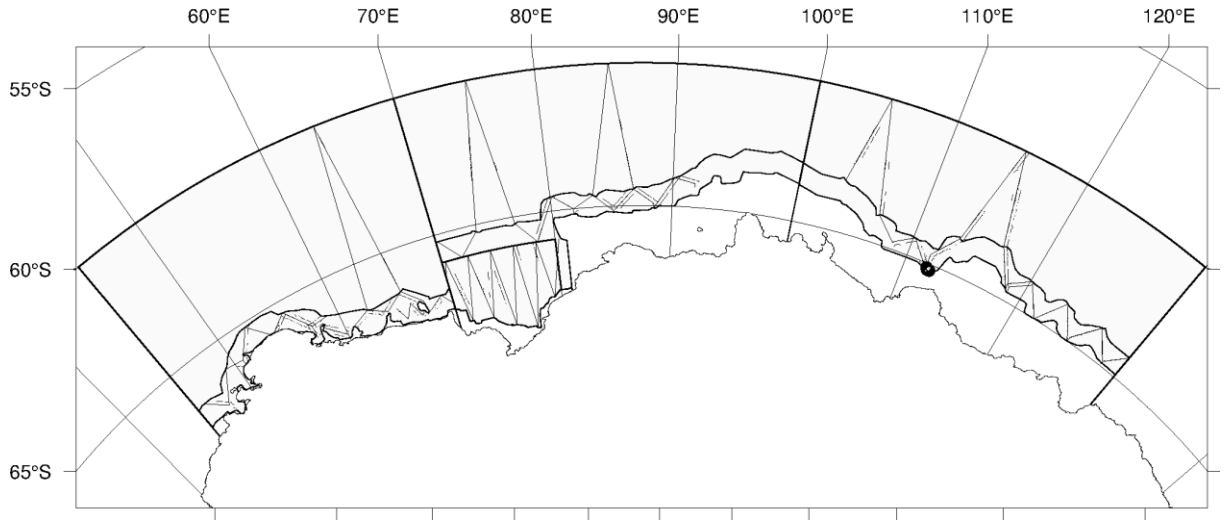

Figure 3b. Geographical distribution of biopsy samples of humpback whales (black opened circle) collected during the NEWREP-A 2016/17 sampling survey. Black bold line of the map was the boundary line for a stratum of the research area. Gray lines were the predetermined track-line. The black line was the track-line of the searching effort. The map using Lambert conic conformal projection.