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Foreword

Remarkable evolution in cetacean studies in recent decades owes much to major journals that have made significant contribution to the development of modern cetology: Discovery Reports, published by the National Institute of Oceanography in the United Kingdom, and Norwegian Whaling Gazette in Norway, as well as The Scientific Reports of the Whales Research Institute in Japan.

The Scientific Reports of the Whales Research Institute was first published in 1948, a year after the Whales Research Institute was established. Aiming to share valuable research findings and scientific knowledge worldwide, the publication was formatted in English since its beginning, quite an ambitious attempt in Japan still recovering from the devastation of World War II.

Since its first publication, a total of 246 scientists contributed 419 scientific papers to The Scientific Reports of the Whales Research Institute. It is widely acknowledged and appreciated that these scientific papers were the foundation for the development of cetacean studies worldwide, and in today's terms, it was a research journal that had a significant impact factor, or high number of citations. Regrettably, however, The Scientific Reports of the Whales Research Institute was discontinued in 1988 with the 39th volume after the institute was reorganized into the Institute of Cetacean Research.

In the 30 years since then, various types of journals on cetacean studies have been published globally, each offering different perspectives on scientific research outcomes. As for Japan, no research journal matching The Scientific Reports of the Whales Research Institute in its quality has been published. It is probably because many domestic cetologists have sought to publish their papers in international research journals based outside Japan.

As the global environment surrounding the issue of whaling became increasingly complex, we have observed a shift in publishing policies among these journals, rejecting papers whose findings are based on specific research methods such as lethal sampling. Because of this, no small numbers of papers submitted by biological scientists using samples collected through lethal surveys, even just for some parts, have been denied proper reviews. While we agree that animal ethics should be given high priority when writing a research paper, if a paper, the research method of which is allowed under domestic and international rules, is rejected, it is a decision made beyond scientific judgment.

Our new journal for cetacean population studies intends to follow the scientific policy of The Scientific Reports of the Whales Research Institute, that is, to contribute to global development of cetacean studies. As long as submitted papers conform to scientifically-accepted animal ethics, we do not make distinctions based on research methods. At the same time, to maintain the journal's neutrality in the complex global environment surrounding whaling issues, the journal will be published from a newly organized committee, rather than as a bulletin type scientific report from a specific research institute. The title of the new journal will be Cetacean Population Studies to be abbreviated CPOPS, and we aim to keep our door wide-open for researchers worldwide, contribute to the scientific development of resource studies for marine mammals especially focusing on cetaceans, and nurture many aspiring scientists.

Seiji Ohsumi, Ph. D.
Chairman
Publication Committee for the Cetacean Population Studies

December 31, 2018

Memory of Dr. Seiji Ohsumi 12 July 1930 – 2 November 2019

We very much regret to inform you that Dr. Seiji Ohsumi, aged 89, Chairman of Governing Council of Cetacean Population Studies (CPOPS) unfortunately died at 7:55 PM on November 2, 2019 due to an acute myocardial infarction.

Dr. Ohsumi had been one of the leading members of the IWC/SC for very long time through the 1960's to the 2010's, serving as head of the Japanese delegation to the IWC/SC during many years in 1990s.

Dr. Seiji Ohsumi was born on July 12, 1930 in Gunma Prefecture, the center of Japan. He had been a temporary member of the Whales Research Institute while he was a graduate student of the Faculty of Agriculture at the University of Tokyo. Subsequently he became a permanent scientist in 1958. In his early days at the WRI he was mainly engaged in field science at numerous places where whaling occurred as well as laboratory analyses under the supervision of the late Professor Masaharu Nishiwaki (senior scientist at WRI) and the late Dr. Hideo Omura (Director general of WRI). In this way he gained biological knowledge about both large and small cetaceans. His first paper in English was published in the Scientific Report of WRI in 1954 [9: 165–177] under his original name of Seiji Kimura and was titled "On the sexual maturity of the sei whale of the Bonin waters".

His Ph.D. thesis submitted to the University of Tokyo, examined age determination with a focus on fin whales and titled "*A study on age determination of the fin whale*", in which he found and confirmed annual deposition rate of growth layer in fin whale earplugs, which was one of most important issues in stock management of fin whales.

Dr. Seiji Ohsumi moved to a government fisheries laboratory in May 1966 and was assigned head of cetacean population studies at the Far Seas Fisheries Research Laboratory (currently, National Research Institute of Far Seas Fisheries) in August 1967. He served as director general at FSFRL from October 1988 to March 1991.

After retirement as a government scientist, he was nominated as a trustee on the board directors of the Institute of Cetacean Research, Tokyo in April 1991. Subsequently, he was nominated as the director general of the ICR in December 1995. He continued to serve in the position of director general until December 2003.

Subsequently Dr. Ohsumi was nominated as a senior scientific adviser in January 2004, and as an honorary scientific adviser in October 2015. He remained in that position until November 2019 and continued to travel from his home in Shinjuku to the ICR almost daily until November 1, 2019. Seiji always said that "I continue to go to the office each day because this is my eternal mission."

During the period through the 1950s to the 2010s, he made tremendous scientific works and published numerous scientific articles on cetaceans over 500 papers including both English and Japanese articles. One of his academically important papers was on the school structure of sperm whales titled "Some investigations on the school structure of sperm whale (*Scientific Report of WRI*, 23: 1–25.)". During his academic years he received numerous numbers of awards including the Royal Norwegian Order of Merit, Special Award of the Mammal Society of Japan, etc.

His public memorial service was held at 13:00 on December 23, 2019 at the Mariners Court Hotel Tokyo (Harumi, Tokyo), jointly hosted by the Institute of Cetacean Research and Seiji Ohsumi's family. The co-mourners were Dr. Yoshihiro Fujise (Director general of ICR), Drs. Masako Osumi (wife, Professor Emeritus of Japan Women's University) and Noriko Osumi (daughter, Professor of the Medical school, Tohoku University).

The next volume, CPOPS *Vol. 3*, will be designated as "*Memorial Volume for Dr. Seiji Ohsumi*", we would request widely to submit paper contributions from various areas and peoples.

June 1, 2020

Hidehiro Kato

Chairman Alternate, Publication Committee for the Cetacean Population Studies

Full paper

A PRELIMINARY STUDY OF EPIGENETIC ESTIMATION OF AGE OF THE ANTARCTIC MINKE WHALE *BALAENOPTERA BONAERENSIS*

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Abstract

Age is one of the most important life history parameters for assessment and management of marine living resources. Counting of the growth layers deposited in the earplugs is the most accepted technique for determining chronological age of baleen whales. However unreadable growth layers form in the earplugs of some individual whales. In such cases, alternative methods of age estimation are required. The objective of the present study was to examine the utility of the DNA methylation technique as a proxy to estimate chronological age in the Antarctic minke whale. For this purpose, skin tissues of a total of 100 Antarctic minke whales sampled in the Pacific region of the Antarctic by JARPAII surveys were used. Earplug-based age data from the same whales were used for calibration purposes. Seven CpG sites in three genes (TET2, CDKN2A and GRIA2) were selected for the analysis. In a previous study, these sites showed significant correspondence between methylation levels and age in humpback whales. Methylation levels of the seven CpG sites were scored successfully. Four CpG sites showed significant regressions with age, which contrasted with the case of the humpback whale where all seven sites showed significant regressions with age. The assay predicted age from skin samples with a standard deviation of 8.865 years. This low precision makes the age estimated by the CpG methylation technique unsuitable for use in population dynamics models such as the statistical catch-at-age (SCAA). Furthermore CpGs methylation levels fluctuated among body positions of the whale, particularly between dorsal (exposure to sunlight) and ventral sides. The precision of the CpG methylation technique for age estimation could be improved by increasing the number of CpG sites showing a good correlation with age, and this work is ongoing. In addition, other factors including variation of CpG methylation levels between different tissues should be examined to further evaluate the utility of the DNA methylation techniques as a proxy of age estimation in baleen whales.

Key words: Antarctic minke whale, age estimation, epigenetics, DNA methylation, proxy of earplugs.

Introduction

Age is one of the most important life history parameters for assessment and management of marine living resources. In baleen whales, age has been determined using a variety of methods such as examination of baleen plates (Nishiwaki, 1951; Zenitani and Kato, 2010), earplugs (Lockyer, 1984)

and tympanic bulla (Christensen, 1995). Counting of the growth layers deposited in the earplugs is the most accepted technique for determining chronological age of baleen whales (Lockyer, 1984). Earplug-based age determination has the advantage that it is time- and cost-efficient, and the technique can be used on available historical samples.

The Antarctic minke whale (*Balaenoptera bonaerensis*) is one of the smallest balaenopterid species, which is widely distributed in the Southern Hemisphere. This species is considered the most abundant baleen whale species, with a total abundance estimated at 515,000 (IWC, 2012). During the assessment of this species, the International Whaling Commission Scientific Committee (IWC SC) successfully applied statistical catch-at-age (SCAA) analyses. A summary history of the application of SCAA to this species was presented by Punt (2014), and an assessment of Antarctic minke whales using SCAA was reported by Punt *et al.* (2014). Among the most important outputs from the SCAA analyses was the information on the age-specific natural mortality and the historical trends of the stocks. The key input data for the SCAA analyses consisted of catches, abundance estimates, length frequency data and age-at-length data. Age data was obtained from reading earplugs of Antarctic minke whales caught during past commercial whaling operations and by scientific surveys by the Japanese Whale Research Program under Special Permit in the Antarctic (JARPA/JARPAII).

While earplug's growth layers reading is considered the most acceptable technique for age determination in whales, unreadable growth layers form in the earplugs of some individual baleen whales (Maeda *et al.*, 2013; George *et al.*, 1999). In the case of the Antarctic minke whale, determining the age by reading earplugs of immature whales is particularly difficult. In such cases, alternative methods of age determination are required.

Indices of chronological age of whales have been developed using molecular approaches. These include length of telomeres (Olsen *et al.*, 2012; 2014), and epigenetic technique based on DNA methylation (Polanowski *et al.*, 2014). Another technique is based on enantiomers of aspartic acid in eye lens, the aspartic acid racemization (AAR) technique, which was successfully applied to Antarctic minke whales (Yasunaga *et al.*, 2017).

The present study focuses on the estimation of age in Antarctic minke whale based on the epigenetic approach. The best studied class of epigenetic change in vertebrates is the methyl group presence or absence at the C5 position of Cytosine residues that are adjacent to Guanidine residues ('CpG sites'). CpG methylation levels play an important role in the control of gene expression, where higher methylation levels ('hypermethylation') generally reduce gene transcription rate. Methylation changes at specific CpGs have been linked to age in mice (Maegawa *et al.*, 2010) and humans (Christensen *et al.*, 2009; Gronniger *et al.*, 2010; Bocklandt *et al.*, 2011; Koch and Wagner, 2011; Hannum *et al.*, 2013). It should be noted that the CpG methylation technique does not provide the chronological age of the individuals but rather a physiological age that can be used as a proxy for chronological age.

The CpG methylation approach was recently applied to known age (from photo-identification studies) humpback whales (Polanowski *et al.*, 2014). These authors assayed 37 cytosines for methylation level in humpback whale skin of which seven had significant age-related profiles. They selected the three most age-information cytosine markers for a humpback whale epigenetic age assay. The assay had a coefficient of estimation (R^2) of 0.787, and predicted age from skin samples with a standard deviation of 2.991 years.

The objective of the present study was to examine the utility of the CpG methylation technique as a proxy to determine chronological age in the Antarctic minke whale, by using the same three informative cytosine markers of the humpback whale (Polanowski *et al.*, 2014). The ages of the Antarctic minke whales examined for this epigenetic study were available from earplug's readings, and these data were used for calibration purposes.

Apart from the investigation of informative CpG sites, several other factors should be considered to further investigate the utility of the CpG methylation technique for determining age of whales for population-level analyses (see summary in IWC, 2017). For example, it was suggested there is a need

to better understand the 'stressors' (e.g. sunlight) that may affect the calibration of the methylation approach. A secondary objective of the present study was therefore to investigate changes of the CpG methylation levels with regard the body positions where the skin samples were obtained, *i.e.* parts with more or less exposure to sunlight.

Materials and methods

Whale sampling

Antarctic minke whales used in the present study were caught in the austral summer seasons 2010/11 and 2011/12 in the Pacific sector of the Antarctic comprised of the area between 130°E–145°W by surveys of the Second Phase of the Japanese Whale Research Program under Special Permit in the Antarctic (JARPAII). For the CpG methylation study a total of 100 whales were selected from the total number of whales caught in the two austral summer seasons. The selection was based on the quality of their age information from earplugs, information used for calibration purposes. The age determination based on earplugs was conducted by a researcher from the Institute of Cetacean Research (ICR) following Lockyer (1984). Each individual age was determined by counting growth layers appearing on the bisected surface of the earplug using a stereoscope microscope, assuming an annual deposition of growth layers (i.e. one pair of dark and pale laminae accumulated per year). For the 100 whales selected, age information from the earplugs was considered as 'Excellent' (very clear Growth Layer Groups (GLGs), and low likelihood of aging reading error) or 'Good' (most of the GLGs are clear, and moderate likelihood of aging reading error). Table 1 shows the number of individuals used in this study by sampling season, sex and age classes.

Tissue sampling and DNA extraction

Skin tissue samples were obtained from each of the 100 whales, from the left lateral part of their body (sampling position '6' in Fig. 1). Tissue samples were stored in 95% ethanol until DNA extraction. Genomic DNA was extracted from 0.05 g of skin tissues using Gentra Puregene kits (QIAGEN).

Table 1. Sample used in the epigenetic study of Antarctic minke whale, by austral summer season, sex and age classes. Age data shown in this table were obtained by earplug readings, and were used for calibration purposes.

Season	Sex	Age					Total
		–10	11–20	21–30	31–40	41–	
2010/2011	F	10	16	9	3	3	41
	M	7	8	8	2		25
2011/2012	F	1	8	11	6	1	27
	M	1	3	1	1	1	7
Total		19	35	29	12	5	100

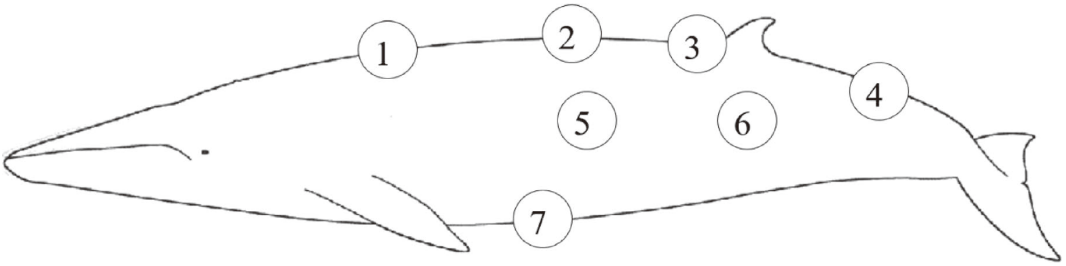

Fig. 1. Whale body positions compared for methylation levels of CpG sites. Additional three positions (8–10, not shown) corresponded to injured parts by cookie cutter shark.

Extracted DNA was stored in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The IWC guidelines for DNA data quality (IWC, 2009) were followed as much as possible (see Kanda *et al.*, 2014).

Identification of age-related CpG sites in the Antarctic minke whale

The procedure for identification of age-related CpG sites and measurement of methylation levels in Antarctic minke whales followed Polanowski *et al.* (2014), and their explanation of procedures is repeated below. The following three genes (seven CpG sites) were selected because they showed significant correspondence between CpG methylation levels and age in humpback whales: TET2 (CpG+16, CpG+21 and CpG+31 sites); CDKN2A (CpG+297, CpG+303 and CpG+309 sites), and GRIA2 (CpG+202 site).

Three candidate regulatory region sequences of Antarctic minke whale (Kishida *et al.*, 2015) were taken from GenBank and BLAT searches of the humpback whale genome. Where candidate genes had a clearly orthologous regulatory region in the humpback whale genome, primers for amplification of Antarctic minke whale sequences were designed by eye based on homologous humpback whale sequences (Table 2).

Measurement of cytosine methylation levels

Methylation levels in the CpG sites were measured with Qiagen PyroMark assays. The pyrosequencing assays were designed using PYROMARK Assay Design Software (Version 2.0.1, Qiagen). Antarctic minke whale DNA was converted using the Epitect Bisulphite Conversion Kit (Qiagen). The assay regions were PCR amplified using a biotin-labelled, HPLC-purified primer and standard sequencing grade primer (Table 2). Amplification reactions consisted of 12.5 μl PYROMARK mastermix, 2.5 μl Coral Load, 1 μl each of 5 μM forward and 5 μM reverse primers, 2 μl of bisulphite converted template DNA and 6 μl of water. Thermocycling conditions were 15 min at 95°C followed by 45 cycles of 30 s at 95°C, 30 s at 56°C and 30 s at 72°C and a final extension step of 10 min at 72°C. Pyrosequencing was performed on a PYROMARK 24 Pyrosequencing System (Qiagen). The PYROMARK Q24 software gave percentage methylation values for each CpG site.

Comparison between methylation level of CpG sites and age determined by earplug readings

The methylation levels for each of the seven CpG sites were compared to the ages determined by earplug readings, for n=100 individuals. Linear regression was used to investigate how much of the variation in CpG site methylation was explained by age differences. The accuracy and precision of the Antarctic minke whale epigenetic age assays were assessed with a Leave One Out Cross Validation (LOOCV) (Picard and Cook, 1984) analysis for the seven methylation sites combined.

Table 2. Primers used for amplification of Antarctic minke whale sequences in the identification of age-related CpG sites by the PyroMark assay.

Sequence Name	Sequence (5' to 3')
Gria2_F-Bio	Biotin-GGGTGAGTGTGTGAGTGTA
Gria2_R	AAACCCTATCTCCCAAATCCTAC
Gria2_SQ	ACTAAATACAACTTCCAAC
TET2_F	GTGGTTAAAGTAAATAGAAGGT
TET2_R-Bio	Biotin-CAAAAACACTCCCCAATTTC
TET2_SQ	GGTTAAAGTAAATAGAAGGTG
Cdkn2a_F	AGAGATTTTTGGTAAAGGGGAGAT
Cdkn2a_R-Bio	Biotin-CCCCATATACCTTTCAATCCTCC
Cdkn2a_SQ	TTGGGGAGTTTTTAGAT

Table 3. Data of Antarctic minke whales examined for variation of methylation levels among body positions (see Fig. 1).

Whale No.	Sex	Body length (m)	Body position (Figure 1)
1	Male	5.61	1–7; 8–10
2	Female	6.56	1–7; 8–10
3	Male	8.66	1–7; 8–10
4	Male	8.03	1–7; 8–10
5	Female	9.56	1–7; 8–10
6	Female	9.30	1–7; 8

Table 4. Information of additional sampling positions (8–10) that corresponded to parts of the body injured or with Cookie cutter shark marks. D: dorsal side; L: lateral side; and V: ventral side.

Whale No.	Injured parts	Cookie cutter shark marks
1	8 (D)	9 (L), 10 (V)
2	–	8 (D), 9 (V), 10 (L)
3	9 (D)	8 (V), 10 (D)
4	–	8 (V), 9 (L), 10 (L)
5	–	8 (L), 9 (L), 10 (L)
6	8 (L)	–

Variation of the methylation levels of CpG sites with body position

Six Antarctic minke whales sampled during the 2016/2017 New Scientific Whale Research Program in the Antarctic Ocean (NEWREP-A) survey in the sector comprised of the area between 45°–150°E were used for this experiment. Skin samples were collected from four positions of the dorsal side (positions 1–4), two positions of the lateral side (positions 5–6), and one position of the ventral side (position 7) (Fig. 1, Table 3). Also, in the case of five whales (all except whale No. 6 in Table 3), additional sampling was carried out at three positions (8–10) that corresponded to parts of the body injured or with Cookie cutter shark (*Isistius brasiliensis*) marks (Table 4). Cookie cutter sharks live in the depths of all the oceans near the equator. Their top teeth are small, pointy and sharp to grasp hold of the whale or other prey's skin. Turning in a circle, this shark carves a round chunk of flesh out with its larger razor-sharp, serrated or saw-like bottom teeth. In a flash, the cookie cutter scoops out the meal. Its preys are left with an almost perfectly round mark that looks like someone used a round cookie cutter on its body (Compagno, 1984). In the case of whale No. 6, only one additional position was investigated (position 8; Table 3). In total, 58 skin samples were collected for this experiment.

Tissue preservation, DNA extraction and DNA-M procedures were the same as explained earlier. Methylation levels of the seven CpG sites in each of the six whales were compared among the seven body positions shown in Fig. 1.

Results

Identification of age-related epigenetic markers in Antarctic minke whale

The PCR amplifications using a biotin-labelled primer set for the three genes were checked for quality by the agarose gel. The three genes for all individuals were amplified successfully. After pyrosequencing assay, the quality checks by PYROMARK Q24 software were evaluated as high (passed) for the three genes of all individuals and methylation levels for each CpG site were assessed.

Comparison between cytosine methylation level and age determined by earplug readings

The regressions of age and methylation levels in each of the seven CpG sites are shown in Fig. 2. The highest R^2 was observed in site TET2_CpG+31 (0.1874) and the lowest in site CDKN2A_CpG+297 (< 0.0001). Four CpG sites had a significant regression relationship with age (TET2_CpG+16, P=0.014; TET2_CpG+21, P=0.009; TET2_CpG+31, P=6.88e-06; and CDKN2A_CpG+309, P=0.005). The other three sites (GRIA2_CpG+202, CDKN2A_CpG+297 and CDKN2A_CpG+303) had a non-significant regression relationship with age (P > 0.05).

The precision of the Antarctic minke whale epigenetic age assay as assessed by the LOOCV is shown in Fig. 3. The overall precision was estimated as the standard deviation of the mean difference between known and estimated ages, which was 8.865 years.

Variation of the methylation levels of CpG sites with body position

Fig. 4 shows the variation of the methylation levels of CpG sites at different positions in the whale body of six individual Antarctic minke whales. This analysis was made for the three genes and seven CpG sites (excepting some few for which amplification failed). Methylation level of the seven CpG sites fluctuated with body positions regardless of the body length of the whales. In particular, the three TET2's CpG sites showed that the difference between the maximum and the minimum values of methylation levels was 50% or more among body positions. The ratio of maximum and the minimum methylation level of the TET2's sites were 2.1–3.2 fold in the whale No. 2; 1.3–1.7 fold in the whale No. 5; and 1.6–2.4 fold in the whale No. 6 (Table 3; Fig. 4). The average methylation level per indi-

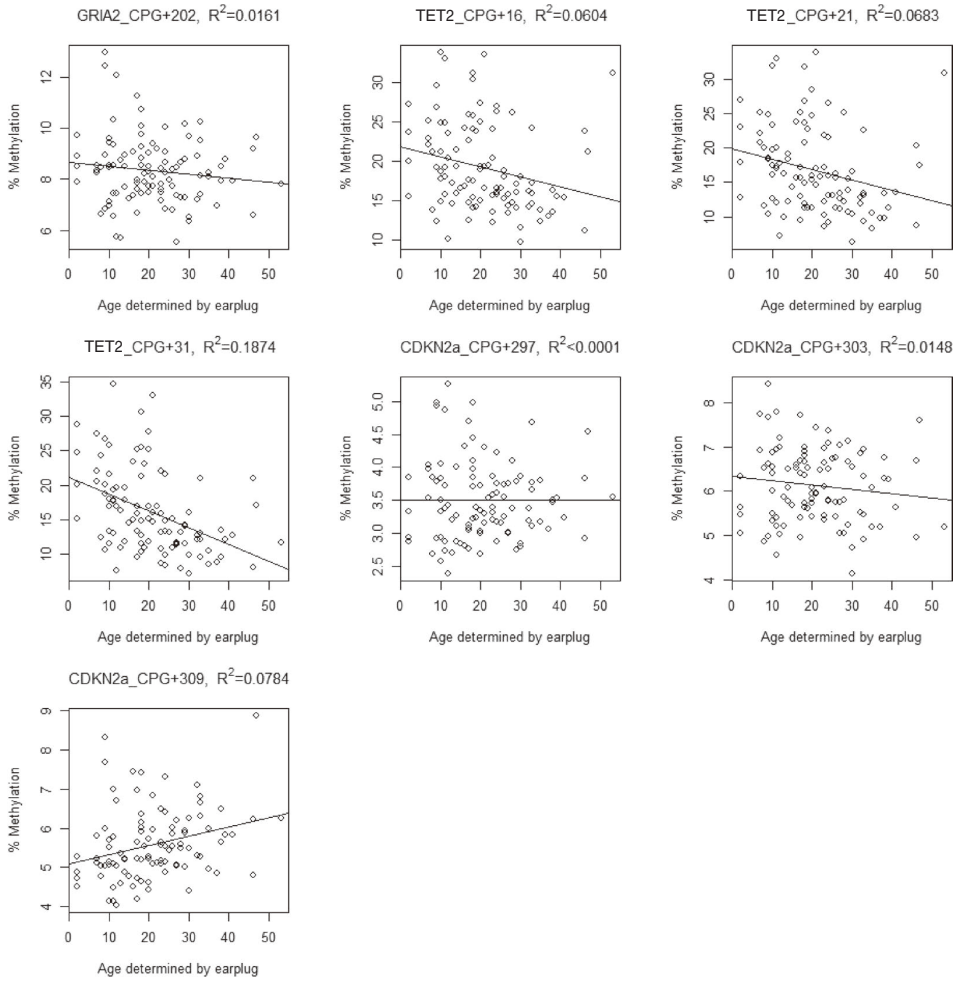

Fig. 2. Regressions of CpG methylation level and age determined by earplug readings at seven sites selected for Antarctic minke whale. CpG methylation level was measured at each site by a PyroMark assay in n = 100 whales.

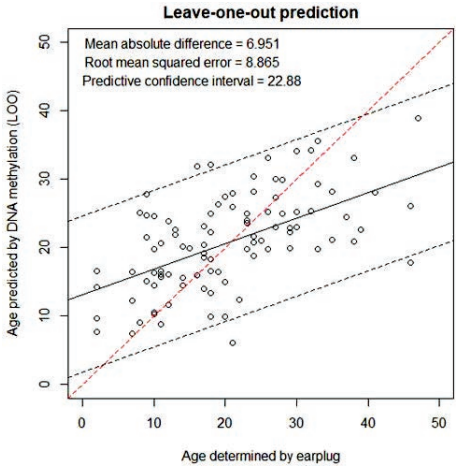

Fig. 3. The precision of the Antarctic minke whale epigenetic age assay by a Leave One Out Cross Validation (LOOCV) analysis for seven CpG methylation sites combined. 95% confidence limits for age prediction are shown.

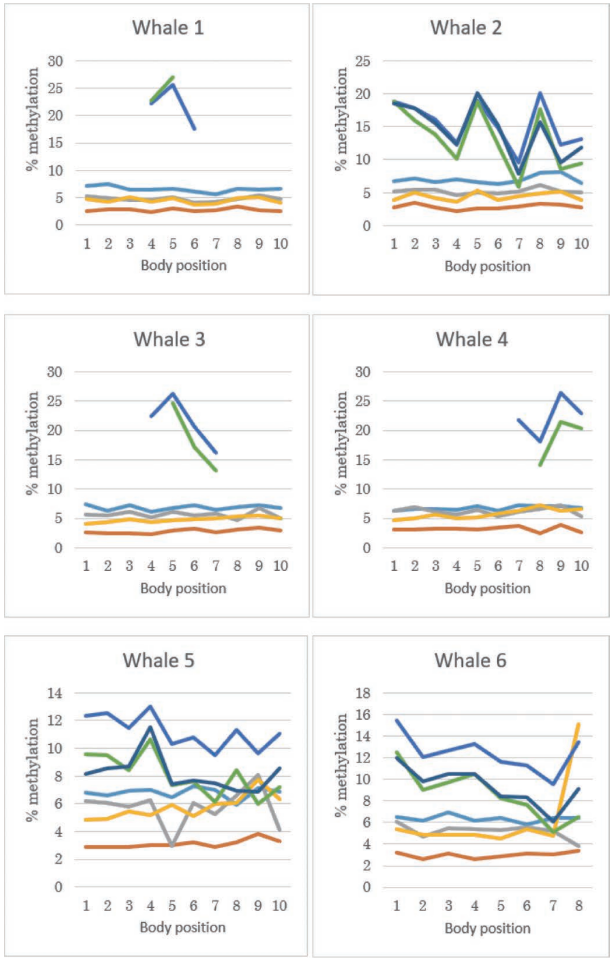

Fig. 4. Comparison of CpG methylation levels at multiple body positions in six individual Antarctic minke whales: (▬) GRIA2_CpG+202, (▬) TET2_CpG+16, (▬) TET2_CpG+21, (▬) TET2_CpG+31, (▬) CDKN2A_CpG+297, (▬) CDKN2A_CpG+303 and (▬) CDKN2A_CpG+309. See Figure 1 for explanation of body positions.

vidual, per TET2's site was 12.5% in the four dorsal positions, 11.5% in the two lateral positions and 7.5% in the ventral position. The three TET2's sites in three individuals (whales No. 2, 5 and 6) showed the lowest methylation level in body position seven (ventral side), compared with the other positions (Table 3; Fig. 4).

Regarding additional sampling positions (8–10) that corresponded to parts of the body with Cookie cutter shark marks, the average methylation level per individual, per TET2's site in the whales No. 2 and 5 was 10.2%. This value was lower than the average methylation level in the four dorsal positions or in the two lateral positions, and higher than in the single ventral position.

Discussion

This study provided a good opportunity to compare the utility of the same CpG sites to determining age in two baleen whale species. The present study took advantages of a substantial number of Antarctic minke whales for which age had been determined independently by earplug readings. Such information was of great utility for calibration purposes.

As noted earlier chronological age is important for assessment purposes related to the Antarctic minke whale such as that conducted using SCAA (Punt *et al.*, 2014). Such models require precise information on the age of individual whales. In this regard the precision of the age estimates by the CpG methylation approach was low in both humpback (standard deviation of 2.991 years, Polanowski *et al.*, 2014) and Antarctic minke (standard deviation of 8.865 years) whales. It should be noted that the LOOCV estimates of standard deviation in both species are not comparable as the analysis in humpback whales was based on three sites while the Antarctic minke whale analysis was based on seven sites. In any case, the low precision in both cases makes the age estimated by the CpG methylation approach unsuitable for use in population dynamics models such as the SCAA. Kitakado (2016) showed that the precision of methylation-based recruitment in the Antarctic minke whale is much worse than that for earplug-based readings, with the methylation-based results hardly better than those without any age information at all.

On the other hand the CpG methylation technique does not provide the chronological age of the individuals but rather a physiological age that can be used as a proxy for chronological age. Thus physiological and metabolic differences between species, means that the technique will need to be calibrated for each species (IWC, 2016). This study made a contribution in this regard as the performance of the same genetic markers was compared between two different species of baleen whales. In fact, some differences were observed between the two species in the pattern of methylation levels with respect to the age. For example in the case of the humpback whale all seven loci showed significant regression with age while in the case of the Antarctic minke whale only four of those loci showed a significant regression relationship. Tanabe *et al.* (2020) concluded that age-related CpGs can differ even between closely related species, and that it is necessary to find species-specific age-related CpGs for estimating the age of animals using this technique.

This present study found that CpG methylation levels vary substantially among different parts of the body from which skin samples were obtained. In particular differences were observed between dorsal (body parts exposed to sunlight) and ventral (body parts not exposed to sunlight). Gronniger *et al.* (2010) showed that aging and sun exposure are associated with comparably small, but significant changes in the DNA methylation patterns of human epidermis and dermis samples. There is a need to better understand the 'stressors' (e.g. sunlight) that may affect the calibration of the methylation approach and further studies should be conducted in the future. On the other hand, the effects on methylation levels of acquired external injures including Cookie cutter shark marks might be minimal for the Antarctic minke whales.

The screening of a substantial number of CpG sites based on Human BeadChip microarray has

already started for the Antarctic minke whale, and results will be presented in the near future. Precision of the CpG methylation technique for age estimation could be improved by increasing the number of CpG sites showing a good correlation with age.

Apart from increasing the number of CpG sites to increase the precision of the age estimates, other aspects should be considered to further evaluate the utility of the CpG methylation approach as a proxy of chronological age in baleen whales. For example, correlation between chronological age and methylation profile varies a great deal among different tissues (Horvath, 2013). In addition to skin, biopsy samples typically include connective tissue and the lipid filled fat cells and these tissues should also be investigated (Arner *et al.,* 2015). These studies should continue in the future using a large number of CpG sites and whales.

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Photo 1. Antarctic minke whale

Full paper

DENSITY DISTRIBUTION OF SEVERAL MAJOR WHALE SPECIES IN THE INDO-PACIFIC REGION OF ANTARCTIC USING JARPA AND JARPAII SIGHTING DATA OBTAINED THROUGH 1987/88–2008/09 SEASONS

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Abstract

This paper examined the geographical distribution of several whale species in the Indo-Pacific region of the Antarctic during the austral summer. The analyses were based on sighting data collected systematically by JARPA and JARPAII surveys in the longitudinal sector of 35°E–145°W, south of 60°S, between 1987/88 and 2008/09. The searching effort comprised a total of 353,134 n.miles. The Antarctic minke whale was the species most frequently sighted, followed by killer, humpback, unidentified beaked, fin, sperm, southern bottlenose, blue, southern right and sei whales. Density index of whales (DIW: no. of individuals sighted/100 n.miles) was calculated using all primary effort and sightings data and its geographical distribution plotted on maps with Lat. 1 degree × Long. 1 degree squares for each species. These maps are more detailed compared to those of the previous maps which used 5° × 5°squares in the 1960s. The geographical distribution was described for each whale species together with some features of their distribution. For example, sei, dwarf minke, humpback and southern bottlenose whales were not sighted in the Ross Sea, distribution areas of southern right whales were limited to the sector 80°E and 135°E, and high-density areas of humpback whales were observed between 80°E and 110°E. The large scale and long-term sighting data set has made a substantial contribution to understanding the geographical distribution patterns and habitat use of whales in the Antarctic ecosystem.

Key words: Antarctic, distribution, baleen whales, toothed whales.

Introduction

One of the main sources of large scale and long-term sighting data for assessing the population status of whale species in the Antarctic is the JARPA (Japanese Whale Research Program under Special Permit in the Antarctic), the first phase of which was conducted between 1987/88 and 2004/05, with its second phase, JARPAII (sighting component), conducted between 2005/06 and 2008/09. One of the features of JARPA and JARPAII is that, unlike the IWC/IDCR (International Whaling Commission/International Decade for Cetacean Research)-SOWER (Southern Ocean Whale and Ecosystem Research) programmes conducted from 1978/79 to 2009/10 (Matsuoka *et al.*, 2003a), surveys have been repeated in the same area and in the same months every second season over a long period. Cur-

rent distribution maps are more detailed for each Area compared to those of the IDCR/SOWER maps which had covered the area only three times for each set of circumpolar data on whales. Therefore, the JARPA and JARPAII surveys facilitate description of the extent of detailed local distribution of whales.

The sighting data collected during the JARPA and JARPAII have been used for studying the distribution patterns and estimated abundance of several large whale species (Kishino *et al.*, 1991; Kasamatsu *et al.*, 2000; Matsuoka *et al.*, 2003b, 2011; Branch *et al.*, 2004; Murase *et al.*, 2002, 2014).

The objective of this study was to investigate the geographical distribution patterns of large whale species in the Indo-Pacific region of the Antarctic during the austral summer feeding season. The study was based on sighting data collected systematically by the JARPA and JARPAII surveys.

Materials and Methods

Research area

The research area comprised the Indo-Pacific region of the Antarctic every year, specifically the IWC Management Areas IIIE (35°–70°E), IV (70°–130°E), V (130°E–170°W) and VIW (170°–145°W), south of 60°S (Fig. 1). Each individual survey was conducted from December to March during austral summer (Fig. 2).

These Areas were divided into two sectors (western sector and eastern sector). Each sector was also divided into two strata (northern and southern strata), along the 60°S latitude line to the line of 45 n.miles from the ice-edge (northern stratum), and ice-edge to 45 n.miles from the ice-edge line (southern stratum), except for the Prydz Bay and the Ross Sea regions. The Prydz Bay is defined as south of 66°S, and the Ross Sea is defined as south of 69°S. There are no stratifications for Areas IIIE and VIW in JARPA. In JARPAII, there are stratifications for Areas IIIE and VIW which are the same as in Area IV (Fig. 3).

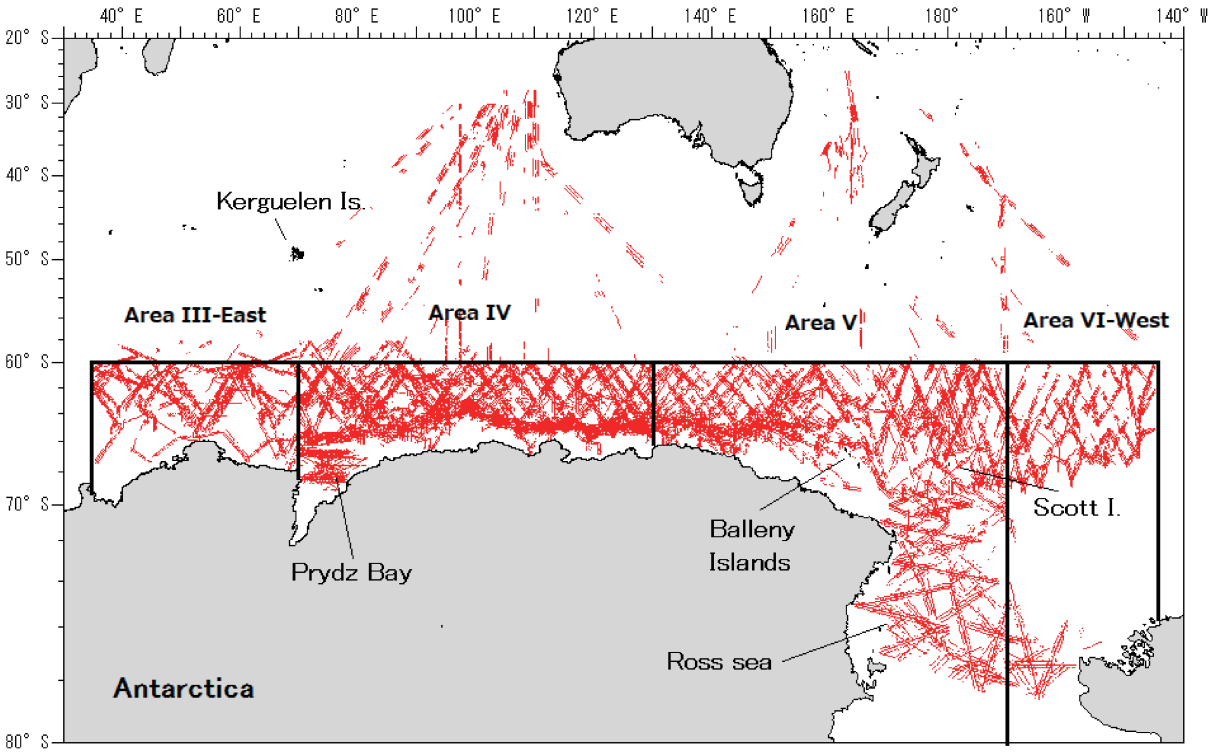

Fig. 1. The main research area of the JARPA and JARPAII surveys between 35°E and 145°W, south of 60°S with searching efforts (red lines) in the period 1987/88–2008/09, including the transit sighting surveys between low latitude and Antarctic regions with the IWC Antarctic Areas for the management of baleen whales (except Bryde's whale).

Sighting data

The collection procedures and analyses of sighting data that have been used in JARPA are very similar to those used in IWC/IDCR-SOWER cruises. Activities aboard the ship are classified into two principal groups: On-effort and Off-effort. On-effort activities are times when full search effort is being executed and conditions (such as weather and sea conditions) are within acceptable parameters to conduct research. Off-effort activities are all activities that are not On-effort. All sightings recorded while the ship is On-effort are classified as Primary sightings. All other sightings are Secondary sightings.

Primary search effort is only conducted in acceptable weather conditions. These conditions are defined as visibility better than 2.0 n.miles, with wind speed less than 20 knots and Beaufort sea state less than 6. These conditions are used as guidelines; in some circumstances, less severe conditions may still be inappropriate for search effort. The sighting procedure in JARPAII (2005/06–2008/09) did not differ substantially from JARPA (Hakamada *et al.*, 2006; Nishiwaki *et al.*, 2014).

The research vessels (ship length averages about 70 meters) were equipped with a top barrel (almost 20 meters from the sea level), from which three men conducted sighting observations. On the upper bridge (almost 11.5 meter from the sea level), a captain, a gunner, a helmsman and a researcher also conducted sightings. The sighting activity was carried out from 30 minutes after sunrise to 30 minutes before sunset. The survey ship speed averages about 11.5 knots.

When a sighting is made, the topman (or upper bridge observer) gives an estimate of the distance and angle to the sighting and the ship turns immediately, regardless of the angle to the sighting. The whales were approached and the species, number of animals and number of calves (if present) determined. In order to save valuable research time, closure to the sighting position of whales that can be positively identified as long-diving species (such as sperm whales or beaked whales) may be abandoned if it is considered that the animals have dived.

In addition, using the round-trip transit sighting surveys were also conducted every season between low latitude and Antarctic regions, although the searching effort was low compared to that south of 60°S (Fig. 1).

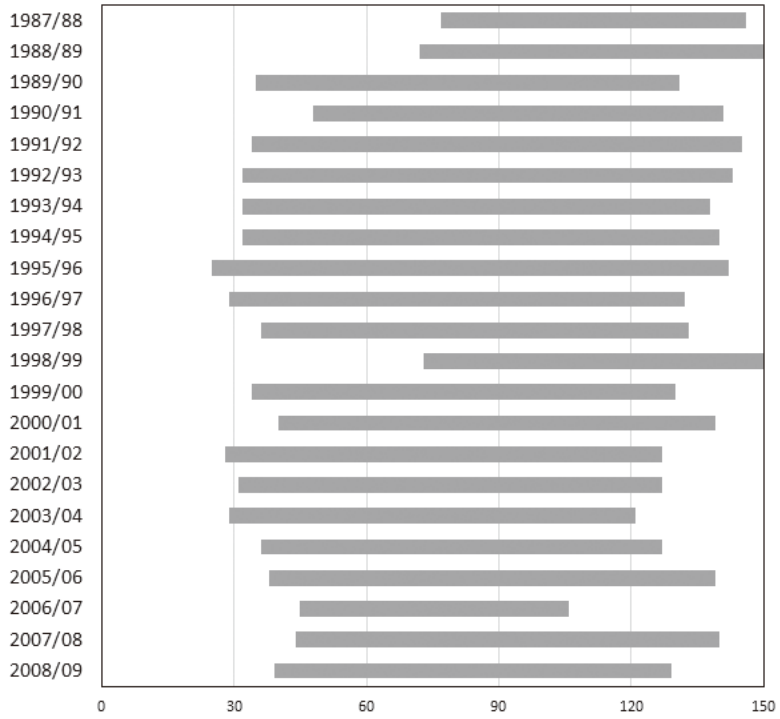

Fig. 2. Start and end dates of JARPA and JARPA II surveys (1989/90–2008/09) in the research area.

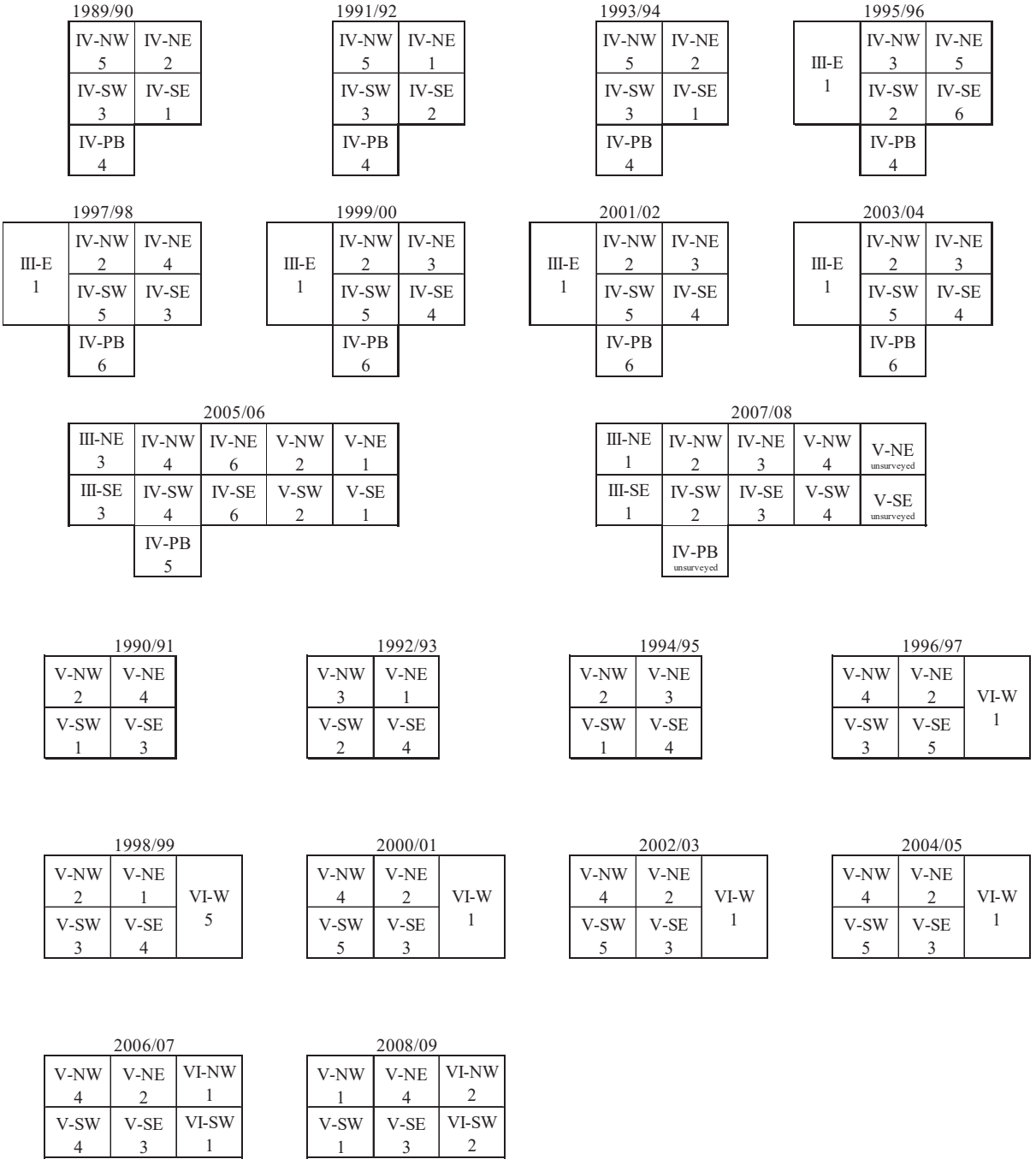

Fig. 3. Survey order by strata for the period from 1989/90 to 2008/09 seasons. Key: III=Area III, IV=Area IV, V=Area V, VI=Area VI, E=East, W=West, NW=North-West, NE=North-East, SW=South-West, SE=South-East, PB= Prydz Bay. A common number in a season indicates that two strata were surveyed in the same period. V-NE, V-SE and IV-PB strata could not be surveyed at all in 2007/08 season (Hakamada and Matsuoka, 2014a).

Density index of whales and mean school size

The Density Index of Whales (DIW), i.e. the number of individual whales sighted per 100 n.miles, was calculated for each Lat.1° × Long.1° grid square. The mean school size (Mss) in this study is the arithmetic mean (i.e. number of animals divided by number of schools).

Results and Discussion

Searching efforts

A total of 353,134 n.miles were surveyed in Areas IIIE, IV, V and VIW, south of 60°S, between 1989/90 and 2008/09. Fig. 4 shows the distribution of the primary searching effort (n.miles). All grid squares were searched and the research area south of 60°S was completely covered.

Distribution of whales

Tables 1a and 1b show a summary of the primary sightings of baleen and toothed whales, respec-

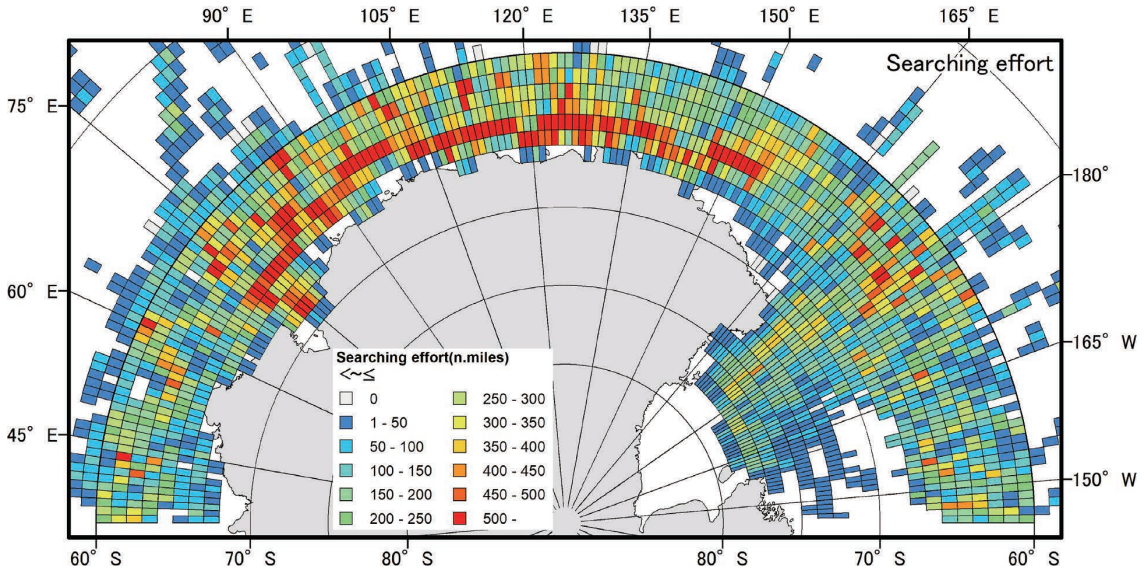

Fig. 4. Distribution of the primary searching effort by Lat.1° × Long.1° squares in JARPA and JARPAII surveys in the period 1987/88–2008/09 seasons.

Table 1a. Summary of baleen whale species sighted in the Indo-Pacific region of the Antarctic.

No.	Season	Research area	Effort (n.miles)	Blue whale			Fin whale			Sei whale			Ant. minke whale			Dwarf minke whale			Humpback whale			S. right whale		
				sch.	ind.	calf	sch.	ind.	calf	sch.	ind.	calf	sch.	ind.	calf	sch.	ind.	calf	sch.	ind.	calf	sch.	ind.	calf
1	1987/88	IV	8,860.6	0	0	0	3	3	0	1	1	0	237	719	0	1	2	0	35	76	0	1	1	0
2	1988/89	V	10,806.7	2	3	0	7	16	0	0	0	0	353	768	0	5	5	0	1	2	0	0	0	0
3	1989/90	IV	16,423.2	5	9	0	5	20	0	0	0	0	758	1,968	0	3	3	0	121	210	11	2	2	0
4	1990/91	V	14,660.0	4	6	0	33	67	0	0	0	0	740	1,713	0	6	6	0	58	90	0	0	0	0
5	1991/92	IV	17,844.1	3	3	0	8	34	0	2	2	0	597	2,030	0	0	0	0	177	321	7	26	30	0
6	1992/93	V	13,924.9	7	9	0	15	27	1	2	4	0	1,024	3,228	0	7	7	0	28	56	5	3	4	0
7	1993/94	IV	17,957.3	5	9	0	9	26	0	0	0	0	688	1,619	0	4	4	0	133	220	1	11	14	0
8	1994/95	V	14,047.7	13	20	1	73	241	1	2	5	0	823	2,453	0	6	6	0	131	228	9	0	0	0
9	1995/96	IIIE, IV	21,466.7	9	16	0	60	214	1	0	0	0	887	2,008	0	2	2	0	325	562	10	8	8	0
10	1996/97	V, VIW	17,783.2	7	9	0	37	82	1	1	1	0	853	2,610	0	9	9	0	114	200	3	0	0	0
11	1997/98	IIIE, IV	21,594.4	16	25	0	18	57	0	0	0	0	672	1,373	0	2	2	0	577	1,122	2	34	37	0
12	1998/99	V, VIW	8,066.5	4	7	1	45	222	1	0	0	0	826	2,665	0	3	3	0	106	203	7	0	0	0
13	1999/2000	IIIE, IV	16,341.5	25	53	2	66	356	3	0	0	0	1,507	6,581	0	0	0	0	661	1,269	5	3	3	0
14	2000/01	V, VIW	20,421.3	10	18	0	114	374	0	7	13	0	1,907	4,949	0	27	27	0	191	341	3	2	2	0
15	2001/02	IIIE, IV	19,767.4	17	26	1	143	983	2	1	2	0	1,867	4,374	0	0	0	0	1,219	2,387	5	15	22	1
16	2002/03	V, VIW	18,126.2	5	10	0	52	216	0	8	14	0	2,420	6,531	0	6	6	0	145	228	4	0	0	0
17	2003/04	IIIE, IV	19,287.4	32	61	0	109	446	0	0	0	0	1,092	3,250	0	2	2	0	1,690	3,134	5	1	2	1
18	2004/05	V, VIW	18,486.7	12	16	0	49	118	1	1	1	0	1,663	4,278	0	0	0	0	197	336	2	2	2	0
19	2005/06	IIIE, IV	16,372.7	24	38	2	188	748	1	2	3	0	1,657	4,375	0	0	0	0	1,702	3,200	22	53	73	4
20	2006/07	V, VIW	11,968.8	7	12	1	37	253	0	0	0	0	969	2,169	0	1	1	0	160	283	13	0	0	0
21	2007/08	IIIE, IV	14,575.3	43	84	1	48	134	4	2	2	0	823	1,702	0	0	0	0	1,314	2,536	7	72	96	0
22	2008/09	V, VIW	14,351.4	14	28	1	109	440	2	5	7	0	1,870	4,668	0	0	0	0	339	587	8	0	0	0
-	Total	-	353,134	264	462	10	1,228	5,077	18	34	55	0	24,233	66,031	0	84	85	0	9,424	17,591	129	233	296	6

Table 1b. Summary of toothed whale species sighted in the Indo-Pacific region of the Antarctic.

No.	Season	Research area	Effort (n.miles)	Sperm whale			S. bottlenose whale			Unid. beaked whales			Killer whale		
				sch.	ind.	calf	sch.	ind.	calf	sch.	ind.	calf	sch.	ind.	calf
1	1987/88	IV	8,860.6	6	6	0	3	5	0	87	218	0	20	194	0
2	1988/89	V	10,806.7	81	91	0	2	4	0	65	143	0	31	189	0
3	1989/90	IV	16,423.2	204	215	0	23	46	0	281	514	0	69	859	0
4	1990/91	V	14,660.0	175	188	0	13	26	0	241	421	1	32	870	2
5	1991/92	IV	17,844.1	225	233	0	29	51	0	181	304	1	53	805	0
6	1992/93	V	13,924.9	105	108	0	10	19	0	202	361	0	82	1,130	0
7	1993/94	IV	17,957.3	321	336	0	145	243	0	205	337	0	56	399	1
8	1994/95	V	14,047.7	133	135	0	74	146	1	168	263	0	35	281	1
9	1995/96	IIIE, IV	21,466.7	341	352	0	137	273	1	161	284	0	109	1,282	1
10	1996/97	V, VIW	17,783.2	121	128	0	75	128	0	78	144	1	50	539	4
11	1997/98	IIIE, IV	21,594.4	295	302	0	222	409	0	197	338	0	82	931	9
12	1998/99	V, VIW	8,066.5	49	50	0	23	53	0	35	54	0	35	409	5
13	1999/2000	IIIE, IV	16,341.5	195	204	0	138	251	0	110	188	0	109	2,011	7
14	2000/01	V, VIW	20,421.3	100	106	0	72	121	0	173	272	0	72	1,471	2
15	2001/02	IIIE, IV	19,767.4	269	272	0	126	226	0	134	205	0	79	939	0
16	2002/03	V, VIW	18,126.2	128	129	0	97	168	0	113	154	0	63	953	0
17	2003/04	IIIE, IV	19,287.4	222	223	0	154	274	0	208	338	0	120	1,348	0
18	2004/05	V, VIW	18,486.7	105	108	0	44	78	0	89	159	0	78	1,472	3
19	2005/06	IIIE, IV	16,372.7	181	182	0	88	179	0	135	244	0	100	1,563	3
20	2006/07	V, VIW	11,968.8	63	63	0	51	80	0	66	88	0	44	394	0
21	2007/08	IIIE, IV	14,575.3	280	280	0	79	157	1	102	155	0	62	790	0
22	2008/09	V, VIW	14,351.4	75	76	0	32	61	0	77	140	0	38	788	14
-	Total	-	353,134	3,674	3,787	0	1,637	2,998	3	3,108	5,324	3	1,419	19,617	52

Table 2. Summary of sighting information for the whole research area in the period 1987/88-2008/09, for whale species and month. Sch: number of primary sightings of schools; Ind: number of primary sightings of individuals; Calf: number of calves; Mss: mean school size (Ind./Sch.); DIS: Density Index (schools/100 n.miles); DIW: Density Index (individuals/100 n.miles).

Species	All Areas (IIIE, IV, V and VIW; south of 60S, 35E-145W)						Order of	Dec.	Jan.	Feb.	Mar.
	Sch.	Ind.	Calf	Mss	DIS	DIW	DIW	DIW	DIW	DIW	DIW
Blue whale	286	495	11	1.73	0.081	0.140	8	0.281	0.092	0.101	0.102
Fin whale	1,268	5,209	20	4.11	0.359	1.475	5	1.323	0.794	1.760	3.059
Sei whale	36	59	0	1.64	0.010	0.017	11	0.002	0.004	0.020	0.044
Antarctic minke whale	25,507	69,076	0	2.71	7.223	19.561	1	10.173	14.301	33.331	19.436
Dwarf minke whale	84	85	0	1.01	0.024	0.024	10	0.008	0.031	0.039	0.008
Humpback whale	10,036	18,770	137	1.87	2.842	5.315	3	3.425	4.842	7.337	6.708
Southern right whale	235	298	6	1.27	0.067	0.084	9	0.001	0.014	0.156	0.292
Sperm whale	3,810	3,926	0	1.03	1.079	1.112	6	1.500	1.272	0.992	0.292
Southern bottlenose whale	1,666	3,045	3	1.83	0.472	0.862	7	0.932	0.974	0.787	0.570
Unid. beaked whale	3,175	5,457	3	1.72	0.899	1.545	4	1.864	1.594	1.123	1.209
Killer whale	1,472	20,569	59	13.97	0.417	5.825	2	1.935	5.692	9.303	6.624

tively for each individual survey. Table 2 shows the summary of sighting data for each species such as the number of calves and the observed mean school size. Total DIW for each species were the total numbers of sighted individuals divided by the total effort and multiplied by 100. Monthly DIW were obtained similarly. Figs. 5a–5d show the maps of the DIW for Antarctic blue (*Balaenoptera musculus intermedia*), fin (*B. physalus*) and sei (*B. borealis*), Antarctic minke (*B. bonaerensis*), dwarf minke (*B. a. subsp.*), humpback (*Megaptera novaeangliae*), southern right (*Eubalaena australis*), sperm (*Physeter macrocephalus*), southern bottlenose (*Hyperoodon planifrons*), unidentified beaked (Ziphiidae) and killer (*Orcinus orca*) whales, for each Lat.1° × Long.1° grid square. Figs. 6a and 6b show the longitudinal band of DIW for each species. Figs. 7a and 7b show the latitudinal band of DIW for each species. Fig. 8 shows a plot with time trends of DIW for each species. Fig. 9 shows the monthly change in DIW for most of the species.

A description of the geographical distribution of each whale species is presented and discussed below.

Blue whale

Blue whale ranked 8th for DIW among the ten species sighted in the research area (Table 2). Blue whales were widely distributed in the research area, not only in the northern stratum, but also in the southern stratum. High density values were recorded for this species in Area IIIE, particularly between 45°E and 65°E (Fig. 5a). Blue whales were rarely found in Prydz Bay, but were sighted in the Ross Sea between 70°S and 77°S. A total of 286 schools (495 individuals), including eleven calves, were sighted south of 60°S (Table 2). Observed mean school size was 1.73 individuals. Sighting rate of a mother and calf pair was 3.85% (11 out of 286 schools) which is the highest number compared to other baleen whale species in the Antarctic. A high-density area was observed between 35°E and 65°E (Fig. 6a). The DIW of this species was 0.140 for the whole survey period and the indices were almost stable from December to March (Table 2; Fig. 9). Previous studies have noted that blue whales are more common close to the ice edge than in more northerly waters (e.g. Kasamatsu *et al.*, 2000). This appears to be true for 110°E eastward, although there are blue whale sightings at or around 60°S in the region 45°E–110°E (Figs. 6a and 7a). Similar distribution pattern was observed by Branch (2007).

Two subspecies of blue whales exist in the Southern Hemisphere: the Antarctic (or true) blue whale (*Balaenoptera musculus intermedia*) and the pygmy blue whale (*B. m. brevicauda*) (Mackintosh, 1966; Ichihara, 1966; Rice, 1998). Complete reviews of the spatial and seasonal distributions, as well as densities and movements of blue whales has been provided by Kato *et al.* (1995), Branch (2007) and Branch *et al.* (2007), respectively. These studies indicated that there is little evidence that pygmy blue whales migrate to high latitudes of the Antarctic, with less than 1% of the records south of 52°S being of this subspecies. There have been a couple of genetic studies reporting some population structure in Antarctic blue whales (Sremba *et al.*, 2012, Attard *et al.*, 2016), but there is limited evidence for multiple separate populations in these papers.

The latest abundance estimate of this species (south of 60°S, 35°E–145°W) was 1,223 whales (CV = 0.345) in the 2007/08 and 2008/09 seasons, and the abundance trend was 8.2% (95% CI: 3.9%, 12.5%) between 1995/96 and 2008/09 combined for Areas IIIE, IV, V and VIW, based on JARPA and JARPAII data (Matsuoka and Hakamada, 2014). In this study, no formal analysis has been conducted but it is probable that the trend of DIW is increasing (Fig. 8). There is a need for continued monitoring of the abundance and abundance trends of this species, especially because it provides an excellent opportunity to improve our understanding of the dynamics of baleen whale populations recovering from low levels.

Fin whale

Fin whales ranked 5th for DIW among the ten species sighted in the research area. A total of 1,268 schools (5,209 individuals), including 20 calves, were sighted (Table 2). Observed mean school size

was 4.11 individuals. This species was more frequently encountered in Areas V and VIW than in Areas IIIE and IV in both northern and southern strata. High density areas were observed in Areas IIIE and IV, particularly between 55°E and 80°E, and in Area V between 163°E and 170°W (Figs. 5a and 6a). Fin whales tended to be distributed more northerly than blue whales (Fig. 7a). The DIW of this species was 1.475 for the whole period and the indices increased from December to March (Fig. 9).

In the summer feeding grounds in the Antarctic, fin whales occur year-round, but a higher density is found from November to May (Kasamatsu *et al.*, 1996; Mackintosh, 1966). These whales can be found as far south as 65–70°S, but most of the population seems to occur north of 60°S (Miyashita *et al.*, 1995). Catches occurred throughout the Antarctic, but most whales (~73%) were taken in IWC Management Areas II and III (Kasamatsu *et al.*, 1996). Sighting data of this study suggest that the fin whale's spatial distribution varies across ocean basins. In this study, no formal analysis has been conducted but it is probable that there is an increasing trend of DIW (Fig. 8). Historically more blue whales were caught than fin whales in the earlier years of pelagic whaling, and thus fin whales are likely less depleted than blue whales now, given their much higher sighting rates. There have been a couple of genetic studies on population structure for fin whales in the management Areas III, IV, V and VI (e.g. Goto and Taguchi, 2019), but these studies did not find any genetic structure in the research area.

Sei whale

The sei whale was rarely sighted in the research area. A total of 36 schools (59 individuals) were sighted south of 60°S (Table 2), with no calves observed. Observed mean school size was 1.64 individuals. Sei whales occurred more frequently in Areas V and VIW than in Areas IIIE and IV in the northern strata (Fig. 5a). High density areas were observed in Area VW, particularly between 150°E and 160°E (Fig. 6a). Sei whales were tended to distribute more northerly between 40°S and 45°S compared to fin and blue whales (Fig. 7a). The DIW of this species was 0.017 for the whole research period.

In summer, sei whales do not venture into higher latitude waters near the Antarctic continent as much as some other baleen whales. Most of the population occurs between 40°S and 60°S, usually north of the Antarctic Convergence (Miyashita *et al.*, 1995). Juveniles are found further north than mature individuals. Occurrence in low latitude wintering grounds has been recorded from March to December, but abundance peaks from June/July to August/September (Horwood, 1987). In late spring and summer, abundance peaks in November between 30°S and 50°S. As the seasons progress, relatively more whales are observed south of 40°S and abundance between 50°S and 60°S increases consistently until March (Horwood, 1987). The results in the present study are consistent with those of previous studies.

Antarctic minke whale

The Antarctic minke whale was the most frequently sighted species throughout the surveys. A total of 25,507 schools (69,076 individuals) were sighted south of 60°S (Table 2). No calves were observed. Observed mean school size was 2.71 individuals. High density areas were observed along the ice-edge, especially between 140°E and 160°E, in the Ross Sea and the Prydz Bay (Fig. 5b). Antarctic minke whales were widely and evenly distributed in the area south of 60°S (Figs. 6a and 7a). The DIW of this species for the whole research period was the highest of all recorded species (19.561). The indices increased from December to February and decreased in March (Fig. 9).

In the austral summer, the majority of Antarctic minke whales congregate in the Southern Ocean, with the greatest densities being close to and within the pack ice, and lower densities with increasing distance from the ice (Kasamatsu *et al.*, 2000; Hakamada and Matsuoka, 2014a; Herr *et al.*, 2019), including some north of 60°S. Antarctic minke whales are noticeably well adapted to living within the ice (Ainley *et al.*, 2007), but the exact proportion of these whales found within the pack ice, and in polynyas, is currently a source of debate. It is possible that a large proportion of the population exists

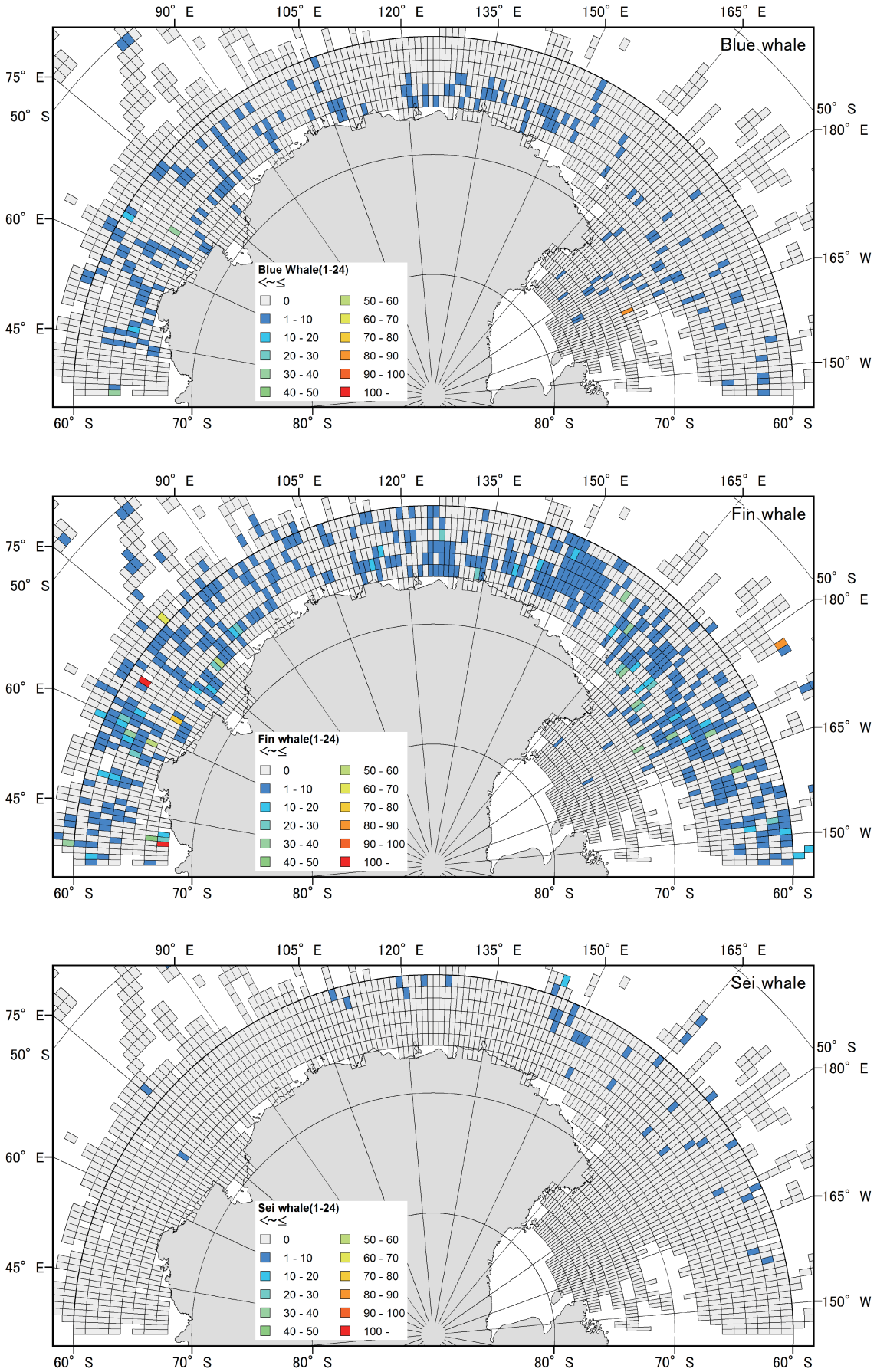

Fig. 5a. DIW of blue, fin and sei whales in the Indo-Pacific region of the Antarctic, based on one degree squares (whole research period).

within the pack ice, out of reach of ship-based sighting surveys (Murase *et al.*, 2005; 2014; Shimada and Kato, 2007; Williams *et al.*, 2014).

There have been a couple of genetic studies reporting some population structure in Antarctic minke whales which indicated at least two stocks in the research areas: P-stock originating from breeding grounds in the western South Pacific and I-stock originating in the Indian Ocean. The study also suggested that the two stocks overlap geographically in a wide area of the Antarctic located at 130°E–165°E, which changes by year and sex depending on the krill availability (Pastene and Goto, 2016).

Dwarf minke whale

Distribution of dwarf minke whale was limited within the research area. There were two separate areas of distribution between 120°E and 147°E, and between 165°E and 170°W in the northern stratum (mainly between 48°S and 63°S), south of Australia and New Zealand (Figs. 5b, 6a and 7a). The dwarf minke whale has a white band on the flipper that distinguishes it from the Antarctic minke whale, but it was only recently identified as separate from Antarctic minke whales (Best, 1985; Rice, 1998; Pastene *et al.*, 2010). Based on previous information, only a small percentage of minke whales in the Antarctic (south of 60°S) are dwarf minke whales. For example, in the IDCR/SOWER surveys from 1993/94–1997/98, only 0.2% of the identified sightings were dwarf minke whales (2 out of 906) (Branch and Butterworth, 2001). Based on this, no formal analysis has been conducted but it is probable that less than 0.2% of the minke whales south of 60°S are dwarf minke whales. In this study, only 0.129 % (85 out of 66,031) of the identified sightings were dwarf minke whales south of 60°S. Kato *et al.* (in press) further examined this aspect by a more extended data set.

Humpback whale

The humpback whale held the 3rd rank among the ten species sighted in the research area. A total of 10,036 schools (18,770 individuals), including 137 calves, were sighted (Table 2). Observed mean school size was 1.87 individuals. Humpbacks were widely distributed in the research area in both northern and southern strata. They were rarely found within Prydz Bay and no sightings occurred south of 73°S in the Ross Sea. This is a very curious difference in their distribution compared to blue whales and Antarctic minke whales. The current distribution map of this species suggests that humpback whales are encountered more frequently in the sector 80°E–110°E from the ice-edge to 60°S because of its high productivity (Figs. 5b and 6a). A high density area was observed in the north between 58°S and 65°S (Fig. 7a). In the 80°E–110°E sector, large scale distribution changes and a "Shift in baleen whale dominance" from Antarctic minke to humpback whales was observed between 85°E and 110°E (Matsuoka *et al.*, 2011; Matsuoka and Hakamada, 2014; Murase *et al.*, 2014; Hakamada and Matsuoka, 2014b). The DIW of this species was 5.315 for the whole survey period. Indices increased from December to February and decreased in March (Fig. 9).

There have been a couple of genetic studies reporting some population structure of humpback whales in the Antarctic (Pastene *et al.*, 2019), which suggested the core distribution areas for each of the 'D: western Australia stock' and 'E1: eastern Australia stock' and possible mixing areas around 130°E.

IDCR/SOWER circumpolar cruises encountered humpback whales more frequently in the sectors 20°E–40°E, 80°E–100°E and 150°E–180° (Branch, 2011). It has been suggested that such changes are related to changing oceanographic and krill environment conditions such as the effect of regime shift in global sea-surface temperatures in relation to El Nino-southern oscillation events (Watanabe *et al.*, 2014; Naganobu *et al.*, 2014). This suggestion should be further investigated in the future.

Southern right whale

A total of 235 schools (298 individuals), including six calves, were sighted (Table 2). Distribution area of this species was limited at certain longitudes of the sector 80°E–135°E, south of Western

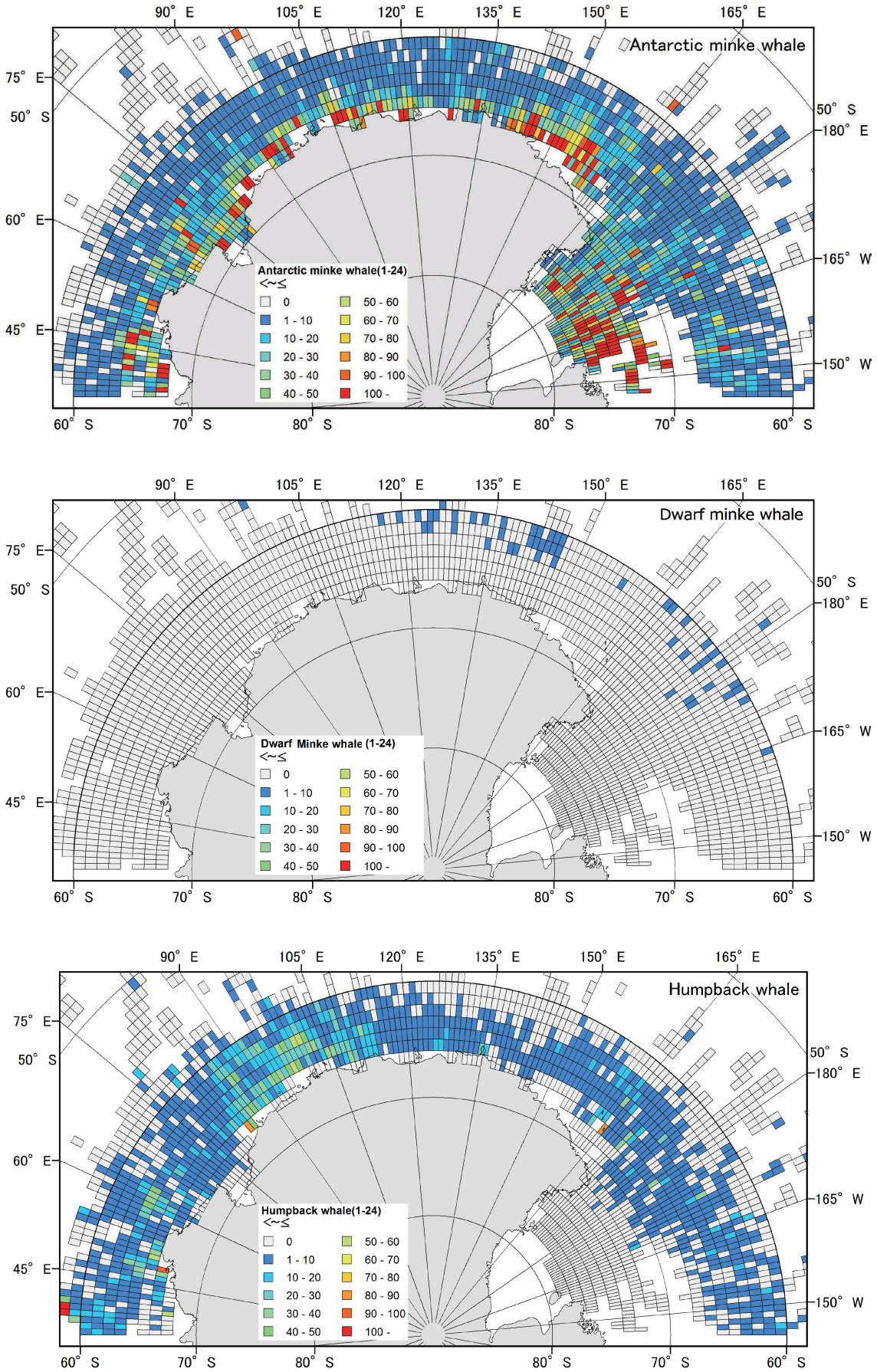

Fig. 5b. DIW of Antarctic minke, dwarf minke and humpback whales in the Indo-Pacific region of the Antarctic, based on one degree squares (whole research period).

Australia (Figs. 5c and 6a). Sighting rate of mother and calf pairs was 2.54% (6 out of 235 schools) which is highest number compared to other baleen whale species in the Antarctic. Southern right whales tended to be distributed more northerly between 40°S and 45°S and more southerly between 62°S and 65°S (Fig. 7a).

The DIW of this species was 0.084 for the whole survey period. Indices increased from December to March (Fig. 9). In summer, southern right whales migrate south, but generally not as far south as other baleen whale species. Southern right whales appear to occur near the subtropical convergence in summer (January to March) at around 40°S–50°S (Ohsumi and Kasamatsu, 1985), but there are records of these animals much further south, for example, around 60°S–65°S, south of Australia (Bannister *et al.*, 1999; 2008). The population estimate for the coastal area of Western Australia was 2,400 in 2006 (Bannister, 2008). A current estimate in Area IV south of 60°S is 1,557 individuals (95% CI, 871–2,783), based on JARPAII data for the 2007/08 season (Matsuoka and Hakamada, 2014). This indicates that southern right whales migrate and make the Antarctic region an important feeding ground in the Austral summer.

Sperm whale

The sperm whale held the 6th rank among the ten species sighted in the research area. A total of 3,810 schools (3,926 individuals) were sighted. No calves were observed (Table 2). Of these sightings, most were single large males (96.5%) and, consequently, the observed mean school size was 1.03. Sperm whales were widely distributed in the research area with high density values being recorded between 35°E and 100°E and between 170°E and 170°W, in the mouth of the Ross Sea (Figs. 5c and 6b). These whales tended to be concentrated on the Antarctic continental slope, on the southern Kerguelen Plateau, and around the mouth of the Ross Sea, where the depth was usually between 1,000 and 4,000 m. This species was rarely found within Prydz Bay or in the Ross Sea (Fig. 5c). Latitudinally, sperm whales tended to be distributed between 40°S and 45°S and between 61°S and 73°S (Fig. 7b). There were no sightings south of 74°S in the Ross Sea. In this study, no formal analysis has been conducted but it is probable that the trend of DIW was stable (Fig. 8). The DIW of this species was 1.081 for the whole research period. The indices decreased from December to March (Fig. 9).

Southern bottlenose whales

The southern bottlenose whale was ranked 7th among the ten species sighted in the research area. A total of 1,666 schools (3,045 individuals), including three calves, were sighted (Table 2). These whales were widely distributed in the research area but were rarely sighted within Prydz Bay and in the Ross Sea. High density values of this whale were observed between 35°E and 70°E (Figs. 5c and 6b). Southern bottlenose whales were latitudinally distributed between 47°S and 70°S (Fig. 7b). Observed mean school size was 1.83 individuals. The DIW of this species was 0.862 for the whole survey period. Indices decreased from December to March (Fig. 9).

Unidentified beaked whales

Unidentified beaked whales held the 4th rank among the ten species sighted in the research area. A total of 3,175 schools (5,457 individuals), including three calves, were sighted (Table 2). The sightings were recorded as unidentified species but confirmed as beaked whales. These ‘unidentified beaked whales’ possibly included the southern bottlenose whale (*Hyperoodon planifrons*), Arnoux’s beaked whale (*Berardius arnuxii*), strap-toothed whale (*Mesoplodon layardii*), Grey’s beaked whale (*M. grayi*) and Cuvier’s beaked whale (*Ziphius cavirostris*). The distribution pattern of the unidentified beaked whales was consistent with that of southern bottlenose whales (Fig. 5d).

Killer whale

Killer whales held the 2nd rank among the ten species sighted in the research area. A total of 1,472

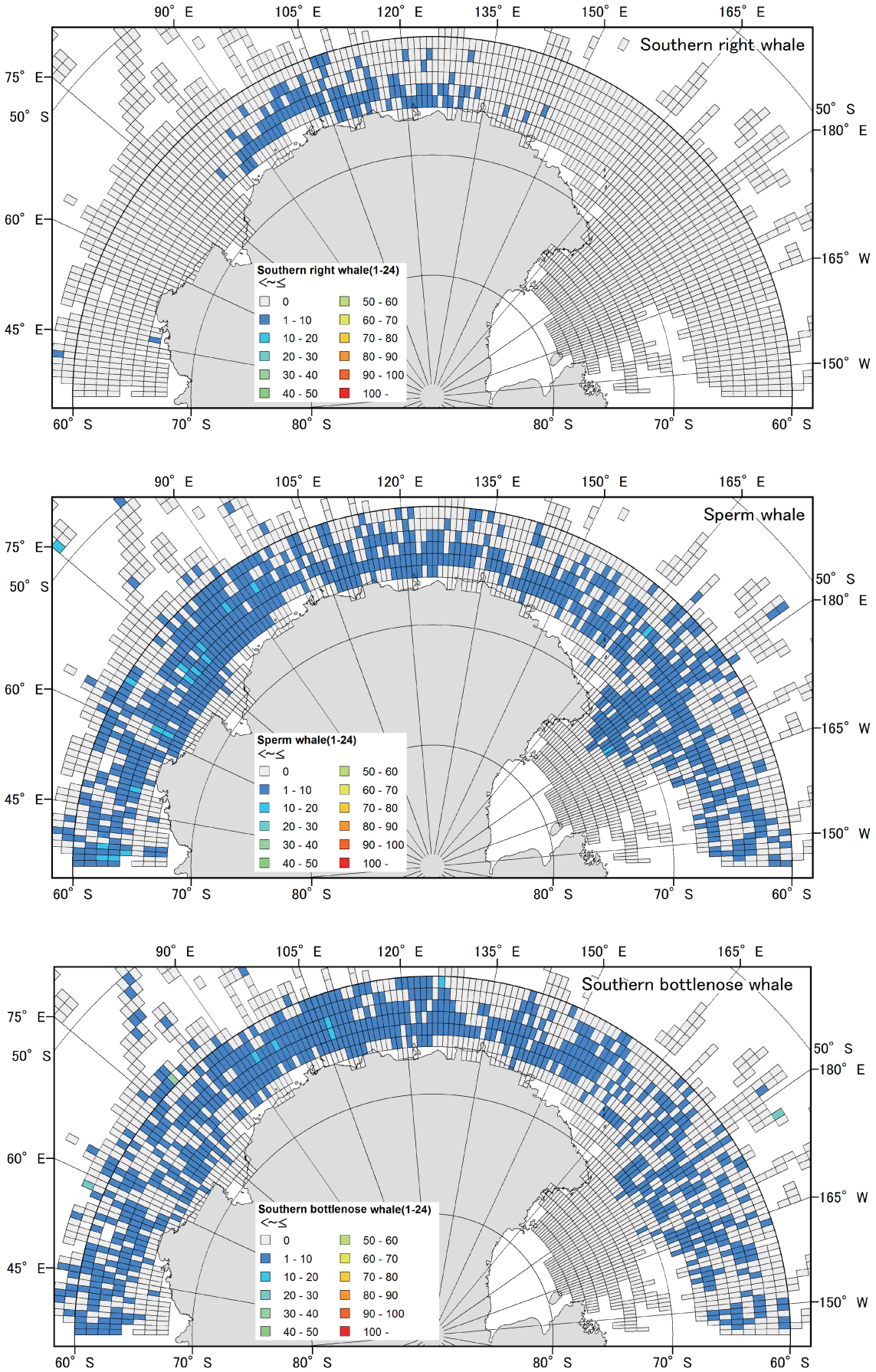

Fig. 5c. DIW of southern right, sperm and southern bottlenose whales in the Indo-Pacific region of the Antarctic, based on Lat.1° × Long.1° squares (whole research period).

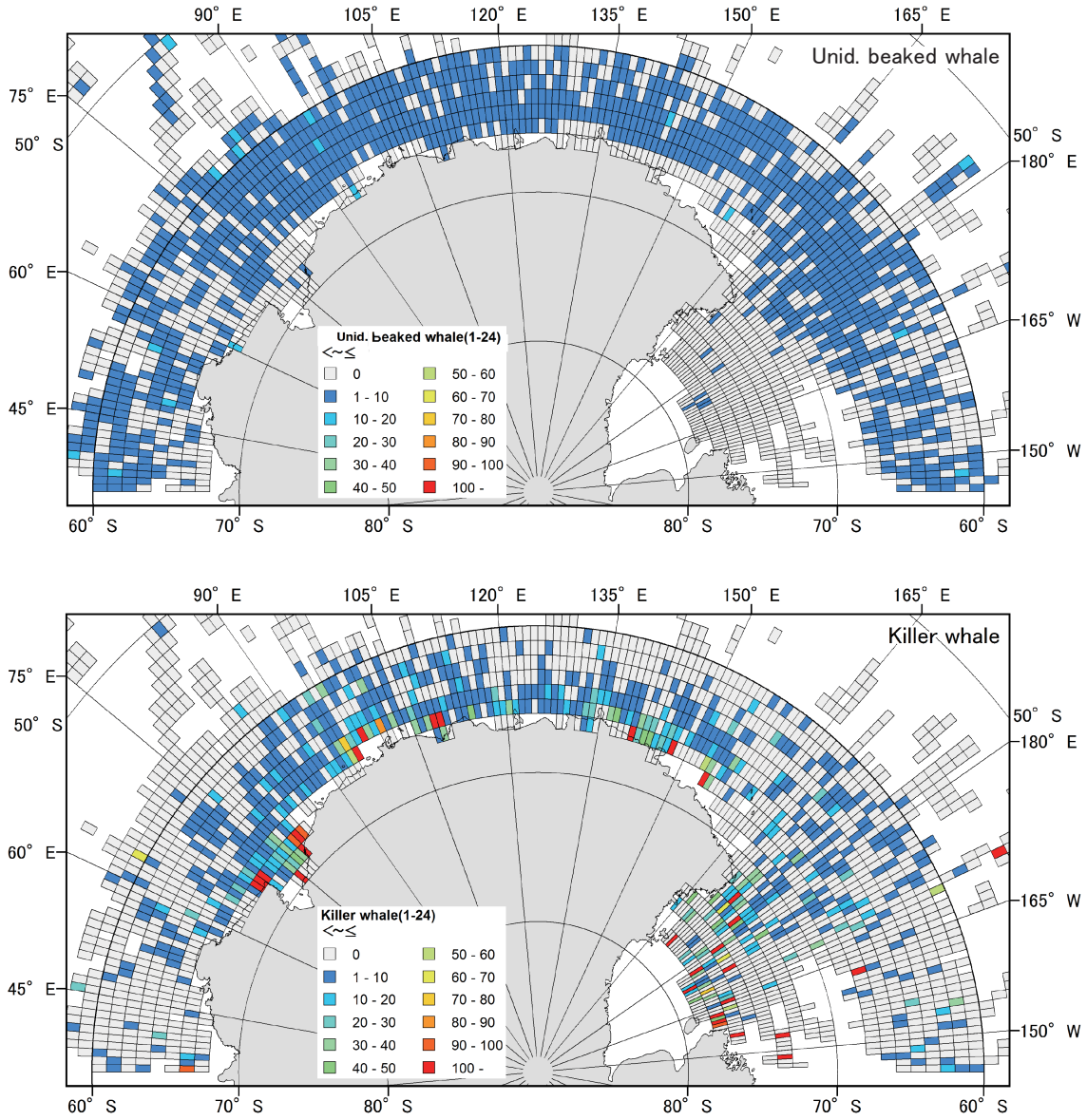

Fig. 5d. DIW of unidentified beaked and killer whales in the Indo-Pacific region of the Antarctic, based on Lat.1° × Long.1° squares (whole research period).

schools (20,569 individuals), including 59 calves, were sighted (Table 2). Observed mean school size was 13.97 individuals. The DIW of this species was 5.825 for the whole study period (Table 2). These whales were widely distributed in the research area longitudinally and latitudinally (Figs. 6b and 7b), and were more frequently sighted in the southern stratum. High density areas were observed near the ice-edge or within Prydz Bay and in the Ross Sea where the pack-ice had melted on the continental shelf slope in February (Figs. 5d, 6b, 7b and 9). Killer whale abundance in the Antarctic was estimated by Branch and Butterworth (2001), and appears to be far higher than in any other ocean in the world. In this study, no formal analysis has been conducted but it is probable that the trend of DIW was stable (Fig. 8). The large school size of killer whales considered to be fish eaters were often sighted in the Prydz Bay and the Ross Sea. Further analyses will be required to definitively identify the killer whale type (fish eater or marine mammal eater etc.) as part of ongoing Antarctic ecosystem research.

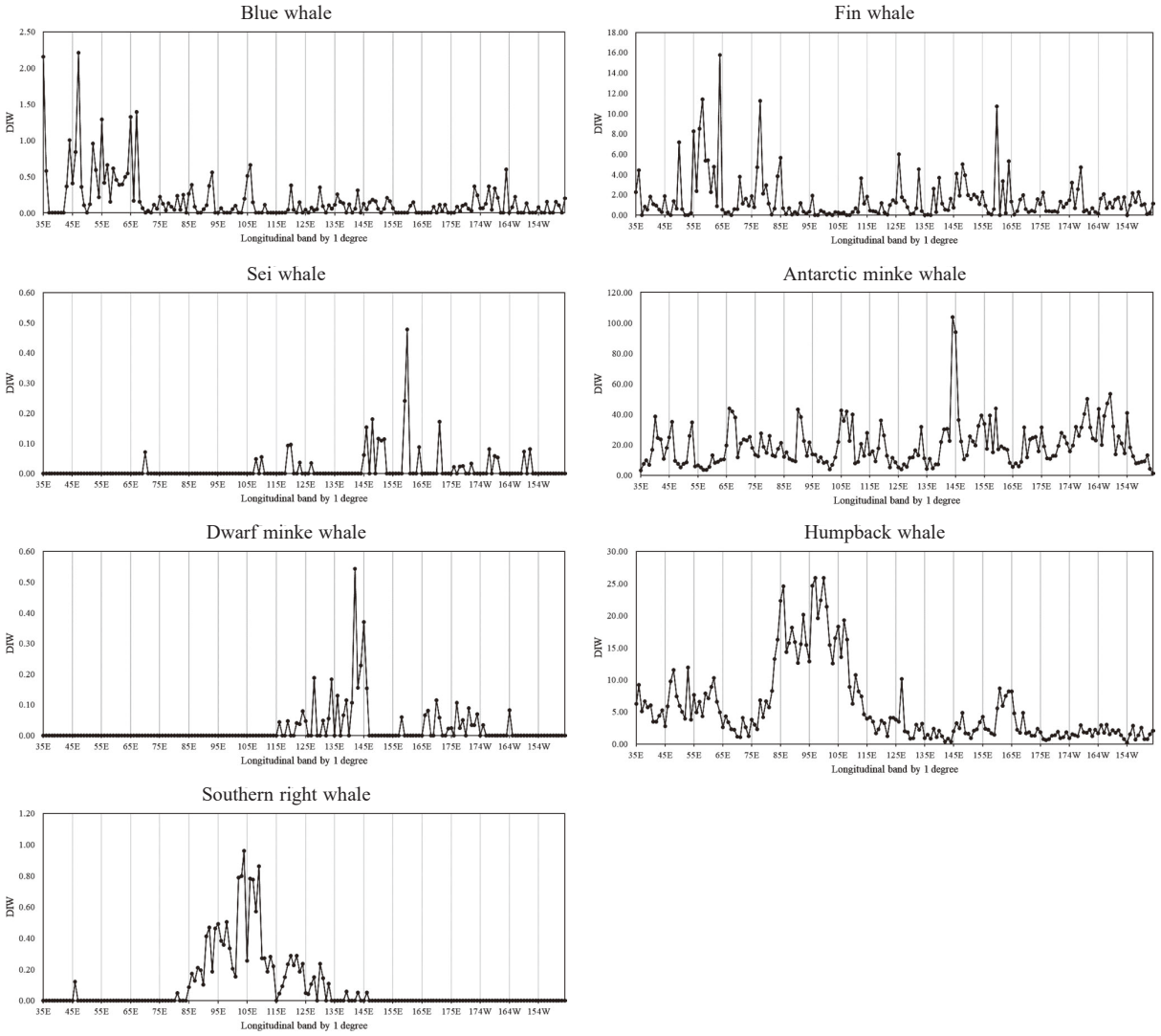

Fig. 6a. The longitudinal band of DIW for blue, fin, sei, Antarctic minke, dwarf minke, humpback and southern right whales in the Indo-Pacific region of the Antarctic, based on Lat.1° × Long.1° squares (whole research period).

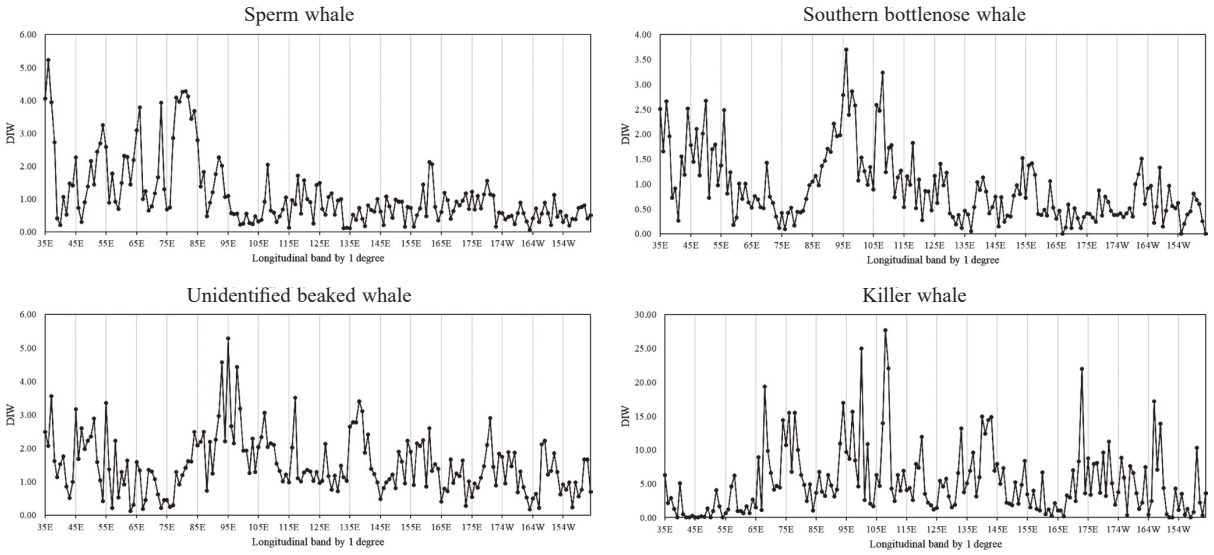

Fig. 6b. The longitudinal band of DIW for sperm, southern bottlenose, unidentified beaked and killer whales in the Indo-Pacific region of the Antarctic, based on Lat.1° × Long.1° squares (whole research period).

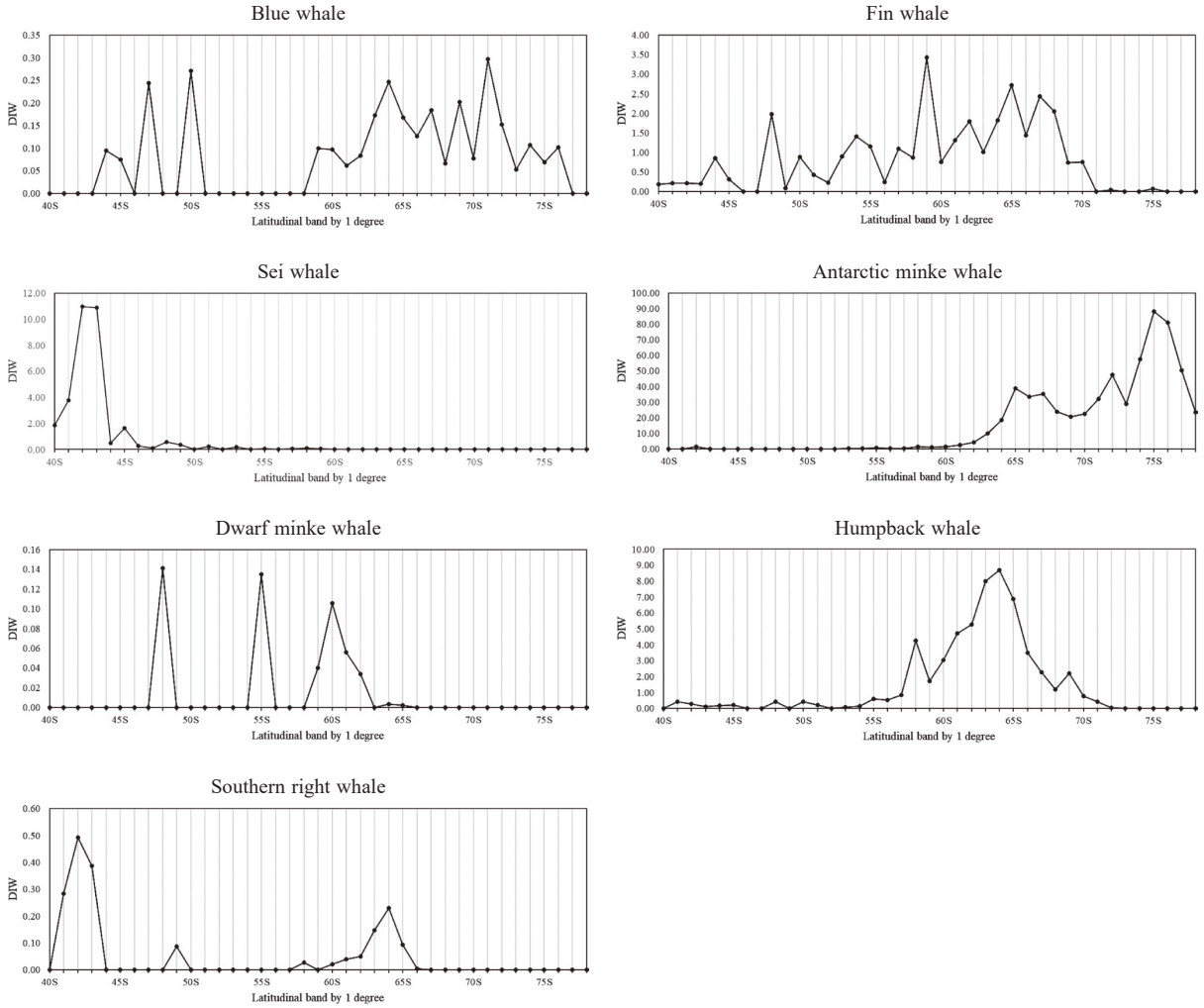

Fig. 7a. The latitudinal band of DIW for blue, fin, sei, Antarctic minke, dwarf minke, humpback and southern right whales in the Indo-Pacific region of the Antarctic, based on Lat.1° × Long.1° squares (whole research period).

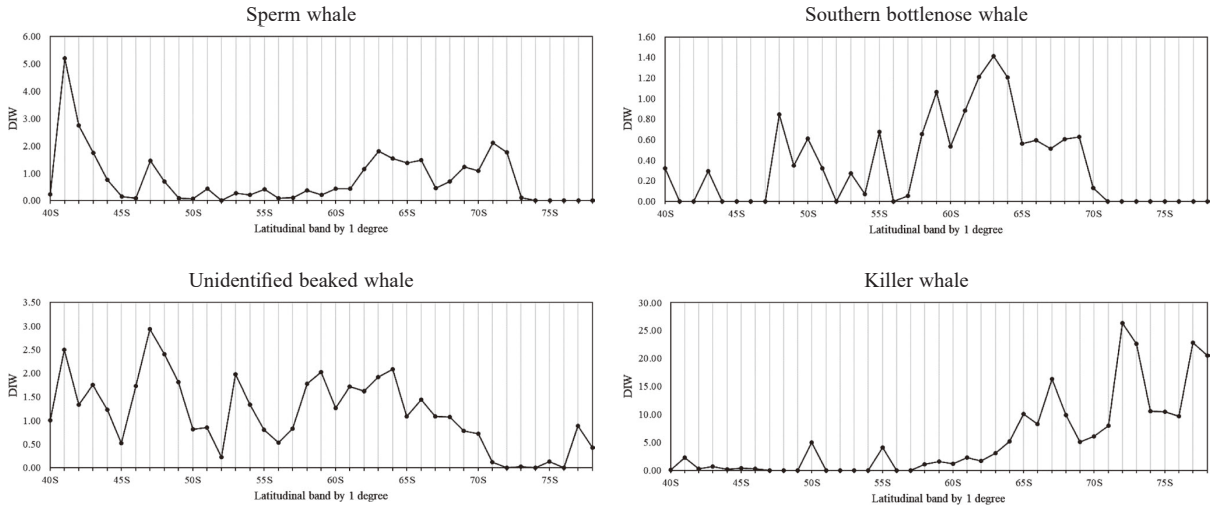

Fig. 7b. The latitudinal band of DIW for sperm, southern bottlenose, unidentified beaked and killer whales in the Indo-Pacific region of the Antarctic, based on Lat.1° × Long.1° squares (whole research period).

Fig. 8. Plot with time trends in DIW for each species and unidentified beaked whales in the Indo-Pacific region of the Antarctic (south of 60°S) during 1987/88 to 2008/09 seasons.

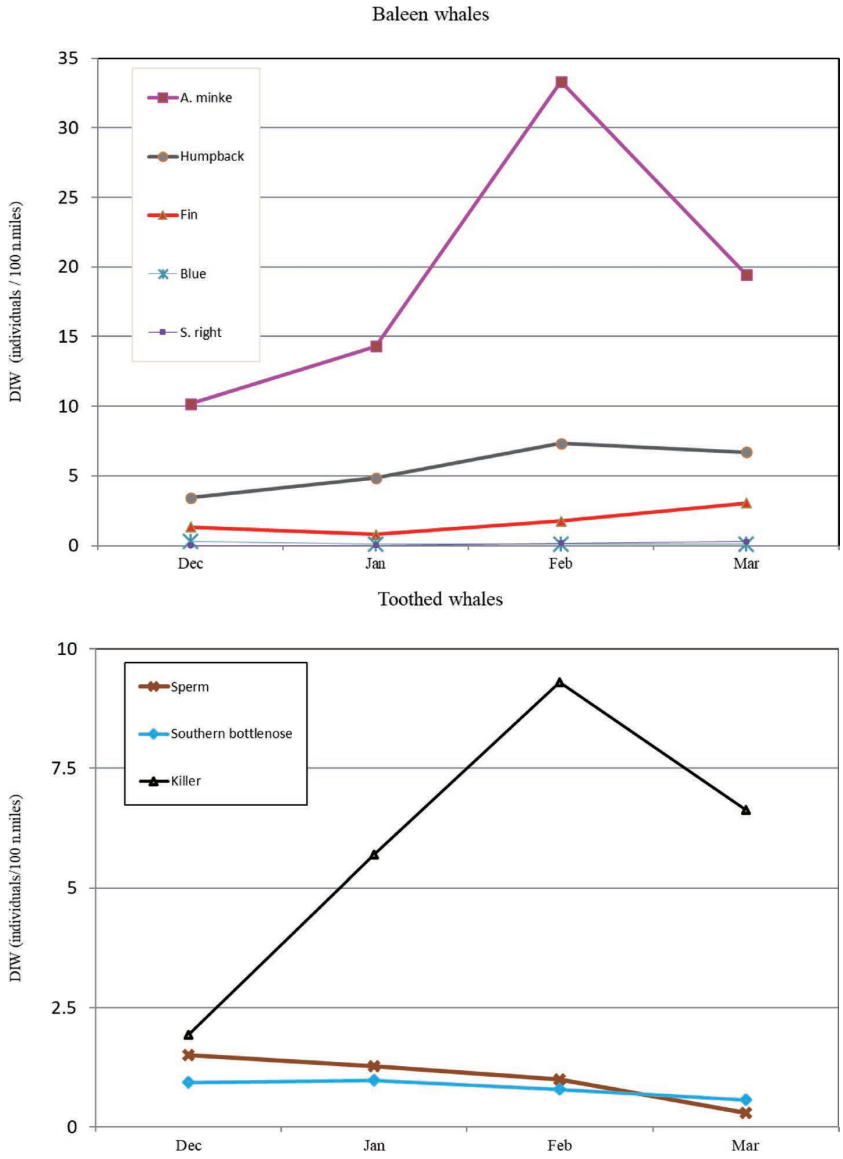

Fig. 9. Yearly trend in DIW for baleen (top) and toothed (bottom) whales in the the Indo-Pacific region of the Antarctic (south of 60°S) during 1987/88 to 2008/09 seasons.

Conclusions

Importance of monitoring whale populations

Most large whales were heavily exploited during the past century and most of the stocks in the Southern Hemisphere were substantially depleted. In the Antarctic Ocean, catches of southern right, humpback, blue, fin and sei whales were prohibited in 1932, 1963, 1964, 1976 and 1978, respectively. Eighty years have passed since the southern right whale has been protected, and more than 50 years have passed since the humpback whale and blue whale have been protected. In the coastal waters of South America, South Africa and along the east and west coasts of Australia, significant recovery of southern right whales and humpback whales in these breeding areas has been recently reported. On the other hand, the information on the present status of pelagic species, such as blue, fin and sei whales was limited. The IWC/IDCR-SOWER cruises have covered the same area every year for 6 years however this is insufficient for monitoring the ecosystem. On the other hand, JARPA and JARPAII have been monitoring baleen whale species populations by large-scale and long-term line transect surveys for over 30 years in Areas IV and V. However, the survey years is still not enough to detect

precise yearly trends for whale populations. For this reason, the JASS-A (Japanese Abundance and Stock structure Surveys in the Antarctic) was started from the 2019/2020 season as a successor to the Japanese Antarctic survey programs such as JARPAII and NEWREP-A (New Scientific Whale Research Program in the Antarctic Ocean), in order to continue to provide additional information about the recovery of whale stocks (Government of Japan, 2019).

Relationship between distribution and oceanographic conditions

There was a common pattern for several whale species to concentrate mainly in the sector 80°E–110°E, south of 60°S. This area is characterised by a large meander (rise to 61°S and slow-moving down to 63°S) of the southern boundary of the Antarctic Circumpolar Current (ACC), which seems to be caused by large-scale upwelling with nutritious bottom waters, resulting from the bottom shape of the southern Kerguelen Plateau (Watanabe *et al.*, 2014; Naganobu *et al.*, 2014). The BROKE Australian Antarctic survey previously indicated the possibility of large-scale upwelling between 80°E and 100°E (Bindoff *et. al.*, 2000). In the JARPA 1999/2000 cruise, a high density of Euphausiids was reported between 100°E and 120°E (Murase *et al.*, 2002). Humpback, southern right, large male sperm and southern bottlenose whales used this longitudinal section between 80°E and 100°E as their key feeding area from December to March. It is necessary to further investigate the relationship between whale distribution and oceanographic condition shifts such as the effect of regime shift in global sea-surface temperatures in relation to El Nino-southern oscillation events (Matsuoka *et al.*, 2003; Watanabe *et al.*, 2014; Naganobu *et al.*, 2006; 2014).

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Photo 1. Two surfacing Antarctic blue whales during biopsy experiment in the Antarctic.

Photo 2. Five fin whales surfacing in the Antarctic.

Photo 3. Surfacing sei whale.

Photo 4. Seven Antarctic minke whales surfacing near pack-ice.

Photo 5. Surfaced humpback whale in the Antarctic.

Photo 6. Surfacing southern right whale during biopsy experiment in the Antarctic.

Photo 7. Surfacing sperm whales near pack-ice in the Antarctic.

Photo 8. Surfacing southern bottlenose whales in the Antarctic.

Photo 9. Killer whales in the Antarctic.

Full paper

CONCENTRATION OF PERSISTENT ORGANIC POLLUTANTS (POPs) IN THREE SPECIES OF BALEEN WHALES IN THE WESTERN NORTH PACIFIC

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Abstract

Concentrations of PCB congeners and DDT, HCH, HCB and CHL isomers in the blubber of five mature males of each of common minke, sei and Bryde's whales taken by the Japanese Whale Research Program under Special Permit in the western North Pacific-Phase II (JARPNII) in 2011 were determined. For comparison, concentrations of these compounds in the blubber of five mature male Antarctic minke whales taken by the Japanese Whale Research Program under Special Permit in the Antarctic-Phase II (JARPAII) in 2010/11 in the Pacific sector of the Antarctic were also determined. Concentrations of PCBs were highest among organochlorines in the whales from the western North Pacific, and were much higher than PCBs concentrations in the Antarctic minke whales. Concentrations of HCB, DDTs and CHLs in Antarctic minke whales were higher or of the same order as North Pacific sei and Bryde's whales but much lower than those in North Pacific common minke whales. Differences are explained by the different trophic levels of the species and or the source of the pollutants. The accumulation of detectable 112 PCB congeners in the whale blubber samples was investigated by principal component analysis (PCA). Two significant factors, in which 72.5% (PC1) and 8.5% (PC2) of the total variance in the data were found. These were attributed to possible trophic level and pollution sources. The main component isomers from pesticide products originating in DDTs and HCHs were comparatively lower, although high levels of trans-chlordane contained in an insecticide were not detected in the whales from the western North Pacific. These results suggest that in the western North Pacific, a great deal of time has passed since the release of DDTs, HCHs and CHLs into the environment.

Key words: common minke whale, sei whale, Bryde's whale, North Pacific, organic pollutants, POPs.

Introduction

Persistent organic pollutants (POPs) are organic compounds that are resistant to environmental degradation through chemical and biological processes, therefore they have the potential to be transported over long distances. The manufacture, use and import/export of the specified compounds have been strictly restricted since the adoption of the Stockholm Convention on POPs by the United Nations Environment Programme in 2001 (Hagen and Walls, 2005). Among them, polychlorinated biphenyls (PCBs), dichloro-diphenyl-trichloroethanes (DDTs), hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB) and chlordanes (CHLs) are commonly called 'legacy POPs' (French *et al.*, 2006), which had been produced and used in large quantities worldwide since the middle of last century. However,

the legacy POPs remain a major environmental challenge for human health and wildlife risk.

In order to understand the fate of these compounds in the environment, it is necessary to directly monitor POPs levels in sea water and the atmosphere in the ocean areas which play a role as a final sink for POPs (Tanabe *et al.*, 1994). However, such monitoring generally produces unrealistic measures because of their extremely low concentrations and instability in the environment (Tanabe and Subramanian, 2006). Consequently, as a possible integrator of POPs, marine organisms such as mussels (Goldberg, 1986), squids (Yamada *et al.*, 1997) and skipjack tuna (Ueno *et al.*, 2003) have been investigated as bioindicators to monitor the fate and behavior of pollutants in the marine environment. Baleen whales have also been suggested as a suitable bioindicator for monitoring POPs levels in offshore waters, because they are mobile and long-lived animals, characteristics that mean that POPs can be monitored in wide sea areas and integrated in some way over time.

In the western North Pacific, yearly change of legacy POPs concentrations in marine mammals have been reported previously. Tanabe *et al.* (1994) reported temporal variation of legacy POPs in female northern fur seals (*Callorhinus ursinus*) from 1971 to 1988. The PCB and DDT concentrations showed a maximum around 1976 and then decreased until 1988. HCH concentrations decreased moderately in the research period. Aono *et al.* (1997) compared legacy POPs in blubber of common minke whales (*Balaenoptera acutorostrata*) from the western North Pacific in 1987 with those in 1994. Their results showed that the concentrations of DDTs, HCHs and HCB in 1987 were comparable or higher than those in 1994, whereas concentrations of PCBs and CHLs in 1987 were considerably higher than those in 1994. On the other hand, Yasunaga, Hakamada and Fujise (2016) reported that yearly changes of PCB concentrations were not observed in common minke whales from the offshore area in the period 2002–2013. These results imply that PCB concentrations in baleen whales from the western North Pacific decreased until the end of the decade of the 1990's and then stabilized since the 2000's. Marine mammals are good bioindicators of POPs in the marine environment, although there are limitations due to confounding factors such as sex, age, metabolism, and feeding (Tanabe and Subramanian, 2006).

The aim of the present study was to investigate the levels of concentration of legacy POPs in three species of baleen whales in the western North Pacific, common minke, sei (*B. borealis*) and Bryde's (*B. edeni*) whales. Samples from the Antarctic minke whale (*B. bonaerensis*) were used for comparison. Potentially the outputs from this study can provide a better understanding of the current fate and behavior of legacy POPs in the marine environment.

Materials and Methods

Samples

Common minke, sei and Bryde's whales used in this study were sampled during the 2012 survey of the Japanese Whale Research Program under Special Permit in the western North Pacific-Phase II (JARPNII). Common minke whales were sampled in sub-area 7, sei whales in sub-area 9 and Bryde's whales in sub-areas 8 and 9, excluding the EEZ of foreign countries, which were established by the Scientific Committee of the International Whaling Commission (IWC) for management purposes (IWC, 1994) (Table 1, Fig. 1). For the analyses, only mature males were used in this study. The rational for this was as follows: (1) lipophilic POPs levels in whale body increase with age especially at young stage; (2) female whales were not used since accumulation depends on several reproductive processes such as parturition and lactation (Tanabe and Subramanian, 2006). Males of common minke, sei and Bryde's whales were defined as sexually mature by testis weight (larger side) of more than 290 g, 1,090 g and 560 g, respectively (Bando *et al.*, unpublished data). Maturity of one common minke whale could not be identified by testis weight, however its body length (8.16 m) was much longer than the maximum body length of the males older than 14 years (mean 7.54 m; $n = 57$, Zenitani, Kato and Fujise, 2000). For the purpose of POPs analysis, blubber tissues were obtained from the

Table 1. Biological data of mature males of common minke, sei and Bryde's whales sampled in the western North Pacific, and Antarctic minke whales sampled in the Antarctic Ocean (see Fig.1 for definition of sub-areas).

Species	Sampling date	Area	Body length (m)
Common minke whale	May 22 2012	sub-area 7	7.33
	May 22 2012	sub-area 7	7.55
	May 25 2012	sub-area 7	7.40
	May 25 2012	sub-area 7	7.70
	June 1 2012	sub-area 7	8.16
Sei whale	June 18 2012	sub-area 9	13.55
	June 25 2012	sub-area 9	13.74
	July 2 2012	sub-area 9	13.48
	July 16 2012	sub-area 9	14.03
	July 19 2012	sub-area 9	13.48
Bryde's whale	July 22 2012	sub-area 9	12.22
	July 24 2012	sub-area 9	12.69
	July 25 2012	sub-area 9	13.06
	July 26 2012	sub-area 8	13.32
	July 27 2012	sub-area 8	13.38
Antarctic minke whale	January 5 2011	Antarctic Area V*	8.67
	January 16 2011	Antarctic Area V	8.78
	January 16 2011	Antarctic Area V	8.35
	January 31 2011	Antarctic Area V	8.77
	February 2 2011	Antarctic Area V	8.31

*: Area V in the Antarctic is comprised between 130°E and 170°W

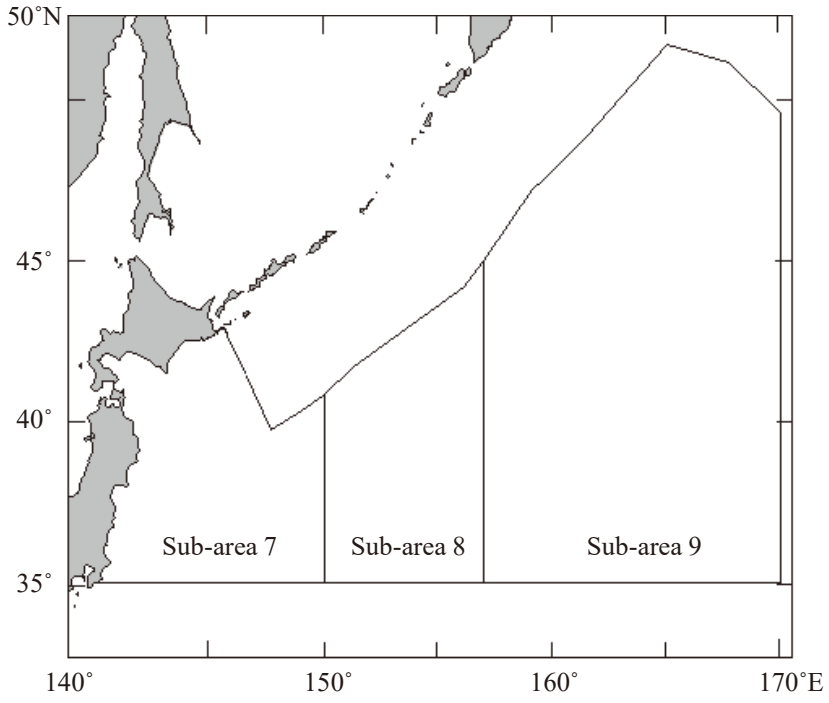

Fig. 1. Subareas surveyed by the JARPNII research excluding the EEZ of foreign countries. Sub-areas definitions are based on IWC (1994).

central lateral part of each whale by researchers on board the research base ship. The blubber samples were stored at −20°C until chemical analyses. Chemical analyses were also conducted on samples from five mature male Antarctic minke whales sampled during the 2010/2011 survey of the Japanese Whale Research Program under Special Permit in the Antarctic-Phase II (JARPAII) in the Pacific sector of the Antarctic (Area V, 130°E-170°W), which were used for comparative purposes (Table 1).

Laboratory analysis

Chemical analyses of the PCBs, DDTs, HCHs, HCB and CHLs were carried at the Miura Institute of Environmental Science using the standard method described by the Environmental Agency of Japan (Japan Environmental Agency, 1998), with some modifications.

Approximately 10 g of blubber were placed into a glass test tube, surrogate standard was added, and then homogenized with 20 mL of acetone and 40 mL of *n*-hexane. Then, the solution was filtered. This extraction procedure was repeated twice. All the solvent layers were combined and washed with 100 mL of 2% sodium chloride solution. The extracts were filtered through 20 g of anhydrous Na_2SO_4 and finally concentrated to 10 mL. Two milliliters of each of the extracts were purified using multilayer silica gel column chromatography composed of two components – 4 g of silica gel impregnated with sulfuric acid (44% mass fraction: 44% H_2SO_4-Si) and 3 g of silica gel impregnated with silver nitrate (10% mass fraction: 10% $AgNO_3$-Si). All the analytes were eluted with 200 mL of 5% dichloromethane/*n*-hexane. After that, the clean-up of the extract was performed by gel permeation chromatography (column: Shodex CLNpak PAE-2000AC) in order to remove remaining hydrocarbons. The mobile phase used acetone with a flow rate of 3.5 mL/min. The eluted solutions were then concentrated.

POPs were determined by a GC-MS (JEOL Ltd., JMS-700; JMS-SX102A). Concentrations of POPs were expressed on a lipid weight basis (ng/g lipid wt.). The quality control of the data was provided throughout the analyses by use of the certified reference material 'Organics in cod liver oil' (NIST 1588a). The results were 1,626.4 ng/g fresh wt. for ∑PCBs, 596 ng/g for *p,p'*-DDE, 236 ng/g for *p,p'*-DDD, 417 ng/g for *p,p'*-DDT, 161.2 ng/g for cis-chlordane, 217.4 ng/g for trans-nonachlor, 67.0 ng/g for cis-nonachlor, 133 ng/g for HCB, and 78.7 ng/g for α-HCH, while the certified values by NIST were 1,789.1 ng/g for fresh wt. ∑PCBs, 651 ± 11 ng/g/ for *p,p'*-DDE, 254 ± 11 ng/g for *p,p'*-DDD, 524 ± 12 ng/g for *p,p'*-DDT, 167.0 ± 5.0 ng/g for cis-chlordane, 214.6 ± 7.9 ng/g for trans-nonachlor, 94.8 ± 2.8 ng/g for cis-nonachlor, 157.8 ± 5.0 ng/g for HCB, and 85.3 ± 3.4 ng/g for α-HCH. Here, ∑PCBs includes PCB congener numbers 28, 31, 44, 49, 52, 66, 87, 95, 101, 105, 110, 118, 128, 132, 138, 149, 151, 153, 156, 159, 163, 164, 170, 180, 182, 183, 187, 190, 194 and 201 (IUPAC numbering). The detection limit was calculated in accordance with the standard method described by the Environmental Agency of Japan (Japan Environmental Agency, 1998), which were 0.3 ng/g lipid wt. (H6CBs), 0.2 ng/g lipid wt. (α-HCH, β-HCH, oxychlordane, trans-chlordane, trans-nonachlor, cis-nonachlor, *p,p'*-DDE, *p,p'*-DDD, T4CBs, P5CBs and O8CBs), 0.1 ng/g lipid wt. (γ-HCH, cis-chlordane, *p,p'*-DDT and H7CBs), 0.08 ng/g lipid wt. (HCB, T3CBs and N9CBs), and 0.04 ng/g lipid wt. (M1CBs, D2CBs and D10CBs).

Statistical analysis

The concentrations of POPs among species were first analyzed by the Kruskal-Wallis test. Pairwise comparisons between species were then performed by the Steel-Dwass post hoc test for multiple comparisons. All differences with $p < 0.05$ were considered statistically significant. Principal component analysis (PCA) was used to assess the difference of composition of detectable PCB congeners in blubber among species caused by habitats, feeding habits and other biological factors. Principal components were derived from standardized data via the correlation matrix. PCA of all 112 PCB congeners were computed on the correlation matrix of the untransformed ng/g lipid wt. data and the projections of the factor scores of each sample along the first 2 principal component axes (PC1 and PC2) were computed. These statistical analyses were executed by SPSS ver.11 for Windows (SPSS Co. Ltd.).

Results and Discussion

Concentration of POPs

Table 2 shows the concentrations of POPs in blubber of common minke, sei, Bryde's and Antarctic minke whales. POPs were detected in all the blubber samples. Relative concentrations of POPs in the blubber of the whale species were significantly higher in the following orders, PCBs: common minke > sei; Bryde's > Antarctic minke whales; HCHs: common minke > sei > Bryde's > Antarctic minke whales; HCB: common minke; Antarctic minke > sei; Bryde's whales; CHLs and DDTs: common minke; sei; Bryde's > Antarctic minke whales ($p < 0.05$).

The POPs concentrations in the sample from the North Pacific whales were clearly higher than those in Antarctic minke whales except for HCB. Concentrations of most POPs such as PCBs levels in environmental samples were highest in northern hemisphere temperate locations (ca. 30-70°N) where anthropogenic usage and atmospheric emissions have been concentrated, whereas relatively volatile chemicals such as HCB levels in environmental samples are related with absolute latitude in both hemispheres due to cold condensation (Iwata *et al.*, 1993; Meijer *et al.*, 2003). The levels of PCBs were highest among POPs in the baleen whales from the northern Pacific, whereas they were comparatively lower than HCB, DDTs and CHLs in Antarctic minke whales. PCBs are still being released into the environment, whereas production and usage were banned in the 1970s in developed countries of the middle latitude of the northern hemisphere (Weber and Goerke, 2003). Furthermore, PCBs with higher lipophilicity are less transportable than the other OCs in the marine environment. Our finding indicates that PCBs may still be of importance for health effects on aquatic organisms in the western North Pacific, even though PCB levels have been decreasing in marine mammals here (Tanabe *et al.*, 1994; Aono *et al.*, 1997; Yasunaga, Hakamada and Fujise, 2016).

In the western North Pacific in particular, PCBs, HCHs and HCB levels in common minke whales were higher than those in sei and Bryde's whales. This result is explained by the different feeding habits of those species. Common minke whales feed mainly on fishes such as Pacific saury and sardine while that sei and Bryde's whales feed mainly on zooplankton and smaller fishes (Konishi *et al.*, 2009). There were no differences in CHLs and DDTs levels among the three species. The effect of diet is particularly significant because the concentration of many persistent pollutants increases through the food web, and therefore tissue concentrations in marine mammals depend on concentrations of the POPs in the food organisms at the various trophic levels. The extent of biomagnification of each chemical is also influenced by other factors such as chemical and physical properties and metabolism (Aguilar, Borrell and Pastor, 1999).

Composition of PCB congeners

Table 3 shows concentrations of PCB congeners in blubber samples of common minke, sei, Bryde's and Antarctic minke whales, and Fig. 2 shows their profiles. The most prevalent congeners in all whale

Table 2. Concentrations of POPs (ng/g lipid wt.) in the blubber of common minke, sei, Bryde's whales from the western North Pacific, and Antarctic minke whales from the Antarctic.

Species	*n*	Fat (%)	PCBs	DDTs	HCHs	HCB	CHLs
Common minke whale	5	29.3 (20.6 - 49.1)	3,100 (1,400 - 8,000)	2,000 (800 - 5,800)	670 (410 - 1,500)	250 (160 - 360)	1,300 (620 - 3,100)
Sei whale	5	73.0 (65.6 - 77.4)	130 (67 - 210)	71 (32 - 130)	91 (30 - 150)	21 (15 - 28)	59 (31 - 90)
Bryde's whale	5	51.9 (17.5 - 79.5)	140 (85 - 200)	82 (36 - 130)	13 (7.0 - 19)	17 (14 - 22)	68 (38 - 88)
Antarctic minke whale	5	61.2 (44.0 - 75.0)	33 (19 - 57)	69 (35 - 110)	0.52 (0.22 - 0.89)	140 (90 - 250)	34 (18 - 59)

Table 3. Average concentrations of PCB congeners (ng/g lipid wt.) in blubber samples of common minke, sei, Bryde's whales from the western North Pacific, and Antarctic minke whales from the Antarctic.

PCB isomers		Common minke whale		Sei whale		Bryde's whale		Antarctic minke whale	
Nos. of Cl	IUPAC No.	ng/g	%	ng/g	%	ng/g	%	ng/g	%
D2CBs	#10,#4	0.41	0.01	0.37	0.29	0.48	0.34	0.063	0.19
	#8,#5	1.2	0.04	0.70	0.55	1.1	0.81	0.054	0.17
	#11	N.D	0.00	N.D	0.00	N.D	0.00	0.053	0.16
	#12,#13	N.D	0.00	N.D	0.00	0.30	0.21	N.D	0.00
	#15	0.37	0.01	0.24	0.19	0.29	0.21	0.030	0.09
	sum	2.0	0.06	1.3	1.0	2.2	1.6	0.20	0.61
T3CBs	#19	N.D	0.00	0.11	0.09	N.D	0.00	N.D	0.00
	#18	3.6	0.12	1.2	0.97	1.2	0.89	0.14	0.44
	#17	0.49	0.02	0.37	0.29	0.47	0.33	0.036	0.11
	#24,#27	0.29	0.01	0.15	0.12	N.D	0.00	0.031	0.09
	#32,#16	0.87	0.03	0.59	0.46	0.76	0.54	0.065	0.20
	#26	0.50	0.02	0.22	0.17	0.26	0.18	0.026	0.08
	#25	0.53	0.02	0.15	0.12	0.23	0.16	N.D	0.00
	#31,#28	5.6	0.18	2.4	1.9	3.3	2.3	0.23	0.70
	#33,#20	3.5	0.11	0.83	0.65	1.1	0.78	0.11	0.33
	#22	0.51	0.02	0.43	0.34	0.62	0.44	0.045	0.14
	#37	N.D	0.00	0.22	0.17	N.D	0.00	N.D	0.00
	sum	16	0.51	6.7	5.3	7.9	5.7	0.69	2.1
T4CBs	#53	2.1	0.07	0.34	0.27	0.30	0.21	0.068	0.21
	#51	0.82	0.03	N.D	0.00	N.D	0.00	N.D	0.00
	#45	0.73	0.02	0.34	0.27	N.D	0.00	N.D	0.00
	#69,#46	N.D	0.00	N.D	0.00	N.D	0.00	0.074	0.22
	#52,#73	100	3.3	8.6	6.8	5.4	3.8	2.0	6.2
	#43,#49	14	0.45	2.4	1.9	1.6	1.2	0.43	1.3
	#47,#48,#75	14	0.44	1.9	1.5	1.3	0.94	0.36	1.1
	#44	5.0	0.16	2.0	1.6	1.2	0.84	0.27	0.82
	#59,#42	2.0	0.07	0.45	0.35	0.52	0.37	0.11	0.35
	#41	2.6	0.08	N.D	0.00	0.46	0.33	0.16	0.50
	#64,#68	0.65	0.02	0.32	0.25	0.35	0.25	0.37	1.1
	#40,#57	1.1	0.03	0.26	0.20	N.D	0.00	0.073	0.22
	#74	63	2.0	3.2	2.5	2.7	1.9	0.67	2.0
	#70,#76	3.0	0.10	0.51	0.40	0.50	0.35	0.093	0.28
	#80	N.D	0.00	N.D	0.00	N.D	0.00	0.11	0.33
	#66	8.0	0.26	2.5	2.0	1.4	0.99	0.26	0.78
	#56,#60	1.9	0.06	0.55	0.43	0.57	0.40	0.058	0.18
	sum	220	7.2	23	18	16	12	5.1	16
P5CBs	#96	1.4	0.05	N.D	0.00	N.D	0.00	N.D	0.00
	#103	1.5	0.05	N.D	0.00	N.D	0.00	N.D	0.00
	#100	1.4	0.05	N.D	0.00	N.D	0.00	N.D	0.00
	#94	1.2	0.04	N.D	0.00	N.D	0.00	N.D	0.00
	#93	2.7	0.09	N.D	0.00	N.D	0.00	0.088	0.27
	#95	59	1.9	6.6	5.2	4.4	3.1	1.8	5.5
	#121,#92	14	0.47	0.78	0.61	0.60	0.43	0.13	0.39
	#89	6.9	0.22	1.5	1.2	N.D	0.00	0.36	1.1
	#84	2.8	0.09	0.85	0.67	N.D	0.00	0.13	0.38
	#90,#101	110	3.4	10	7.8	N.D	0.00	3.1	9.4
	#99	190	6.2	6.0	4.7	6.2	4.4	1.2	3.7
	#119	3.2	0.10	N.D	0.00	N.D	0.00	N.D	0.00
	#97,#86	6.0	0.19	1.6	1.2	1.2	0.83	0.26	0.80
	#125,#87	11	0.35	2.0	1.6	1.5	1.1	0.46	1.4
	#117,#115,#85	N.D	0.00	N.D	0.00	N.D	0.00	0.96	2.9
	#110	18	0.58	1.1	0.82	2.2	1.6	0.22	0.68
	#82	2.5	0.08	0.44	0.35	0.39	0.28	0.090	0.27
	#124	2.0	0.07	N.D	0.00	N.D	0.00	N.D	0.00
	#123,#106	9.8	0.31	0.46	0.36	0.56	0.40	0.15	0.47
	#118	220	7.1	7.6	6.0	9.1	6.5	1.6	4.8
	#114	N.D	0.00	0.34	0.26	0.40	0.28	N.D	0.00
	#122	0.83	0.03	N.D	0.00	N.D	0.00	N.D	0.00
	#105,#127	7.7	0.25	1.6	1.3	1.7	1.2	0.095	0.29
	sum	670	22	41	32	28	20	11	32

H6CBs	#155	6.7	0.22	N.D	0.00	0.58	0.41	N.D	0.00
	#150	1.7	0.05	N.D	0.00	N.D	0.00	N.D	0.00
	#148	2.8	0.09	N.D	0.00	N.D	0.00	N.D	0.00
	#136	28	0.91	1.3	1.0	1.3	0.92	0.55	1.7
	#154	7.6	0.24	N.D	0.00	N.D	0.00	N.D	0.00
	#151	25	0.81	2.7	2.1	3.0	2.1	0.89	2.7
	#135,#144	35	1.1	1.5	1.2	1.6	1.2	0.53	1.6
	#147	4.4	0.14	0.44	0.34	0.47	0.33	0.11	0.32
	#149	240	7.8	8.0	6.3	9.5	6.7	2.5	7.7
	#139,#140	3.7	0.12	N.D	0.00	N.D	0.00	N.D	0.00
	#134	2.1	0.07	N.D	0.00	N.D	0.00	N.D	0.00
	#143,#142,#133	11	0.34	0.40	0.31	0.40	0.28	0.13	0.39
	#146	81	2.6	2.3	1.8	3.3	2.4	0.77	2.4
	#153,#132,#168	640	21	15	12	22	15	3.4	10
	#141	2.5	0.08	0.45	0.36	0.81	0.58	0.19	0.57
	#130	22	0.71	0.54	0.42	0.70	0.50	0.13	0.38
	#137	19	0.60	0.62	0.49	0.79	0.56	0.17	0.51
	#138,#163,#160	450	14	11	8.3	16	11	2.5	7.7
	#129	1.5	0.05	N.D	0.00	N.D	0.00	N.D	0.00
	#166	4.3	0.14	N.D	0.00	N.D	0.00	N.D	0.00
	#159	1.2	0.04	N.D	0.00	N.D	0.00	N.D	0.00
	#128	11	0.35	1.0	0.82	0.97	0.69	0.12	0.37
	#167	18	0.59	0.55	0.43	0.69	0.49	0.18	0.53
	#156	25	0.79	0.67	0.53	1.0	0.74	0.18	0.55
	#157	7.5	0.24	N.D	0.00	N.D	0.00	N.D	0.00
	sum	1,700	53	47	36	62	44	12	38
H7CBs	#188	4.7	0.15	N.D	0.00	N.D	0.00	N.D	0.00
	#184	3.7	0.12	0.23	0.18	0.25	0.18	N.D	0.00
	#179	28	0.89	0.85	0.67	1.0	0.72	0.35	1.1
	#176	7.1	0.23	0.31	0.24	0.34	0.24	0.088	0.27
	#186	13	0.42	N.D	0.00	N.D	0.00	N.D	0.00
	#178	17	0.55	0.54	0.42	0.93	0.66	0.17	0.53
	#175	3.4	0.11	N.D	0.00	0.17	0.12	0.055	0.17
	#187,#182	120	3.9	3.0	2.3	4.7	3.4	0.93	2.8
	#183	43	1.4	0.93	0.73	1.5	1.1	0.19	0.57
	#185	2.0	0.06	N.D	0.00	N.D	0.00	0.052	0.16
	#174	37	1.2	0.85	0.67	1.4	1.0	0.39	1.2
	#177	28	0.90	0.70	0.55	1.2	0.84	0.20	0.62
	#171	11	0.37	0.23	0.18	0.46	0.33	0.053	0.16
	#172,#192	10	0.33	0.25	0.20	0.59	0.42	0.097	0.30
	#180	140	4.4	2.7	2.1	5.3	3.7	1.0	3.1
	#191	1.3	0.04	N.D	0.00	N.D	0.00	N.D	0.00
	#170,#190	48	1.5	0.97	0.76	1.7	1.2	0.27	0.82
	#189	3.3	0.11	N.D	0.00	0.18	0.13	N.D	0.00
	sum	520	21	16	14	24	19	8.6	16
O8CBs	#202	6.4	0.20	N.D	0.00	0.26	0.18	0.084	0.26
	#201	3.9	0.13	N.D	0.00	N.D	0.00	0.070	0.21
	#197	3.4	0.11	N.D	0.00	N.D	0.00	N.D	0.00
	#200	1.8	0.06	N.D	0.00	N.D	0.00	N.D	0.00
	#198	1.1	0.03	N.D	0.00	N.D	0.00	N.D	0.00
	#199	13	0.42	0.26	0.20	0.60	0.42	0.15	0.46
	#203,#196	14	0.44	0.30	0.23	0.49	0.35	0.087	0.27
	#195	3.1	0.10	N.D	0.00	N.D	0.00	N.D	0.00
	#194	9.3	0.30	0.21	0.16	0.30	0.21	0.078	0.24
	#205	1.2	0.04	N.D	0.00	N.D	0.00	N.D	0.00
	sum	57	1.8	0.77	0.60	1.6	1.2	0.47	1.4
N9CBs	#208	2.0	0.07	N.D	0.00	0.19	0.13	0.044	0.13
	#207	2.1	0.07	N.D	0.00	0.23	0.17	0.030	0.09
	#206	2.5	0.08	N.D	0.00	N.D	0.00	0.038	0.11
	sum	6.6	0.21	ND	0.00	0.42	0.30	0.11	0.34
D10CBs	#209	3.7	0.12	0.13	0.10	0.38	0.27	0.037	0.11
Total-PCBs		3,100	100	130	100	140	100	33	100

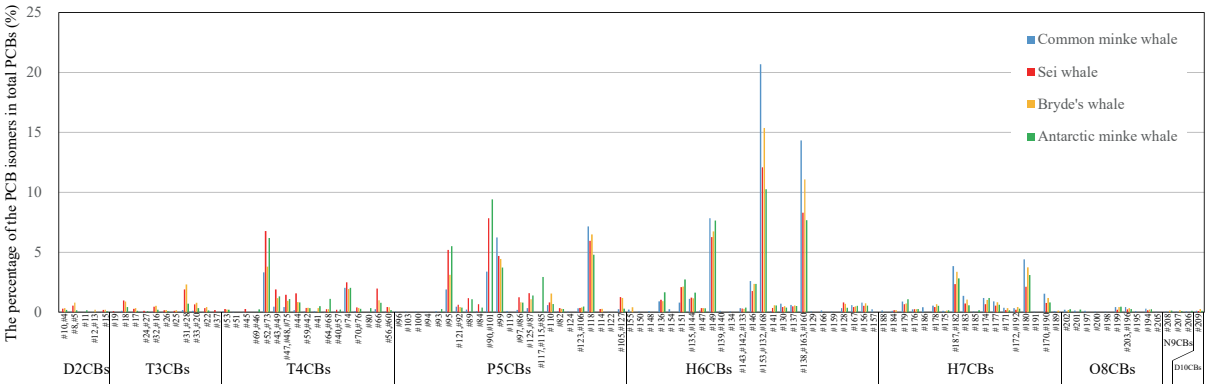

Fig. 2. The percentage profiles of the PCB isomers in total PCBs (%) in blubber of common minke, sei, Bryde's from the western North Pacific, and Antarctic minke whales from the Antarctic.

species were hexachlorobiphenyl CB-153, 132 and 168. In order to clarify features of accumulation of PCB congeners, PCA was conducted using the detectable 112 PCB congeners in common minke, sei, Bryde's and Antarctic minke whales. The results showed that the two principal components (PCs) represented 72.5% (PC1) and 8.5% (PC2) of the variance (Table 4, Fig. 3a, 3b). Focusing on PCB congener with high correlation coefficients of PC1 and low correlation coefficients of PC2, PC1 was positively correlated with mainly higher-chlorinated PCB congeners (hexa- to deca-chlorinated) and part of tetra-chlorinated congeners (Fig. 3a). PC2 was positively correlated with part of di- and tri-chlorinated PCB congeners, although it was negatively correlated with CB-11, tetra- and penta- chlorinated congeners (Fig. 3a).

With the exception of one common minke whale having higher PC1 and lower PC2, the coefficients of PC2 of three whale species in the western North Pacific were clearly higher than those of Antarctic minke whales (Fig 3b), which can be interpreted as geographical difference of their habitats. Then, PC2 might be associated with higher levels of di- (CB-4, 5, 8 and 10) and tri- (CB-16, 25 and 32) chlorinated PCB congeners in the three baleen whales in the western North Pacific whales and higher levels of di- (CB-11), tetra- (CB-46, 69 and 80) and penta- (CB-85, 115 and 117) chlorinated congeners in Antarctic minke whales. Although there was insufficient information given for some isomers, CB-11 was unintentionally produced in certain processes of the diarylide pigment production (Hu and Hornbuckle, 2010), whereas CB-4, 5 and 8 are slightly contained in technical PCB, Kanechlors having made in Japan before the 1960's (Takasuga, Inoue and Ohi, 1995; Jarman, *et al.*, 1998). Furthermore, Vorkamp (2016) established that CB-11 was the most predominant PCB congener in the atmosphere

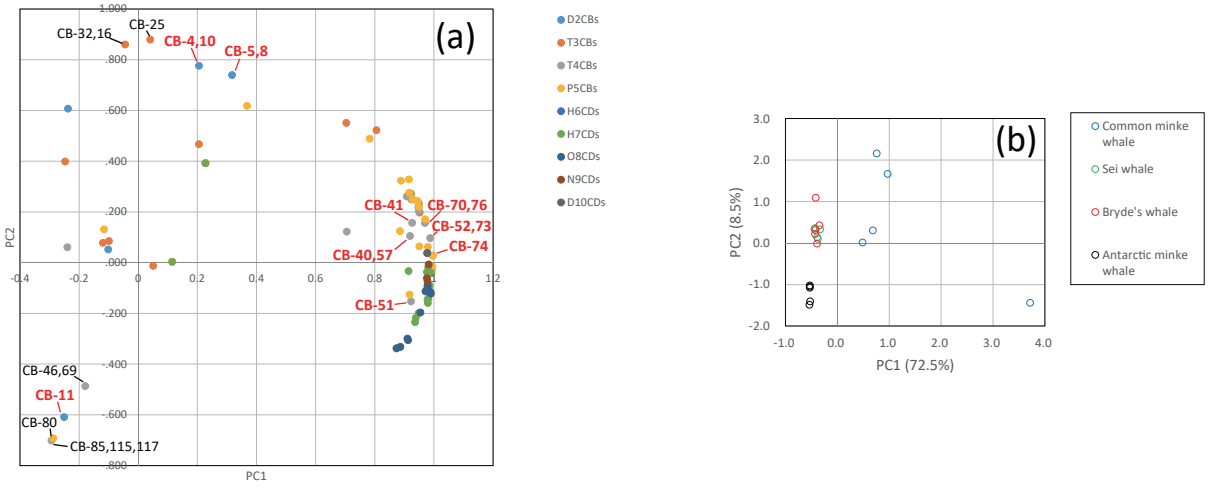

Fig. 3. Distribution of PCB congeners (a) and whale species (b) plotted against the PC1 and PC2 in two-dimensional principal component analysis for the detectable 112 PCB congeners in blubber of common minke, sei, Bryde's whales from the western North Pacific, and Antarctic minke whales from the Antarctic.

Table 4. Factor loadings of a selection of individual PCB isomer concentrations in blubber samples of common minke, sei, Bryde's from the western North Pacific and Antarctic minke whales from the Antarctic to the two principal components (PCs) 1 and 2.

PCB isomers		PC1	PC2
Nos. of Cl	IUPAC No.		
D2CBs	#10,#4	0.206	0.776
	#8,#5	0.318	0.740
	#11	–0.250	–0.609
	#12,#13	–0.101	0.052
	#15	–0.237	0.607
T3CBs	#19	–0.119	0.078
	#17	–0.247	0.399
	#24,#27	0.051	–0.013
	#32,#16	–0.044	0.860
	#26	0.704	0.551
	#25	0.041	0.880
	#31,#28	0.806	0.522
	#33,#20	0.952	0.197
	#22	0.206	0.467
	#37	–0.099	0.085
T4CBs	#53	0.924	0.249
	#51	0.923	–0.153
	#45	0.706	0.122
	#69,#46	–0.179	–0.487
	#52,#73	0.988	0.097
	#43,#49	0.949	0.236
	#47,#48,#75	0.950	0.229
	#44	0.909	0.261
	#59,#42	0.924	0.272
	#41	0.927	0.157
	#64,#68	–0.239	0.061
	#40,#57	0.920	0.105
	#74	0.997	0.028
	#70,#76	0.970	0.156
	#80	–0.293	–0.701
	#66	0.950	0.203
	#56,#60	0.948	0.218
P5CBs	#96	0.874	–0.338
	#103	0.951	0.064
	#100	0.918	–0.126
	#94	0.874	–0.338
	#93	0.980	0.062
	#95	0.971	0.171
	#121,#92	0.996	0.028
	#89	0.917	0.276
	#84	0.783	0.489
	#90,#101	0.95	0.219
	#99	0.995	–0.025
	#119	0.368	0.618
	#97,#86	0.889	0.323
	#125,#87	0.945	0.243
	#117,#115,#85	–0.287	–0.693
	#110	0.929	0.249
	#82	0.886	0.124
	#124	0.887	–0.333
	#123,#106	0.990	–0.121
	#118	0.995	–0.015
	#114	–0.115	0.131
	#122	0.115	0.003
	#105,#127	0.916	0.328
H6CBs	#155	0.933	0.182
	#150	0.931	–0.183
	#148	0.874	–0.338
	#136	0.990	–0.082
	#154	0.996	–0.062
	#151	0.974	0.173
	#135,#144	0.993	–0.083
	#147	0.981	0.097
	#149	0.993	–0.074
	#139,#140	0.874	–0.338
	#134	0.874	–0.338
	#143,#142,#133	0.988	–0.127
	#146	0.990	–0.105
	#153,#132,#168	0.990	–0.020
	#141	0.912	0.248
	#130	0.982	–0.155
	#137	0.984	–0.149
	#138,#163,#160	0.991	–0.010
	#129	0.928	–0.172
	#166	0.950	–0.245
	#159	0.788	0.053
	#128	0.961	0.191
	#167	0.983	–0.156
	#156	0.991	–0.106
	#157	0.985	–0.147
H7CBs	#188	0.228	0.392
	#184	0.915	–0.033
	#179	0.984	–0.116
	#176	0.980	–0.159
	#186	0.228	0.392
	#178	0.949	–0.199
	#175	0.937	–0.235
	#187,#182	0.986	–0.040
	#183	0.984	–0.110
	#185	0.992	–0.041
	#174	0.980	–0.112
	#177	0.986	–0.090
	#171	0.982	–0.141
	#172,#192	0.979	–0.146
	#180	0.981	–0.019
	#191	0.115	0.003
	#170,#190	0.977	–0.037
	#189	0.939	–0.218
O8CBs	#202	0.989	–0.116
	#201	0.990	–0.122
	#197	0.874	–0.338
	#200	0.913	–0.306
	#198	0.887	–0.332
	#199	0.979	–0.097
	#203,#196	0.979	–0.090
	#195	0.955	–0.196
	#194	0.972	–0.113
	#205	0.911	–0.299
N9CBs	#208	0.979	–0.074
	#207	0.983	–0.007
	#206	0.977	–0.061
D10CBs	#209	0.978	0.038
% total variance		72.5	8.5

of the Antarctic region, because only this congener is ubiquitously present globally, although other lower chlorinated congeners are easily degraded in the atmosphere. Therefore, composition of lower chlorinated congeners in the whale body would be especially influenced by environmental factors such as the distance from anthropogenic sources and latitude.

With the exception of one common minke whale having higher PC1 and lower PC2, coefficients of PC1 were in the order of common minke > Bryde's = sei >Antarctic minke whales, which is consistent with trophic levels based on their feeding ecology reported in previous studies (Konishi *et al.*, 2009; Tamura and Konishi, 2009). In the western North Pacific baleen whales, the second most prevalent congeners in common minke, sei and Bryde's whales were hexachlorobiphenyl CB-138, 163 and 160. This was negatively correlated with lower-chlorinated PCB congeners (di- to penta-chlorinated) which are rapidly metabolized and eliminated in the marine environment although they have higher mobility and therefore more available to aquatic organisms (de Boer *et al.*, 2001). This suggests that PC1 can explain the persistence of PCB congeners through the food chain in the marine environment.

In previous studies, hexa-chlorinated CB-153 was the most prevalent congener, followed by CB-138 in small cetaceans such as Dall's porpoise, striped dolphin and finless porpoise from the western North Pacific (Minh *et al.*, 2000), whereas CB-153 was also the most prevalent congener, followed by CB-128 in common minke whales from the Northeast Atlantic (Kleivane and Skaare, 1998). The difference in the secondary dominant congeners is mainly because CB-128 is partly contained in Aroclors which had been used as PCB products in Europe, whereas it is contained in lesser amounts in Kanechlors which had been used in Japan (Kannan, Maruya and Tanabe, 1997). This suggests that levels of CB-128 in baleen whales would reflect their habitat.

Table 5. Average concentrations (ng/g lipid wt.) and percentages (%) of DDT isomers in the blubber samples of common minke, sei, Bryde's whales from the western North Pacific and Antarctic minke whales.

Species	*n*		*p,p'*-DDE	*p,p'*-DDD	*p,p'*-DDT	Total-DDTs
Common minke	5	average	1,600	330	99	2,000
whale		%	(78.5)	(16.1)	(4.9)	
Sei whale	5	average	44	18	7.9	71
		%	(62.5)	(24.7)	(11.1)	
Bryde's whale	5	average	61	13	7.8	82
		%	(74.5)	(15.8)	(9.5)	
Antarctic minke	5	average	48	6.4	16	69
whale		%	(70.1)	(9.3)	(22.5)	

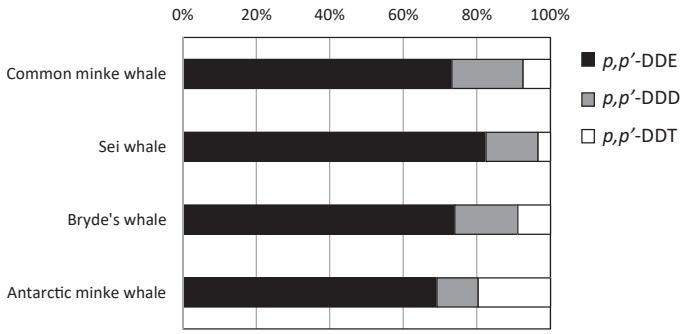

Fig. 4. Compositions of DDTs in the blubber of common minke, sei, Bryde's whales from the western North Pacific, and Antarctic minke whales.

Composition of DDT, HCH and CHL isomers

The averages of percentage of *p,p'*-DDT in the whale species in this study were in the order of Antarctic minke whale (20.0%) > Bryde's whale (8.7%) ≈ common minke whale (7.4%) > sei whale (3.3%) (Table 5; Fig. 4). The *p,p'*-DDE/total DDT ratio is widely used as an indicator of the time elapsed since releasing technical DDT into the environment (Borrell and Aguilar, 1987), because technical DDTs which have been used to control malaria are composed of almost all *p,p'*-DDT (De Jager *et al.*, 2006), and *p,p'*-DDT released in the environment is changed to *p,p'*-DDE in the animal body and the environment (Okonkwo *et al.*, 2008). Our results are consistent with previous reports that *p,p'*-DDE/total DDT ratios in male Antarctic minke whales had decreased for 1984/1985 (ca. 40%) and 1992/1993 (ca. 25%) (Aono *et al.*, 1997), and that of mature male common minke whales sampled in sub-area 7 for 1996 and 1998 was 8.9% (calculated by mean of *p,p'*-DDT 184 ng/g lipid wt. and total DDT 2,060 ng/g lipid wt.) (Fujise *et al.*, 2000). This suggests that a technical DDT might have been slightly used in the southern hemisphere.

In the ratios of the three baleen whale species in the western North Pacific, that of sei whale was higher than those of the other whales. Notably, that of sei whale was higher than that of Bryde's whale in spite of the same levels of DDTs (Table 2) and trophic position. The production and usage of DDTs had been discontinued in the early 1970's in most developed countries, whereas they have been continued until now for malaria and leishmaniasis control in the tropical developing countries (De Jager *et al.*, 2006). Moreover, van den Berg, Manuweera and Konradsen (2017) estimated DDT usage globally declined by 30 percent from 2001 to 2014, mainly because of the reduction in India which holds 84% of the total global stockpile. Our result suggests that the lower *p,p'*-DDT ratio of sei whales compared to common minke and Bryde's whales in the northern hemisphere might be the distance of their habitat to the major technical DDT sources. This is also consistent with the fact that the ratios of

Table 6. Average concentrations (ng/g lipid wt.) and percentages (%) of HCH isomers in blubber samples of common minke, sei, Bryde's whales from the western North Pacific and Antarctic minke whales.

Species	*n*		*α*-HCH	*β*-HCH	*γ*-HCH	Total-HCHs
Common minke	5	average	21	650	7.9	670
whale		%	(3.2)	(96.7)	(1.2)	
Sei whale	5	average	3.3	85	1.1	91
		%	(3.6)	(93.2)	(1.2)	
Bryde's whale	5	average	0.59	12	0.28	13
		%	(4.7)	(98.4)	(2.2)	
Antarctic minke	5	average	0.12	0.10	0.37	0.52
whale		%	(22.9)	(19.7)	(69.8)	

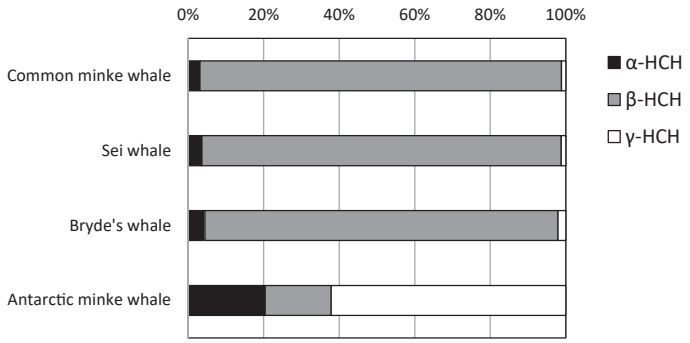

Fig. 5. Compositions of HCHs in the blubber of common minke, sei, Bryde's from the western North Pacific, and Antarctic minke whales.

common minke whale in this study was lower than that of common minke whale collected in 2006 from the Korean coast (ca. 35-40%) (Moon *et al.*, 2010).

The averages of percentage of *β*-HCH accounted for over 90% of total HCHs in common minke, sei and Bryde's whales from the western North Pacific, whereas the percentage of *γ*-HCH was the dominant isomer of HCHs in Antarctic minke whales (Table 6; Fig. 5). An odorless *γ*-HCH purified from technical HCH has been produced and used worldwide since the 1950's, although the technical HCH (*α*-HCH: 53-70%, *β*-HCH: 3-14%, *γ*-HCH: 10-18% and others) having a persistent bad smell and taste had been produced as a pesticide in Europe and other countries in the late 1940's and 50's (Vijgen *et al.*, 2011). After then, the mass-production base of *γ*-HCH moved to China, Russia and India, now leaving only a small number of producing countries (India and Romania) (Vijgen *et al.*, 2011). It is noted that *γ*-HCH is produced from 8 times the amount of technical HCH to it and that the residue was not properly treated in the producing countries. Therefore, HCH emissions have been almost all only *γ*-HCH in the southern hemisphere, suggesting that this may be reflected in the HCH levels in Antarctic minke whales.

In the three whale species in the western North Pacific, *β* -HCH is much higher than *γ*-HCH, whereas *β*-HCH is a minor component in a technical HCH. Because *γ*-HCH is rapidly metabolized, the *β*-HCH is consistently found in higher concentrations in mammals (Willett, Ulrich and Hites, 1988).

The percentages of CHL isomers in common minke, sei and Bryde's whales from the western North Pacific were in the order of *trans*-nanochlor > *cis*-nonachlor, whereas those in Antarctic minke whales were in the order of *trans*-nanochlor > oxychlordane (Table 7; Fig. 6). Technical CHLs were used for insecticide in Japan until 1986, of which 60-70% consisted of *cis*- and *trans*-chlordane (Loganathan *et al.*, 1993). *Trans*-chlordane was not detected in all four whale species in this study, whereas *cis*-chlordane was detected at relatively low levels. Ueno *et al.* (2003) reported that their *trans*-chlordane /

Table 7. Average concentrations (ng/g lipid wt.) and percentages (%) of CHL isomers in blubber samples of common minke, sei, Bryde's whales from the western North Pacific and Antarctic minke whales.

Species	*n*		oxychlordane	*trans*-chlordane	*cis*-chlordane	*trans*-nonachlor	*cis*-nonachlor	Total-CHLs
Common minke whale	5	average	150	N.D.	33	950	210	1,300
		%	(11.1)	(0.0)	(2.5)	(71.1)	(15.7)	
Sei whale	5	average	7.6	N.D.	3.3	36	12	59
		%	(12.9)	(0.0)	(5.6)	(61.4)	(20.1)	
Bryde's whale	5	average	4.2	N.D.	9.8	39	14	68
		%	(6.2)	(0.0)	(14.5)	(57.8)	(21.0)	
Antarctic minke whale	5	average	11	N.D.	1.3	16	4.8	34
		%	(33.2)	(0.0)	(3.9)	(48.9)	(14.2)	

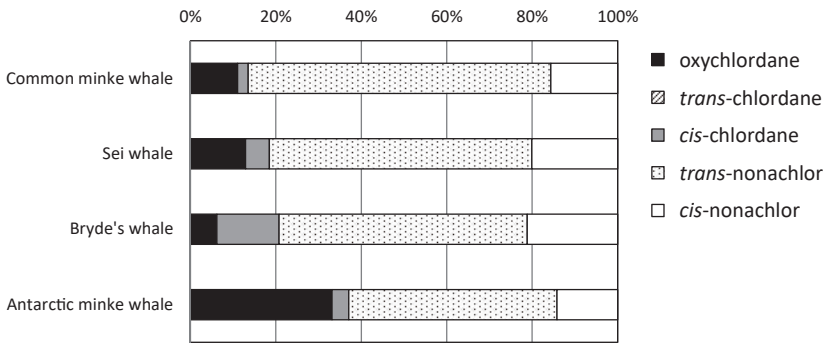

Fig. 6. Compositions of CHLs in the blubber of common minke, sei, Bryde's from the western North Pacific, and Antarctic minke whales.

trans-nanochlor ratios are in the range of 2.9-3.4, and decreasing ratio in the environment means that time has passed after technical CHLs release. This implies that the four baleen whale species from the western North Pacific and Antarctic would not have been recently exposed to new technical CHLs.

Conclusion

To examine the accumulation features and current status of POPs in the western North Pacific, the present study determined the concentrations of these compounds in the blubber of common minke, sei and Bryde's whales. The study found that there have been no recent anthropogenic inputs of legacy POPs in the western North Pacific, however, the POPs compositions in the whales might reflect their usage and fate. Understanding the historical usage and fate of these compounds may help predict their future behavior in the marine environment.

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Photo 1. Common minke whale

Photo 2. Sei whale

Photo 3. Bryde's whale

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