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Cover photo: Measuring the body of an Antarctic minke whale aboard a research base ship in the Antarctic (top); cetacean sighting activities in the Antarctic (middle); deployment of a Conductivity Temperature Depth (CTD) profiler in the Antarctic (below).

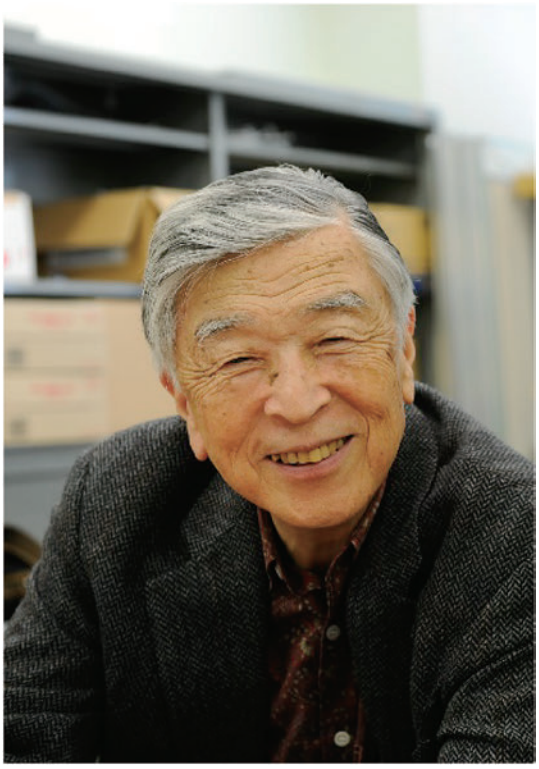
TECHNICAL REPORTS OF THE INSTITUTE OF CETACEAN RESEARCH

TEREP-ICR
No. 3

The Institute of Cetacean Research (ICR)
Tokyo, 2019

Obituary

This issue of TEREP-ICR is respectfully dedicated to Dr. Seiji Ohsumi, who died on 2 November 2019 at the age of 89 years old, to his long time devotion to cetacean research and important scientific contributions to the biology, conservation and management of this group of animals. Dr. Ohsumi was Director General of the Institute of Cetacean Research in the period 1995–2003, and at the time of his death was Honorary Scientific Adviser of the institute. Dr. Ohsumi was one of the leading members of the Scientific Committee of the International Whaling Commission between the 1960's and the 2010's. We will miss him deeply.



Foreword

It is a pleasure for me to introduce the third issue of the Technical Reports of the Institute of Cetacean Research (TEREP-ICR-3). At the outset I would like to inform that, as an effect of the change in the whaling policy of the Government of Japan, the whale research programs under special scientific permit, NEWREP-A in the Antarctic and NEWREP-NP in the western North Pacific, ceased on 30 June 2019. As explained in earlier issues, research under these programs was the main and first priority of the ICR. From now on, Japan will conduct whale research programs based on non-lethal methods in both the North Pacific and the Antarctic Oceans. In addition, collection of biological samples and data will continue based on whales caught for commercial purposes in Japan's territorial waters and Exclusive Economic Zone. The ICR will have an important role in designing and implementing new research programs, and our scientists will continue analyzing samples and data collected from previous and new research programs. Consistent with its stated objectives, TEREP-ICR will continue describing and reporting on the process, progress, and results of technical or scientific research, or the state of technical or scientific research programs conducted by the ICR.

Similar to TEREP-ICR-1, TEREP-ICR-2 was widely distributed to approximately 120 individual scientists from Japan and 30 foreign countries. It was also distributed to approximately 160 research institutions (including universities, research institutes, public libraries, museums and aquariums), both in Japan and foreign countries. As a result of disseminating information on ICR's research activities through the TEREP-ICR, an increasing number of international scientists are showing interest in conducting research in collaboration with the ICR. Based on this, I believe that TEREP-ICR are contributing to achieve its stated objectives.

I sincerely hope that this third issue of the TEREP-ICR will continue contributing to an increased understanding of the technical and research activities conducted by the ICR among the national and international scientific community.

Dr. Yoshihiro Fujise
Director General ICR
Tokyo, December 2019

Editorial

Welcome to the third issue of the Technical Reports of the Institute of Cetacean Research (TEREP-ICR-3).

This third issue contains seven technical reports and one commentary article. The first report summarizes the results of research on assessment and management of Antarctic minke whales. The second report shows the progress of the research on age estimation in Antarctic minke whale based on aspartic acid racemization. The third report explains the importance of $g(0)$ for estimating abundance of large whales in the Antarctic. The fourth report summarizes the technical aspects of ICR's oceanographic and krill surveys in the Antarctic. The next two reports in this issue deal with research in the North Pacific. The first one summarizes the information on population genetic structure of the North Pacific sei whale, which is of importance for management purposes. The second one summarizes the information on spatial and temporal distribution of large whales in the western North Pacific, emphasizing the importance of long-term monitoring research programs. The technical report note explains the platform and equipment used during the ICR's satellite tracking experiments on large whales.

The commentary article deals with the issue of Japan's withdrawal from the International Whaling Commission and the implications of this for the whale research conducted by the ICR.

This third TEREP-ICR issue also includes sections to outline the contribution of ICR scientists to national and international meetings in 2019 as well as their contribution in terms of peer-reviewed publications up to December 2019.

I hope you will find this third issue informative and useful.

Dr. Luis A. Pastene
Editor TEREP-ICR
Tokyo, December 2019

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Technical Report (not peer reviewed)

What do we know about whales and the ecosystem in the Indo-Pacific region of the Antarctic? Part 1: Summary of results on assessment and management of Antarctic minke whales

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ABSTRACT

The Institute of Cetacean Research has conducted whale research under special scientific permit in the Antarctic since the austral summer season of 1987/88. The research was conducted systematically under different research programs such as JARPA and JARPAII, and more recently, under the NEWREP-A. These research programs employed both lethal and non-lethal methods. NEWREP-A ceased after the 2018/19 austral summer season as an effect of Japan's decision to withdraw from the International Whaling Commission and start commercial whaling within its Exclusive Economic Zone. This paper summarizes the most relevant outputs from the Japanese research programs under special scientific permit in the Indo-Pacific region of the Antarctic related to the assessment and management of Antarctic minke whales.

INTRODUCTION

Japan conducted systematic research on whales and the Antarctic ecosystem for more than 30 years. The first research program was the Japanese Research Program under Special Permit in the Antarctic (JARPA), which was followed by JARPAII and subsequently by the New Scientific Whale Research Program in the Antarctic Ocean (NEWREP-A). The Institute of Cetacean Research (ICR) has been the institution in charge of designing and implementing those research programs. Tamura *et al.* (2017) provided details on the objectives, sampling and analytical methodology of these three research programs. Several international review workshops (e.g. IWC, 2015) discussed and evaluated the large amount of data and results from those research programs.

As an effect of the change in the whaling policy of the Government of Japan, NEWREP-A ceased from 1 July 2019. From now on, Japan will conduct whale research in the Antarctic based solely on non-lethal methods. At this point, it was considered important to summarize the knowledge on whales and the Antarctic ecosystem accumulated so far by the Japanese whale research in the Antarctic.

The objective of this paper was to summarize the most relevant outputs from the Japanese research programs under special scientific permit in the Indo-Pacific region of the Antarctic related to the assessment and management of Antarctic minke whales.

SURVEYS, DATA AND SAMPLES

Surveys were conducted in the Indo-Pacific region of the Antarctic, which correspond to the International Whaling Commission (IWC)'s management Areas III, IV, V and VI (Figure 1). Survey and sampling methodologies of the Japanese whale research programs in the Antarctic were explained in Pastene *et al.* (2014) and Tamura *et al.* (2017). A list of data and samples collected by the Japanese whale research programs in the Antarctic was available from the IWC (2015).

The most relevant research outputs for assessment and management (i.e. taxonomy/stock structure, abundance and biological parameters) of Antarctic minke whales are presented in the next section.

MAIN RESEARCH OUTPUTS

Taxonomical aspects

Until relatively recently, only one species of minke whale was thought to exist: *Balaenoptera acutorostrata* Lacèpéde, 1804. However, Rice (1998) reviewed morphological (e.g. Williamson, 1959; van Utrecht and van der Spoel, 1962; Kasuya and Ichihara, 1965; Omura, 1975;

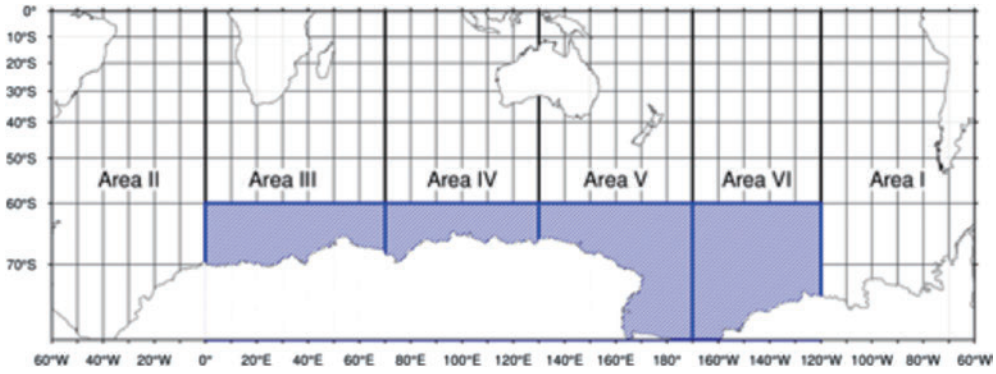

Figure 1. Research area of the JARPA, JARPAII and NEWREP-A in the Indo-Pacific region of the Antarctic.

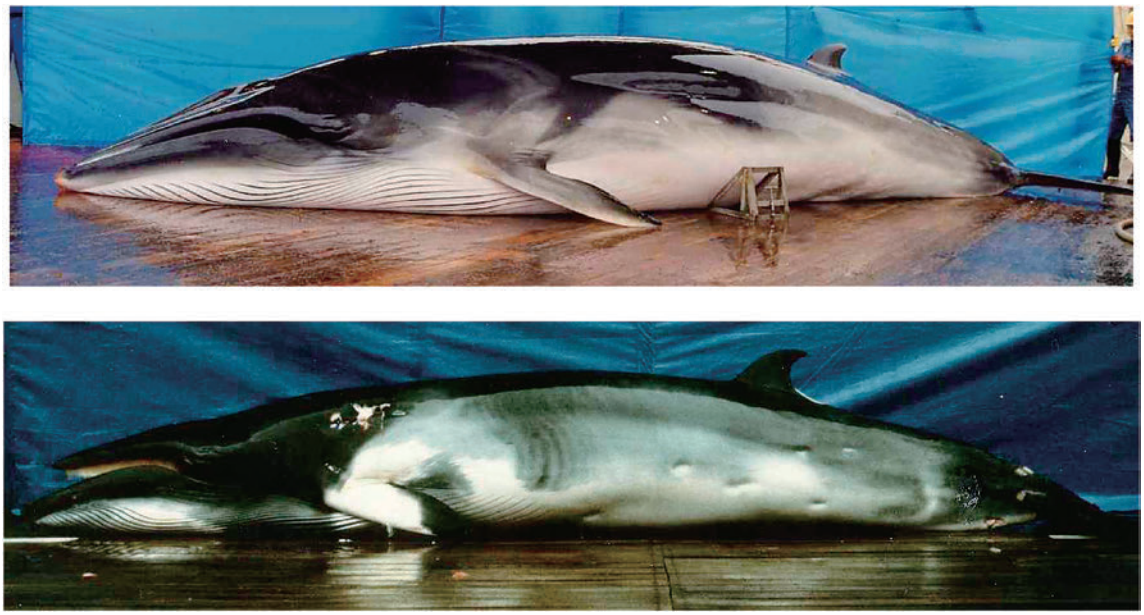

Figure 2. External appearance of Antarctic minke (above) and dwarf minke (below) whales.

Best, 1985) and genetic (e.g. Wada *et al.*, 1991; Arnason *et al.*, 1993; Pastene *et al.*, 1994) data collected from extant minke whale populations and re-specified two species, the Antarctic minke whale *B. bonaerensis* Burmeister, 1867, which is restricted to the Southern Hemisphere, and the common minke whale *B. acutorostrata* Lacèpéde, 1804, which is distributed globally.

In the Southern Hemisphere the common minke whale is known as 'diminutive' or 'dwarf' minke whale (Best, 1985; Arnold *et al.*, 1987). Currently, the dwarf minke whale is considered an un-named sub-species of *B. acutorostrata*. A large amount of biological and genetic information was obtained from 16 dwarf minke whales caught by earlier JARPA surveys in the Indo-Pacific region of the Antarctic. Earlier genetic analyses based on those samples and mitochondrial DNA (mtDNA) showed that dwarf and North Pacific minke whales were more similar to each other than they were to the Antarctic minke whale (Wada *et al.*, 1991; Pastene *et al.*, 1994).

Kato and Fujise (2000) reported on the morphology, growth and life history of dwarf minke whales based on 16 animals sampled by JARPA in the Indo-Pacific region of the Antarctic. Results of their analyses confirmed several of the morphological and morphometric characters reported previously, and the striking differences with the Antarctic minke whale. The mean body length at physical maturity was estimated at 7.0 m and 6.6 m for females and males, respectively. The age and length at sexual maturity in females was 7–10 years and 6.0–6.5 m, respectively. Furthermore, the authors stated that the conception of the dwarf minke whale is highly concentrated in mid-winter. Some additional morphological and morphometric analyses of minke whales worldwide, including dwarf minke whales, were conducted by Nakamura *et al.* (2014) and Kato *et al.* (2015).

On the basis of the morphological differences documented by Best (1985) and Arnold *et al.* (1987) and genetic differences (Wada *et al.*, 1991) the IWC Scien-

tific Committee (IWC SC) recognized the existence of two southern minke whales and agreed that the two minke whales in the Southern Hemisphere should definitively be considered separately for management purposes (IWC, 1991). In 1993, after examining the genetic information provided by Pastene *et al.* (1994), the IWC SC recommended the inclusion of the dwarf minke whale in the *Schedule*, so that catch limits for Antarctic minke whales recognise the distinction between the two southern minke whales (IWC, 1994).

In 2000, the IWC SC again recognised the two species but deferred a decision on other nominal taxa until the completion of a worldwide review of genetic and non-genetics information for minke whales (IWC, 2001). Subsequent worldwide genetic analyses of minke whales provided further evidence for the separation of the two species, *B. bonaerensis* and *B. acutorostrata*, and at least three sub-species of the common minke whale as recognized by Rice (1998) using mtDNA sequences (Pastene *et al.*, 2010) and microsatellite DNA (Glover *et al.*, 2013).

While additional genetic and morphological/morphometric studies are required to further elucidate the taxonomic status within *B. acutorostrata*, including that of the dwarf minke whale, there is agreement on the taxonomic status of *B. bonaerensis*. Figure 2 shows the external morphological appearance of Antarctic minke and dwarf minke whales.

Stock structure of the Antarctic minke whale

Detailed information on stock structure of the Antarctic minke whale was provided by Taguchi *et al.* (2017). The main findings are summarized here.

The primary data sources for studies of stock structure in this species were from the JARPA and JARPAII programmes, both of which had stock identity as one of their objectives. Studies based upon these samples have proved to be more useful than those from the commercial period, given the wider geographical coverage and the more random sampling design. In contrast, the commercial whaling samples were taken mainly from areas of high density near the ice-edge (Pastene, 2006). Initially, the JARPA genetic studies on stock structure were based on mtDNA Restriction Fragment Length Polymorphism (RFLP), and considerable genetic heterogeneity in Areas IV and V was found (Pastene *et al.*, 1993; 1996).

Consideration of a suite of information from mtDNA and microsatellite DNA (Pastene *et al.*, 2006), morphometrics for 10 external measurements (Fujise, 1995; Hakamada, 2006) and mean length at physical maturity (Bando *et al.*, 2006) led to the conclusion that Antarctic minke whales in the feeding grounds between Areas IIIE and VIW do not comprise a single stock (IWC, 2008). Rather, the results are consistent with the occurrence of at least two genetic stocks in these feeding grounds, which are probably related to the two proposed breeding areas in the eastern Indian Ocean and western South Pacific (Kasamatsu *et al.*, 1995). The following names have been proposed for these stocks: Eastern Indian Ocean Stock (I-Stock) and Western South Pacific Ocean Stock (P-Stock) (Pastene, 2006). The analyses of the JARPA data also suggested an area of transition in the region around 150°–165°E, across which there is an as yet undetermined level and range of mixing (IWC, 2008).

Given additional data from JARPAII, the analyses of stock structure were refined in two ways: the first involved additional laboratory work for additional genetic markers, and the second involved modified analytical measures. All the Antarctic minke whales taken by JARPAII between 2005/06 and 2010/11 were sequenced for a 340 bp-segment of the control region of the mtDNA (instead of the mtDNA RFLP used in JARPA), and genotyped using 12 microsatellite loci (instead of the six used in JARPA). Results of the heterogeneity test for both markers showed statistically significant genetic differences between whales in the two sectors, western (35°–130°E) and eastern (165°E–145°W), confirming that different stocks inhabit the Indian and Pacific sectors of the Antarctic (the I- and P-stocks). Microsatellite DNA analyses showed more dispersal amongst males than females and some degree of annual variation (Pastene and Goto, 2016).

Schweder *et al.* (2011) developed an integrated approach for estimating longitudinal segregation of two stocks using various sources of data: morphometric, microsatellite and mtDNA data. This approach revealed that the soft boundary (or transition area) suggested previously could vary by year and sex. A joint likelihood function was defined to estimate mixing proportions and apply statistical tests without assuming any baseline populations. The approach was originally applied to the JARPA data (Schweder *et al.*, 2011) and subsequently to JARPA and JARPAII data (Kitakado *et al.*, 2014). The results of this approach confirmed the occurrence of at least two stocks (I and P) in the Indo Pacific sector of the Antarctic. Furthermore, the results indicated that the spatial distribution of the two stocks had soft boundaries in Area IVE (100°–130°E) and VW (130°–165°E), which changes by year. Results also suggested possible sex differences in the pattern of distribution of the two stocks (Kitakado *et al.*, 2014).

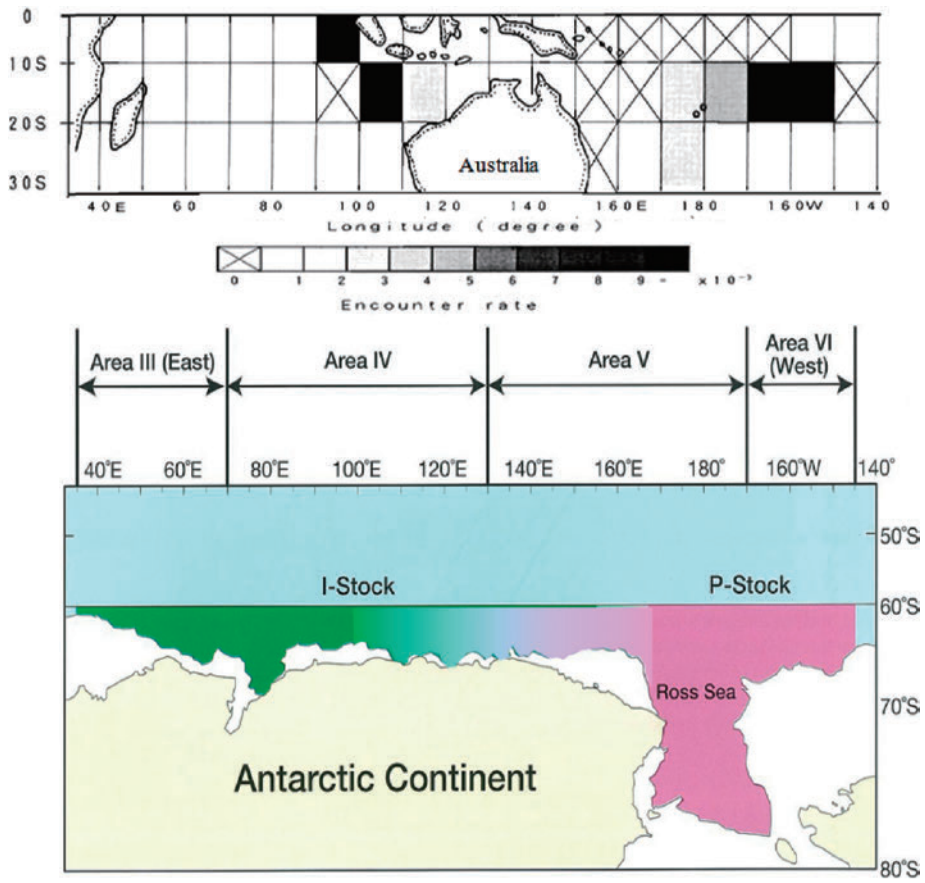

Figure 3. Hypothesis on stock structure of the Antarctic minke whale. The upper figure shows the encounter rates of Antarctic minke whales in 10° squares of latitude and longitude in waters 0°–30°S during October (Kasamatsu *et al.*, 1995). The high sighting densities in the eastern Indian Ocean and western South Pacific could correspond to breeding grounds of this species. At least two stocks (I- and P-stocks) occur in the research area of JARPA and JARPAII, which mix through a transition area. The transition area and the mixing rate appears to change by year and sex (Pastene and Goto, 2016).

Consequently, the structure of Antarctic minke whale in Areas IIIE to VIW appears to be more complex than originally thought. In summary, the IWC SC agreed (IWC, 2008) that there are (at least) two stocks with a wide mixing area that may change by year and sex. The extent of the I-stock to the west of 35°E and that of P-stock to the east of 145°W cannot be investigated due to a lack of appropriate samples.

The identification of spatial and temporal boundaries of stocks has been important for the interpretation of the biological parameters estimated of this species in the Antarctic (i.e. Punt *et al.*, 2014; Bando, 2017), and should also be important for the interpretation of abundance and abundance trends in the near future.

Figure 3 shows a schematic representation of the stock structure hypothesis derived from different analyses based on JARPA and JARPAII data.

Abundance of Antarctic minke whales

Detailed information on abundance estimates of Antarctic minke whales was provided by Hakamada and Matsuoka (2017). The main findings are summarized here.

Hakamada *et al.* (2013) estimated abundance and abundance trends of Antarctic minke whales in the Indian sector (Area IV) and Pacific sector (Area V) based on Japanese dedicated sighting surveys (JARPA and JARPAII), under the assumption of $g(0)=1$. Abundance estimates for the Indian sector range from 16,562 (CV=0.542) in 1997/98 to 44,945 (CV=0.338) in 1999/00, while those for the Pacific sector range from 74,144 (CV=0.329) in 2004/05 to 151,828 (CV=0.322) in 2002/03. Estimates of the annual rates of increase in abundance are 1.8% with a 95% CI of [−2.5%, 6.0%] for the Indian sector and 1.9% with a 95% CI of [−3.0%, 6.9%] for the Pacific sector.

Adjustments to allow for the $g(0)$ being less than 1 were made by the application of a regression model, developed from the results of the Okamura–Kitakado (OK) method estimate of minke whale abundance from the IDCR/SOWER surveys, which provides estimates of $g(0)$ from the statistics of the minke whale school size distri-

bution in a stratum. With this adjustment, abundance estimates increased by an average of 32,333 (106%) for the Indian sector and 89,245 (86%) for the Pacific sector, while the estimates of annual rates of increase and their 95% CIs changed slightly to 2.6% [−1.5%, 6.9%] for the Indian sector and 1.6% [−3.4%, 6.7%] for the Pacific sector. See Figure 4 for the abundance trends in the two sectors.

In 2012 the IWC SC agreed to a new best abundance estimate for Antarctic minke whales in Antarctic open waters south of 60°S, based on IDCR/SOWER sighting data. The estimates were 720,000 based on the sighting data collected during the CPII (1985/86–1990/91) with 95% CI (512,000, 1,012,000), and 515,000 based on the sighting data collected during the CPIII (1992/93–2003/04) with 95% CI (361,000, 733,000). No significant statistical differences were found between the CPII and CPIII estimates (IWC, 2013).

The estimates in the Indian sector (Area IV) were 55,237 (CV: 0.17) in CPII and 59,677 (CV: 0.34) in CPIII. In the Pacific sector (Area V) were 300,214 (CV: 0.13) in CPII and 183,915 (CV: 0.11) in CPIII.

By considering the 95% CIs of the estimates it can be suggested that the stocks of Antarctic minke whales are broadly stable with at most a slight decline, therefore the conclusion on trends is similar to that derived from JARPA and JARPAII data in Areas IV and V.

Abundance of Antarctic minke whales should be interpreted in the near future in the context of additional information on stock structure of the species in the Indo-Pacific region of the Antarctic.

Biological parameters of Antarctic minke whale

Detailed information on biological parameter estimates of Antarctic minkes whale was provided by Bando (2017). The main findings are summarized here.

Estimates of biological parameters of Antarctic minke whales based on samples obtained during the JARPA, are summarized in Table 1 (IWC, 2008; Bando, 2017).

The grouping of samples for the estimates of biological parameters considered the information on stock structure summarized above.

Age dependent natural mortality was estimated from the Statistical Catch-at-Age (SCAA) model. The pattern was similar for the I and P stocks with natural mortality being higher in young and old animals. It was calculated as 0.048 (for age=15) to 0.107 (for age=35) for the I-

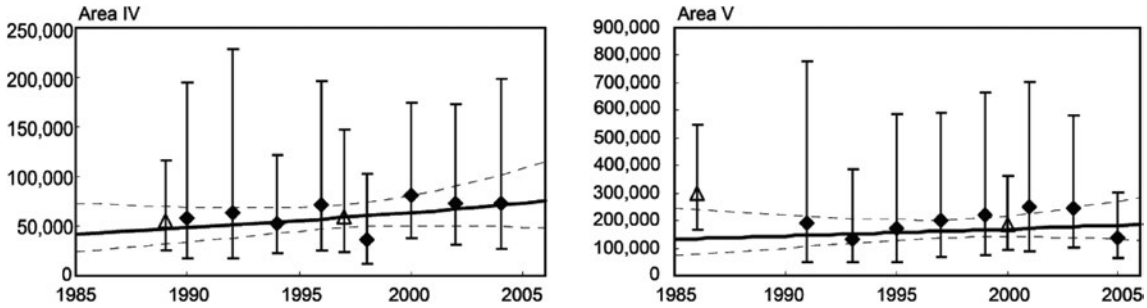

Figure 4. The best-case estimates of annual abundance of Antarctic minke whale in the Indian sector (Area IV) and Pacific sector (Area V) together with their 95% CIs. The IDCR/SOWER estimates for a common northern boundary for CPII and CPIII are shown by the open triangles. Confidence intervals include allowance for additional variance. The dashed curves indicate the 95% CIs for the exponential model (after Hakamada *et al.*, 2013).

Table 1

Summary of biological parameters of Antarctic minke whales estimated according to one stock structure hypothesis (after IWC, 2008).

		I-stock (Area IIIE+IV+VW)		P-stock (Area VE+VIW)	
		Male	Female	Male	Female
Length at sexual maturity (m)	*Lmov*		8.40m		8.30m
	Lm50%	7.29m	8.16m	7.17m	7.97m
Age at sexual maturity	*tmov*		7.9		8.4
	tm50%	5.3	7.6	5.4	8.0
Length at physical maturity (m)	50%mature	8.32m	9.12m	8.22m	8.73m
Age at physical maturity	50%mature	16.0	21.2	17.0	20.6
Growth curve		$y = 8.61(1\text{-}e^{-(0.27x+0.54)})$	$y = 9.16(1\text{-}e^{-(0.23x+0.49)})$	$y = 8.45(1\text{-}e^{-(0.29x+0.51)})$	$y = 8.93(1\text{-}e^{-(0.21x+0.59)})$
Percentage of matured females pregnant			92.9%		85.4%
Foetal sex ratio (male %)			51.8%		46.8%
Mean litter size			1.007		1.013

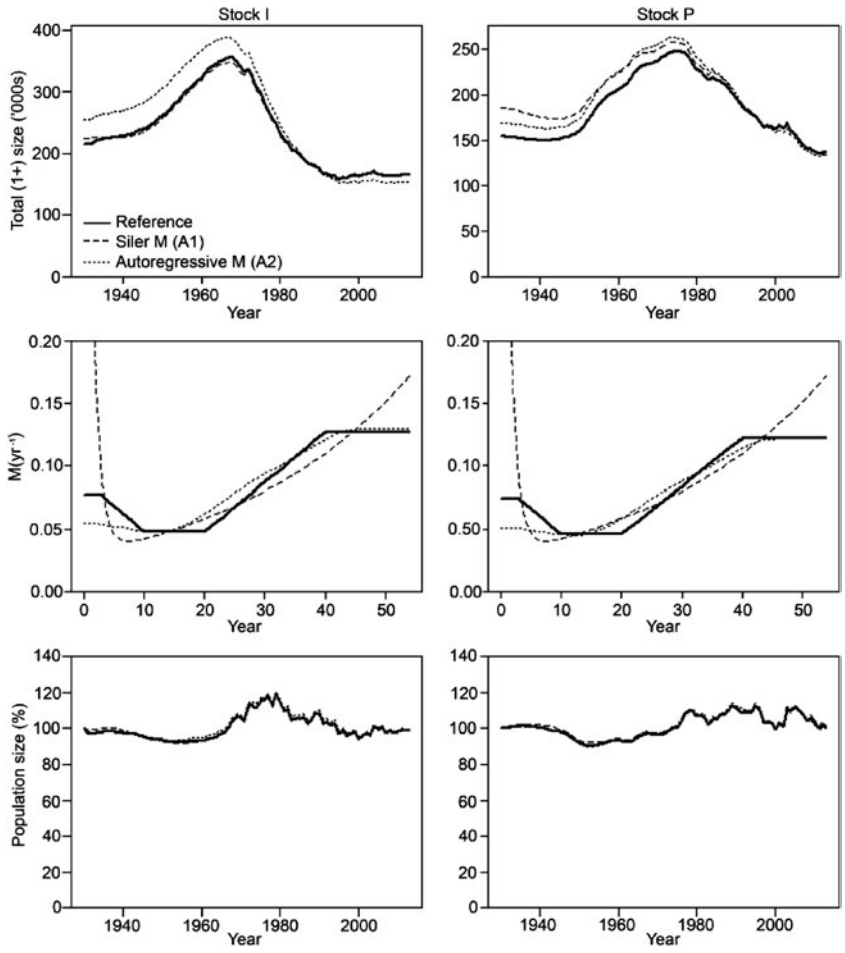

Figure 5. Time-trajectories of total (1+) population size (upper panels), age-specific natural mortality (center panels), and total (1+) population size relative to carrying capacity (lower panels) for three ways to model natural mortality (the Siler model, autoregressive and piecewise linear) of two stocks of Antarctic minke whale (from Punt *et al.*, 2014). Biological data obtained from both JARPA and JARPAII were used in the SCAA.

stock and 0.046 (for age=15) to 0.103 (for age=35) for P-stock (Figure 5) (Punt *et al.*, 2014). Time-trajectories derived from SCAA showed that total population size of the Antarctic minke whale increased until 1970's and then declined until 2000's for both stocks (Figure 5) (Punt *et al.*, 2014).

CONCLUSIONS

The Japanese whale research programs under special permit have provided important information on stock structure, biological parameters and abundance of Antarctic minke whales in the Indo-Pacific region of the Antarctic. Such information is key for the assessment of the species and the application of management procedures such as the IWC's Revised Management Procedure (RMP) in the future. Continuation of the systematic monitoring of the stocks of Antarctic minke whales is recommended.

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Technical Reports of the Institute of Cetacean Research (2019) pp. 9–13

Technical Report (not peer reviewed)

Age estimation of Antarctic minke whales based on aspartic acid racemization: technical development

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ABSTRACT

At present, examination of earplugs is the only practicable means to obtain age data at the annual scale in baleen whales. It is the only method providing age data accurate enough for population-level analyses such as the Statistical Catch-at-Age Analysis (SCAA) of Antarctic minke whales. Because there are a number of whales sampled with unreadable earplugs, the feasibility of using other techniques for age determination is being investigated by scientists at the Institute of Cetacean Research with the aim of determining the age of those samples where earplugs does not work. One of those techniques is based on enantiomers of aspartic acid in eye lens, the aspartic acid racemization (AAR) technique. This paper presents a brief review of the technical development of the AAR technique at the Institute of Cetacean Research, and the preliminary results of using this technique for age determination of Antarctic minke whales.

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 advantages that it is time- and cost-efficient, and the technique can be used on available historical samples; however, unreadable growth layers form in some individuals baleen whales (Maeda *et al.*, 2013; George *et al.*, 1999). In such cases alternative methods of age determination are required.

Indices of chronological age of whales have been developed using molecular approaches. For example length of telomeres, nucleoprotein caps flanking DNA (Olsen *et al.*, 2012), and methylation levels at the C5 position of cytosine residues adjacent to guanidine residues (Polanowski *et al.*, 2014) were examined in relation to age using skin of humpback whales (*Megaptera novaeangliae*) in the Gulf of Maine. The feasibility of the DNA methylation technique to determine age in Antarctic minke whales (*Balaenoptera bonaerensis*) has been evaluated by scientists at the Institute of Cetacean Research (ICR) (Goto and Inoue, 2018). Currently, these epigenetic approaches face challenges such as measurement of indices error and whale specific biological processes, which makes it difficult to interpret the age of whales.

Regarding biochemical approaches, the Advanced Glycation End (AGE) products was proposed as one of the possible factors underlying the functioning of the biological clock for mammals (Severin *et al.*, 2013), however no relationship was observed between AGE levels and age in bowhead whales (Rosa, 2006). Another technique is based on enantiomers of aspartic acid in eye lens, the aspartic acid racemization (AAR) technique, which is being developed at the ICR.

This paper presents a brief review of the technical development of the AAR technique at the ICR, and shows preliminary results of using this technique for age determination of Antarctic minke whale.

PRINCIPLE OF THE AGE ESTIMATION BY THE ASPARTIC ACID RACEMIZATION TECHNIQUE

The AAR technique for determining age is based on temporal changes of the ratio of D and L-enantiomers of aspartic acid in mammals (Helfman and Bada, 1975) (Figure 1). The technique is based on the principle that D-aspartic acid accumulates logarithmically with age, and the crystalline in the core of the lens has been conserved chemically since it is formed at the fetus stage and is metabolically inactive for all of the animal's life (Masters

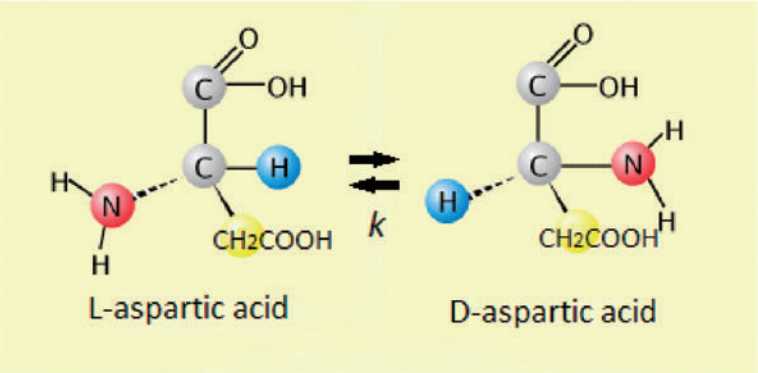

Figure 1. Scheme of racemization of aspartic acid.

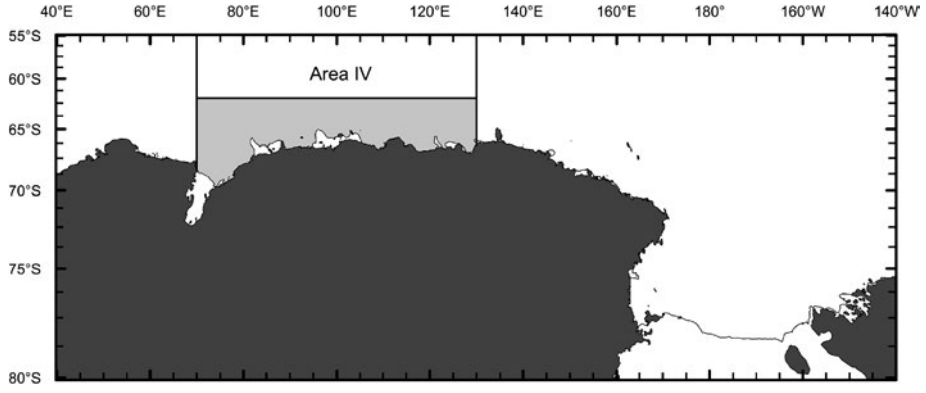

Figure 2. Research area of JARPAII in the 2005/2006 and 2007/2008 austral summer seasons.

et al., 1977).

Masters *et al.* (1977) and Bada *et al.* (1980) proposed that the racemization of amino acids follows a first-order reversible rate law, where the racemization equation is:

$$2k_{\mathrm{Asp}} \cdot t = \mathrm{Log_e}\left[(1+D/L)/(1-D/L)\right] - \mathrm{Ln}\left\{\left[1+(D/L)_0\right]/\left[1-(D/L)_0\right]\right\}. \quad (1)$$

A linear regression model is constructed using this expression, where D/L is the ratio of D- and L-aspartic acids, t is any given time during racemization, and the logarithmic term at t=0 describes the amount of D-aspartic acid formed at birth.

RESEARCH ON ASPARTIC ACID RACEMIZATION AT THE INSTITUTE OF CETACEAN RESEARCH

At the ICR, the feasibility of using the AAR technique to determine the age of Antarctic minke whales has been investigated (see details in Yasunaga *et al.*, 2017). The JARPA and JARPAII research programs in the Antarctic have provided sufficient biological information on this species that makes assumptions and extrapolations of key parameters used in this technique unnecessary. For example k_{Asp} and $(D/L)_0$ in Eq. (1) can be determined by direct comparison with earplug-based estimated ages, and $(D/L)_0$ can be estimated using available foetal samples.

Samples

Antarctic minke whales used in this study were sampled in the Indo region of the Antarctic, which corresponds to the International Whaling Commission's (IWC)'s management Area IV (70°–130°E), south of 62°S (Figure 2). Whales were sampled during the austral summer seasons 2005/2006 and 2007/2008 by JARPAII surveys.

At the field, scientists collected the left eyeball from 20 foetuses and lens samples from 18 female Antarctic minke whales. The samples were stored in polyethylene bags at −80°C until analysis.

Preparation of lens of Antarctic minke whale

Lenses of all samples (foetuses and females) were rinsed first with phosphate-buffered saline (Figure 3). Then their outermost layers were removed with mucus, and the cores were dissected out using a surgical knife. Approximately 10 mg of core samples were homogenized with 1 ml of tris-buffer (200mM Tris, 150mM NaCl, pH 8.0) using an ultrasonic homogenizer. The homogenate was centrifuged at 15,000×*g* for 15 min at 4°C, and 100 µl of the supernatant was then desalted with acetone and air-dried. They were hydrolyzed in the gas-phase HCl (6N-HCl) for 7 h at 108°C (Pico Tag Work Stations, Waters, Tokyo) (Figure 4). The hydrolysates were evaporated

Figure 3. Lens of Antarctic minke whale rinsed with phosphate buffer.

Figure 4. Hydrolysis of lens of Antarctic minke whale using Pico Tag Work Stations (Waters, Tokyo).

under reduced pressure.

Laboratory procedures

The Asp *D*/*L* was determined using HPCL (Alliance® HPLC systems e2696, Waters) with a Nova-Pak ODS column (3.9 mm×300 mm, Waters) using fluorescence detection (344 nm excitation wavelength and 443 nm emission wave length) (Figure 5). Elution was carried out with an isocratic adsorption of 3% acetonitrile +3% tetrahydrofuran/0.1M acetate buffer pH 6.0 at a flow rate of 0.8 ml/min and column temperature of 23°C. Then, 70 µl of borate buffer (0.1M, pH 10.4), 5 µl of n-tert-butyloxycarbonyl-l-cysteine and 5 µl of *o*-phthalaldehyde were successively added to 5 µl of the hydrolysate dissolved in 0.1 N-HCl. The measured Asp *D*/*L* was calibrated against real ratios in the standard solutions and then it was corrected for hydrolysis effect under our laboratory conditions, which was represented as $(D/L)_{act}$.

Figure 5. HPCL system (Alliance® HPLC systems e2696, Waters) used for fluorescence detection.

Results

Table 1 shows $(D/L)_{act}$ and the age index calculated for the 18 female Antarctic minke whales. The $(D/L)_0$ which is one of two specific coefficients of AAR, was determined using lenses of foetuses at various developing stages because their $(D/L)_{act}$ can be approximated to those at birth. However, precision of the $(D/L)_0$, which is estimated using foetuses was unsatisfactory ($(D/L)_0$=0.0134; SE=3.78×10^{-4}; 95% confidential interval 0.0121–0.0147).

Taken together, the slope of the age estimation equation was examined for two cases. Figure 6 shows the relationships between the age indexes and earplug ages. The single outlier at 40-years-old for which Cook's distance exceeded 2 was eliminated in the first regression analysis. The two equations (cases) of age estimation are shown below as Eq. (2) and Eq. (3). In Eq. (1) linear regression analysis were re-performed to determine k_{Asp} substituting $(D/L)_0$=0.0134 using dataset of the $(D/L)_{act}$ and the earplug ages in the 17 whales as follows:

$$\begin{aligned} &\mathrm{Log_e}\left\{\left[1+(D/L)_{act}\right]/\left[1-(D/L)_{act}\right]\right\} \\ &=1.79\times10^{-3}\times\text{earplug age (year)}+0.0268, \\ &\therefore p<0.001,\ r^2=0.890,\ k_{Asp}=8.94\times10^{-4}, \\ &\mathrm{SE}\ (2k_{Asp})=1.52\times10^{-4}. \end{aligned} \quad (2)$$

In Eq. (3) linear regression analysis was performed to determine k_{Asp} and $(D/L)_0$ in Eq. (1) using dataset of the $(D/L)_{act}$ and the earplug ages in the 17 whales as follows:

Table 1
The actual D/L ratios of aspartic acid in lens and AAR ages of Antarctic minke whales.

Sample No.	Sex	Earplug Age	$(D/L)_{act}$	$Log_e[(1+D/L)/(1-D/L)]$	Age estimated by the AAR*		
					Age	SE	95% CI
05/06-AM348	F	14	0.0223	0.0446	10.6	1.5	8.1–14.2
05/06-AM349	F	21	0.0328	0.0656	19.8	2.2	16.1–25.0
05/06-AM352	F	9	0.0222	0.0444	10.6	1.5	8.2–14.2
05/06-AM361	F	26	0.0432	0.0865	28.9	2.9	24.2–35.7
05/06-AM372	F	40	0.043	0.086			
05/06-AM382	F	7	0.0173	0.0347	6.3	1.2	4.4–9.1
05/06-AM386	F	8	0.019	0.038	7.8	1.3	5.6–10.8
05/06-AM398	F	7	0.0164	0.0329	5.6	1.2	3.7–8.2
05/06-AM488	F	4	0.0136	0.0272	3.1	1	1.4–5.3
05/06-AM498	F	4	0.0151	0.0302	4.4	1.1	2.6–6.8
05/06-AM517	F	3	0.0106	0.0212	0.5	0.8	−0.9–2.3
05/06-AM539	F	1	0.0124	0.0249	2.1	0.9	0.5–4.1
05/06-AM565	F	2	0.0174	0.0348	6.4	1.2	4.4–9.1
05/06-AM592	F	4	0.0169	0.0337	5.9	1.2	4.0–8.6
05/06-AM603	F	6	0.0173	0.0346	6.3	1.2	4.3–9.1
05/06-AM615	F	7	0.0167	0.0333	5.8	1.2	3.8–8.5
05/06-AM630	F	5	0.0152	0.0305	4.5	1.1	2.8–7.0
05/06-AM634	F	3	0.0125	0.025	2.2	0.9	0.7–4.2

* Ages estimated by the AAR were derived from Eq. (3)

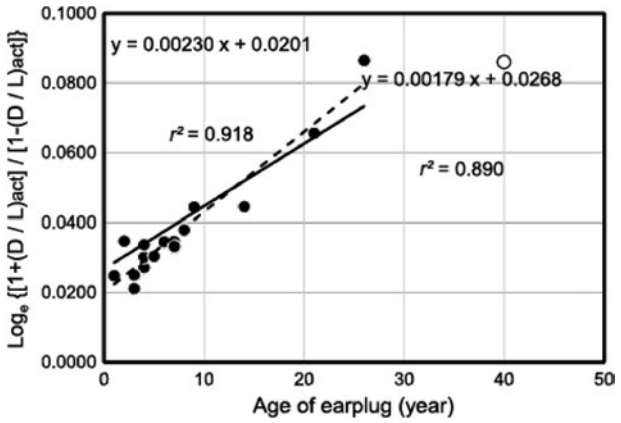

Figure 6. Relationship between age indexes, $Log_e\{[1+(D/L)_{act}]/[1-(D/L)_{act}]\}$, and ages of earplugs of Antarctic minke whales: open circle was excluded from simple linear regression analysis as an outlier, and a solid and broken line is calculated by Eq. (2) and Eq. (3), respectively.

$$\begin{aligned}&Log_e\left\{\left[1+(D/L)_{act}\right]/\left[1-(D/L)_{act}\right]\right\}\\&=2.30\times10^{-3}\times\text{earplug age (year)}+0.0201,\\&\therefore p<0.001,\ r^2=0.918,\ k_{Asp}=1.15\times10^{-3},\\&\text{SE}\,(2k_{Asp})=1.71\times10^{-4},\ \text{SE (intercept)}=1.72\times10^{-3}.\end{aligned}\quad (3)$$

Squared correlation coefficient of the Eq. (3) was higher than that of the Eq. (2), and the SE of $2k_{Asp}$ and intercept of the Eq. (3) were lower than those of the Eq. (2). Therefore, Eq. (3) is considered more precise and accurate to estimate ages of whales than Eq. (2).

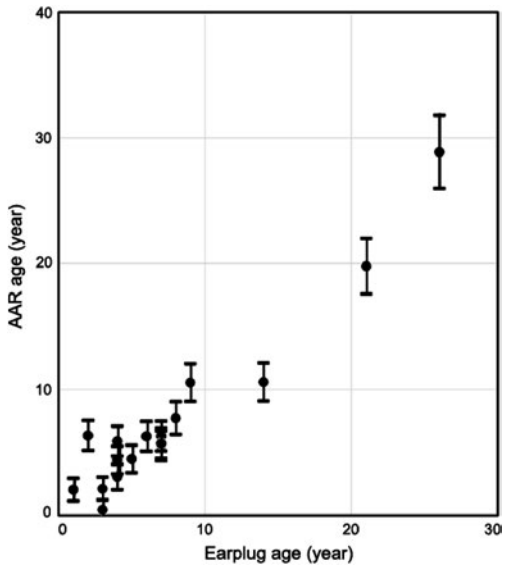

Figure 7. Relationship between ages estimated by AAR and earplugs (averages and SEs estimated by bootstrap simulation) in Antarctic minke whales.

Finally, the standard errors were calculated for the ages estimated by AAR. Ages estimated from Eq. (3) and $(D/L)_{act}$ in each whale, including their SEs and 95% confidence intervals estimated by bootstrap simulation, are shown in Table 1. Figure 7 shows the relationships be-

tween ages estimated from earplugs, and ages (including SEs and 95% confidence intervals), estimated by the AAR. The range of SEs of the AAR-based age estimates was 0.9 and 2.9 years, and they were within the 95% confidence interval, except for one case.

CONCLUSIONS

This study was successful in developing the AAR technique for the Antarctic minke whale. The application of this technique can complement the age estimation of individuals of this species based on earplug reading, especially for young animals with unreadable earplugs. A few issues including bias due to cataracts require further consideration in the future.

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Technical Reports of the Institute of Cetacean Research (2019) pp. 14–20

Technical Report (not peer reviewed)

A note on *g*(0) estimates derived from vessel-based sighting surveys

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ABSTRACT

This article introduces the concept of *g*(0), the probability of detection of animals at zero distance from the trackline, in the development of abundance estimates of large whales. In the conventional line transect method, it is assumed that *g*(0)=1, meaning that all animals on the trackline are detected. However, this assumption is sometimes violated, which causes underestimation of the abundance. Also this article provides overviews of the methods used to estimate *g*(0) for large baleen whales, and of the current work of the Institute of Cetacean Research to estimate *g*(0) based on dedicated sighting surveys in the Antarctic.

INTRODUCTION

Abundance estimates of animals can be based on total counts (census), sample counts, index counts, among others. The method producing the most accurate estimate is probably the total census of animals in a given area. This is possible when the area is small, the visibility is good, the target species is easily detectable, and when there is no emigration and immigration. However, these conditions are difficult to meet for most of the large whale species. In such cases, the sample counts approach is more appropriate. Under this approach, the abundance estimate is extrapolated from data obtained from a part of the total research area.

Distance Sampling (DS) methods (Buckland *et al.*, 2001) are widely used for estimating whale abundance in large areas. The line transect method is one of those methods, in which detailed surveys are conducted along a trackline that covers part of the entire survey area (Figure 1A). The detection probability of whales is not constant but decreases with the distance from the trackline (Figure 1B). Hence, in conventional line transect methods it is assumed that the detection probability on the trackline, *g*(0), is equal to 1. This means that all whales on the trackline are detected (Buckland *et al.*, 2001).

However, whale schools are not always detected even if they are on the trackline because whales dive and because observers could miss the schools on the trackline. In such cases a *g*(0) estimate is required for correction of abundance estimates.

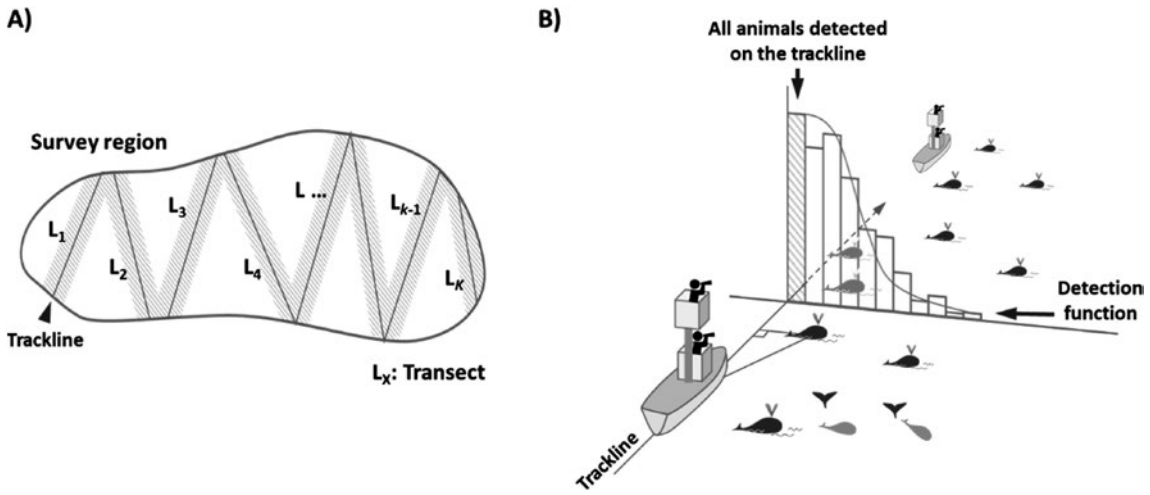

Figure 1. Diagram showing the zigzag tracklines in part of the total research area under the line transect survey (A); and the pattern of detection probability in relation to perpendicular distances (B).

The objective of this article is to introduce the concept of *g*(0) in the development of abundance estimates of large whales. Also this article provides overviews of the methods used to estimate *g*(0) for large baleen whales, and of the current work of the Institute of Cetacean Research (ICR) to estimate *g*(0) based on dedicated sighting surveys in the Antarctic.

AVAILABILITY AND PERCEPTION BIASES

Under the conventional DS methods (Buckland *et al.*, 1993; 2001), *g*(0) is assumed as equal to 1. However, when this assumption is violated, conventional DS estimators result in density and abundance being underestimated. In the case of whales, they are not always detected because they spend time underwater. This is known as 'availability bias' (Figure 2). This bias is potentially the largest problem for long-diving species that spend little time at the surface when the boat or ship surveys are conducted. For diving species, it may be necessary to model diving behavior to estimate the availability bias (e.g. Barlow, 1999).

Also, the observers could miss some whales available at the surface for a variety of reasons including visibility such as glare and mist, fatigue or moments of inattention. This is known as 'perception bias' (Figure 2). This bias is potentially largest for species that occur as single animals or in small groups and do not show much of their body when surfacing, such as Hector's dolphin and minke whales (Dawson *et al.*, 2004).

Both availability and perception biases may reduce the detection probability. In many cases, there is a combined effect of these on estimates of abundance and density for target species. The term 'visibility bias' is used generically to refer to either or both types of biases (Laake and Borchers, 2004).

If the assumption of *g*(0)=1 is violated then the abundance estimates of the target species will be underestimated. For example, Marsh and Sinclair (1989) estimated that 83.3% of dugons (*Dugong dugon*) were beneath the water surface and unavailable for detection, while the observer team only missed 2–17% of the visible dugongs within a 200 m strip. Laake and Borchers (2004) noted that the availability bias should not be ignored if it occurs, because it can be a substantial source of bias that may be much larger than perception bias.

Therefore estimates of *g*(0) for cases of availability and perception biases are important and data required for such estimates should be collected.

DATA REQUIRED FROM THE FIELD TO ESTIMATE *g*(0) IN CASE OF LARGE WHALES

The ICR conducts sighting surveys based on the distance sampling for estimating abundance of large whales. In general, the surveys follow the Requirements and Guidelines for Conducting Surveys and Analysing Data within the Revised Management Scheme of the International Whaling Commission Scientific Committee (IWC SC) (IWC, 2012). Details of the procedures are found in Hakamada and Matsuoka (2017).

These surveys have been implemented on predetermined zigzag tracklines. The start point is randomly selected for each survey. The design takes care not to follow physical features such as isobaths that may be correlated with whale distribution and their migration. For each whale school sighted, the species is identified and other data are recorded such as sighting position, school

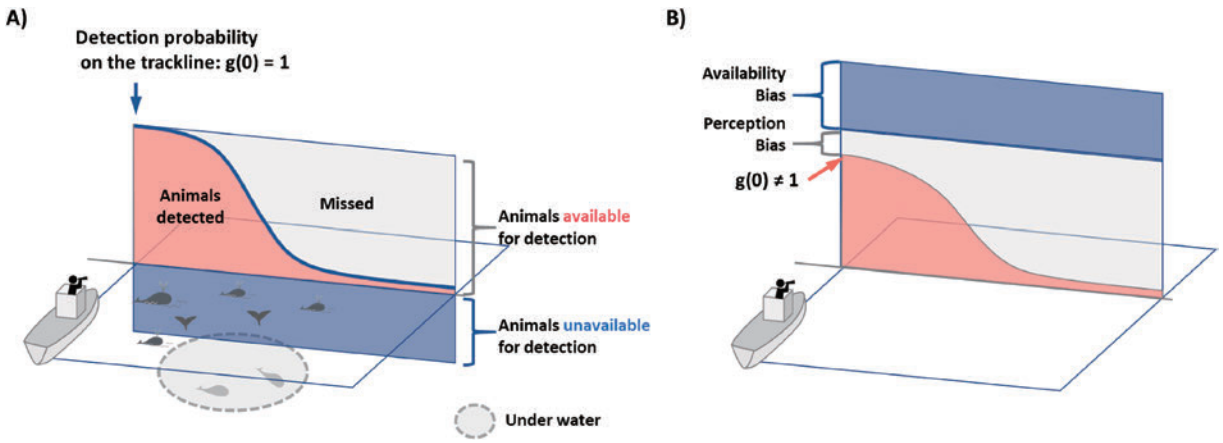

Figure 2. Diagram showing the detection probability with assumed *g*(0)=1 (A); and the case of considering availability and perception biases when *g*(0) is different from 1 (B) (modified from https://workshops.distancesampling.org/stand-intermed-2018/slides/mrds1-g0.pdf).

size, weather information, etc.

Sighting data are collected from two platforms available in the same vessel, which allows accounting for animals missed on the trackline. Sighting surveys are conducted by using a top barrel platform (TOP) and an independent observer platform (IOP). Sighting surveys are conducted from two platforms independently, following established procedure protocols (Butterworth and Borchers, 1988; Palka, 1995; Matsuoka *et al.*, 2003; IWC, 2012).

Under the IO mode, sighting surveys are conducted from the TOP and the IOP. Personnel at the upper bridge of the vessels record the sighting information from the two platforms and determine if the same sighting is made from the two platforms or if some sightings are missed from one of the platforms.

Figure 3 shows the research vessel *Kaiyo-Maru* No.7 (KY7) equipped with TOP, IOP and upper bridge. The vessel has instruments allowing contact between TOP and upper bridge, and between IOP and upper bridge.

During the survey, one or more observers are always in the TOP, and one observer is in the IOP. Other observers are in the upper bridge getting sighting information from the two platforms. Observers in the top barrel and IOP report the sightings to the upper bridge observers, but that information is not interchanged between TOP and IOP.

Figure 4 shows a diagram of double-platform sighting survey and judgment of duplicate status. Here, a single whale appears three times, which is detected by platform A (TOP). On the other hand, platform B (IOP) detects the same whale only during the last surfacing (A3). Observers in the upper bridge receive sighting information (e.g. distance, angle, species and school size) from platform A at the first sighting (A1) and track this single whale until platform B observed it. They make a judgment concerning the duplicate status (same whale sighted by the two platforms).

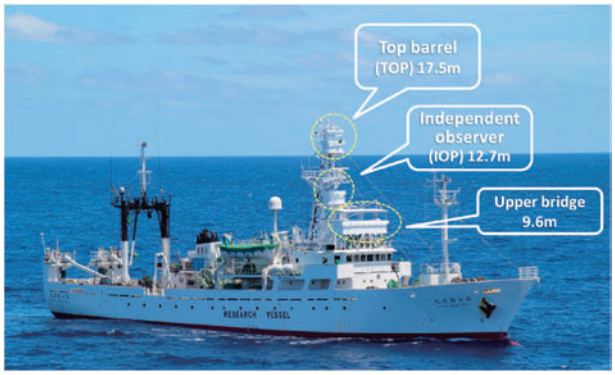

Figure 3. Survey vessel equipped with three platforms for conducting surveys required for *g*(0) estimate.

ANALYTICAL APPROACHES TO ESTIMATE *g*(0)

MRDS method

The method called 'Mark-Recapture Distance Sampling' (MRDS) is one of the methods employed to estimate *g*(0), which consists of two models: a multiple covariate Mark-Recapture (MR) model (for estimating observer detection rates) and a multiple covariate Distance Sampling (DS) model (for estimating the variation of detection probabilities with distance from the vessel) (Buckland and Turnock, 1992; Alpizar-jara and Pollock, 1996; Quang and Becker, 1997; Borchers *et al.*, 1998; Innes *et al.*, 2002; Laake and Borchers, 2004; Borchers *et al.*, 2006, Burt *et al.*, 2014). The MRDS method deals only with perception bias.

The probability that either or both observers detect a school (or animals) is given by:

$$\hat{P}_{\cdot}(y,z) = \hat{P}_1(y,z) + \hat{P}_2(y,z) - \hat{P}_1(y,z)\hat{P}_2(y,z),$$

where y is the perpendicular distance corrected and z denotes the covariate. Although observers 1 and 2 are considered independent from each other under the IO mode survey, the detection probability of observers can be correlated because of factors such as school size. This heterogeneity is denoted in the probabilities of detection using a logistic form for the detection function:

$$P_{l|3-l}(y,z) = \frac{\exp\left(\beta_0 + \beta_1 y + \sum_{k=1}^{K} \beta_{k+1} z_k\right)}{1 + \exp\left(\beta_0 + \beta_1 y + \sum_{k=1}^{K} \beta_{k+1} z_k\right)},$$

where, l can take the values 1 or 2 to represent the observers, $\beta_0, \beta_1 \cdots \beta_K$ represent the parameters to be esti-

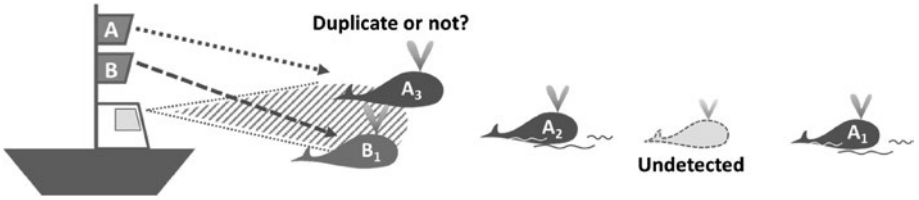

Figure 4. Diagram of the double-platform sighting survey conducted by the ICR.

mated, and K is the number of covariates. When perpendicular distance differed between duplicates, the average distance of the duplicate pair are used.

On the other hand, the detection probability away from the trackline is described and estimated by the DS model as follows:

$$\text{Half-normal: } f(y,z) = \exp\left(-\frac{y^2}{2\sigma(z)^2}\right),$$

$$\text{Hazard-rate: } f(y,z) = 1-\exp\left[\left(\frac{-y}{\sigma(z)}\right)^{-b}\right],$$

where σ and b are the parameters of each functional form.

Both models can include covariates (e.g. school size, Beaufort, etc.). In addition, the likelihoods can be treated as MR model and DS model separately or as combination of those models. The best model usually is selected using the Akaike Information Criterion (AIC; Akaike, 1973).

The MRDS method do not consider the availability bias. Hence, the availability bias should be accounted by analyses of data from additional experiments (e.g. Heide-Jørgensen *et al.*, 2010).

Hazard probability model: 'OK' method

This method deals with both availability and perception biases. The method, called the 'OK' method, was developed by Okamura *et al.* (2003) by extending the model of Skaug and Schweder (1999), and by combining the merits of the models of Schweder *et al.* (1997) and Cooke (2001). Okamura *et al.* (2003) expanded the concept of the hazard probability model (Skaug and Schweder, 1999) to avoid the use of external information for considering diving behavior (the conventional and simple hazard probability models require an estimate of the surfacing rate based on external data).

The hazard probability function $Q(x,y)$ for an observer u (u=A or B) is assumed as:

$$Q_u(x,y) = \mu_u \exp\left\{-\left(\frac{x}{\sigma_u}\right)^{\gamma_1} - \left(\frac{y}{\sigma_u}\right)^{\gamma_2}\right\},$$

where $0<\mu_u\leq 1$, $\sigma_u>0$, $\gamma_1, \gamma_2>0$, μ_u is the level parameter of the hazard probability function, σ_u is the scale parameter, and γ_1 and γ_2 are the shape parameters (Skaug and Schweder, 1999). The corresponding detection function from this hazard function is explicitly expressed as:

$$g_u(x) = 1-\exp\left\{-\left(\frac{\lambda}{v}\right)\int_0^\infty Q_u(x,y)\,dy\right\}$$

$$= 1-\exp\left[-\lambda v^{-1} c_2^u \exp\left\{-\left(\frac{x}{\sigma_u}\right)^{\gamma_1}\right\}\right],$$

where λ is surfacing intensity, v is constant vessel speed and $c_2^u=\sigma_u\mu_u\gamma_2^{-1}\Gamma(\gamma_2^{-1})$, with Γ being the gamma function.

The 'OK' method is a more general and comprehensive method that integrates the merits of previous hazard probability models.

ONGOING STUDIES IN THE ANTARCTIC

The 'OK' method explained above (Okamura *et al.*, 2003; Okamura and Kitakado, 2012) was used previously for estimating Antarctic minke whale (*Balaenoptera bonaerensis*) abundance from the IDCR/SOWER data collected from the 1985/86 to the 2003/04 austral summer season. In that study $g(0)$ was estimated less than 1 for Antarctic minke whales (0.327 to 0.793). Using this result, the abundance estimates of Antarctic minke whale based on JARPA data were corrected by application of a regression model (Hakamada *et al.*, 2013).

Preliminary results of the MRDS method on Antarctic minke whales

Data were obtained during the surveys conducted from

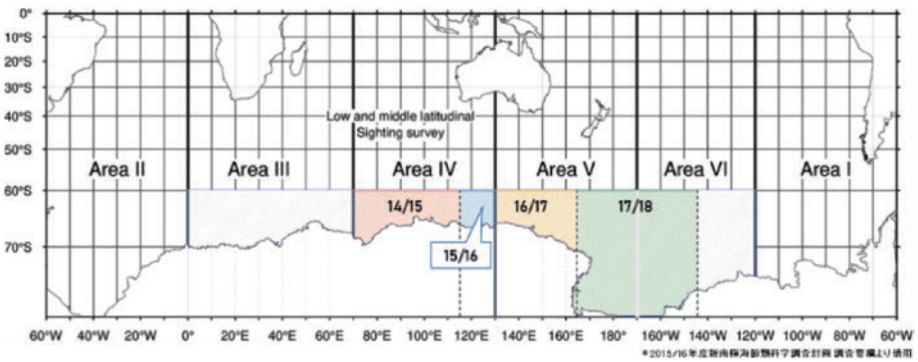

Figure 5. Research area of the study to estimate $g(0)$ of Antarctic minke whale based on MRDS.

December to February from the 2014/15 to the 2017/18 austral summer seasons in Antarctic Area IV, V, and VI (Figure 5). As indicated above, the MRDS method for estimating $g(0)$ requires data collected from IO mode survey.

The data comprised a total of 215 schools, including 63 schools which were observed from TOP and IOP (Figure 6, Table 1). Mean school size was 2.23. Also sightings with perpendicular distance of more than 1.5 n.miles, and those observed when Beaufort status was higher than six, were excluded from the dataset.

Table 2 shows the AIC values after fitting explanatory variables to the DS and MR models. For the DS model, school size and cue were the most important explanatory variables. For the MR model, platform and school size were the most important explanatory variables. For the best model, detection probability on the trackline was 0.676 (CV=0.092) for the TOP, 0.429 (CV=0.145) for the IOP, and 0.810 (CV=0.066) for the pooled platforms. The estimated detection function plots are shown in Figure 7.

The detection probability on the trackline for the Antarctic minke whale was similar to the result from the previous study based on the 'OK' method and IDCR-SOWER datasets (Okamura and Kitakado, 2012). In addition, this result was similar to that found for North Pacific common minke whale. For this species the $g(0)$ for TOP and upper bridge was estimated at 0.798 by the 'OK' method using the IO passing mode sighting survey data (Okamura *et al.*, 2010).

Future studies

The estimate of $g(0)$ for Antarctic minke whale based on the MRDS method was similar to that obtained by the 'OK' method. The estimate considered covariates that affect the detection functions. However, the analysis only accounts for perception bias and did not account for availability bias. Hence, some additional investigations are needed to estimate whale diving pattern as in the case of Heide-Jørgensen *et al.* (2010). They considered availability correction factors using data collected from external experiment survey. Also, model development is needed, for example, correction for non-uniform density of animals within strata using data collected from external experiment survey, and the use of the Bayesian hierarchical approach to incorporate habitat use into a detection model within a mark-recapture distance sampling framework (Oyster *et al.*, 2018), is required. These studies are ongoing at the ICR.

ACKNOWLEDGEMENTS

I thank the researchers, captains, officers and crew members who have participated in all surveys. This work would not have been possible without the substantial efforts of all of them.

Table 1

Number of sightings of Antarctic minke whales made by each platform in each survey. Note that the number of unique sightings is the number of sightings seen by observer 1 plus the number seen by observer 2, minus duplicates.

Survery ID	Number of sightings by observers			Number of unique sightings
	Platform A (TOP)	Platform B (IOP)	Detected by both (Duplicate)	
YS1_1415	14	10	6	18
YS2_1415	6	1	1	6
YS3_1516	47	22	14	55
KY7_1617	6	6	3	9
YS3_1617	14	10	4	20
KY7_1718	10	11	5	16
YS2_1718	79	42	30	91
Total	176	102	63	215

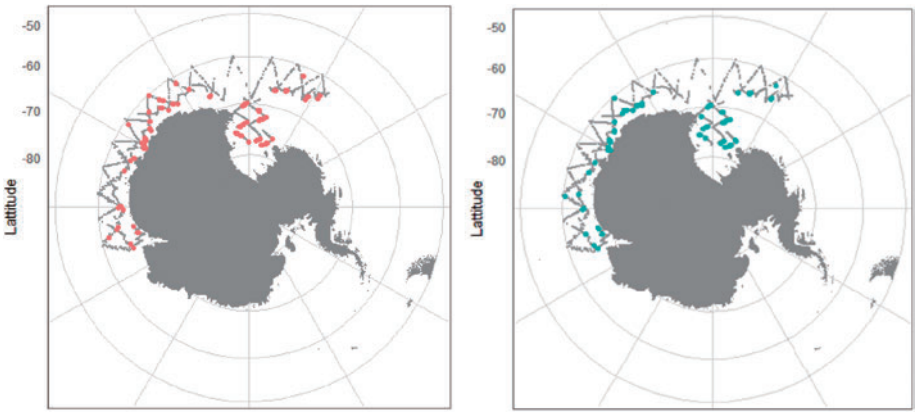

Figure 6. Tracklines and sighting position of Antarctic minke whale observed by each platform (left: TOP; right: IOP). Gray lines indicate tracklines.

Table 2

Akaike Information Criterion (AIC) values after fitting explanatory variables to the Distance Sampling (DS) and Mark-Recapture (MR) models. This table shows a part of all result of model fitted for reference. The final models chosen are given in bold.

Model ID	DS model		MR model	AIC_{DS}	AIC_{MR}	AIC	ΔAIC
	Key*	Covariate	Covariate				
35	hn	beaufort	distance	141.03	472.79	613.82	64.13
37	hn	cue	distance	134.88	472.79	607.67	57.98
31	hn	size	distance	126.90	472.79	599.69	50.01
32	hn	size+beaufort	distance	128.65	472.79	601.44	51.76
33	hn	size+cue	distance	115.75	472.79	588.54	38.85
47	hn	size+cue	distance+platform	115.57	437.56	553.13	3.44
103	hn	size+cue	distance+size	115.57	469.05	584.63	34.94
131	hn	size+cue	distance+beaufort	115.57	473.72	589.30	39.61
145	hn	size+cue	distance+cue	115.57	474.53	590.11	40.42
54	**hn**	**size+cue**	**distance+platform+size**	**115.57**	**434.11**	**549.69**	**0.00**
61	hn	size+cue	distance+platform+beaufort	115.57	438.48	554.06	4.37
68	hn	size+cue	distance+platform+cue	115.57	439.53	555.10	5.42
40	hr	size+cue	distance	118.10	472.79	590.89	41.21
159	hr	size+cue	distance+platform+size	118.10	434.11	552.21	2.53

*: hn denotes a Half-normal function; hr denotes a Hazard-rate function.

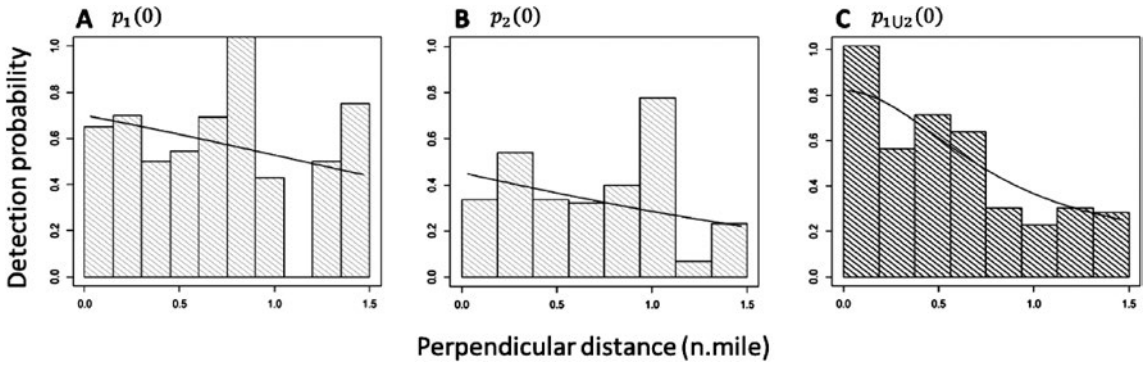

Figure 7. Detection function plots for the TOP (A), IOP (B) and both platforms pooled (C) by the MRDS method.

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Technical Reports of the Institute of Cetacean Research (2019) pp. 21–31

Technical Report (not peer reviewed)

Dedicated whale sighting vessels as a platform for krill and oceanographic research in the Indo-Pacific region of the Antarctic

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ABSTRACT

The Institute of Cetacean Research has conducted research on the ecosystem in the Indo-Pacific sector of the Antarctic, as part of the JARPA, JARPAII and NEWREP-A whale research programs. As part of the ecosystem research, oceanographic and krill surveys have been carried out in a systematic manner using dedicated sighting surveys as platform. Krill are key species in the Antarctic ecosystem and changes in its abundance have effects on predators and the whole ecosystem. Changes in the oceanographic conditions will affect distribution and krill biomass and in turn the abundance and distribution of whales. Changes in oceanographic conditions might indicate effects of climate changes. This paper describes the technical aspects of the krill and oceanographic surveys conducted by the Institute of Cetacean Research from dedicated sighting vessels.

INTRODUCTION

The Institute of Cetacean Research (ICR) has conducted research on the ecosystem in the Indo-Pacific sector of the Antarctic in a systematic manner for a considerable number of austral summer seasons. The ecosystem research involved oceanographic and krill surveys, which were conducted as part of the JARPA (1987/88–2004/05), JARPAII (2005/06–2013/14) and NEWREP-A (2015/16–2018/19) whale research programs.

Oceanographic and krill research is important to understand changes in the Antarctic ecosystem. Krill are key species in the Antarctic ecosystem and changes in their abundance have effects on predators and the whole ecosystem. Changes in the oceanographic conditions will affect distribution and krill biomass and in turn the abundance and distribution of whales. Changes in oceanographic conditions might indicate effects of climate changes. Fujise and Pastene (2018) presented scientific evidence for historical and current changes in the Antarctic ecosystem. Historical changes were consistent with the 'krill surplus hypothesis' (Laws, 1977; 1985) while current changes were interpreted as the reverse of this hypothesis.

This paper describes the technical aspects of the krill and oceanographic surveys conducted by the ICR from dedicated sighting vessels. Oceanographic and krill survey plans and results have been presented and discussed at annual meetings of the International Whaling Commission Scientific Committee (IWC SC) as well the Convention for the Conservation of Antarctic Marine Living Resources Working Group on Ecosystem Monitoring and Management (CCAMLR-EMM). Surveys have been designed and conducted by following technical advice of experts from these meetings. Also this paper shows as an example, the results of the oceanographic and krill surveys conducted by NEWREP-A in the 2018/19 austral summer season.

DEDICATED SIGHTING VESSELS

Oceanographic and krill surveys were conducted from dedicated whale sighting surveys. A total of three research vessels were used during the NEWREP-A, which covered the IWC management Areas using zigzag tracklines (Figure 1, Table 1): *Yushin-Maru* No.2 (*YS2*; 747GT, Figure 2), *Yushin-Maru* No.3 (*YS3*; 742GT, Figure 3) and *Kaiyo-Maru* No.7 (*KY7*; 649GT, Figure 4). The transducers of Quantitative Echosounder (EK80; Simrad, Norway) were hull-mounted at a depth of 4.3 m below the sea surface in the case of *YS2* and *YS3*. The transducers of Quantitative Echosounder (EK60; Simrad, Norway) were hull-mounted at a depth of 4.7 m below the sea surface in the case of *KY7*. The electrical winch (TS-F2, Tsurumi-Seiki Co., Ltd., Japan, Figure 5) was set at the right side deck in the case of *YS2* and *YS3*.

INSTRUMENTS USED FOR KRILL SURVEY

All three survey vessels were mounted with Quantitative Echosounders for acoustic data recording. These data are

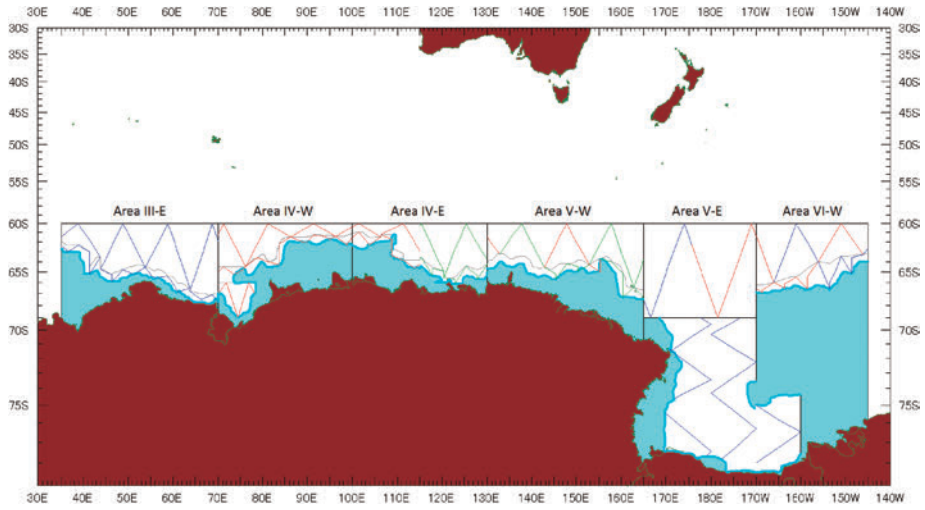

Figure 1. Research area and tracklines. Blue line: surveyed trackline of *Yushin-Maru* No. 2, Green line: surveyed trackline of *Yushin-Maru* No.3, Red line: surveyed trackline of *Kaiyo-Maru* No.7, Gray line: boundary between northern and southern research area, Turquoise line: ice edge, Brown: Antarctic continent.

Table 1

Research area and vessels covered by NEWREP-A. *YS2*: *Yushin-Maru* No. 2, *YS3*: *Yushin-Maru* No. 3, *KY7*: *Kaiyo-Maru* No. 7.

Area	Season			
	2015/16	2016/17	2017/18	2018/19
III-NE				*YS2*
III-SE				*YS2*
IV-NW				*KY7*
IV-SW				*KY7*
Prydz Bay				*KY7*
IV-NE	*YS3*			*KY7*
IV-SE	*YS3*			*KY7*
V-NW		*YS3, KY7*		
V-SW		*YS3, KY7*		
V-NE			*YS2, KY7*	
V-SE			*YS2*	
VI-NW			*YS2, KY7*	
VI-SW*			*YS2, KY7*	
VI-SW*			*YS2*	

*Southern of 69°S

Figure 2. *Yushin-Maru* No.2 (*YS2*).

Figure 3. *Yushin-Maru* No.3 (*YS3*).

used to estimate distribution and biomass of krill in the research area. Such estimate requires information on krill species and krill body length, which is obtained from net sampling. Two types of net sampling were used, Isaacs-Kidd Midwater Trawl (IKMT) and small ring net sampling.

The IKMT sampling was carried out by *KY7* while the small ring net was used by *YS2* and *YS3*. Due to its small size, the small ring net cannot be used for obtaining representative information of some quantitative traits (e.g. number of individuals and length frequency distribution of krill in the area) but the qualitative information (e.g. the species occurring in the echo-signs) can be obtained.

Quantitative Echosounder

All three vessels steamed on the predetermined tracklines and acoustic data were recorded continuously. The usual navigation speed of *YS2* and *YS3* was approxi-

Figure 4. *Kaiyo-Maru* No.7 (*KY7*).

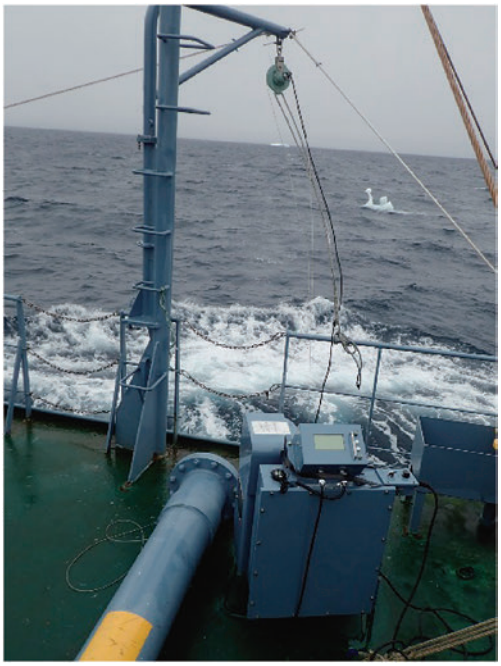

Figure 5. The electrical winch at *Yushin-Maru* No. 3.

mately 11.5 knots. The speed of *KY7* was 11.0 knots. All Quantitative Echosounders operated with frequencies at 38 kHz, 120 kHz and 200 kHz. Maximum data recording depth was set at 500 m.

Standard calibration of Quantitative Echosounders was made using a standard method (Demer *et al.*, 2015) in the vicinity of Japan and also at the research area before starting the research. This was done for every survey to determine the likely effective acoustic sampling range and the potential for detecting krill for multiple frequencies over the required survey depth.

Also calibration between EK80 (*YS2*, *YS3*) and EK60 (*KY7*) Quantitative Echosounders was conducted when *YS3*, *YS2* and *KY7* passed nearby following the procedure of Simmonds and MacLennan (2005) (Figure 6).

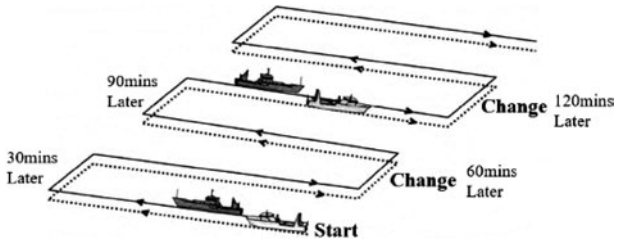

Figure 6. Procedure for the calibration of EK80 and EK60 Quantitative Echosounders (Based on Simmonds and MacLennan, 2005).

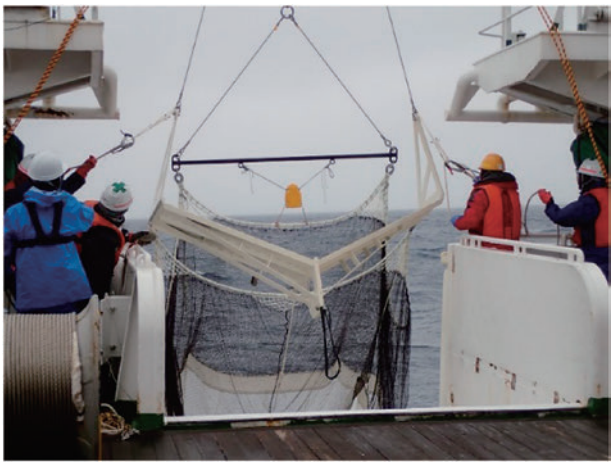

Figure 7. IKMT sampling net at the *Kaiyo-Maru* No. 7.

IKMT sampling

As noted above, the *KY7* was equipped with an IKMT designed by Nippon-Kaiyo Co., Ltd. Japan (Figure 7). The purpose of IKMT sampling was the collection of qualitative and quantitative information for krill (e.g. determination of the species occurring in echo signs and representative numbers and krill length frequencies). The sampling was conducted during day time. The IKMT was 3.66 m in mouth diameter and 18.43 m length. For comparative purposes with the small ring net, 0.5 mm mesh size was used for IKMT.

Data Storage Tag (DST) was installed at the mouth of the IKMT to record actual depth of the net. On average, it took approximately 11 minutes per haul excluding time for setting. The target depth of net sampling was set based on depth of echo sign but the maximum depth was 200 m. The depth of the mouth was monitored at the bridge by PI sensor (PI32; Simrad, Norway). Towing speed of IKMT was 1.0 m/s.

Small ring net sampling

All three vessels were equipped with a small ring net that had a mouth opening of 1.0 m across, and 2.4 m or 3.0 m length. Mesh size was 0.5, 1.5 or 4.5 mm (Nippon-Kaiyo Co., Ltd. Japan, Rigosha & Co., Ltd. Japan, Figure 8) (Tra-

than *et al.*, 2001). At the time of the sampling the vessels stopped their engines.

The target depth of net sampling followed echo backscattering to a maximum of 200 m. If a Light Emitting Diode (LED) and digital compact camera were attached to the net, the maximum depth was switched to 100 m because of their pressure capacity. The depth of the mouth was estimated visually from the angel of the wire with a protractor. The accurate depth of the mouth was confirmed by DST record in the laboratory. If DST were not used, the accurate depth of the mouth was checked by PI sensor at the field. Hauling speed of the net was approximately 1.0 m/s depending on sea state.

The small ring net sampling was conducted to identify the species targeted by echo signs. Notably, the purpose of using this net in *KY7*, was to check efficiency of the small ring net by comparing the samples with those obtained by IKMT. If the small ring net samples collected sufficient amount of samples to examine length frequency of krill and the composition of plankton samples was similar to those collected by IKMT, the result of the small ring net are useful. The small ring net hauls were carried out as in *YS2* and *YS3*, but were only conducted where IKMT collected swarms of krill.

Sample treatment

All plankton samples were kept in bottles with 10% formalin and/or frozen at −20°C for further analysis in the laboratory. Preliminary standard measurements (AT) of krill sampled, were carried out onboard the vessels. The AT is measured from the front of the eye to the tip of the telson, the thin, tapered triangular plate at the end of the abdomen (CCAMLR, 2011, Figure 9).

Recording depth of net

A Data Storage Tag (DST Centi-ex, Star Oddi Co., Ltd., Iceland, Figure 10) was put at the mouth of the small ring nets and IKMT for recording temperature and depth at one second intervals. Temperature Depth Recorders (TDR, AL1, Ishida Engineering, Japan, Figure 11) and PI sensors were used as an alternative to DST in 2015/16 and 2017/18 NEWREP-A, respectively. The TDR was put in mouth of the net for recording temperature and hydraulic pressure. The PI sensor was put on the small ring net

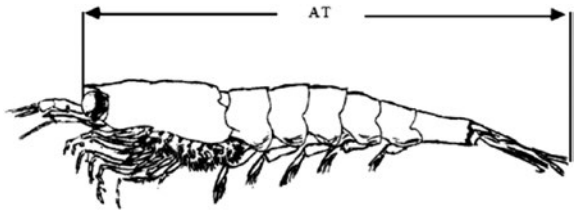

Figure 9. Measurement of total body length (AT) of krill (CCAMLR, 2011).

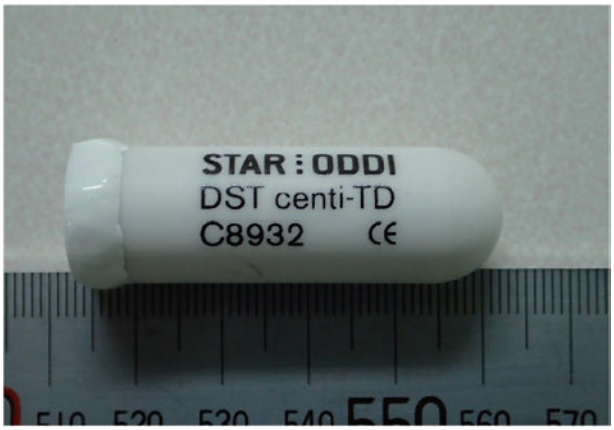

Figure 10. The Data Storage Tag (DST).

Figure 11. The Temperature Depth Recorder (TDR).

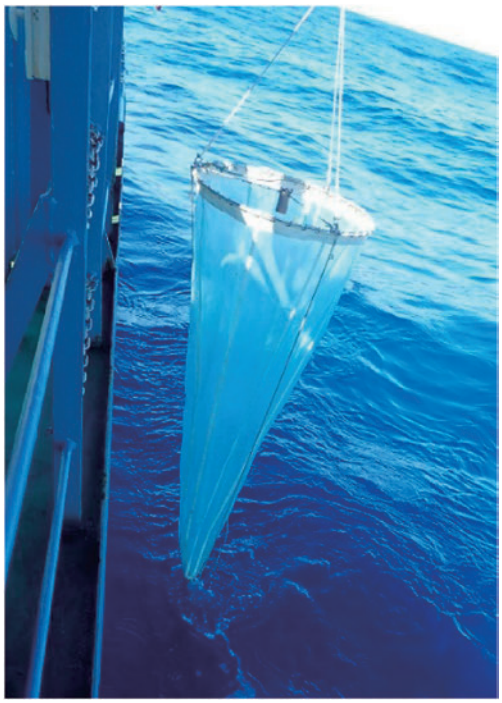

Figure 8. Small ring net at the *Yushin-Maru* No. 2.

Figure 12. LED with camera used for attracting krill in the small sampling net.

and depth read from the display directly at one minute intervals.

Attracting krill

In some surveys small net sampling was conducted with LED and a movie recorded the appearance of the mouth. Almost in all net sampling, a maximum 3,000 lumen LED (FIX NEO 3000 DX, Fisheye Co., Ltd., Japan), digital compact camera (TG-4 Tough, Olympus Co., Ltd., Japan) and housing system (Nauticam TG3, Fisheye Co., Ltd., Japan) (Figure 12) was put in mouth of net (Wiebe *et al.*, 2004). The lighting system was not used after the 2015/16 NEWREP-A following recommendations by CCAMLR specialists (CCAMLR, 2016).

INSTRUMENTS USED FOR OCEANOGRAPHIC SURVEYS

Oceanographic observations were based on Japan Meteorological Agency (1999) standards and conducted in parallel with krill surveys.

CTD casting

Hydraulic pressure, temperature, salinity, chlorophyll-*a* and dissolved oxygen were recorded from sea surface to 500 m depth using Conductivity-Temperature-Depth profiler (CTD). The CTD SBE 19 plus V2 SeaCAT (Sea-Bird Scientific Inc., USA, Figure 13) was used by the *YS2* and *YS3*, and the SBE 19 plus SeaCAT (Sea-Bird Scientific Inc., USA) was used by the *KY7*. The instruments were descended using an electrical winch with diameter of 3 mm and an overall length of 1,000 m wire. Normally CTD was descended to 500 m. The data from the CTD was uploaded for conversion by following the manual. All CTD were calibrated before every cruise by Sea-Bird Scientific Inc.

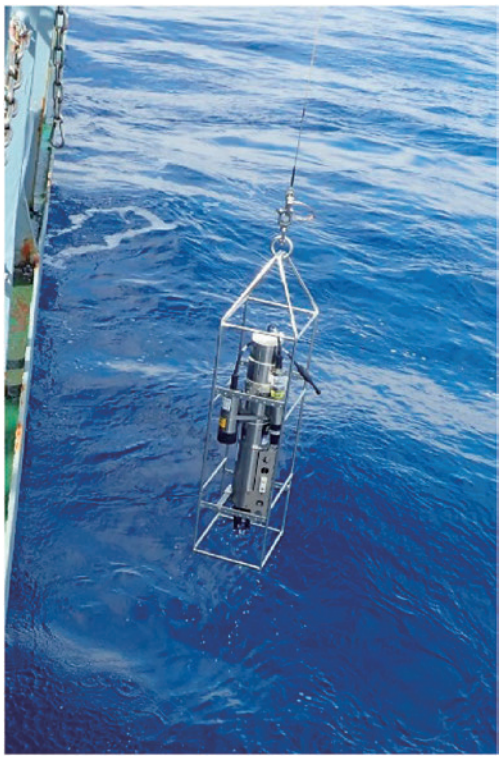

Figure 13. CTD at *Yushin-Maru* No. 2.

Seawater sampling

The seawater sampling was carried out for calibrating the CTD sensors. Niskin water sampling bottles Model-1010 1.2L (General Oceanics, Inc., USA, Figure 14) were used by *YS2* and *YS3* and Model-1010 1.7L (General Oceanics, Inc., USA, Figure 15) was used by *KY7*. The bottles were dropped to depths from 0 to 200 m and sampling was conducted every 20 m. Depth information of sampling bottles was based on the angle of the wire while operating. Accurate depths of sampling bottle were recorded at the laboratory from the DST or PI sensor.

The seawater was kept in freezers for subsequent analysis in the laboratory. The sea water was kept in two bottles. One was a 250 mL clarity seawater bottle (WOCE type 5419-C, Rigosha & Co. Ltd., Japan) for salinity calibration, which was stored at 4°C. The other bottle was for chlorophyll-*a* calibration. 118 mL of seawater from the bottles was filtrated (Whatman 233303 GF/F 25 mm, GE Healthcare, USA) (Figure 16). The filter paper was kept in 8 mL centrifugal tubes (60.452, Sarstedt AG & Co., Germany) filled with dimethylformamide. The tubes were stocked in freezer about −20°C (Saito, 2007). After the cruises, salinity was measured by Autosal Salinometer OSIL 8400B (Ocean Scientific International Ltd, UK) and chlorophyll-*a* was measured by TURNER 10AU Field and Laboratory Fluorometer (Turner Designs, Inc., USA).

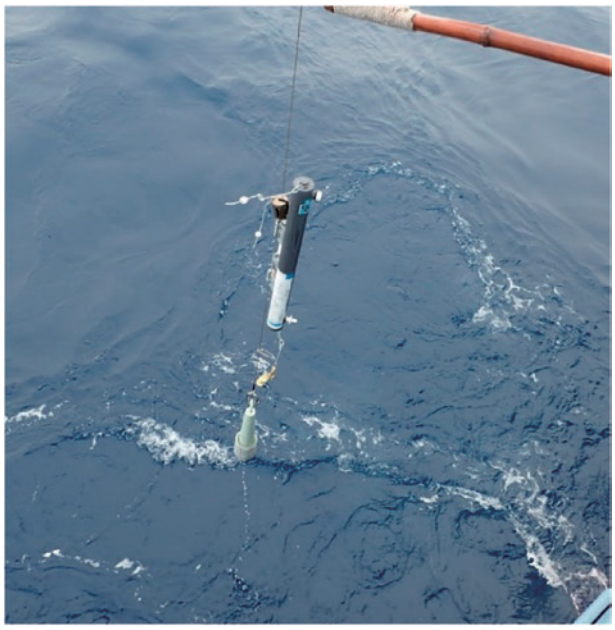

Figure 14. Niskin water sampling bottle at *Yushin-Maru* No. 2.

Figure 15. Niskin water sampling bottle at *Kaiyo-Maru* No. 7.

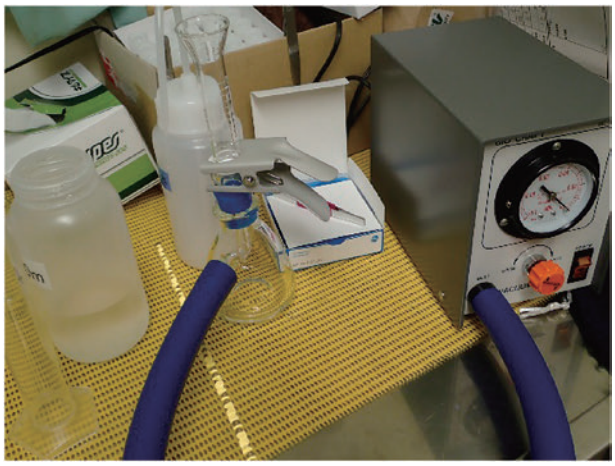

Figure 16. Filtrating seawater with filter paper.

HOW THE KRILL AND OCEANOGRAPHIC SURVEYS ARE CONDUCTED FROM A WHALE SIGHTING VESSEL?

The trackline was designed for the main purpose of abundance estimates of large whales based on the DISTANCE sampling (Buckland *et al.*, 2015). It followed the accepted guidelines by the IWC SC for the International Decade for Cetacean Research/Southern Ocean Whale and Ecosystem Research (IDCR/SOWER) cruises (Matsuoka *et al.*, 2003). The trackline consisted of a zigzag course changing direction at each 5°00′ longitudinal degree intervals in the northern stratum and at 2°30′ longitudinal degree intervals in the southern stratum. The trackline in the Ross Sea was set zigzag in north and south to westward or eastward. The zigzag course changed direction at 1°30′ latitudinal degree intervals. Also the trackline in the Prydz Bay changed direction in northward or southward at 2°30′ longitudinal degree intervals. A randomized start point was determined based on the IWC SC guidelines (IWC, 2012).

The sighting surveys were conducted during the daytime from an hour after sunrise to an hour before sunset. The vessel would move through the tracklines at a speed of 11.0–11.5 knots. The survey stopped when the sea conditions and weather were not appropriate for sighting activities. When the conditions improved, the vessel restarted the survey from the same position on the trackline. Three researchers participated onboard the vessels, two in charge of the sighting survey and one in charge of the krill and oceanographic surveys.

Details of the sighting survey procedures used by the ICR can be found in Hakamada and Matsuoka (2017).

Krill survey

The Quantitative Echosounder data were recorded continuously while vessels steamed on the predetermined tracklines, day and night. Only the acoustic data obtained when the vessels were on the trackline are used for krill abundance estimates.

All net sampling was conducted following the echo sign by the Quantitative Echosounder (Figure 17). When the researcher confirmed a swarm of krill and decided to tow the net, the sighting survey was stopped and preparation for net sampling was made as soon as possible. In principle, net sampling was conducted once a day in the 2015/16 and 2016/17 NEWREP-A. In order to increase the number of samples, net sampling was conducted after each confirmation of echo signs in the 2017/18 and 2018/19 NEWREP-A.

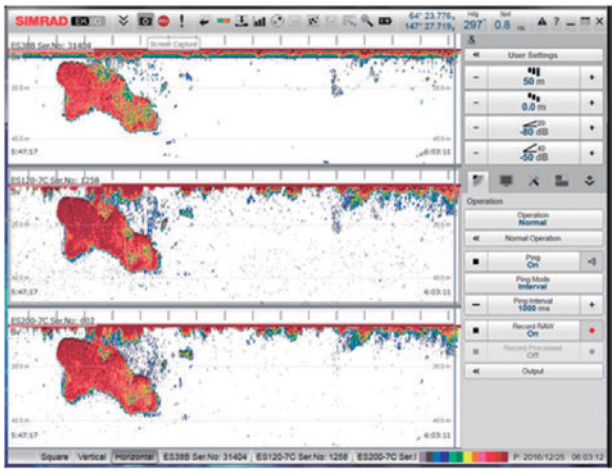

Figure 17. Echo sign of Antarctic krill by the EK80 of *Yushin-Maru* No. 3.

During the IKMT sampling, the vessel steamed approximately at 2.0 knots. In the case of small ring net sampling, the vessels stopped their engines when reconfirming echo sign. Both sampling was conducted with careful confirmation of krill swarm location by monitoring of the Quantitative Echosounder. When the net sampling was completed, the vessel re-started sighting surveys from the same position on the trackline that had stopped for the sampling. Preliminary analyses of the sample were carried out immediately after sampling.

Oceanographic survey

It was planned that oceanographic observations using CTD casting would cover the whole area, and for this reason the geographical position of the CTD casting stations would not necessarily coincide with the position of the net sampling. The CTD casting stations were positioned at approximately 60 n.miles on the trackline. The vessels stopped their engines when they arrived at the CTD casting station. Once observations were completed, they re-started the sighting surveys from the same position on the tracklines where the vessels had stopped their engines.

To calibrate the CTD sensors, the stations for seawater sampling were the same as some CTD casting station. Each station was positioned approximately within a radius of 120 n.miles in entire research area for covering the entire area.

Net sampling, CTD casting and seawater sampling were conducted during day time and smooth sea states.

RESULTS OF THE 2018/19 KRILL AND OCEANOGRAPHIC SURVEY

As an example, this section shows the results of the 2018/19 NEWREP-A in management Areas III-E and IV. The surveys were conducted by the research vessels *YS2* and *KY7*.

Quantitative Echosounder

Calibrations of Quantitative Echosounders were made on 30 October 2018 by *YS2* and on 7 November 2018 by *KY7* in the vicinity of Japan before departure for the Antarctic. During the calibrations, the vessel's engines were stopped for anchoring at sea bottom 37 m for *YS2* and 48 m for *KY7*. Calibrations in the survey area were made on 13 December 2018 by *YS2* and on 6 December 2018 by *KY7* before starting the survey on the tracklines. During the calibrations, the vessel's engines were stopped and the drifting speed was approximate 0.7 knots because anchoring at that depth could not be achieved in the Antarctic. The data from the Quantitative Echosounders was recorded according to setting of calibration in Antarctic.

The Quantitative Echosounders calibration (EK80 in *YS2* and EK60 in *KY7)* was conducted on 12 November 2018 in Japanese coastal areas. The methodology of Simmonds and MacLennan (2005) was followed. The Quantitative Echosounder data were recorded by *YS2* and *KY7* moving in formation with one in the lead and the another about 400 m astern, far enough to the side to be clear of the leader vessel's wake. The two vessels took the lead in turns and exchanged positions at the end of each transect. Both research vessels recorded Quantitative Echosounders data while shifting the leaders every 30 minutes four times for two hours. As a result, calibrations between EK80 and EK60 were recorded shallow over 200 m sea bottom backscattering constantly by all three frequencies, 38 kHz, 120 kHz and 200 kHz.

Table 2 shows a summary of the total effort spent on the Quantitative Echosounder survey. This survey was conducted for a total of 7,195 n.miles along the tracklines (3,365 n.miles in Area III-E, 2,267 n.miles in Area IV-W, 509 n.miles in Prydz Bay and 1,055 n.miles in Area IV-E).

IKMT sampling

IKMT sampling was conducted at a total of 22 stations by *KY7* (10 stations in Area IV-W, seven stations in the Prydz Bay and five stations in Area IV-E, Tables 2, 3 and Figure 18). Logistical considerations were also taken into account to decide whether to proceed with net sampling such as the sea state, sea ice as well as other survey priorities.

Small ring net sampling

The small ring net sampling was conducted at a total of 54

Table 2
Research item and summary in the 2018/19 NEWREP-A.

Area	Quantitative Echosounder		IKMT stations	Small ring net stations	CTD casting stations	Seawater sampling stations	DST stations
	Days	n.miles					
III-NE	39	2,175	—	17	42	5	17
III-SE	32	1,191	—	28	27	2	28
IV-NW	15	1,254	3	0	19	3	3
IV-SW	17	1,013	7	6	23	2	14
Prydz Bay	6	509	7	1	11	2	7
IV-NE	8	587	1	2	8	1	3
IV-SE	9	468	4	0	14	1	4
Total	72*	7,195	22	54	144	16	76

*The survey was conducted some areas at same day

Table 3
Summary of IKMT sampling in 2018/19NEWREP-A.

Area	Antarctic krill		Ice krill		Bigeye krill		*Euphausiids*		Copepoda		Other Zooplankton		Fish	
	Stations	%	Stations	%	Stations	%	Stations	%	Stations	%	Stations	%	Stations	%
IV-NW	2	67	0	0	1	33	0	0	0	0	0	0	1	33
IV-SW	6	86	0	0	0	0	1	14	0	0	2	29	0	0
Prydz Bay	6	86	1	14	0	0	0	0	1	14	1	14	1	14
IV-NE	0	0	0	0	0	0	0	0	1	100	1	100	0	0
IV-SE	2	50	0	0	0	0	3	75	1	25	2	50	0	0
Total	16	73	1	5	1	5	4	18	3	14	6	27	2	9

Figure 18. Antarctic krill sample of IKMT.

stations by *YS2* and *KY7* (45 stations in Area III-E, six stations in Area IV-W, one station in the Prydz Bay and two stations in Area IV-E, Tables 2, 4 and Figure 19). Because of weather, sea ice conditions or survey priority reasons, some net sampling stations were skipped. Calibration of the flow meters was conducted on 10 December 2018 by *YS2* and on 6 December 2018 and 30 January 2019 by *KY7* respectively.

Sample contents of the net

A total of 11 species, including three euphausiid species: Antarctic krill (Figure 20), ice krill *E. crystallorophias* (Figure 21) and bigeye krill *Thysanoessa macrura* (Figure 22) and fish (Figure 23 and 24), were identified in the 73 net sampling contents. Several other species, *Hydromedusae*, *Siphonophorae*, *Polychaeta*, *Pteropoda*, *Copepoda* (Figure 25), *Amphipoda* and *Chaetognatha* were confirmed by small ring net sampling and IKMT sampling. There were no contents at three net sampling stations.

Distribution of krill

Tables 3 and 4 show the summary of frequencies of occurrence of krill species and *Copepoda* sampled by the IKMT and small ring nets, respectively. Antarctic krill was sampled at 48 stations in entire of survey area. Distribution of surface water temperature was in the range of −1.7°C to 1.9°C. The Antarctic krill were sampled at depths

Table 4
Summary of small ring net sampling in 2018/19NEWREP-A.

Area	Antarctic krill		Ice krill		Bigeye krill		*Euphausiids*		Copepoda		Other Zooplankton	
	Stations	%	Stations	%	Stations	%	Stations	%	Stations	%	Stations	%
III-NE	7	41	0	0	6	35	0	0	12	71	16	94
III-SE	21	75	0	0	3	11	0	0	18	64	20	71
IV-NW	—	—	—	—	—	—	—	—	—	—	—	—
IV-SW	4	67	0	0	0	0	0	0	0	0	1	17
Prydz Bay	0	0	0	0	0	0	0	0	0	0	1	100
IV-NE	0	0	0	0	0	0	0	0	1	50	1	50
IV-SE	—	—	—	—	—	—	—	—	—	—	—	—
Total	32	59	0	0	9	17	0	0	31	57	39	72

Figure 19. Antarctic krill sample of small ring net.

Figure 20. Antarctic krill.

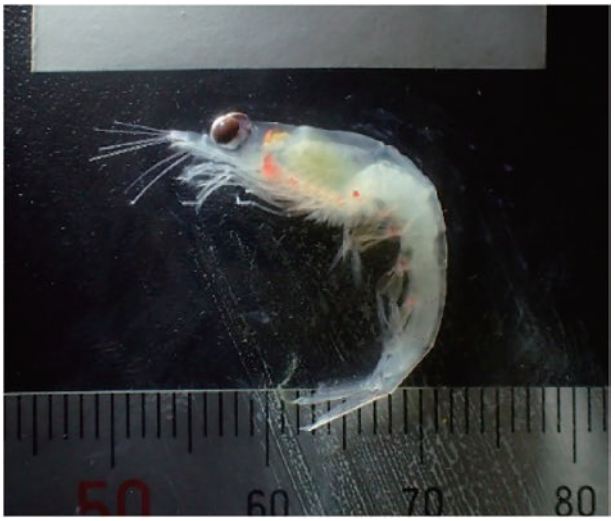

Figure 21. Ice krill.

Figure 22. Bigeye krill.

of 19 m to 146 m, depth of sea bottom range was 178 m to 5,240 m. The range of body length was from 12 mm to 59 mm. The incubating Antarctic krill was sampled at two stations in Area III-SE. Ice krill was sampled at a station in the Prydz Bay, distribution of surface water temperature was 1.6°C. They were sampled at depths of 130 m, depth of sea bottom was 670 m. Bigeye krill were sampled at ten stations, distribution of surface water temperature

Figure 23. Antarctic silverfish (*Pleuragramma antarcticum*).

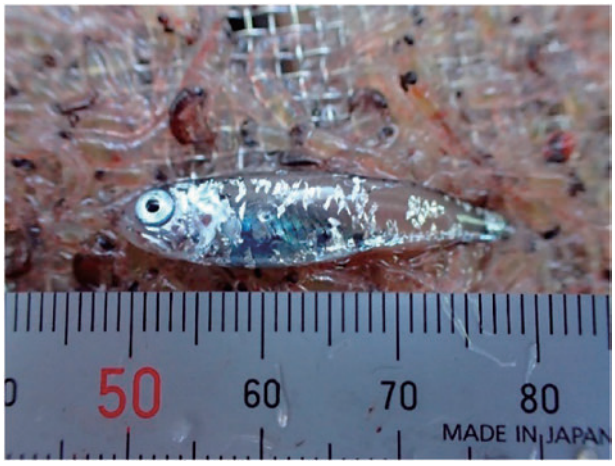

Figure 24. *Myctophidae.*

Figure 25. *Copepoda.*

was in the range of −1.4°C to 1.0°C. They were sampled at depths of 35 m to 146 m, depth of sea bottom range was 2,100 m to 5,240 m. The range of body length was from 10 mm to 25 mm.

Comparison of IKMT and small ring net

Simultaneous samplings with the small ring net and IKMT were conducted at seven stations by *KY7*. In three cases, Antarctic krill was sampled by both IKMT and small ring net. However, for the other four cases, the results from both nets were not consistent. In the 2016/17 and 2017/18 NEWREP-A, a total of seven cases of simultaneous sampling occurred, two cases were consistent for both nets (Wada *et al.* 2017; 2018). These results indicate that it is difficult to collect representative krill samples by the small ring net. However, the small ring net can contribute to obtaining qualitative information on the distribution of krill species and it requires less time in comparison to IKMT sampling.

Oceanographic observation

The oceanographic observation by CTD casting was conducted at 144 stations by *YS2* and *KY7* (69 stations in Area III-E, 42 stations in Area IV-W, 11 stations in the Prydz Bay and 22 stations in Area IV-E, Table 2). The seawater sampling was conducted at 16 stations at the same locality where CTD castings were taken. Seven stations in Area III-E and five stations in Area IV-W, two stations in the Prydz Bay and two stations in Area IV-E were sampled (Table 2). A total of 176 seawater samples were taken then kept in clear bottles for salinity calibration. For chlorophyll-*a* calibration samples were filtered and stored with dimethylformamide.

ANALYSES OF DATA

The main objective of krill and oceanographic surveys was to estimate the abundance of Antarctic krill acoustically, and to obtain the length frequency distribution and maturity stage of Antarctic krill in the survey area.

Quantitative Echosounder data is used for estimating krill abundance. The software Echoview 9 (Echoview Software Pty. Ltd., Australia) is used for calculating backscattering of krill after noise removal. Estimate value of krill abundance is obtained from this backscattering in each research area. This estimate requires identification of krill species and body length obtained by the IKMT sampling and small net sampling.

The oceanographic data is used for identifying structure of ocean currents likely Upper Circumpolar Deep Water, Lower Circumpolar Deep Water and Shelf Water based on CTD data. These currents are assumed in relation to the distribution of krill and predators.

The analyses are being conducted by the ICR and several external research organizations.

ACKNOWLEDGEMENTS

I would like to thank the Government of Japan for providing support for this survey, and the captains and crew members of *YS2*, *YS3* and *KY7* for their effort at the field. I also thank several scientists that supported the design and development of the krill and oceanographic surveys.

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Genetic study of stock structure in North Pacific sei whales

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ABSTRACT

The Institute of Cetacean Research has conducted genetic analyses of stock structure in North Pacific sei whales based on historical genetic samples, and samples collected more recently by the IWC-POWER (biopsy samples) and JARPNII program. Results have contributed to the *in-depth assessment* of North Pacific sei whales conducted by the IWC Scientific Committee. This paper presents details of the research area, samples, genetic approaches, and main results of the genetic analyses of stock structure of sei whales in the pelagic area of the North Pacific. Results of the genetic analyses are consistent with the hypothesis of a single stock of sei whales in the pelagic area of the North Pacific surveyed (140°E–130°W).

INTRODUCTION

Sei whale, *Balaenoptera borealis*, (Figure 1) is a large baleen whale species inhabiting all of the major open oceans (Horwood, 1987; Rice, 1998). Sei whales live up to 60 years of age and their maximum body length is 20 m. Sei whales migrate from winter breeding grounds in low latitude to high latitudinal areas in summer, where they feed on various prey species (e.g. Nemoto, 1959; Nemoto and Kawamura, 1977).

This species was one of the major targets of commercial whaling worldwide (Horwood, 1987). In the North Pacific the main period of exploitation was from 1963 to 1973 (Allen, 1980), and they were caught in waters off California, Canada, Japan, Kamchatka and the Kuril Islands by pelagic fleets (Horwood, 2018).

Delineation of the stock structure of the species and the estimation of abundance by stock are key information required for management and conservation. A pioneering work on stock structure of North Pacific sei whales was carried out by Wada and Numachi (1991) using three polymorphic allozyme loci. The study reported no evidence of temporal and spatial genetic heterogeneity in samples collected in the area east of 160°E, suggesting the existence of only a single stock in the area.

The most comprehensive genetic study was performed by Kanda *et al.* (2009) using 17 microsatellite DNA loci and 487bp of mitochondrial DNA (mtDNA) control region sequences. The study used a total of 790 specimens collected during the past commercial whaling in 1972–1973 and during the JARPNII surveys in 2002–2007. Results of the study showed no evidence of significant genetic differences in areas between 140°E and 135°W of the North Pacific.

Subsequently, Kanda *et al.* (2013) examined not only spatial but also temporal genetic differences of the North Pacific sei whales. The study showed no evidence of temporal (40 years apart between recent and past samples) and spatial (the research area divided into western (140°–170°E), central (170°E–150°W) and eastern (150°–130°W) areas) genetic differences. Results were consistent with those found by Kanda *et al.* (2009). Furthermore, Kanda *et al.* (2015a) considered that spatial genetic differentiation should be tested using samples

Figure 1. North Pacific sei whale (*Balaenoptera borealis*).

collected in the same year in order to eliminate temporal negative biases. Consequently, they examined genetic variations at 16 microsatellite loci using only samples collected during summer in 2010, 2011 and 2012. Again, the results of the study failed to find evidence of multiple stocks of sei whales in the North Pacific.

This paper presents details of the research area, samples, genetic approaches, and main results of the genetic analyses of stock structure of sei whales in the pelagic area of the North Pacific, updated after the work by Kanda *et al.* (2015a). This paper also provides information on some basic concepts of population genetic statistics.

MATERIALS AND METHODS

Research area

The genetic analyses were conducted based on three sampling areas in the pelagic region of the North Pacific: western (140°–150°E), central (150°E–180°) and eastern (180°–130°W) areas (Figure 2). Genetic samples were collected from whales taken during past commercial whaling operations as well during more recent surveys of the JARPNII research program. Biopsy samples obtained during the IWC-Pacific Ocean Whale and Ecosystem Research (IWC-POWER) were also used (see details below).

Samples and laboratory procedures

Samples and DNA extraction

A total of 1,748 genetic samples of sei whales obtained during past commercial whaling in 1972–1973 (n=312), the JARPNII in 2002–2016 (n=1,354) and the IWC-POWER (biopsy samples) in 2010–2013 (n=82) was subjected to DNA extraction (Table 1).

Total genomic DNA was extracted from 0.05 g of skin tissue, preserved in 99% ethanol at room temperature or stored frozen at −20°C, using the standard phenol-chloroform method (Sambrook *et al.*, 1989) or using Gentra Puregene kits (QIAGEN). Extracted DNA was stored in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

MtDNA sequencing

See an explanation of mtDNA sequencing analyses in Taguchi *et al.* (2017).

Approximately 500 base pairs of partial control region were amplified by the polymerase chain reaction (PCR) using a set of primers MT4 (Árnason *et al.*, 1993) and Dlp 5R (5'-CCA TCG AGA TGT CTT ATT TAA GGG GAA C-3'). PCR was performed with an initial denaturation step at 95°C for 5 minutes, followed by 30 cycles of 30 seconds at 94°C, 30 seconds at 50°C and 30 seconds at 72°C, with a final extension step at 72°C for 10 minutes. PCR products were purified using MicroSpin S-400HR columns (Pharmacia Biotech). Cycle sequencing was performed using BigDye terminator cycle sequence Kit (Applied Biosystems) and the PCR primers, following the protocols of the manufacturer. The cycle sequencing products were purified using AutoSeq G-50 spin Columns (Pharmacia Biotech). The labeled sequencing fragments from tissue samples collected until 2004, during 2005–2010, and

Table 1

Number of genetic samples of North Pacific sei whales used in this study. Sample size for the present data analyses is shown in parenthesis (mtDNA sequences, microsatellite genotypes).

Sample source	Sampling area		
	Western	Central	Eastern
Past commercial whaling		181 (175, 177)	131 (128, 121)
JARPNII	30 (30, 30)	1324 (1322, 1323)	
IWC-POWER		3 (3, 3)	79 (75, 75)
Total	30 (30, 30)	1508 (1500, 1503)	210 (203, 196)

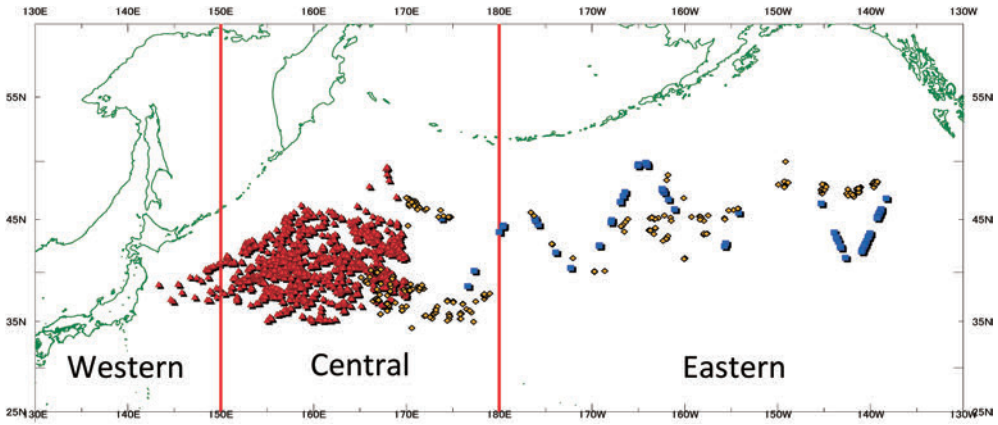

Figure 2. Sampling position of sei whales used in the genetic analyses. Color indicates sample source: yellow: past commercial whaling; red: JARPNII; and blue: IWC-POWER.

from 2011 were resolved using an ABI PRISM 377, ABI PRISM 3100 and ABI3500 Genetic Analyzers (Applied Biosystems), respectively.

Microsatellite DNA genotyping

See an explanation of microsatellite DNA analyses in Taguchi *et al.* (2017).

All individuals sampled in JARPNII and IWC-POWER surveys were genotyped at 17 nuclear microsatellite loci: EV1, EV14, EV21, EV94, EV104 (Valsecchi and Amos, 1996), GT011 (Bérubé *et al.*, 1998), GT23, GT211, GT271, GT310, GT575 (Bérubé *et al.*, 2000), GATA28, GATA53, GATA98, GATA417, GGAA520 (Palsbøll *et al.*, 1997), and DlrFCB17 (Buchanan *et al.*, 1996). The past commercial whaling samples were genotyped at 15 microsatellite loci (EV14 and GATA417 were not used). Primer sequences and PCR cycling profiles generally followed those of the original authors. The multiplex PCR amplifications were performed in 15 µl reaction mixtures containing 10–100 ng of DNA, 5 pmole of each primer, 0.625 units of Ex Taq DNA polymerase (Takara Shuzo), 2mM of each dNTP, and 10x reaction buffer containing 20mM $MgCl_2$ (Takara Shuzo), with 94°C for 2 minutes, followed by 30 cycles at 94°C for 20 seconds/54–61°C for 45 seconds/72°C for 1 minute, and a post-cycling extension at 72°C for 10 minutes.

PCR products from tissue samples collected until 2013 were run on a 6% polyacrylamide denaturating gel (Long Ranger) with internal size standard (GENESCAN400HD, Applied Biosystems) using BaseStation100 DNA fragment analyzer (Bio-Rad). Although alleles were visualized using Cartographer software specifically designed for the BaseStation, allelic sizes were determined manually in relation to the internal size standard and sei whale's DNA of known size that were rerun on each gel. The PCR products of samples collected after 2013 were electrophoresed on an ABI 3500 DNA Analyzer (Applied Biosystems), and allele sizes were determined using a 600 LIZ size standard (Applied Biosystems) and GeneMapper v. 4.0 (Applied Biosystems). Microsatellite scores from the latter platform were standardized according to those from the former for each locus.

Data analyses

Three whales re-sampled (two genetic samples obtained from the same individual whale) were excluded from the analysis. Three calves accompanied by their mothers were also excluded from all subsequent analyses, to ensure an independence of the dataset. In addition to this, nine mtDNA sequences data and thirteen microsatellite genotype data sets were also excluded from the present data set due to their low data qualities.

FDR correction (Benjamini and Hochberg, 1995) was used to adjust the significance level for all multiple comparisons in this study.

Considering a long time series of sample collection of over 40 years, annual genetic variations were preliminary examined in each of the sampling areas, which consistently showed no significant genetic differences. Therefore, the genetic data from several years were combined in each sampling area in all subsequent analyses. More detailed descriptions of the temporal analyses were provided in Appendix 7 in Tamura *et al.* (2019).

Genetic diversities

For microsatellite DNA, the departure from Hardy–Weinberg equilibrium (*HWE*: see Box 1) was tested in each locus using the R package '*HWxtest*' (Engels, 2009). A global test across loci combining the observed *P*-values in each locus by Fisher's method was performed using the R package '*metap*' (Dewey, 2018). The inbreeding coefficient (F_{IS}; Weir and Cockerham, 1984) in each locus and across loci was estimated using the R package '*Demerelate*' (Kraemer and Gerlach, 2017). The number of alleles (*A*) and expected heterozygosity (H_E: see Box 2) in each locus and across loci were estimated using the program ARLEQUIN v. 3.5.1.2 (Excoffier and Lischer, 2010).

For mtDNA, haplotype (*h*) and nucleotide (π) diversities (Nei, 1987) (see Box 2) with sample standard deviations were estimated using the program ARLEQUIN.

Genetic differentiation and structuring

The F_{ST}-like estimates for microsatellites and conventional pairwise F_{ST} for mtDNA (see Box 3) were calculated to measure the genetic differentiation between sampling areas using 10,000 random permutations of the original dataset in the program ARLEQUIN. A probability test implemented in the program GENEPOP (Rousset, 2008) was used to detect the genetic heterogeneity in microsatellite allele frequency among sampling areas. Difference in mtDNA haplotype frequency among sampling areas was also tested using the Monte Carlo simulation-based chi-square test of independence (Roff and Bentzen, 1989) in R.

Bayesian clustering analysis was performed using microsatellite data to infer the most likely number of clusters using STRUCTURE 2.3.4 (Pritchard *et al.*, 2000). The analysis was conducted with ten independent runs for $K = 2–3$. All runs were performed with 100,000 Markov chain

Box 1. Hardy–Weinberg equilibrium

The genetic composition of a population is usually described in terms of allele frequency, number of alleles and heterozygosity. Allele and genotype frequencies attain an equilibrium, referred to as the Hardy–Weinberg equilibrium (*HWE*), in the next generation in infinitely large and random mating populations when there are no mutation, immigration or selection. When any of the assumptions underlying *HWE* are violated, then deviations from the equilibrium genotype frequencies will be observed. Thus, the *HWE* are widely used to detect if the population has non-random mating, migration or selection.

For a single locus with two alleles, *HWE* genotype frequencies can be expected according to the relationship:

$$p^2 + 2\mathrm{pq} + q^2 = 1$$

where p and q are the respective allele frequencies; p^2=frequency of one allele homozygotes, q^2=frequency of the other allele homozygotes, and $2pq$=frequency of heterozygotes.

To determine if the observed genotype frequencies deviate from the frequencies expected from the *HWE* relationship statistically, the difference in number of genotypes between them is tested using a χ^2 test.

As an example, consider a single microsatellite locus GATA53 with alleles 198 and 202 in ten sei whales (See Table 2.1 in Box 2), hence three genotypes (198/198, 202/198, 202/202) are found. From the observed number of respective genotypes, it is possible to calculate p and q, and to estimate the *HWE* genotype frequencies based on the allele frequencies calculated from the observed genotype frequencies, as shown in Table 1.1.

Table 1.1

Calculation of allele frequencies based on the observed number of genotypes, and the genotype frequencies expected from the *HWE* in a microsatellite locus GATA53 of sei whales.

Genotypes	Observed						Expected	
	Number of genotypes (*O*)	Genotype frequency	Number of alleles		Allele frequency		Genotype frequency	Number of genotypes (*E*)
			198	202	198	202		
198/198	1	1/10 0.1	2×1 2		2/20 0.1		p^2=0.3×0.3 0.09	0.09×10 0.9
202/198	4	4/10 0.4	1×4 4	1×4 4	4/20 0.2	4/20 0.2	$2pq$=2×0.3×0.7 0.42	0.42×10 4.2
202/202	5	5/10 0.5		2×5 10		10/20 0.5	q^2=0.7×0.7 0.49	0.49×10 4.9
Total	10	1.0	6	14	p=0.3	q=0.7	1.00	10.0

Monte Carlo repetitions and 10,000 burn-in length using the admixture model with correlated allele frequencies. The web-based program STRUCTURE HARVESTER (Earl and vonHoldt, 2012) was used to estimate the mean posterior probability of the data (Also see Goto *et al.* (2017) for detailed basic concept of the STRUCTURE analysis).

RESULTS AND DISCUSSION

All 17 microsatellite loci were polymorphic in the entire dataset of 1,729 sei whales from the North Pacific, which ranged from 22 alleles at EV14 to 3 alleles at GATA53 (Table 2). The mtDNA control region sequences of 1,733 sei whales from the North Pacific contained 39 variable nucleotide sites and a single alignment gap defining 84 haplotypes.

Genetic diversities

The H_E at each locus and across loci were not largely different among sampling areas (Table 2), and the estimates across loci ranged from 0.632 in the western area to 0.639 in the eastern area (Table 2). Significant deviations from *HWE* were not observed at any loci and across loci in each sampling area after FDR correction (Table 2), which suggested that North Pacific sei whales in each of the sampling areas derived from a single breeding population.

Regarding mtDNA, both h and π were similar among sampling areas, which ranged from 0.908 in the western area to 0.927 in the eastern area, and from 0.789% in the western area to 0.803% in the central area, respectively (Table 3). These observations were consistent with the

Box 2. Estimating genetic diversity

Genetic diversity is characterized by allele frequencies at each locus, which can be influenced by population size, gene flow, reproductive system, natural selection, genetic mutation or genetic linkage. In natural environment, populations are commonly fragmented into subpopulations in different size partly separated from each other by barriers to migration, and this may change the genetic diversity among populations.

Observed heterozygosity (H_O) is defined as the proportion of individuals heterozygous across a set of loci (h_j), or the proportion of loci for which an individual is heterozygous (h_i). This estimate can be obtained by direct count of heterozygotes, as shown in Table 2.1. Overall estimates are averages across the loci used in the study.

Table 2.1

Sub-set of data used in this study involving seventeen microsatellite loci genotyped in each of ten sei whales.

Microsatellite loci (*j*)	Individual (*i*)										h_j
	SEI-001	SEI-002	SEI-003	SEI-004	SEI-005	SEI-006	SEI-007	SEI-008	SEI-009	SEI-010	
EV21	120124	120120	120120	118120	120126	120122	120120	120124	118122	120124	0.7
GGAA520	217221	221221	217229	225229	225229	217233	213225	221229	217229	221225	0.9
GATA98	102110	094098	098106	102102	102102	094102	098102	102102	094106	098102	0.7
GT211	115115	115115	115117	115117	115115	115115	115115	115115	115119	115121	0.4
EV14	155167	159179	155167	157167	155167	161161	155155	155159	155155	155155	0.6
GATA53	202202	198198	198202	202202	202202	202202	198202	202202	198202	198202	0.4
EV1	130146	130146	144162	146146	148152	146146	148158	148152	146148	130138	0.8
EV94	219221	217217	217219	213221	217225	217219	217219	221223	217219	217221	0.9
GT23	116118	116126	116116	116116	112116	116116	116116	116118	116116	116120	0.5
GT575	140146	138146	146148	138138	138138	138146	138148	146146	138148	138138	0.6
GATA417	220220	224224	212240	216232	216224	212228	220224	224224	212220	216220	0.7
GT310	108110	108108	108110	108108	108110	110110	108108	108108	108108	108114	0.4
EV104	134140	134144	134140	140140	140144	134134	134142	140142	140144	140142	0.8
GATA28	228232	228232	224232	216236	232232	212220	224232	216232	232232	212232	0.8
GT271	096096	096096	096096	096096	096096	094096	094096	094096	096096	096096	0.3
GT011	123127	123123	123123	123123	123129	123129	123123	123127	123123	123123	0.4
DlrFCB17	199203	203207	205221	199215	199207	199203	209215	183205	201203	203211	1.0
h_i	0.765	0.471	0.765	0.471	0.647	0.588	0.647	0.647	0.647	0.765	0.641

Genotypes are represented by 6-digits codes (each allele is coded by 3-digits), and heterozygotes are indicated with red color. In this example, 109 of 170 assayed genotypes are heterozygous (overall H_O=0.641).

Expected heterozygosity (H_E) is defined as the expected proportion of individuals heterozygous from observed allele frequencies, assuming the population is in *HWE* (see Box 1), as shown in Table 2.2. In the case of two alleles at a locus with their respective frequencies of *p* and *q*, the expected heterozygosity $H_E=2pq$. When there are more than two alleles, it is simpler to calculate H_E as follows:

$$H_E = 1 - \sum_{i=1}^{k} p_i^2$$

where p_i is the frequency of the *i*-th allele, and *k* is the number of alleles. H_E is normally used rather than H_O since it is less affected by sampling.

comparable genetic diversities among sampling areas observed in microsatellite DNA.

Genetic differentiations and structuring

Pairwise F_{ST}-like estimates for microsatellites did not show any genetic differentiations between sampling areas (Table 4), which was consistent with the pairwise conventional F_{ST} estimates for mtDNA (Table 4). Heterogeneity tests also showed no differences in microsatellite allele (P=0.679, *d.f.*=34, χ^2=29.69) and mtDNA haplotype

Table 2.2

Allele frequencies and three statistics of genetic diversity, i.e. number of alleles (A), observed (H_O) and expected (H_E) heterozygosity, per microsatellite loci based on the data set in Table 1.1

Microsatellite loci	Alleles (i)										A	H_O	H_E
	allele 1	allele 2	allele 3	allele 4	allele 5	allele 6	allele 7	allele 8	allele 9	allele 10			
EV21	0.10	0.60	0.10	0.15	0.05						5	0.7	0.60
GGAA520	0.05	0.20	0.25	0.20	0.25	0.05					6	0.9	0.79
GATA98	0.15	0.20	0.50	0.10	0.05						5	0.7	0.68
GT211	0.80	0.10	0.05	0.05							4	0.4	0.35
EV14	0.50	0.05	0.10	0.10	0.20	0.05					6	0.6	0.69
GATA53	0.30	0.70									2	0.4	0.42
EV1	0.15	0.05	0.05	0.35	0.20	0.10	0.05	0.05			8	0.8	0.80
EV94	0.05	0.40	0.25	0.20	0.05	0.05					6	0.9	0.73
GT23	0.05	0.75	0.10	0.05	0.05						5	0.5	0.42
GT575	0.50	0.05	0.30	0.15							4	0.6	0.64
GATA417	0.15	0.15	0.25	0.30	0.05	0.05	0.05				7	0.7	0.80
GT310	0.70	0.25	0.05								3	0.4	0.45
EV104	0.30	0.40	0.15	0.15							4	0.8	0.71
GATA28	0.10	0.10	0.05	0.10	0.10	0.50	0.05				7	0.8	0.71
GT271	0.15	0.85									2	0.3	0.26
GT011	0.80	0.10	0.10								3	0.4	0.34
DlrFCB17	0.05	0.20	0.05	0.25	0.10	0.10	0.05	0.05	0.10	0.05	10	1.0	0.86
Means											5.1	0.64	0.60

The averaged statistics over the seventeen loci were shown at the bottom (overall H_E=0.60).

For haplotypic data such as mitochondrial DNA, a simple estimate of diversity, which is equivalent to H_E for diploid data, called as **haplotype diversity (*H*)** can be also calculated as $H=1-\sum_{i=1}^{k} p_i^2$, where k is the number of haplotypes, and p_i is the frequency of haplotype i (Nei, 1987). In the case of full sequence comparison, **nucleotide diversity (π)**, which is equivalent to H at the nucleotide level, is given by $\pi=\sum_{ij} x_i x_j \pi_{ij}$, where p_{ij} is the proportion of different homologous nucleotide sites between haplotypes i and j, and x_i and x_j are the frequencies of haplotypes i and j, respectively (Nei, 1987).

Box 3. Measuring genetic differentiation

F-statistics, which is first introduced by Wright (1951), is one of important estimators to describe population genetic structure. This statistics partitions overall genetic diversities into components within and among populations, and can be calculated using heterozygosity as the following equations (Nei, 1987):

$$F_{IS} = (h_s - h_i) / h_s$$
$$F_{IT} = (h_t - h_i) / h_t$$
$$F_{ST} = (h_t - h_s) / h_t$$

where h_i is the H_O averaged across all populations, h_s is the H_E averaged across all populations, and h_t is the H_E for the total population. Two of which, i.e. F_{IS} and F_{ST}, are widely used as estimators to describe genetic structure. The F_{IS} (inbreeding coefficient) can be positive indicating a deficiency of heterozygotes when inbreeding or population subdivision occur within populations, while negative values indicating an excess of heterozygotes will be observed when outbreeding occurs within populations. The F_{ST} (fixation index) is a common estimator for genetic differentiation among populations, and varies from 0 (no differentiation between populations) to 1 (fixation of different alleles in populations).

Table 2
Summary statistics for 17 microsatellite loci in North Pacific sei whale in each sampling area.

Microsatellite loci	Western					Central					Eastern				
	n	*A*	H_E	*HWE*	F_{IS}	*n*	*A*	H_E	*HWE*	F_{IS}	*n*	*A*	H_E	*HWE*	F_{IS}
EV21	30	5	0.706	0.667	−0.136	1501	6	0.644	0.867	−0.004	196	6	0.651	0.295	−0.011
GGAA520	30	7	0.789	0.648	−0.014	1499	9	0.802	0.965	0.004	193	9	0.792	0.833	0.059
GATA98	30	5	0.686	0.441	−0.070	1485	7	0.734	0.792	−0.005	159	6	0.748	0.043	0.050
GT211	30	4	0.295	1.000	0.099	1503	6	0.308	0.019	0.016	194	4	0.316	0.124	−0.078
EV14	30	12	0.818	0.625	0.105	1326	22	0.865	0.846	0.001	74	14	0.861	0.862	−0.020
GATA53	30	3	0.413	0.291	−0.050	1500	3	0.494	0.990	0.003	192	3	0.508	0.819	0.015
EV1	30	12	0.814	0.814	0.017	1502	16	0.836	0.583	0.010	196	17	0.835	0.407	0.034
EV94	30	6	0.655	0.222	0.085	1502	8	0.685	0.519	−0.023	196	6	0.702	0.287	−0.032
GT23	30	6	0.554	0.335	−0.146	1503	14	0.606	0.308	0.017	195	11	0.600	0.741	0.008
GT575	30	4	0.603	0.378	−0.108	1503	5	0.593	0.657	0.024	190	6	0.577	0.816	0.061
GATA417	30	7	0.791	0.956	−0.055	1326	9	0.782	0.449	−0.005	75	8	0.785	0.561	0.049
GT310	30	3	0.513	1.000	0.026	1502	5	0.483	0.160	−0.020	196	4	0.483	0.364	−0.077
EV104	30	5	0.743	0.847	−0.078	1500	10	0.726	0.878	0.003	191	8	0.703	0.678	0.017
GATA28	30	9	0.821	0.082	0.027	1503	11	0.812	0.559	0.016	196	10	0.825	0.719	−0.015
GT271	30	3	0.242	1.000	−0.105	1503	4	0.140	0.579	0.006	196	3	0.130	1.000	0.022
GT011	30	4	0.415	0.889	−0.128	1503	6	0.449	0.899	0.006	196	4	0.458	0.751	0.020
DlrFCB17	30	14	0.890	0.310	−0.049	1502	20	0.872	0.106	0.012	188	17	0.882	0.165	0.023
Overall		6.41	0.632	0.939	−0.034		9.47	0.637	0.790	0.003		8.00	0.639	0.753	0.007

Table 3
Summary statistics for mtDNA control region in North Pacific sei whale in each sampling area.

	Western					Central					Eastern				
mtDNA	*n*	*h*	S.D.	π (%)	S.D.	*n*	*h*	S.D.	π (%)	S.D.	*n*	*h*	S.D.	π (%)	S.D.
	30	0.908	0.036	0.789	0.454	1500	0.925	0.004	0.803	0.446	203	0.927	0.010	0.797	0.445

Table 4
Pairwise F_{ST} between sampling areas. Upper and below diagonals indicate results from microsatellite and mtDNA.

Sampling area	Western	Central	Eastern
Western		−0.0002	−0.0010
Central	−0.0007		−0.0014
Eastern	0.0004	0.0002	

(P=0.699, χ^2=142.09) frequencies among sampling areas.

The clustering patterns in each *K* estimated by the program STRUCTURE also did not infer a distinct genetic structuring of this species (Figure 3). These results suggested absence of genetic structure among sei whales distributed in the pelagic area of the North Pacific investigated in this study, which was also supported by the fact that genetic diversities were not different among sampling areas in both markers, and there were no departures from *HWE* in each of sampling areas.

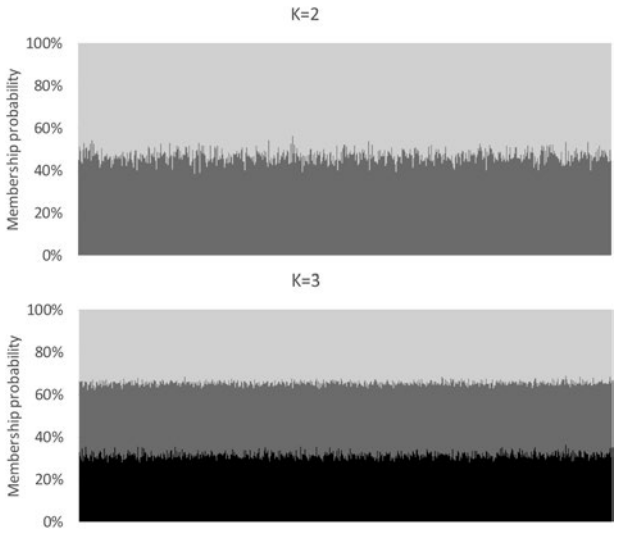

Figure 3. Bar plot of membership probabilities in STRUCTURE analyses at *K*=2–3 for North Pacific sei whales. Each individual is characterized by a thin vertical line, which is divided into *K* colored segments on the basis of the individual's membership fractions in *K* clusters.

CONCLUSIONS

The present pairwise F_{ST} estimates and heterogeneity tests did not show any significant genetic differentiations among the three sampling areas for both genetic markers. This was also supported by the STRUCTURE analysis suggesting a lack of genetic structure of this species. These findings implied that the North Pacific sei whale consists of a single breeding population at least in the pelagic areas surveyed. This inference was consistent with the comparable genetic diversities, i.e. h, π, H_E, among sampling areas as well as the results from the tests of *HWE* and F_{IS} estimates in this study. Kanda *et al.* (2015b) suggested that a single stock of sei whale occurs in the entire North Pacific based on genetic and non-genetic evidence. However, this study did not covered the coastal areas in both sides of the North Pacific. Additional genetic samples from the coastal areas are required to confirm this hypothesis.

ACKNOWLEDGEMENTS

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Technical Reports of the Institute of Cetacean Research (2019) pp. 41–51

Technical Report (not peer reviewed)

Distribution of blue, fin, humpback and North Pacific right whales in the western North Pacific based on JARPN and JARPNII surveys (1994–2014)

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ABSTRACT

This paper examines the geographical and temporal patterns of distribution of blue, fin, humpback and North Pacific right whales in the western North Pacific. The analyses were based on sighting data collected systematically by JARPN and JARPNII surveys during May to September in the years 1994–2014. A total of 269,728.1 n. miles was surveyed in the entire research period. The Density Index (No. of individual whales sighted/100 n. miles) was calculated and its geographical and temporal (monthly) distribution plotted for each individual species. Fin whale was the species most frequently sighted, followed by humpback, blue and right whales. The geographical and temporal pattern of distribution was described for each whale species. The large sighting data set collected systematically by JARPN and JARPNII has made a substantial contribution to understanding the pattern of geographical distribution and habitat use of whales in the western North Pacific ecosystem.

INTRODUCTION

One of the main sources of sighting data for assessing the population status of whale species in the western North Pacific was the JARPN, which was conducted between 1994 and 1999, and its second phase JARPNII conducted between 2000 and 2014.

The sighting data collected by JARPN and JARPNII have been used for studying the distribution pattern and abundance estimation of several large whale species in the western North Pacific (Okamura *et al.*, 2001; Matsuoka *et al.*, 2009). Details of the sighting survey procedures in JARPN and JARPNII are available in Fujise (2000) and Tamura *et al.* (2009), respectively.

This technical report summarizes the Institute of Cetacean Research (ICR)'s investigation of the pattern of geographical and temporal distribution of blue (*Balaenoptera musculus*), fin (*B. physalus*), humpback (*Megaptera novaeangliae*) and North Pacific right (*Eubalaena japonica*) whales in the western North Pacific. The investigation was based on sighting data collected systematically by JARPN and JARPNII surveys in the period 1994–2014.

MATERIALS AND METHODS

Research area

The research area comprised the western part of the North Pacific, specifically the International Whaling Commission (IWC) management sub-areas 7, 8, 9 and 11 (used for management of the common minke whale), north of 35°N, excepting the 200 n.mile EEZ's of foreign countries (Figure 1).

Sighting data

Primary sighting and effort data were collected by JARPN (1994–1999) and JARPNII (2000–2014) systematic surveys. Surveys were conducted by sighting and sampling vessels (SSV) (vessels engaged in sighting and sampling of whales) and dedicated sighting vessels (SV) (vessels engaged only in sighting of whales). Sighting data obtained by SSVs and SVs were combined for the calculation of the Density Index (see below).

Sighting procedures

The sighting procedures for SSVs and SVs in JARPNII (2000–2014) was not significantly changed with regard the procedures in JARPN (1994–1999) with only some minor changes made (see details in Matsuoka *et al.*, 2016). The research vessels were equipped with a top barrel, where three top men conducted sightings. On the upper bridge, a captain, a gunner, a helmsman and a researcher also conducted the sightings. The sighting activity was conducted under acceptable weather conditions (see below), from 60 minutes after sunrise to 60

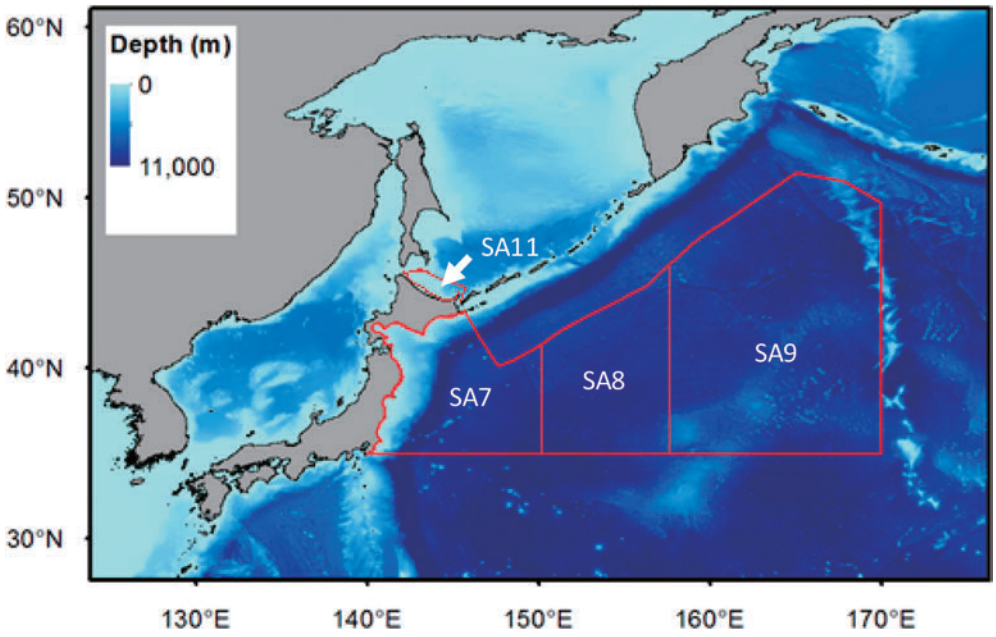

Figure 1. Research area in the western North Pacific indicating the sub-areas used for management of common minke whale.

minutes before the sunset.

Survey modes

SSV

Searching was conducted under two survey modes: NSC (Normal Search Closing) mode and NSS (Normal Search Closing Special). The NSC mode corresponds to 'closing mode' conducted under the normal weather conditions defined as visibility of 2 n.miles or more and wind velocity 4 or below. The NSS corresponds to 'closing mode' conducted under more unfavorable weather conditions but under which, the collection of whale samples was possible (Tamura *et al.*, 2009).

SV

Searching was conducted under two survey modes: ASP and NSP modes. The ASP mode corresponds to 'closing mode' conducted under normal weather conditions defined as visibility of 2 n.miles or more and wind velocity 4 or below. The NSP mode corresponds to 'passing mode' conducted under the normal weather conditions defined above (Matsuoka *et al.* 2016).

Cruise track design

For the main survey, the zigzag-shaped track line was established to cover the survey area. Furthermore, the 'Special Monitoring Survey (SMS)' was adopted in areas where the density of whales was expected to be high (see Fujise, 2000; Tamura *et al.*, 2009). The vessels conducted sighting surveys 6 and 4 n.miles apart from each other in the main and SMS surveys, respectively. These data were combined for the analyses.

Confirmation of the sightings

When a sighting was made, the vessels closed the school immediately in order to identify the species, estimate the school size and get other biological information such as the number of calves, estimated body length, etc. Surface temperatures were recorded at the location of each whale sighting.

Density Index of Whales

The Density Index of Whales (DIW) (the number of individual whales sighted by 100 n.miles) was calculated by each Lat.1°× Long.1°grid squares, for each species. Calculations were made for the entire research period (1994–2014) as well by month. For the calculation sighting data collected by the SSV and SV were combined.

RESULTS AND DISCUSSION

Searching efforts

A total of 269,728.1 n.miles were surveyed in sub-areas 7, 8, 9 and 11 (Figure 1) between 1994 and 2014 (including transit surveys in the Sea of Japan). Figure 2 show the research area and the primary searching effort (n. miles) by Lat.1°×Long.1° grid square.

Geographical and temporal distribution pattern of whales

Table 1 shows the summary of all primary whale sightings

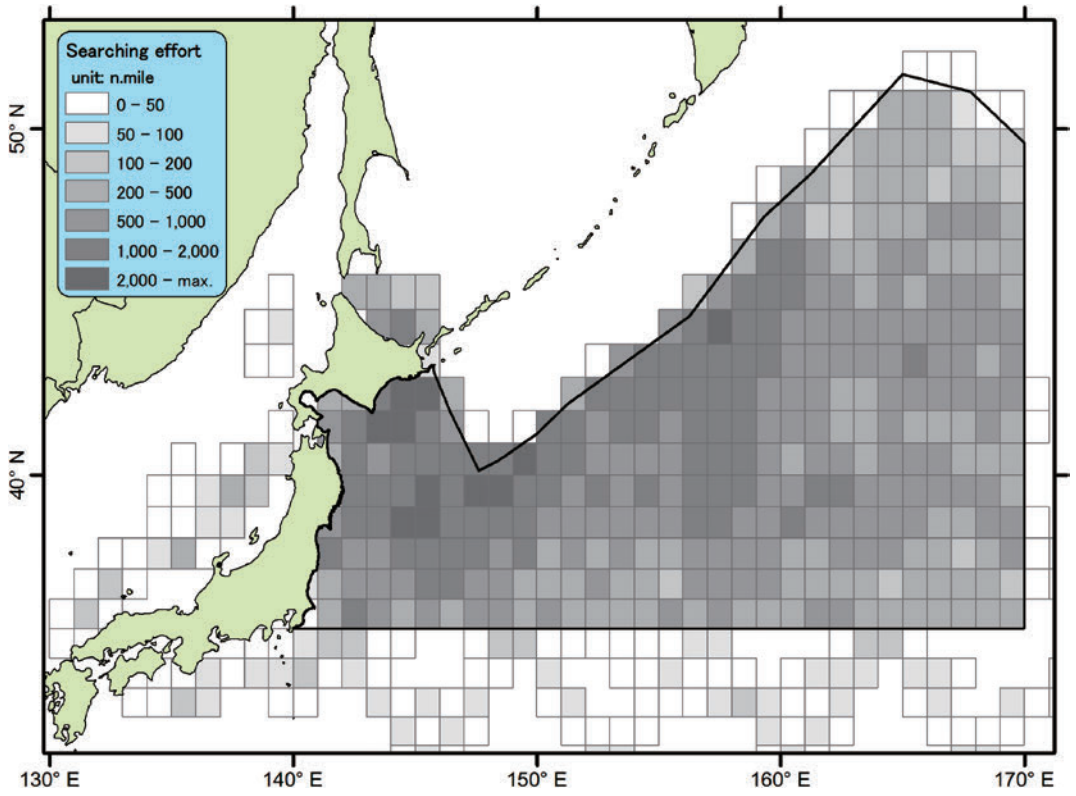

Figure 2. The searching effort by each Lat.1°× Long.1° grid square by JARPN and JARPNII surveys during 1994 to 2014 (including transit surveys in the Sea of Japan).

Table 1

Summary of all primary sightings of blue, fin, humpback and North Pacific right whales during the JARPN and JARPNII in the entire period (1994–2014) including transit surveys to and from the research areas.

Species	Indices						
	Sch.	Ind.	Calf	Mss	DIS	DIW	WT
Blue whale	374	508	23	1.36	0.14	0.19	3.0–25.8°C
Fin whale	799	1,125	37	1.41	0.30	0.42	2.9–26.9°C
Humpback whale	492	685	42	1.39	0.18	0.25	2.8–24.1°C
N.P. right whale	48	68	9	1.42	0.02	0.03	2.7–17.0°C

Sch.: number of the primary sightings of schools; Ind.: number of the primary sightings of individuals; Calf: number of calves; Mss: mean school size (Ind./Sch.); DIS: Density Index of Schools (schools/100 n.miles); DIW: Density Index of Whales (individual whales/100 n.miles); WT: range of surface temperature of the species sighting positions.

for blue, fin, humpback and North Pacific right whales in the entire period (1994–2014), including transit survey in the Sea of Japan. Fin whale (799 schools/1,125 individuals including 37 calves) was the species most frequently sighted (DIW: 0.42). Next was the humpback whale (492 schools/685 individuals including 42 calves) (DIW: 0.25). It was followed by the blue whale (374 schools/508 individuals including 23 calves) (DIW: 0.19) and North Pacific right whale (48 schools/68 individuals including 9 calves) (DIW: 0.03).

Pattern of geographical distribution

Figures 3a, 3b, 3c and 3d show the geographical distribution of the DIW of blue, fin, humpback and North Pacific right whales, respectively, for the entire period (1994–2014).

Blue whale (Figure 3a)

Blue whales were widely distributed mainly north of 35°N in sub-areas 8 and 9 from May to September. Surface temperature ranged from 3.0°C to 25.8°C (Table 1). A high-density area was observed north of 45°N, southeast off the Kamchatka Peninsula in sub-area 9.

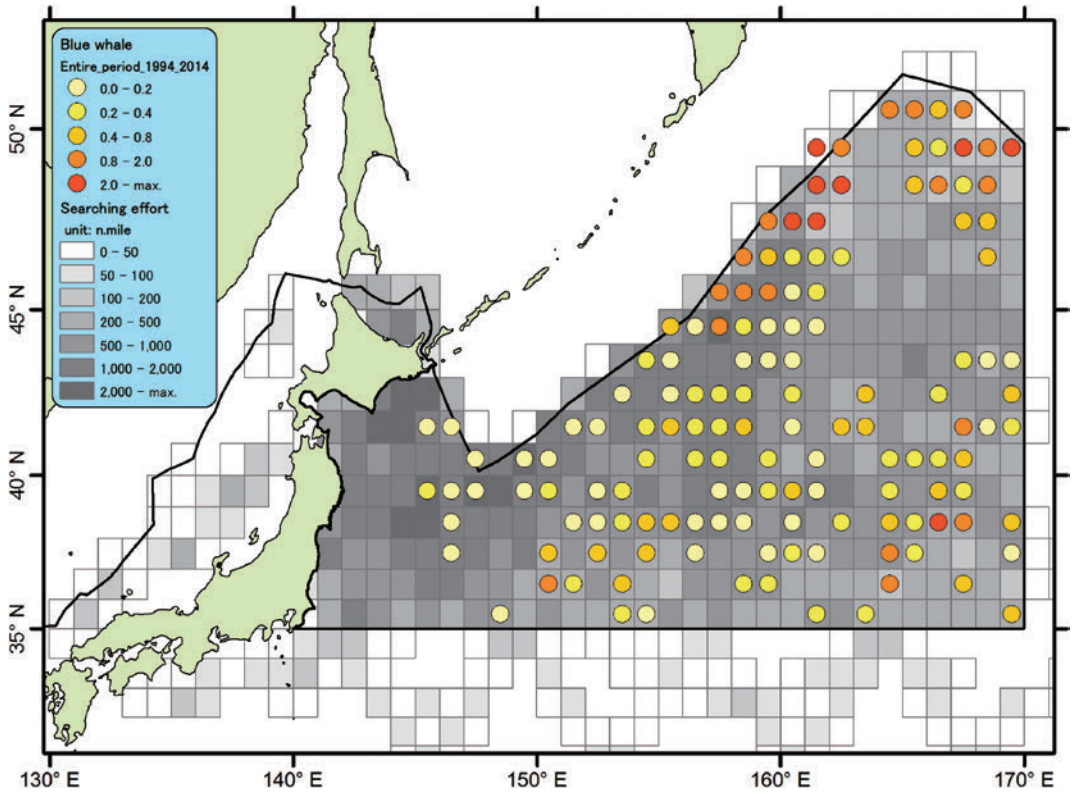

Figure 3a. Distribution of DIW of blue whales by Lat.1°× Long.1° grid squares. The DIW was calculated using JARPN and JARPNII sighting data in the entire period (1994 to 2014).

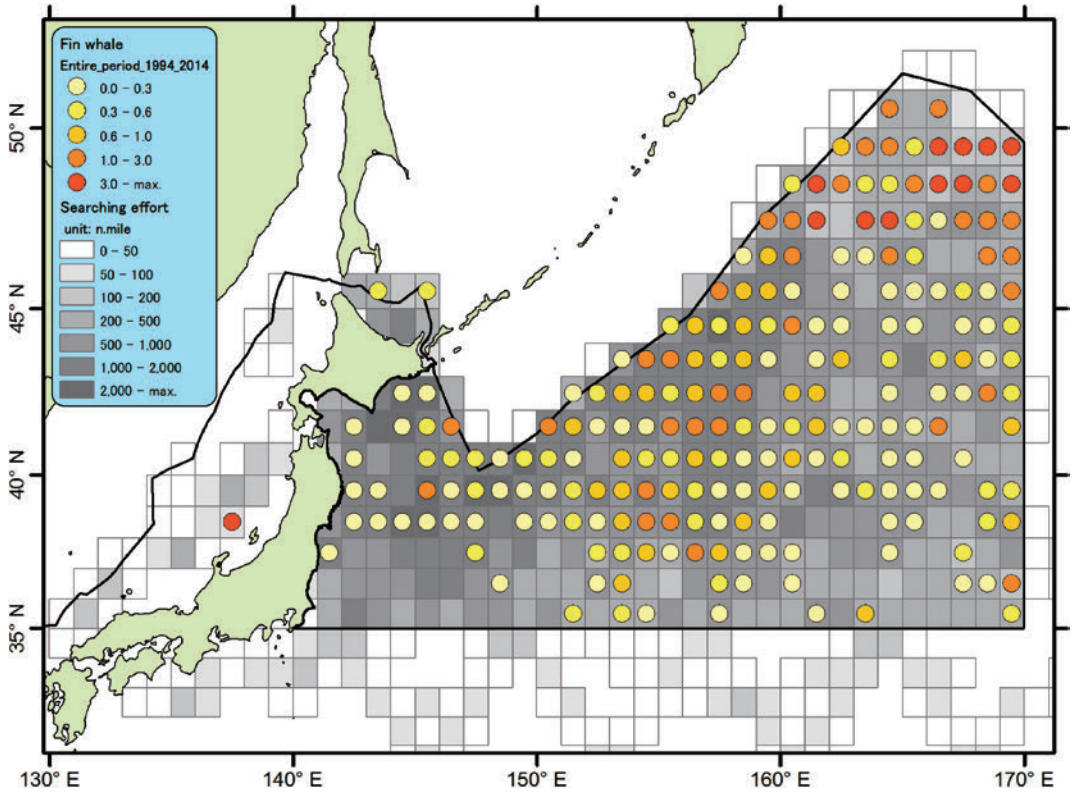

Figure 3b. Distribution of DIW of fin whales by Lat.1°× Long.1° grid squares. The DIW was calculated using JARPN and JARPNII sighting data in the entire period (1994 to 2014).

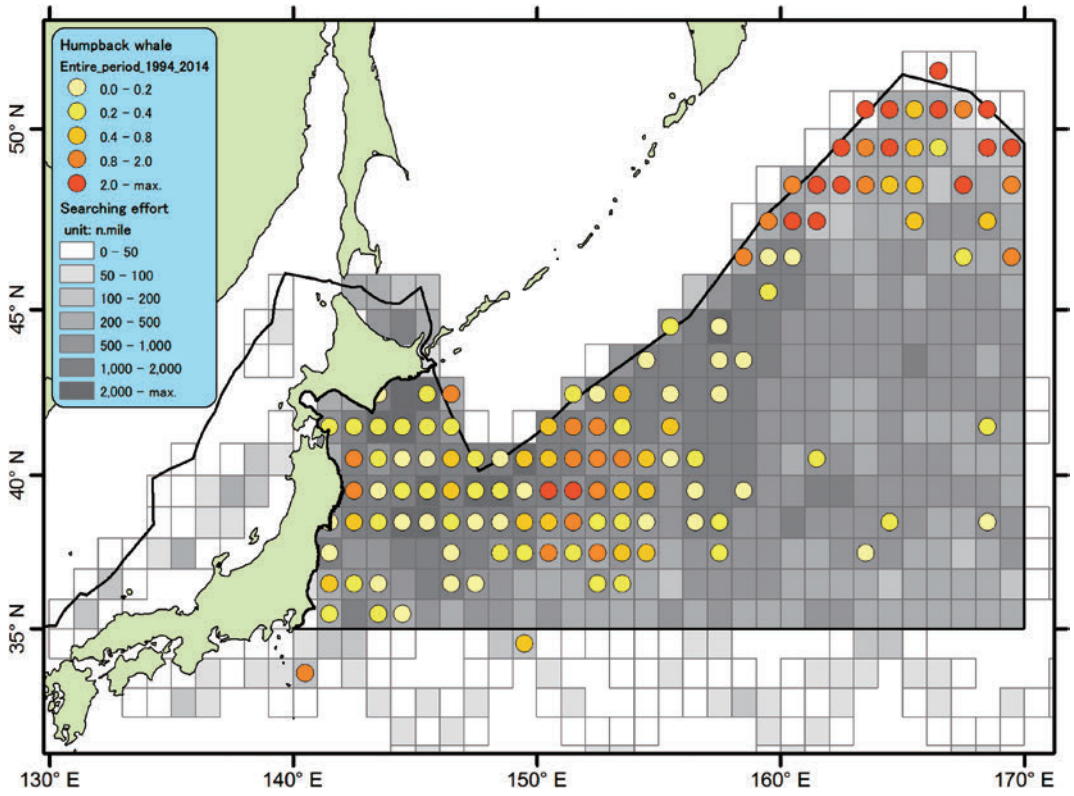

Figure 3c. Distribution of DIW of humpback whales by Lat.1°×Long.1° grid squares. The DIW was calculated using JARPN and JARPNII sighting data in the entire period (1994 to 2014).

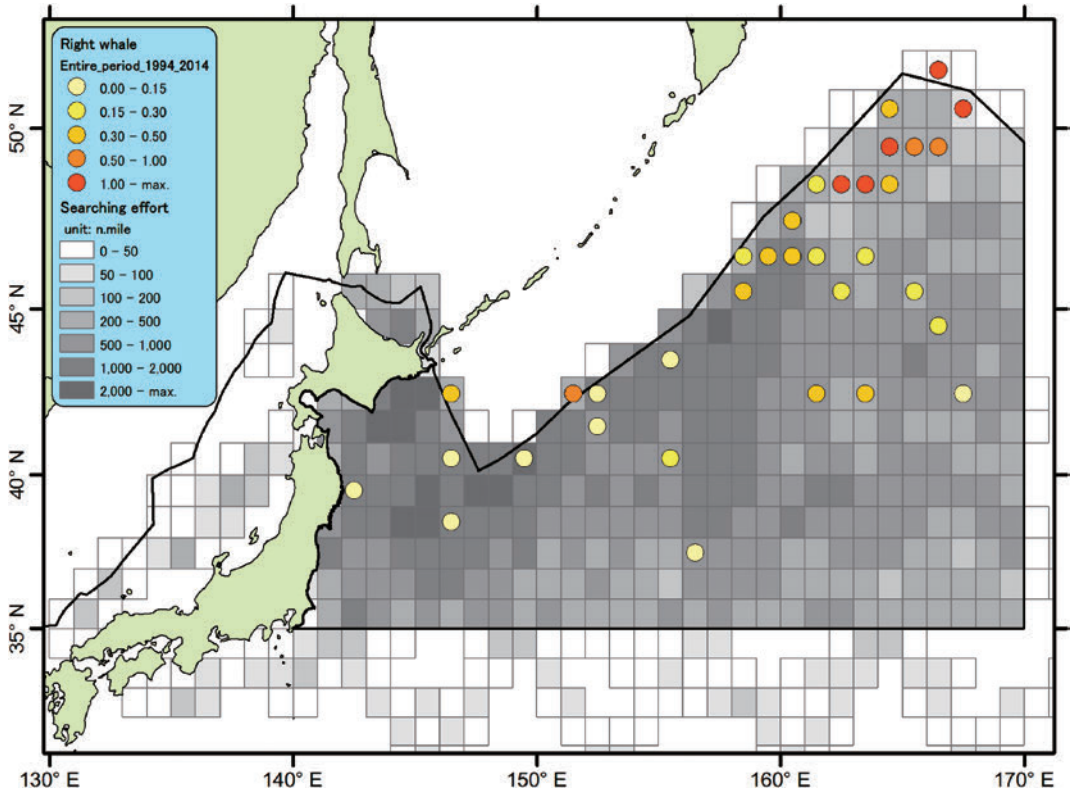

Figure 3d. Distribution of DIW of North Pacific right whales by Lat.1°×Long.1° grid squares. The DIW was calculated using JARPN and JARPNII sighting data in the entire period (1994 to 2014).

Blue whales were caught in the past around the rim of the western North Pacific. In summer, they concentrated along the edge of the continental shelf and along the south side of the Aleutian Archipelago. The catch data showed a distribution gap between sub-areas 7 and 9 in summer (Nishiwaki, 1966). However, JARPNII data showed no such gap. The previous gap may have been caused by regulation of the whaling operations between coastal (land base type) and offshore (mother ship type) whaling. Further, according to sighting data collected by the Japanese Scouting Vessel (JSV) between 1966 and 1990, blue whales were not sighted in sub-areas 7, 8 and 9 in June. However, JARPN and JARPNII sighting data suggested that this species was widely distributed in those sub-areas in June in the period 1994–2014.

The design-based abundance of this species in sub-areas 7, 8 and 9 was estimated as 38 whales during May to June (2009) and as 958 whales during July to August (2008) (Hakamada and Matsuoka, 2016).

Fin whale (Figure 3b)

Fin whale was the most frequently sighted species. This species was mainly sighted in sub-areas 8 and 9, and the distribution pattern was similar to that of the blue whale. They were mainly distributed north of 37°N in sub-areas 7, 8 and 9 from May to September. Surface temperature ranged from 2.9°C to 26.9°C (Table 1). A high-density area was observed north of 45°N in sub-area 9.

Fin whales were caught in the past along the outer shelf and south of the Aleutian Islands. A distribution gap between sub-areas 7 and 9 was previously observed for this species in summer (Nishiwaki, 1966). However, as in the case of the blue whale, the present results showed no such gap. The previous gap may have been caused by regulation of the whaling operations between coastal (land base type) and offshore (mother ship type) whaling.

The design-based abundance of this species in sub-areas 7, 8 and 9 was estimated as 413 whales during May to June (2009) and as 3,958 whales during July to August (in 2008) (Hakamada and Matsuoka, 2016).

Humpback whale (Figure 3c)

Humpback whales were mainly distributed north of 37°N in sub-areas 7, 8 and 9 from May to September. Surface temperature ranged from 2.8°C to 24.1°C (Table 1). High density areas were observed north of 35°N in sub-areas 7 and 8, and north of 45°N in sub-area 9.

The design-based abundance of this species in sub-areas 7, 8 and 9 was estimated as 1,136 whales during May–June (2009) and as 392 whales during July–August (2008) (Hakamada and Matsuoka, 2016).

North Pacific right whale (Figure 3d)

North Pacific right whale was the least frequently sighted species. This species was mainly distributed north of 37°N in sub-areas 7, 8 and 9 from May to September. Surface temperature ranged from 2.7°C to 17.0°C (Table 1).

According to Miyashita *et al.* (1995), there were no sightings in waters outside of the Okhotsk Sea, north of 40°N. However, present results confirmed the existence of this species in the offshore region during 1994–2014 from May to September.

The design-based abundance of this species in sub-areas 7, 8 and 9 was estimated as 1,147 whales during May–June (2011–2012) and 416 whales during July–August (2008) (Hakamada and Matsuoka, 2016).

Pattern of temporal distribution

A more detailed description of the monthly distribution of the species is presented here based on Figures 4a, 4b, 4c and 4d, which show the monthly changes in the density index of blue, fin, humpback and North Pacific right whales, respectively. As a whole, the main distribution of blue, fin, humpback and North Pacific right whales moved northward from 35°N to 45°N from May–August, which coincided with the results of previous large-scale distribution pattern reported by Miyashita *et al.* (1995).

Blue whale (Figure 4a)

A northward migration pattern was observed for this species. The main distribution from 35°N to 40°N in May to June moved north of 40°N in July to August in sub-areas 8 and 9.

Fin whale (Figure 4b)

A northward migration pattern of fin whale was also observed. The main distribution from 35°N to 40°N during May–June moved north of 40°N during July–August in sub-areas 8 and 9. There were almost no fin whale sightings in the west of 144°E in June, and west of 150°E in July.

Humpback whale (Figure 4c)

A northward migration pattern of humpback whales was also observed. The main distribution from 37°N to 43°N in sub-area 7 during May to June moved to north of 45°N during July–August in sub-areas 8 and 9.

The present results revealed that the distribution pattern of this species changed from that reported in previous studies. Humpback whales were not sighted in

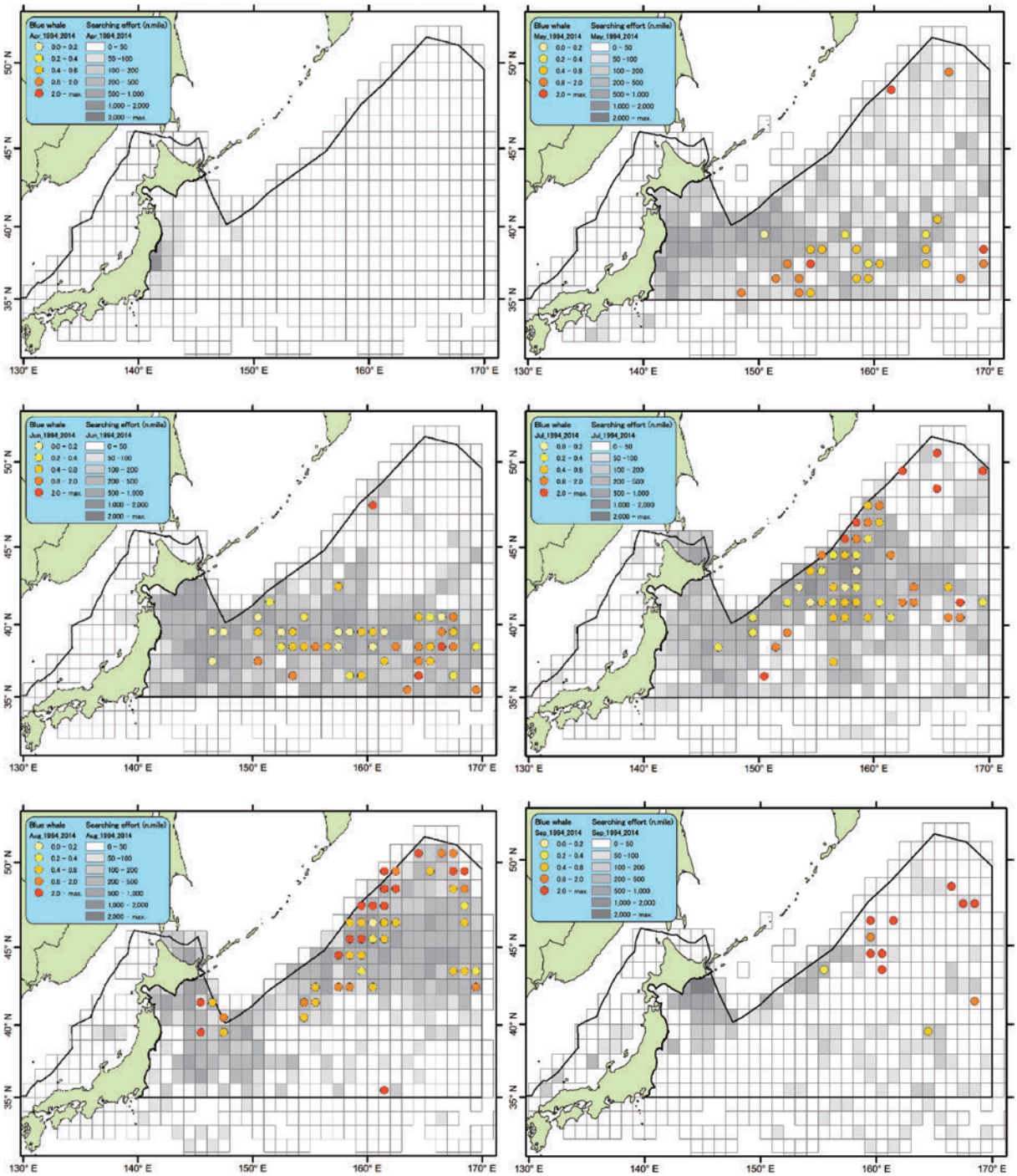

Figure 4a. Monthly change of DIW of blue whales during JARPN and JARPNII from 1994 to 2014 surveys, by Lat.1°×Long.1°square. Top left: April; Top right: May; Middle left: June; Middle right: July; Bottom left: August; Bottom right: September.

sub-areas 7 and 8 in May and June by the JSV surveys (1966–1990), however they were present in those sub-areas and period by the JARPN and JARPNII surveys in 1994–2014.

North Pacific right whale (Figure 4d)

A northward migration pattern of this species was observed. The main distribution area was north of 42°N during July to August in sub-area 9. The distribution pattern of this species was reported using historical catch and JSV data (Omura, 1986; Miyashita and Kato, 1998; Clapham *et al.*, 2004). The present results confirm the existence of this species in sub-areas 7, 8 and 9 during 1994–2014, and the distribution pattern in May–September is similar to that reported previously.

There are two migration routes along both sides of the

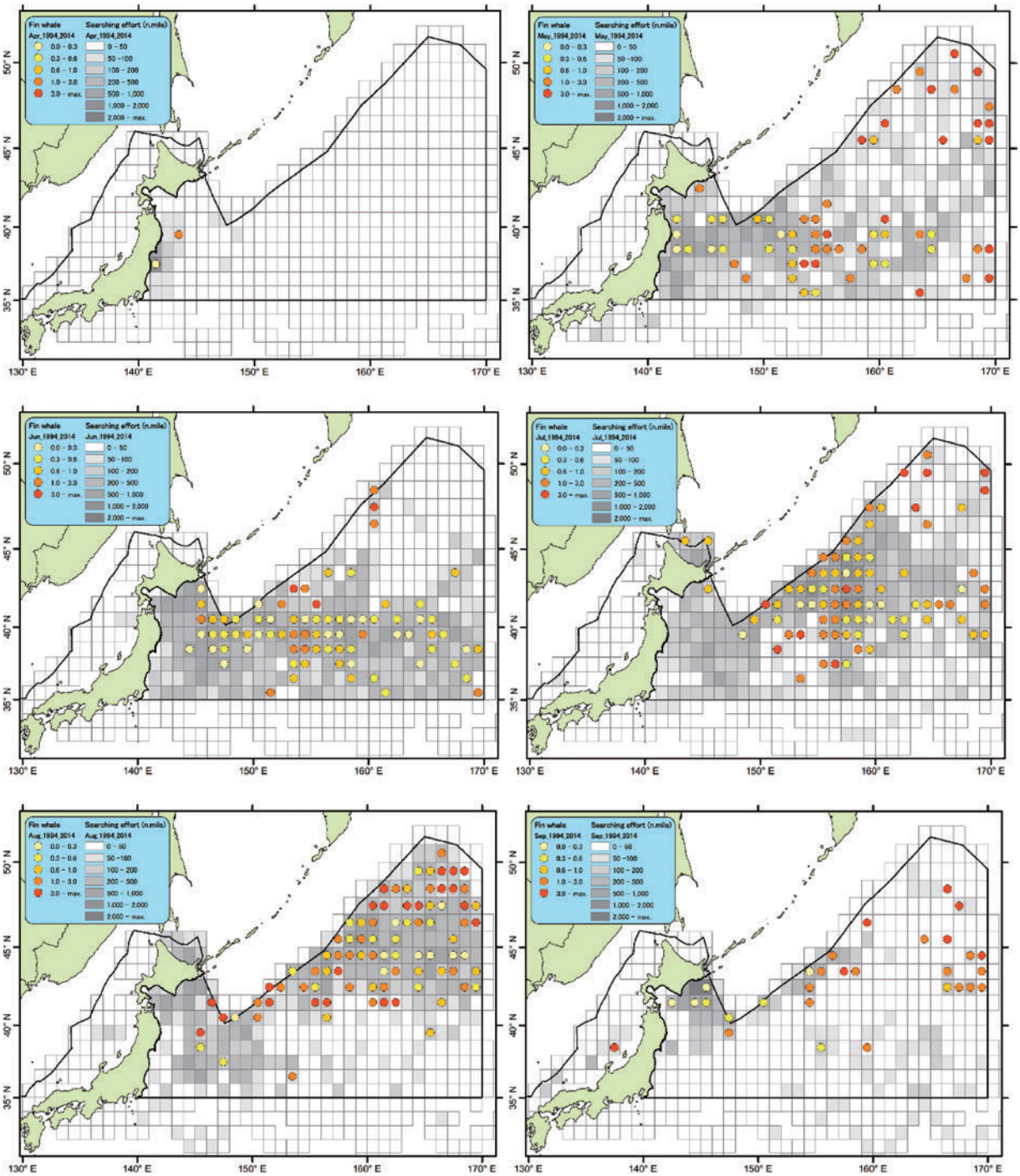

Figure 4b. Monthly change of DIW of fin whales during JARPN and JARPNII from 1994 to 2014 surveys by Lat.1°× Long.1°square. Top left: April; Top right: May; Middle left: June; Middle right: July; Bottom left: August; Bottom right: September.

Japanese main Island, based on historical whaling data (Omura, 1986). Several scientists have suggested that the Kuril Islands and Kamchatka coasts are likely to be major summer feeding regions, based on historical and recent new information (Matsuoka *et al.*, 2009; Brownell *et al.*, 2001; Clapham *et al.*, 2004; Sekiguchi *et al.*, 2014). A northward migration pattern was also observed from the Pacific to the Sea of Okhotsk during the winter to summer in recent analyses using Japan and Russian sighting data (Matsuoka *et al.*, 2018).

CONCLUSIONS

Sighting data of large whales in the western North Pacific, collected systematically by JARPN and JARPNII for a long period of time, were valuable in providing information on the geographical and temporal pattern of distribution of

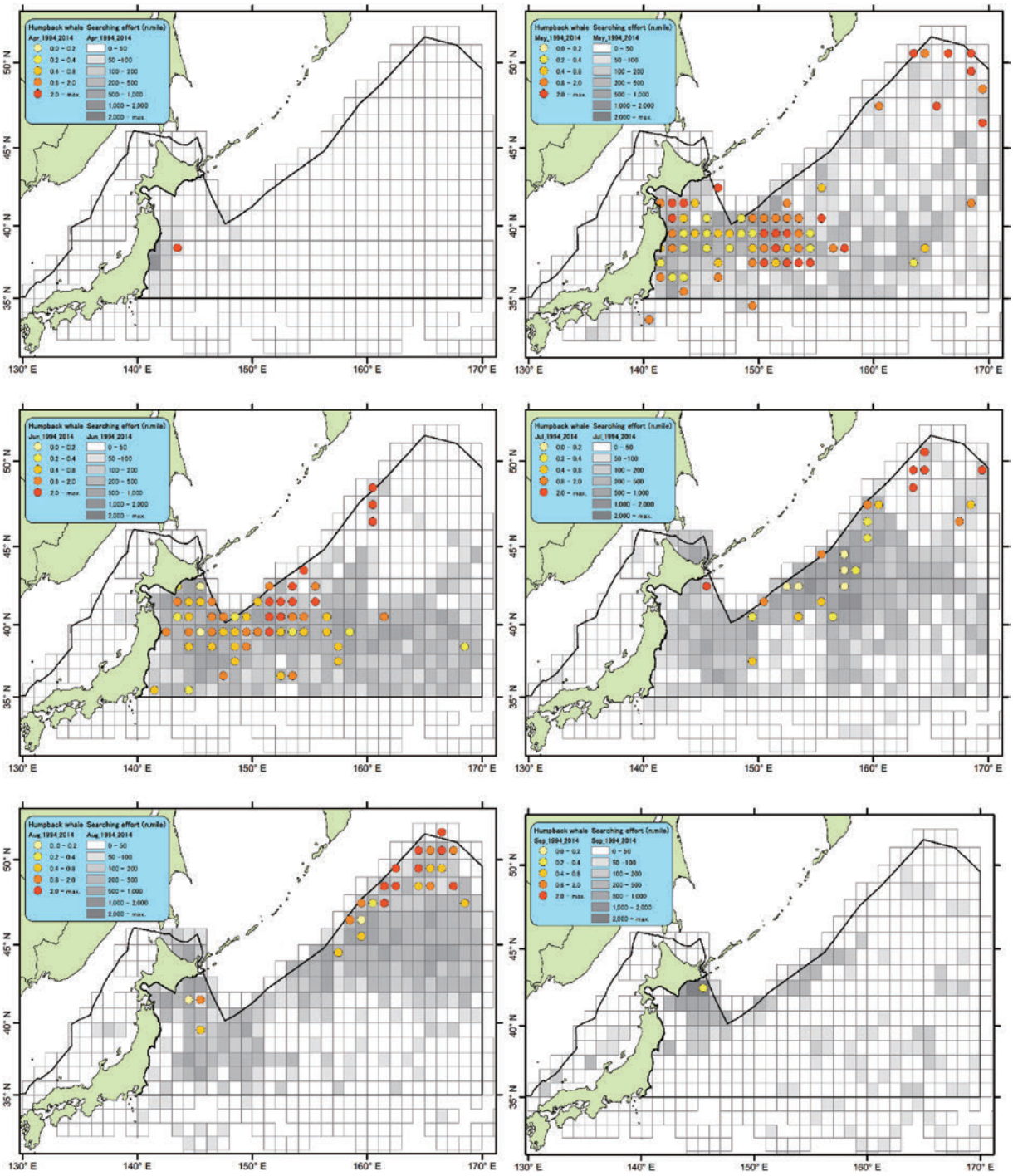

Figure 4c. Monthly change of DIW of humpback whales during JARPN and JARPNII from 1994 to 2014 surveys by Lat.1°×Long.1°square. Top left: April; Top right: May; Middle left: June; Middle right: July; Bottom left: August; Bottom right: September.

blue, fin, humpback and North Pacific right whales in this oceanic basin. The data indicate that some species have been expanding their distribution in recent years. The same sighting data have been used for abundance estimates of these species, however, future studies on distribution and abundance should take into consideration the available information on stock structure.

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We thank all cruise leaders, captains, officers, crew and researchers involved on the JARPN and JARPNII surveys for their effort to collect sighting data.

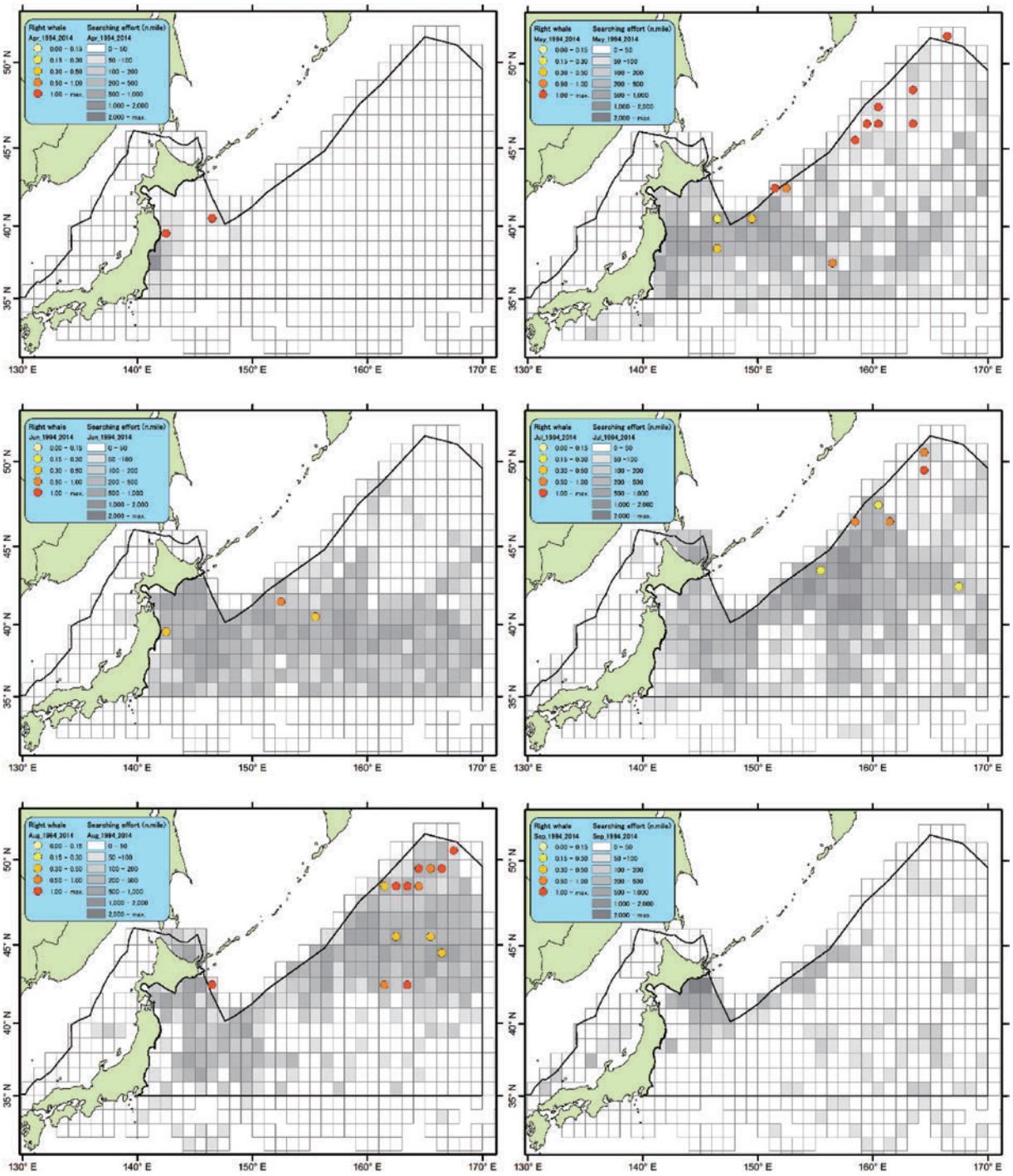

Figure 4d. Monthly change of the Density Index (number of primary sightings of whales/100 n.mile) of North Pacific right whales during JARPN and JARPN II from 1994 to 2014 surveys by Lat.1°× Long.1°square. Top left: April; Top right: May; Middle left: June; Middle right: July; Bottom left: August; Bottom right: September.

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Technical Reports of the Institute of Cetacean Research (2019) pp. 52–54

Technical Report-Note (not peer reviewed)

The platform and equipment for satellite-monitored tagging experiments in NEWREP-A and NEWREP-NP

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In recent years, satellite monitored tags have been recognized as crucial tools for behavioural studies of wild animals. These tools have been used for monitoring movement patterns of free-ranging animals such as marine mammals and sea turtles (e.g. Hoenner *et al*., 2012; McIntyre, 2014; Lee *et al*., 2017). However, the attachment of the tags on the body of large whales is a challenging issue, as attachment of the tag needs to be made from moving vessels on moving whales.

Recently satellite tags housed in stainless steel and air-pressured tagging guns have been used successfully for the long-term tracking of large whales to investigate their migration routes (e.g. Kennedy *et al.*, 2014; Garrigue *et al*., 2015; Panigada *et al*., 2017). From the analysis of time series location data, the changes of movement speed and the swimming angle can be calculated, and the latest modelling approach enables the definition of behaviour of the animals, such as foraging or migrating (Jonsen *et al*., 2005; 2013; Silva *et al*., 2013; 2014; Prieto *et al*., 2014; Dulau *et al*., 2017).

Satellite tracking of baleen whales was part of the objectives of NEWREP-A and NEWREP-NP, and the Institute of Cetacean Research (ICR) have been working on the development of tags, and attachment systems to be used from the platform of large vessels. This paper reports on the platform and instruments of the satellite monitored tagging experiments conducted during NEWREP-A and NEWREP-NP surveys including a description of the tags, the attachment system and the vessels from where the experiments were conducted. Results of the experiments will be summarized in future TEREP-ICR issues.

Satellite monitored tags

The ICR used the so-called Type C tags. The anchoring systems and electronic packages are consolidated and embedded in the body of the animals with an external antenna (IWC, NOAA and ONR, 2019). This Type C tags have been used for relatively large whales. Geographical locations are obtained by satellite transmissions, such as Argos system operated by Collected Localisation Satellite (CLS). Specifically the ICR used the SPOT177 tags (113 mm with triangle stop plate; Wildlife Computers Inc.) (Figure 1).

SPOT177 was designed to track the positions of the whales. The anchor was designed by ICR (*ca*. 100 mm in ISOD-type ver.2) for stabilizing the tags at implantation, and it was screwed on the anterior of the tag-housing (Figure 1 and 2). The penetration into the body was almost 200 mm (from the stopper at the end of tags to the

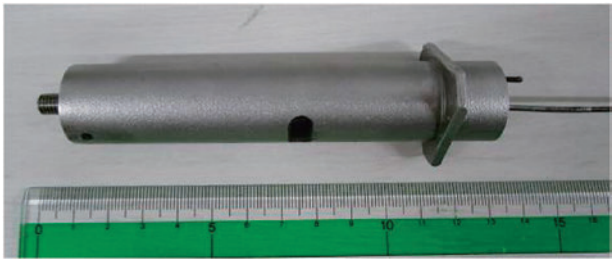

Figure 1. Satellite monitored tag SPOT177 (Wildlife Computers Inc.)

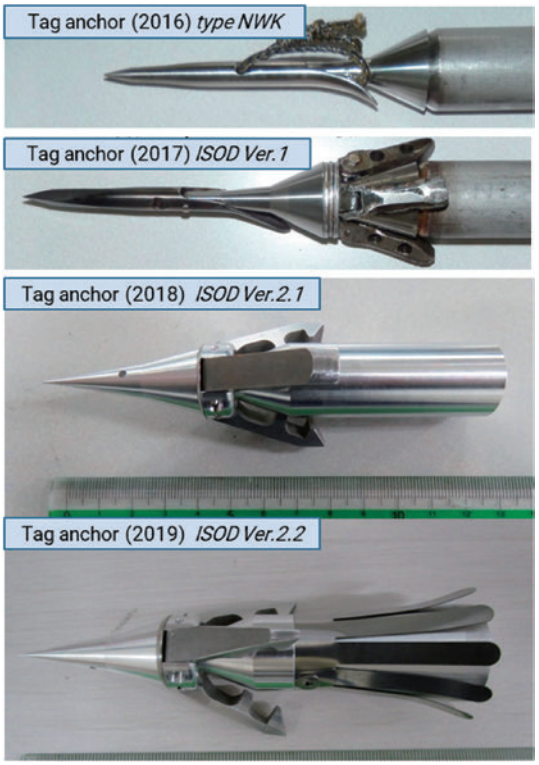

Figure 2. The anchors designed by the ICR for SPOT type tags.

tip of the anchor). The ending part of the tag was connected to a pipe-like LK-Carrier (made of polycarbonate designed by LK-ARTS, Skutvik, Norway) (Figure 3) with a timing release, and inserted into the barrel of the Airgun. LK-Carrier also works as a float in case deployment failure occurs at sea. The anchors and tags were sterilized before their use. Once deployment was confirmed as successful, a saltwater sensor activated the tag that started transmitting when the dry switch sensor surfaced.

Airgun

The Air Rocket Transmitting System (LK-ARTS), which was developed by Lars Kleivane in cooperation with Restech-Norway, was used for launching the tags in NEWREP-A and NEWREP-NP (Figure 4). LK-ARTS is designed to launch satellite tags, sensor packages, biopsy darts etc. by compressed air. The entire body is made of alumite and the barrel (launching tube) and the posterior stock (shoulder support) are detachable, so that the length can be changed depending on the tag shapes. Total length is approximately 1,350 mm with a 780 mm launching tube. An optical sight and a pressure manometer are connected. The air cylinder with hose is connected to the valve of the LK-ARTS in the Figure 4. Air pressure is easily controlled by the valve on the base. This allows shooting at different distances.

Figure 3. A set for SPOT177 attachment. Anchor, tag and LK-Carrier are connected.

Vessels

The tags were deployed from the bow deck (6.5 m height from sea surface) of the R/V *Yushin-Maru*-type vessels (*ca*.720 GT) with length of about 70 m (Figure 5). The use of this type of vessels has the advantage of being able to conduct tag attachments far from shore and under rough sea condition. In parallel to tagging, skin biopsies were also collected from the same individual whales for genetic analyses using a Larsen gun system (Larsen, 1998).

During the tagging experiments, the part of the whale body where the tags were attached was recorded (Figure 6). Also, photos of the attached tags were taken to in-

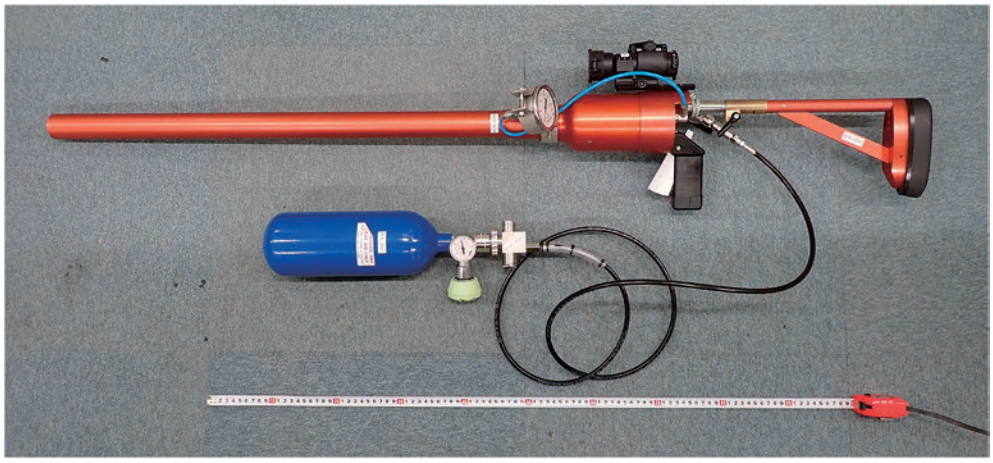

Figure 4. The Air Rocket Transmitting System (LK-ARTS) launching system.

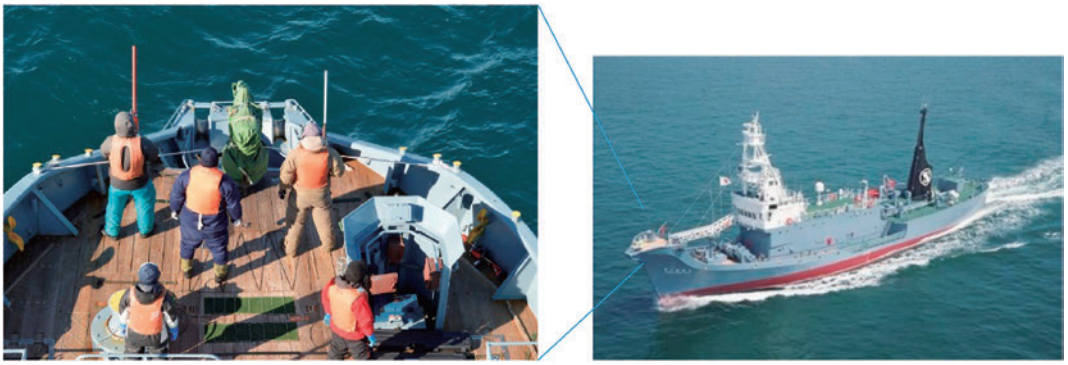

Figure 5. *Yushin-Maru*-type vessel and its bow deck.

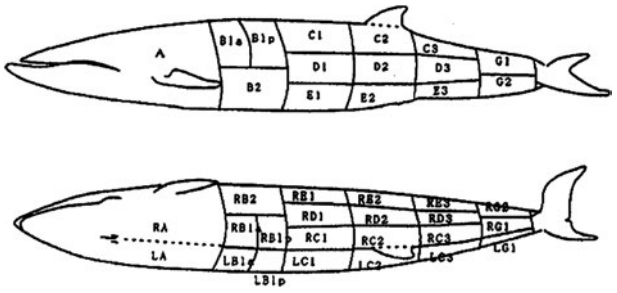

Figure 6. Code of whale body area used for recording the location of tags deployment.

vestigate the relationship between the depth of penetration and the radio transmissions for modification of the anchor and improvement of the attachment techniques.

Target whale species

The platform and equipment described in this note have been used in all NEWREP-A (2015/16–2018/19) and NEWREP-NP (2017–2019) survey years. The satellite-monitored tags were deployed on the Antarctic minke whales in the Indo-Pacific region of the Antarctic, and on common minke, sei, fin and blue whales in the western North Pacific. The tracking results will be reported in future TEREP-ICR issues.

ACKNOWLEDGEMENTS

We thank Lars Kleivane, Shingo Minamikawa and Shigetoshi Nishiwaki for providing technical advice on the development and implementation of tagging equipment. Thanks are also due to the crews on board of the vessels for their assistance during the field works.

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Technical Reports of the Institute of Cetacean Research (2019) pp. 55–58

Commentary

The views expressed here are those of the author and do not necessarily reflect the views of the Institute of Cetacean Research

Withdrawal of Japan from the International Convention for the Regulation of Whaling: implications for the whale research conducted by the Institute of Cetacean Research

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INTRODUCTION

On 26 December 2018, Japan announced its withdrawal from the International Convention for the Regulation of Whaling (ICRW), which came into effect on 30 June 2019. From this same date Japan ceased its two whale research programs under special scientific permit conducted under Article VIII of the ICRW, the NEWREP-A (New Scientific Whale Research Program in the Antarctic Ocean) and the NEWREP-NP (New Scientific Whale Research Program in the western North Pacific). From 1 July 2019, Japan started commercial whaling on common minke, Bryde's and sei whales within its territorial sea and Exclusive Economic Zone (EEZ).

As reported by Tamura *et al.* (2017), the Institute of Cetacean Research (ICR) was in charge of designing and implementing both NEWREP-A and NEWREP-NP, and research under these two programs was the main and first priority of the ICR. Now that these research programs have ceased, what is the future of the ICR and its research on whales? This note attempts to respond to this question.

This author's view is that the basis for responding to the question above can be found i) in the statement from Japan at the International Whaling Commission Scientific Committee (IWC SC) in 2019 regarding Japan's withdrawal from the IWC; and ii) in Japan's research activities aimed to calculate sustainable catch limits for commercial whaling in its EEZ.

STATEMENT FROM JAPAN AT THE IWC SCIENTIFIC COMMITTEE REGARDING JAPAN'S WITHDRAWAL FROM THE IWC

At the 2019 annual meeting of the IWC SC, Japan made a statement regarding its future involvement in the work of the IWC SC (IWC, 2019). The main points of the statement are summarized below:

- Japan will continue research programs with non-lethal methods in both the North Pacific and the Antarctic Ocean.
- Japan will collect fisheries-dependent scientific data through commercial whaling within its EEZ.
- Japan will provide the IWC SC scientific findings derived from the activities described above.
- Japan will provide the IWC SC standard statistics in relation to its commercial whaling.
- As for the data collected through its special permit programs (JARPA/JARPAII, NEWREP-A, JARPN/JARPNII, NEWREP-NP), Japan will continue to be engaged in their analyses and provide the IWC SC with scientific findings thereon.
- Japan will continue to provide information on its DNA register for large whales on a voluntary basis.
- Japan is prepared to continue the IWC-POWER (Pacific Ocean Whale and Ecosystem Research) in the North Pacific.

Given their previous experience with NEWREP-A and NEWREP-NP, this author considers that ICR scientists have the potential to play a substantial future role in the research activities listed above.

CATCH LIMIT CALCULATION FOR SUSTAINABLE WHALING IN THE WESTERN NORTH PACIFIC

In its IWC SC statement, Japan noted that catch limits of common minke, Bryde's and sei whales will be calculated in line with the Revised Management Procedure (RMP), 'taking into account relevant scientific progress achieved by the IWC SC such as outputs from *Implementation Reviews* and *In-depth Assessment*.' While such scientific progress should be taken into account, Japan's withdrawal from the ICRW means that it is now the sole mandate and responsibility of Japan to calculate sustainable catch limits for the target whale species in the western North Pacific, and to allocate such quotas internally within Japan.

In fact, Japan established an *ad-hoc* domestic group

composed of scientists from several Japanese research organizations and officials from the Fisheries Agency of Japan (FAJ) with the aim to carrying out the calculations of catch limits of common minke, Bryde's and sei whales in line with the RMP. The main work of the *ad-hoc* group was:

i) to summarize the key information on stock structure (required to define management areas), abundance and catch history of the three species of baleen whales;
ii) to run the Norwegian Catch Limit Algorithm (CLA) computer code for a tuning level of 0.6;
iii) to investigate the robustness of the catch limits calculated by the CLA to some uncertainties by the so-called *Implementation Simulation Trials* (*ISTs*) process (in the case of common minke and Bryde's whales where stock structure is complex).

The final catch quotas were determined by the FAJ after the calculations of catch limits by the domestic *ad-hoc* group were reviewed by a team of international experts. Based on those catch quotas, which were lower than the calculated catch limits, commercial whaling operations started from 1 July 2019 in Japan's EEZ. Participation of ICR scientists was key in the successful work of the *ad-hoc* group.

Japan's work on catch limits (including the *ISTs*) will continue to be based on the best available science; hence, the catch limits will be revised from time to time to reflect the latest scientific information. In this context, a domestic Steering Group was recently established to deal with the required data and analyses associated with future updates of the calculations of catch limits. The Terms of References (TORs) of this Steering Group involve among others, i) the identification of relevant input data to update the catch limit calculations in line with the RMP (i.e. abundance estimates, genetic and non-genetic data for refining the current stock structure hypotheses, etc.); ii) the identification of biological data (i.e. age, reproductive data) and the process required to improve/optimize the use of the current RMP and; iii) the research of alternative management procedures to the RMP.

This author considers that, given the contribution and experience acquired by ICR scientists during the work of the previous *ad-hoc* group, the contribution of ICR scientists to the work of the new Steering Group would remain substantial.

IMPLICATIONS FOR THE WHALE RESEARCH CONDUCTED BY THE ICR

From the points made in Japan's statement at the IWC SC and the TORs and activities proposed for the domestic Steering Group on sustainable whaling in the western North Pacific it is possible to highlight some research activities relevant for the future work of the ICR.

1. Research programs with non-lethal methods in both the North Pacific and the Antarctic Ocean

Japan presented the outline of a new research program in the Indo-Pacific region of the Antarctic at the 2019 IWC SC (GOJ, 2019a) and at the Convention on the Conservation of Antarctic Marine Living Resources's Working Group on Ecosystem Monitoring and Management (CCAMLR EMM) (GOJ, 2019b) meetings. The research program, called JASS-A (Japanese Abundance and Stock structure Surveys in the Antarctic), has two main research objectives i) the study of the abundance and abundance trends of large whale species, and ii) the study of the distribution, movement and stock structure of large whale species. JASS-A also has several secondary research objectives related to oceanographic conditions, marine debris and whale biology. The research program will be based on systematic sighting surveys utilizing the line transect method, to be conducted alternatively in IWC Areas III, IV, V and VI, south of 60°S by one or two specialized vessels, during a tentative period of eight austral summer seasons. Analyses related to main and secondary objectives will be conducted based on new as well previous data collected by JARPA/JARPAII and NEWREP-A in the same research area.

In the western North Pacific, dedicated sighting surveys are being planned with the aim of obtaining sighting data for abundance estimation of large whale species, with emphasis given to common minke, Bryde's and sei whales, the species that are the current target of commercial whaling. Also the surveys will collect biopsy samples, which are important for genetic analyses to elucidate/refine stock structure hypotheses.

Surveys of the IWC-POWER program have been conducted successfully until 2019. Japan in consultation with the IWC should decide on the survey plan for 2020, and on the future of the POWER after 2020. The importance of such surveys is that they provide sighting data (and biopsy samples) from areas not covered by the Japanese dedicated sighting surveys.

The ICR could play a key role in the research activities based on non-lethal methods given the substantial experience of its scientific staff in both field sighting surveys and in analytical procedures related to abundance estimates and stock structure research.

2. Collection of fisheries-dependent scientific data through commercial whaling

Japanese scientists have already identified the data and samples to be collected during commercial whaling operations. The data involve data/samples relevant for applying/optimizing the RMP as explained above (genetic samples for stock structure analyses, biological samples for age and reproductive status determination), and other data/samples for more generic use.

As is the case for non-lethal research noted above, ICR scientists also have substantial experience in collecting data and biological samples during past whale research programs under special permit. To keep consistency and the quality of the sampling process, ICR scientists should be involved in sampling during commercial whaling operations in collaboration with scientists from the National Research Institute of Far Seas Fisheries and government inspectors.

3. Analyses of data and samples from previous whale research programs under special permit

In the case of the Antarctic the analyses by JASS-A under its main and secondary objectives will be conducted on pooled data with those data/samples collected by JARPA/JARPAII and NEWREP-A. The analyses of pooled data will contribute with important information not only for the assessment of several large whale species but also for studies on the ecosystem.

The analyses of samples and data obtained by JARPN, JARPNII and NEWREP-NP in the western North Pacific will be continued, and those analyses should be conducted in conjunction with data and samples collected during commercial whaling operations. In this case emphasis should be given to analyses relevant for assessment and management of common minke, Bryde's and sei whales.

Because the whale research programs under special permit were designed and implemented by the ICR, it is natural to assume that the ICR would play an important role in this particular research activity in the future.

4. DNA registry of large whales

Given the expertise and accumulated experience, the ICR should continue developing and implementing the DNA registry of large whales for monitoring of the domestic market under the supervision of the FAJ. Technical updates of the registry should be presented by the ICR to the annual meetings of the IWC SC on a voluntary basis.

5. Catch limit calculations for sustainable whaling

ICR scientists have the potential to contribute to the work of the Steering Group in areas of their expertise such as, i) the design of dedicated sighting surveys; ii) the estimates of abundance; iii) collection and analyses of genetic samples to refine the information on stock structure of the key species; and iv) running the CLA and designing/implementing the *ISTs*.

Data accumulated by the ICR through its previous whale research programs are likely to play a key role in the process. Data available involve not only those key for the application of the current RMP (stock structure, abundance, and catch history) but also some biological data such as age and reproductive status, which can be used for the improvement or optimization of the current RMP, and for developing of new catch limit calculation methods.

FUTURE RELATIONSHIP BETWEEN JAPAN AND THE IWC SCIENTIFIC COMMITTEE AND OTHER RESEARCH ORGANIZATIONS

Japan will have observer status for its future participation in the work of the IWC SC. As usual, ICR scientists should continue participating in the annual meetings and intersessional workshops of the IWC SC either as part of Japan's observer delegation or as Invited Participants. Following domestic consultation, ICR scientists may present results of the analyses mentioned above to the annual meetings.

ICR scientists have been engaged in meetings of other international organizations related to the conservation and management of marine living resources i.e. in meetings of the North Atlantic Marine Mammal Commission Scientific Committee (NAMMCO SC), CCAMLR's working groups, North Pacific Marine Science Organization (PICES), etc. Their future participation in these international fora should be encouraged.

CONCLUSION

The ICR and its scientists can play an important role in the future whale research activities of Japan. The basis for this conclusion are given in the research plans and guidelines outlined at Japan's statement regarding its withdrawal from the IWC, and on the TORs of the recently established Steering Group on sustainable commercial whaling in Japan's EEZ. This conclusion is not surprising given the ICR's substantial accumulated experience of approximately 30 years investigating whales and their ecosystem in both the Antarctic and the western North Pacific. The ICR scientists have the field and analytical expertise (based on lethal and non-lethal methods) to continue responding to biological and ecological ques-

tions on whales, and contribute in this way to the future conservation and management of this group of animals.

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International meetings

Participation of scientists of the Institute of Cetacean Research in International Meetings in 2019

34th International Symposium on the Okhotsk Sea & Polar Oceans, 2019

The Okhotsk Sea & Polar Oceans Symposium is organized annually by the City of Mombetsu. This symposium promotes the advancement of all ice-related studies including biology and fisheries, in the Sea of Okhotsk.

The 2019 symposium was held at the Mombetsu Culture Hall, Mombetsu, Hokkaido, Japan from 17 to 20 February. Two scientists from the Institute of Cetacean Research (ICR) (Kato and Tamura) participated in the symposium by presenting an 'Outline of the second whale scientific permit survey off the coast of the Okhotsk Sea under the NEWREP-NP program.'

Mombetsu Culture Hall, Mombetsu, Japan.

Annual meeting of the International Whaling Commission Scientific Committee (IWC SC)

The International Whaling Commission (IWC) is an international body set up by the terms of the International Convention for the Regulation of Whaling (ICRW), which was signed in Washington, D.C., United States, on 2 December, 1946 to 'provide for the proper conservation of whale stocks and thus make possible the orderly development of the whaling industry.' One of the important components of the IWC is the Scientific Committee (SC), which meets annually.

The 2019 meeting of the IWC SC was held at the Safari Park Hotel & Casino, Nairobi, Kenya from 10 to 23 May. A total of ten scientists from the ICR participated in the meeting (Fujise, Kato, Pastene, Tamura, Matsuoka, Goto, Hakamada, Yasunaga, Taguchi and Takahashi). They presented five documents at plenary sessions, five documents at the *Ad hoc* Working Group on Abundance Estimates, Stock Status and International Cruises, two documents at the Working Group on Ecosystem Modelling, two documents at the Working Group on Environmental Concerns, one document at the Working Group on Stock Definition and DNA Testing, and two documents at the Sub-Committee on Other Southern Hemisphere Whale Stocks.

The report of the IWC SC meeting can be found in the website of the IWC (https://iwc.int/home).

Safari Park Hotel & Casino, Nairobi, Kenya.

In 2019, ICR scientists also participated in the First Intersessional Workshop on the *Implementation Review* for western North Pacific common minke whales, held at the Fisheries Agency of Japan (FAJ)'s Crew House, Tokyo, Japan from 25 February to 1 March. Pastene, Goto, Hakamada, Taguchi, Inoue and Takahashi from the ICR participated in the workshop

The report of this meeting can be found in the website of the IWC (https://iwc.int/home).

Annual meeting of the Convention on the Conservation of Antarctic Marine Living Resources—Working Group on Ecosystem Monitoring and Management (CCAMLR-EMM)

The Convention on the Conservation of Antarctic Marine Living Resources (CCAMLR) is part of the Antarctic Treaty System. The Convention was opened for signature on 1 August 1980 and entered into force on 7 April 1982 thereby establishing the Commission for the Conservation of Antarctic Marine Living Resources. The goal is to preserve marine life and environmental integrity in and

near Antarctica. It was established in large part in response to concerns that an increase in krill catches in the Southern Ocean could have a serious impact on populations of other marine life which are dependent upon krill for food. The CCAMLR has a Scientific Committee and several Working Groups including the Working Group on Ecosystem Monitoring and Management (EMM), which meet annually.

The 2019 meeting of the CCAMLR-EMM was held at the Concarneau Marine Station, Concarneau, France from 24 June to 5 July. One scientist from ICR participated in the meeting (Pastene). He presented the documents 'Distribution and possible areas of spatial mixing of two stocks of humpback whales, a krill predator, in the Indo-Pacific region of the Antarctic revealed by genetic analyses' and 'Outline of a research program to investigate the abundance, abundance trends and stock structure of large whales in the Indo-Pacific region of the Antarctic, including a survey plan for the 2019/20 austral summer season.' The presentations of these documents were made under the agenda item 'Krill predator biology, ecology and population dynamics.'

The report of the meeting can be found in the website of the CCAMLR (https://www.ccamlr.org/).

Concarneau Marine Station, Concarneau, France.

FAO Expert Meeting to Develop Technical Guidelines to Reduce Bycatch of Marine Mammals

The Food and Agriculture Organization of the United Nations (FAO) is a specialized agency of the United Nations that leads international efforts to defeat hunger. The FAO was formed on 16 October 1945 and has its headquarters in Rome, Italy. As of August 2018, the FAO has 197 member states. The agency is directed by the Conference of Member Nations, which meets every two years. The Conference elects a council of 49 member states that acts as an interim governing body, and the Director-General, that heads the agency. FAO is composed of six departments: Agriculture and Consumer Protection, Economic and Social Development, Fisheries and Aquaculture, Forestry, Corporate Services, Human Resources and Finance and Technical Cooperation.

The Expert Meeting to 'Develop technical guidelines to reduce bycatch of marine mammals in capture fisheries' was held at the FAO headquarters in Rome, Italy on 17-19 September 2019. Twenty-nine fisheries and bycatch experts and observers from FAO Members participated in the Meeting: Argentina, Australia, Brazil, Canada, Chile, Denmark, Iceland, Japan, Norway, Russian Federation, Sweden, and the United States of America. The meeting was also attended by experts from various regional and international organizations: Convention on the Conservation of Migratory Species of Wild Animals (CMS), International Council for the Exploration of the Seas (ICES), International Whaling Commission (IWC), North Atlantic Marine Mammal Commission (NAMMCO), and NGOs, including Marine Stewardship Council (MSC), Whale and Dolphin Conservation (WDC), and the World Wildlife Fund (WWF). Kato, Advisor of ICR, participated in the meeting as an expert.

The report of the meeting can be found in the website of FAO (http://www.fao.org/home/en/).

FAO headquarters, Rome, Italy.

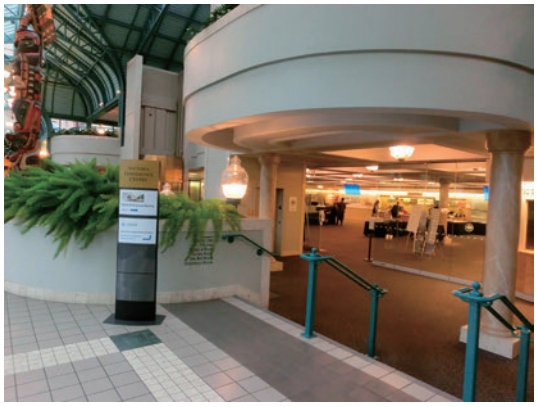
Annual meeting of the North Pacific Marine Science Organization (PICES)

The North Pacific Marine Science Organization (PICES) is an intergovernmental science organization established in 1992 to promote and coordinate marine scientific research in the North Pacific Ocean and its adjacent seas, and to provide a mechanism for information and data exchange among scientists in its member countries. Its present members are Canada, Japan, People's Republic of China, Republic of Korea, the Russian Federation, and the United States of America.

The 2019 meeting of the PICES was held at the Victoria,

BC, Canada from 16 to 27 October. One scientist from ICR participated in the meeting (Tamura). He presented the study 'Estimation of prey consumption by marine mammals in the PICES regions -Update of Hunt *et al.* (2000)-' as an oral presentation at the session 'Implications of Prey Consumption by Marine Birds, Mammals, and Fish in the North Pacific.' Konishi, Matsuoka and Hakamada were co-author in this study. Tamura, Hakamada and Matsuoka were co-authors of another study 'Spatial estimation of prey consumption by sei, Bryde's and common minke whales in the western North Pacific during the summers of 2008–2009: Density surface model approach,' that was also presented at the same session.

The report of the meeting can be found in the website of PICES (https://meetings.pices.int/).



Victoria Conference Centre, Victoria, Canada.

Annual meeting of the North Atlantic Marine Mammal Commission (NAMMCO) Scientific Committee (SC)

The North Atlantic Marine Mammal Commission (NAMMCO) is an international body for cooperation on the conservation, management and study of marine mammals in the North Atlantic. The NAMMCO Agreement was signed in Nuuk, Greenland on 9 April 1992 by Norway, Iceland, Greenland and the Faroe Islands, and entered into force on 8 July 1992. The agreement focuses on modern approaches to the study of the marine ecosystem as a whole, and to better understanding the role of marine mammals in the ecosystem. NAMMCO has a Scientific Committee (SC) which meets annually.

The 2019 NAMMCO SC meeting was held at Tórshavn Marina, Faroe Islands from 29 October to 1 November. Three scientists from ICR participated in the meeting (Pastene, Isoda and Taguchi) as observers from Japan. They presented the 2017–2019 Japan progress report on cetacean research as well an outline of the research program to investigate the abundance, abundance trends and stock structure of large whales in the Indo-Pacific region of the Antarctic (JASS-A).

The report of the meeting can be found in the website of NAMMCO (https://nammco.no/).

Tórshavn Marina, Faroe Islands.

19th World Marine Mammal Conference

The World Marine Mammal Conference (WMMC) is a joint conference between the Society for Marine Mammalogy (SMM) and the European Cetacean Society (ECS). The ECS was founded in January 1987 with the aim to promote and advance the scientific studies and conservation efforts of marine mammals and disseminate information about them to the society and the public. The ECS convenes once a year in a European country, alternating between southern and northern Europe as much as possible, with invited international authorities. The SMM was founded in 1981 and is the largest international association of marine mammal scientists in the world. The mission of the SMM is to promote the global advancement of marine mammal science and contribute to its relevance and impact in education, conservation and management.

The 19th WMMC was held at the Centre de Convencions Internacional de Barcelona (CCIB), Barcelona, Catalonia, Spain from 7–12 December 2019. Two scientists from the ICR participated in the conference (Konishi and Takahashi) presenting the studies 'Changes in sei whale feeding habits in response to fish species replacement in the western North Pacific during 2002–2018,' and 'A com-

parison of the diet composition derived from skin stable isotopes and stomach contents for two baleen whale species: sei and Bryde's whales in the western North Pacific' as posters at the session 'Foraging Ecology.'

Konishi, Tamura and Bando were co-authors of the study 'Tracing life history of immature Antarctic minke whales: stable isotope oscillation in baleen revealed ontogenetic diet shifts and seasonal migration,' presented as a poster at the session 'Foraging Ecology.' Matsuoka was co-author of the studies 'Estimation of population dynamics for the Antarctic blue whale using Bayesian state-space models' and 'Estimation of relationship between density surface of humpback whales and environmental factors in the North Pacific ocean using IWC-POWER data,' presented as posters at the session 'Population Biology and Abundance' and 'Habitat and Distribution.' Katsumata and Kato submitted the study 'Expansion of wintering ground of the humpback whales in the North Pacific: Beginning of the seasonal migration to around Hachijo Island, Izu Archipelago,' which was presented as a poster at the session 'Habitat and Distribution.'

The Centre de Convencions Internacional de Barcelona (CCIB), Barcelona, Spain.

'Ecopath 35 Years—Making Ecosystem-Based Management Operational' Conference

This conference and associated workshops was held at the Fish and Wildlife Research Institute, St. Petersburg, Florida, USA from 4 to 11 December 2019, to showcase 35 years of progress using the Ecopath approach in fields such as fisheries management, marine conservation, ecosystem dynamics, climate impacts, and ecosystem-based management, as well as to introduce new aspects of the approach.

Tamura and Hakamada were the co-authors of a contributing study presented to the conference titled 'Ecosystem modeling in the western North Pacific from 1994 to 2013 using Ecopath with Ecosim (EwE) with focus on forage fish and baleen whales.'

The Fish and Wildlife Research Institute, St. Petersburg, Florida, USA.

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National meetings

Participation of scientists of the Institute of Cetacean Research in National Meetings in 2019

Annual meeting of the Ecological Society of Japan (ESJ)

The Ecological Society of Japan was founded in 1953 to promote research in all aspects of ecology. The main events organized by the society are the annual meetings held in spring in one of the main cities of Japan. The ESJ currently has an enrollment of over 3,900 members including students and non-professionals. The ESJ works not only on science matters but it also puts effort into dissemination of information among the general public.

The 2019 meeting of the ESJ was held at the Kobe Convention Center, Hyogo from 15 to 19 March. Konishi, a scientist from the Institute of Cetacean Research (ICR) participated in the meeting making an oral presentation of the study 'Relationship between the Antarctic minke whale and sea ice at Southern Ocean by satellite monitored tracking' at the session on Behavior (Isoda and Bando from the ICR were co-authors in this study).

Kobe Convention Center, Hyogo.

Annual meeting of the Japanese Society of Fisheries Science (JSFS)

The Japanese Society of Fisheries Science (JSFS) was established in 1932. It is a non-profit registered society dedicated to the promotion of all aspects of fisheries science. The society fulfills its global commitment by promoting science, striving to achieve sustainable development while recognizing the crucial need of preserving the natural aquatic resources. It also strives to forge relationships with the industry comprising both capture and culture fisheries, the fishing environment, and the concerned trade. The main events organized by the society are the biannual meetings held in spring and autumn in one of the main cities of Japan. In this forum the members present their research activities, exchange information, and create partnerships in vital areas of research.

The 2019 spring meeting of the JSFS was held at the Tokyo University of Marine Science and Technology, Tokyo from 26 to 29 March. Three scientists from the ICR (Bando, Tamura and Konishi) were co-authors in the study 'Estimation of feeding habits of the pregnant Antarctic minke whales from stable isotope ratios in baleen plates,' which was presented as a poster.

Tokyo University of Marine Science and Technology, Tokyo.

Annual Meeting of the Mammal Society of Japan (MSJ)

The Mammal Society of Japan (MSJ) was established in 1987 by uniting two academic organizations, the Mammalogical Society of Japan and the Research Group of Mammalogists, which were founded in 1949 and 1955, respectively. The MSJ currently has an enrollment of over 1,100 members including students and non-professionals. The annual meeting of the society consists of academic sessions (symposia, oral presentations, and poster papers), workshops, and a business meeting.

The 2019 autumn meeting of MSJ was held at Chuo University, Tokyo, from 15 to 18 September. Several scientists from the ICR were co-authors in studies presented to the meeting: 'Growth dependent changes of external morphology of fetus of the Antarctic minke whale' (Bando, Fujise and Kato) (poster presentation); 'Gut microbiome of baleen whale in the Antarctic and western North Pacific' (Tamura) (oral presentation at the session Conservation and Management); 'Estimation of population dynamics for the Antarctic blue whale using Bayesian state-space models' (Matsuoka) (oral presentation at the

Chuo University, Tokyo.

session Conservation and Management); and 'Relationship between density distribution of humpback whales and environmental factors in the North Pacific Ocean using IWC-POWER data' (Matsuoka) (oral presentation at the session Conservation and Management).

Peer-reviewed publications

List of peer-reviewed publications based on the Institute of Cetacean Research (ICR)'s surveys up to 2019

This section presents a list of peer-reviewed publications based on data collected by surveys conducted under special scientific permit (JARPA/JARPAII/NEWREP-A and JARPN/JARPNII/NEWREP-NP), including both lethal and non-lethal techniques. Peer-reviewed publications based on these surveys are focused mainly on topics related to assessment and management of large whales. However samples and data collected by the surveys have also been useful to carry out studies of a more academic-oriented nature. Publications based on such studies are also listed here.

This section also includes a list of peer-reviewed publications resulting from other surveys and research activities, different from special scientific permit surveys.

Publications having as a first author a non-ICR scientist commonly followed a data request or collaboration research agreement with ICR. In a few cases, external scientists used published data from ICR surveys in their analyses and publications, without a formal agreement with ICR. These cases are indicated by an asterisk (*).

JARPA/JARPAII/NEWREP-A surveys

1989 (2)

Kato, H., Hiroyama, H., Fujise, Y. and Ono, K. 1989. Preliminary report of the 1987/88 Japanese feasibility study of the special permit proposal for Southern Hemisphere minke whales. *Rep. int. Whal. Commn* 39: 235–248.

Nakamura, T., Ohnishi, S. and Matsumiya, Y. 1989. A Bayesian cohort model for catch-at-age data obtained from research takes of whales. *Rep. int. Whal. Commn* 39: 375–382.

1990 (8)

Butterworth, D.S. and Punt, A.E. 1990. Some preliminary examinations of the potential information content of age-structure data from Antarctic minke whale research catches. *Rep. int. Whal. Commn* 40: 301–315.

Ichii, T. 1990. Distribution of Antarctic krill concentrations exploited by Japanese krill trawlers and minke whales. *Proc. NIPR Symp. Polar Biol.* 3: 36–56.

Itoh, S., Takenaga, F. and Tsuyuki, H. 1990. Studies on lipids of the Antarctic minke whale. I. The fatty acid compositions of the minke whale blubber oils caught on 1987/88 season. *Yukagaku* 39 (7): 486–490 (in Japanese).

Kasamatsu, F., Kishino, H. and Hiroyama, H. 1990. Estimation of the number of minke whale (*Balaenoptera acutorostrata*) schools and individuals based on the 1987/88 Japanese feasibility study data. *Rep. int. Whal. Commn* 40: 239–247.

Kato, H., Fujise, Y., Yoshida, H., Nakagawa, S., Ishida, M. and Tanifuji, S. 1990. Cruise report and preliminary analysis of the 1988/89 Japanese feasibility study of the special permit proposal for southern hemisphere minke whales. *Rep. int. Whal. Commn* 40: 289–300.

Kato, H., Kishino, H. and Fujise, Y. 1990. Some analyses on age composition and segregation of southern minke whales using samples obtained by the Japanese feasibility study in 1987/88. *Rep. int. Whal. Commn* 40: 249–256.

Nagasaki, F. 1990. The Case for Scientific Whaling. *Nature* 334: 189–190.

Tanaka, S. 1990. Estimation of natural mortality coefficient of whales from the estimates of abundance and age composition data obtained from research catches. *Rep. int. Whal. Commn* 40: 531–536.

1991 (9)

Bergh, M.O., Butterworth, D.S. and Punt, A.E. 1991. Further examination of the potential information content of age-structure data from Antarctic minke whale research catches. *Rep. int. Whal. Commn* 41: 349–361.

Ichii, T. and Kato, H. 1991. Food and daily food consumption of southern minke whales in the Antarctic. *Polar Biol* 11 (7): 479–487.

Kasamatsu, F., Kishino, H. and Taga, Y. 1991. Estimation of southern minke whale abundance and school size composition based on the 1988/89 Japanese feasibility study data. *Rep. int. Whal. Commn* 41: 293–301.

Kato, H., Fujise, Y. and Kishino, H. 1991. Age structure and segregation of southern minke whales by the data obtained during Japanese research take in 1988/89. *Rep. int. Whal. Commn* 41: 287–292.

Kato, H. and Miyashita, T. 1991. Migration strategy of southern minke whales in relation to reproductive cycles estimated from foetal lengths. *Rep. int. Whal. Commn* 41: 363–369.

Kato, H., Zenitani, R. and Nakamura, T. 1991. Inter-reader calibration in age readings of earplugs from southern

minke whale, with some notes of age readability. *Rep. int. Whal. Commn* 41: 339–343.

Kishino, H., Kato, H., Kasamatsu, F. and Fujise, Y. 1991. Detection of heterogeneity and estimation of population characteristics from the field survey data: 1987/88 Japanese feasibility study of the Southern Hemisphere minke whales. *Ann. Inst. Statist. Math.* 43 (3): 435–453.

Nakamura, T. 1991. A new look at a Bayesian cohort model for time-series data obtained from research takes of whales. *Rep. int. Whal. Commn* 41: 345–348.

Wada, S., Kobayashi, T. and Numachi, K. 1991. Genetic variability and differentiation of mitochondrial DNA in minke whales. *Rep. int. Whal. Commn* (special issue) 13: 203–215.

1992 (2)

Nakamura, T. 1992. Simulation trials of a Bayesian cohort model for time-series data obtained from research takes of whales. *Rep. int. Whal. Commn* 42: 421–427.

Tanaka, S., Kasamatsu, F. and Fujise, Y. 1992. Likely precision of estimates of natural mortality rates from Japanese research data for Southern Hemisphere minke whales. *Rep. int. Whal. Commn* 42: 413–420.

1993 (7)

Fujise, Y., Ishikawa, H., Saino, S., Nagano, M., Ishii, K., Kawaguchi, S., Tanifuji, S., Kawashima, S. and Miyakoshi H. 1993. Cruise report of the 1991/92 Japanese research in Area IV under the special permit for Southern Hemisphere minke whales. *Rep. int. Whal. Commn* 43: 357–371.

Hasunuma, R., Ogawa, T., Fujise, Y. and Kawanishi, Y. 1993. Analysis of selenium metabolites in urine samples of minke whale (*Balaenoptera acutorostrata*) using ion exchange chromatography. *Comp. Biochem. Physiol.* 104C (1): 87–89.

Itoh, S., Takenaga, F. and Tsuyuki, H. 1993. Studies on lipids of the Antarctic minke whale. II. The fatty acid compositions of the blubber oils of minke whale and dwarf minke whale caught on 1988/89 and 1989/90 seasons. *Yukagaku* 42 (12): 1007–1011 (in Japanese).

Iwata, H., Tanabe, S., Sakai, N. and Tatsukawa, R. 1993. Distribution of persistent organochlorines in the oceanic air and surface seawater and the role of ocean on their global transport and fate. *Environ. Sci. Technol.* 27: 1080–1098.

Kasamatsu, F., Yamamoto, Y., Zenitani, R., Ishikawa, H., Ishibashi, T., Sato, H., Takashima, K. and Tanifuji, S. 1993. Report of the 1990/91 southern minke whale research cruise under scientific permit in Area V. *Rep. int. Whal. Commn* 43: 505–522.

Nakamura, T. 1993. Two-stage Bayesian cohort model for time-series data to reduce bias in the estimate of mean natural mortality rate. *Rep. int. Whal. Commn* 43: 343–348.

Pastene, L.A., Kobayashi, T., Fujise, Y. and Numachi, K. 1993. Mitochondrial DNA differentiation in Antarctic minke whales. *Rep. int. Whal. Commn* 43: 349–355.

1994 (3)

Kimoto, H., Endo, Y. and Fujimoto, K. 1994. Influence of interesterification on the oxidative stability of marine oil triacylglycerols. *JAOCS* 71 (5): 469–473.

Pastene, L.A., Fujise, Y. and Numachi, K. 1994. Differentiation of mitochondrial DNA between ordinary and dwarf forms of southern minke whale. *Rep. int. Whal. Commn* 44: 277–281.

Yoshioka, M., Okumura, T., Aida, K. and Fujise, Y. 1994. A proposed technique for quantifying muscle progesterone content in the minke whales (*Balaenoptera acutorostrata*). *Can. J. Zoo.* 72: 368–370.

1995 (3)

Fukui, Y., Mogoe, T., Terawaki, Y., Ishikawa, H., Fujise, Y. and Ohsumi, S. 1995. Relationship between physiological status and serum constituent values in minke whales (*Balaenoptera acutorostrata*). *Journal of Reproduction and Development* 41 (3): 203–208.

Ishikawa, H. and Amasaki, H. 1995. Development and physiological degradation of tooth buds and development of rudiment of baleen plate in Southern minke whale, *Balaenoptera acutorostrata*. *J. Vet. Med. Sci.* 57 (4): 665–670.

Kasamatsu, F., Nishiwaki, S. and Ishikawa, H. 1995. Breeding areas and southbound migrations of southern minke whales *Balaenoptera acutorostrata*. *Mar. Ecol. Prog. Ser.* 119: 1–10.

1996 (7)

Bakke, I., Johansen, S., Bakke, O. and El-Gewely, M.R. 1996. Lack of population subdivision among the minke whales (*Balaenoptera acutorostrata*) from Icelandic and Norwegian waters based on mitochondrial DNA sequences. *Marine Biology* 125: 1–9.

Butterworth, D.S. and Geromont, H.F. 1996. On the provision of advice on the effect on stock(s) of scientific permit catches, with particular reference to proposed research catches of minke whales from Antarctic Area IV. *Rep. int. Whal. Commn* 46: 653–655.

Butterworth, D.S., Punt, A.E., Geromont, H.F., Kato, H. and

Miyashita, T. 1996. An ADAPT approach to the analysis of catch-at-age information for Southern Hemisphere minke whales. *Rep. int. Whal. Commn* 46: 349–359.

Fukui, Y., Mogoe, T., Jung, Y.G., Terawaki, Y., Miyamoto, A., Ishikawa, H., Fujise, Y. and Ohsumi, S. 1996. Relationships among morphological status, steroid hormones, and post-thawing viability of frozen spermatozoa of male minke whales (*Balaenoptera acutorostrata*). *Marine Mammal Science* 12 (1): 28–37.

Iga, K., Fukui, Y., Miyamoto, A., Ishikawa, H. and Ohsumi, S. 1996. Endocrinological observations of female minke whales (*Balaenoptera acutorostrata*). *Marine Mammal Science* 12 (2): 296–301.

Matsuoka, K., Fujise, Y. and Pastene, L.A. 1996. A sighting of a large school of the pygmy right whale, *Caperea marginata* in the southeast Indian Ocean. *Marine Mammal Science* 12 (4): 594–597.

Pastene, L.A., Goto, M., Itoh, S. and Numachi, K. 1996. Spatial and temporal patterns of mitochondrial DNA variation in minke whale from Antarctic Areas IV and V. *Rep. int. Whal. Commn* 46: 305–314.

1997 (3)

Aono, S., Tanabe, S., Fujise, Y., Kato, H. and Tatsukawa, R. 1997. Persistent organochlorines in minke whale (*Balaenoptera acutorostrata*) and their prey species from the Antarctic and the North Pacific. *Environmental Pollution* 98: 81–89.

Fukui, Y., Mogoe, T., Ishikawa, H. and Ohsumi, S. 1997a. Factors affecting *in vitro* maturation of minke whale (*Balaenoptera acutorostrata*) follicular oocytes. *Biology of Reproduction* 56: 523–528.

Fukui, Y., Mogoe, T., Ishikawa, H. and Ohsumi, S. 1997b. *In vitro* fertilization of *in vitro* matured minke whale (*Balaenoptera acutorostrata*) follicular oocytes. *Marine Mammal Science* 13 (3): 395–404.

1998 (7)

Ichii, T., Shinohara, N., Fujise, Y., Nishiwaki, S. and Matsuoka, K. 1998. Interannual changes in body fat condition index of minke whales in the Antarctic. *Mar. Ecol. Prog. Ser.* 175: 1–12.

Ito, S., Takenaga, F. and Tsuyuki, H. 1998. Lipids in Antarctic minke whales III. Site and sexual differences of muscle lipids. *J. Jpn. Oil Chem. Soc.* 47 (2): 191–194 (in Japanese).

Kida, M.Y. 1998. Morphology of the tracheobronchial tree and the route of the pulmonary artery in the fetal minke whale (*Balaenoptera acutorostrata*). *Okajimas Folia Anat. Jpn.*75 (5): 251–258.

Mogoe, T., Fukui, Y., Ishikawa, H. and Ohsumi, S. 1998a. Morphological observations of frozen-thawed spermatozoa of Southern minke whales (*Balaenoptera acutorostrata*). *Journal of Reproduction and Development* 44 (1): 95–100.

Mogoe, T., Fukui, Y., Ishikawa, H. and Ohsumi, S. 1998b. Effects of diluent composition and temperature on motility and viability after liquid storage and cryopreservation of minke whale (*Balaenoptera acutorostrata*) spermatozoa. *Marine Mammal Science* 14 (4): 854–860.

Nagasawa, K., Yamada, T.K. and Ishikawa, H. 1998. Measurements of the skeleton of a minke whale (*Balaenoptera acutorostrata*) from the Antarctic Ocean. *Bulletin of the Yamagata Prefectural Museum* 20: 1–19.

Terabayashi, T. and Kawanishi, Y. 1998. Naturally occurring ganglioside lactones in minke whale brain. *Carbohydrate Research* 307 (3): 281–290.

1999 (4)

Bannister, J.L., Pastene, L.A. and Burnell, S.R. 1999. First record of movement of a southern right whale (*Eubalaena australis*) between warm water breeding grounds and the Antarctic Ocean, South of 60°S. *Marine Mammal Science* 15 (4): 1337–1342.

Butterworth, D.S., Punt, A.E. Geromont, H.F., Kato, H. and Fujise, Y. 1999. Inferences on the dynamics of Southern Hemisphere minke whales from ADAPT analyses of catch-at-age information. *J. Cetacean Res. Manage.* 1 (1): 11–32.

Ishikawa, H., Amasaki, H., Dohguchi, H., Furuya, A. and Suzuki, K. 1999. Immunohistological distributions of fibronectin, tenascin, type I, III and IV collagens, and laminin during tooth development and degeneration in fetuses of minke whale, *Balaenoptera acutorostrata*. *J. Vet. Med. Sci.* 61 (3): 227–232.

Thomson, R.B., Butterworth, D.S. and Kato, H. 1999. Has the age at transition of southern hemisphere minke whales declined over recent decades? *Marine Mammal Science* 15 (3): 661–682.

2000 (5)

Asada, M., Horii, M., Mogoe, T., Fukui, Y., Ishikawa, H. and Ohsumi, S. 2000. *In vitro* maturation and ultrastructural observation of cryopreserved minke whale (*Balaenoptera acutorostrata*) follicular oocytes. *Biology of Reproduction* 62 (2): 253–259.

Kasamatsu, F., Matsuoka, K. and Hakamada, T. 2000. Interspecific relationships in density among the whale community in the Antarctic. *Polar Biol* 23 (7): 466–473.

Mogoe, T., Suzuki, T., Asada, M., Fukui, Y., Ishikawa, H. and Ohsumi, S. 2000. Functional reduction of the Southern minke whale (*Balaenoptera acutorostrata*) testis during the feeding season. *Marine Mammal Science* 16 (3): 559–569.

Rosenbaum, H.C., Brownell, R.L., Brown, M.W., Schaeff, C., Portway, V., White, B.N., Malik, S., Pastene, L.A., Pateneude, N.J., Baker, C.S., Goto, M., Best, P.B., Clapham, P.J., Hamilton, P., Moore, M., Payne, R., Rowntree, V., Tynan, C.T., Bannister, J.L. and DeSalle, R. 2000. Worldwide genetic differentiation of *Eubalaena*: questioning the number of right whale species. *Molecular Ecology* 9: 1793–1802.

Wei, H. and Fukui, Y. 2000. Fertilizability of ovine, bovine or minke whale (*Balaenoptera acutorostrata*) spermatozoa intracytoplasmically injected into bovine oocytes. *Zygote* 8 (3): 267–274.

2001 (3)

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