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Cover photo: School of Antarctic minke whales.

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**TECHNICAL REPORTS OF THE
INSTITUTE OF CETACEAN RESEARCH**

TEREP-ICR

No. 1

The Institute of Cetacean Research (ICR)

Tokyo, 2017

Foreword

It is a pleasure for me to introduce the first issue of the Technical Reports of the Institute of Cetacean Research (TEREP-ICR). The main objective of the TEREP-ICR is to describe and report on the process, progress, or results of technical or scientific research, or the state of a technical or scientific research program conducted by the Institute of Cetacean Research (ICR). To inform a wide audience of the field and analytical research activities of the ICR, the TEREP-ICR will be written in English and will be published in December of each year.

In principle the TEREP-ICR will include two kinds of articles: research articles and commentary articles. In addition, the TEREP-ICR will publish in each annual issue a summary of international meetings in which ICR scientists have participated, and an updated list of peer reviewed publications based on ICR research. The main focus of the research articles will be on biological, ecological, stock assessment and resource management research, but studies related to social science will be also included. Articles will be based on data collected by surveys under Special Permit Scientific research, and other surveys conducted by ICR.

TEREP-ICR is a non-peer reviewed journal, and articles included will be considered as 'non-archival' publications, so that the authors of articles published in the TEREP-ICR will be free to publish them elsewhere in peer-reviewed venues with or without modifications.

I sincerely hope that the TEREP-ICR will contribute to increasing understanding of the technical and research activities conducted by the ICR among the international scientific community.

Dr. Yoshihiro Fujise
Director General ICR
Tokyo, December 2017

Editorial

Welcome to the first issue of the Technical Reports of the Institute of Cetacean Research (TEREP-ICR). TEREP-ICR is a non-peer reviewed journal and therefore the contents of the articles published here will be primarily the responsibility of the respective authors. Work by the ICR's editorial team is limited to making suggestions of topics for articles and to keeping editorial consistency among the different articles. This first issue contains eight research articles and one commentary article. The first research article is an overview of the research programs conducted at the ICR, which provides a general background for the subsequent technical research articles focused this time on ICR's main research topics including stock structure, abundance, biological parameters, feeding ecology and environmental pollutants, in both the Antarctic and western North Pacific. This first issue also includes routine sections to outline the contribution of ICR scientists to international meetings in 2017 as well as their contribution in terms of peer reviewed publications up to 2017.

Dr. Luis A. Pastene
Head of Science ICR
Editor TEREP-ICR
Tokyo, December 2017

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Technical Report (not peer reviewed)

An overview of the research programs on large whales conducted by the Institute of Cetacean Research

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ABSTRACT

This paper summarizes the research programs and related activities conducted by scientists of the Institute of Cetacean Research (ICR). ICR conducts biological and ecological research of whales and their ecosystem through different research programs: special permit scientific programs involving lethal and non-lethal techniques; dedicated sighting survey programs; DNA register for large whales and market molecular monitoring programs; and cetacean stranding record programs. This paper summarizes those research programs, including their objectives and methodologies. Outputs of specific studies based on data collected will be published in the annual series of the Technical Reports of ICR (TEREP-ICR).

INTRODUCTION

The Institute of Cetacean Research (ICR) was founded in 1987 as a foundational juridical person whose legal status is authorized by the Minister of Agriculture, Forestry and Fisheries of the Government of Japan. ICR has the purpose of contributing to the appropriate management and utilization of marine resources by conducting research on marine mammals centered on whales, and investigations of the international situation concerning large whales.

The Survey and Research Division of ICR is in charge of the biological and ecological research of whales and their ecosystem. The division has well-equipped laboratories to study whale biology, environmental pollutants and molecular ecology, and includes a number of national and international scientists, who conduct specific studies in the context of the objectives of the research programs conducted by the institute.

Outputs of the research are available in a substantial number of peer-reviewed publications and scientific documents presented to national and international meetings, mainly to meetings of scientific committees of international organizations in charge of the conservation and management of marine resources including large whales, for example working groups of the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) and the Scientific Committee (SC) of the International Whaling Commission (IWC).

The Technical Reports of the ICR (TEREP-ICR) will publish outputs of specific studies conducted by ICR

scientists. As an introduction to such outputs, this paper summarizes the research programs conducted by the institute, including their objectives and methodologies.

RESEARCH PROGRAMS

Special permit scientific research

ICR has been in charge of implementing some special permit research programs under Article VIII of the International Convention for the Regulation of Whaling (ICRW). These research programs involve both lethal and non-lethal components. Among the former component, a limited number of whales have been taken. The research plans have been presented and commented on by the IWC SC, and data and results obtained have been reviewed by workshops of specialists sponsored by the IWC SC and by the IWC SC itself.

Antarctic

The first program was the Japanese Whale Research Program under Special Permit in the Antarctic (JARPA), which was conducted between the austral summer seasons of 1987/88 and 2004/05. The JARPA had four main objectives: a) estimation of biological parameters to improve the stock management of the Southern Hemisphere minke whale; b) elucidation of the role of whales in the Antarctic marine ecosystem; c) elucidation of the effect of environmental changes on cetaceans; and d) elucidation of the stock structure of Southern Hemisphere minke whales to improve stock management. The second phase of JARPA, JARPAII, started with two feasibility surveys in

the seasons 2005/06 and 2006/07. The first full survey started in the 2007/08 season. The objectives of the JARPAII were the following: a) monitoring the Antarctic ecosystem (whale abundance trends and biological parameters; krill abundance and the feeding ecology of whales; effects of contaminants on cetaceans; and cetacean habitat); b) modeling competition among whale species and future management objectives (constructing a model of competition among whale species; and new management objectives including the restoration of the cetacean ecosystem); c) elucidation of temporal and spatial changes in stock structure; and d) improving the management procedure for Antarctic minke whale stocks. See details of the objectives, methodology and outputs from JARPAII in Pastene *et al.* (2014).

The current New Scientific Whale Research Program in the Antarctic Ocean (NEWREP-A), started in the austral summer season 2015/16 as a 12-year long research program. NEWREP-A has two main objectives: a) improvement in the precision of biological and ecological information for the application of the Revised Management Procedure (RMP) to the Antarctic minke whales; and b) investigation of the structure and dynamics of the Antarctic marine ecosystem through building ecosystem models. In order to attain the first objective, four sub-objectives were set: (i) abundance estimates for Antarctic minke whales taking into account of $g(0)$ and additional variance; (ii) improvement of precision of biological and ecological parameters; (iii) refinement of stock structure hypotheses of Antarctic minke whale in Areas III–VI for the implementation of the RMP; and (iv) specification of RMP *Implementation Simulation Trials (ISTs)* for the Antarctic minke whales (GOJ, 2015). In order to attain the second objective, four sub-objectives were set: (i) ecological research (krill abundance estimate and oceanographic observation); (ii) abundance estimate of some cetacean species as input data for ecosystem modelling; (iii) estimation of prey consumption by the Antarctic

minke whale and its nutritional condition; (iv) ecosystem modelling (spatial interaction among baleen whales and consideration of predators-prey system and allometric reasoning). See details of the objectives and methodology of NEWREP-A in GOJ (2015).

An outline of the research area, survey methodology and data being obtained by NEWREP-A are given below.

Research area

The research area of NEWREP-A is shown in Figure 1. The research area comprises the Indian and western South Pacific regions of the Antarctic, which involves IWC Management Areas III, IV, V and VI.

Research activities in the field based on lethal techniques

The lethal part of NEWREP-A involves the sampling of a limited number of Antarctic minke whales ($n=333$ whales annually).

Sampling methodology

Track-lines for sighting and sampling of Antarctic minke whales are generally designed south of 60°S. Survey courses are established in offshore and ice edge waters of the research area by the line transect method. Two or three sampling and sighting vessels advance along parallel track-lines 7n. miles apart, at a standard speed of 11.5 knots. Basically, each of the sampling and sighting vessels change the track-line order every day to avoid possible sighting bias produced by fixed positions. The starting point each day is set at the most advanced position where one of the vessels ended the surveys on the previous day. A maximum of two Antarctic minke whales per school sighted is sampled randomly.

All whales are taken using explosive grenades to attain instantaneous death in line with existing norms of whale killing methods. If instantaneous death is not achieved by the primary killing method, a suitable secondary method, such as a large caliber rifle or another grenade is chosen,

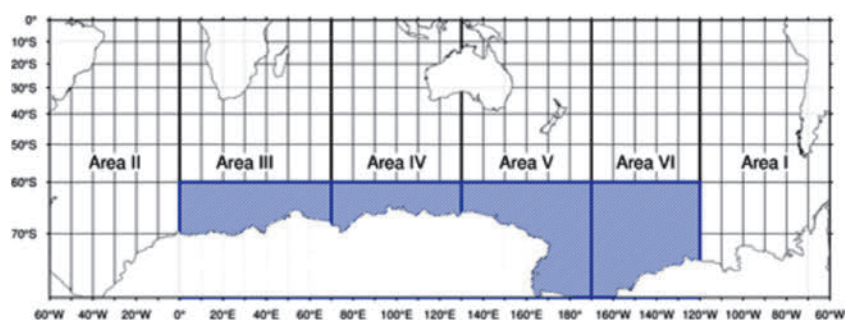


Figure 1. Research area of NEWREP-A.



Figure 2. Biological survey of Antarctic minke whale under the NEWREP-A.

depending on the condition of the whale.

Biological surveys

Sampled whales are immediately transported to the research base vessel, where biological measurements and sampling are carried out in a systematic manner by researchers (Figure 2). The following main data/samples are obtained on board for each whale sampled (see also Table 1). Samples and data collected are required for the analyses related to the main objectives and sub-objectives of NEWREP-A.

1. Morphometrics for studies on stock structure.
2. Body weight, blubber thickness and total fat weight measurements for nutritional studies.
3. Reproductive organs (testis and ovaries) for determination of sexual maturity.
4. Earplugs for age determination.
5. Stomach contents (qualitative and quantitative) for studies on feeding ecology.
6. Other biological samples such as ocular lenses for age determination and tissues for genetic studies on stock structure.

Research activities in the field based on non-lethal techniques

Dedicated sighting surveys

Data collected by these surveys are required for the abundance estimation of whales. Sighting surveys are conducted by the Line Transect Method. Survey protocols follow the IWC SC's Requirements and Guidelines for Conducting Surveys and Analyzing Data within the Revised Management Scheme (IWC, 2012) and are conducted under the oversight of the IWC SC. Sighting protocols are the same as those in the former International Decade for Cetacean Research/Southern Ocean Whale and Ecosystem Research (IDCR/SOWER) (Matsuoka *et al.*, 2003).

In general, the sighting surveys cover areas south of 60°S. Surveys are planned to cover one IWC Management Area (one of the Areas III to VI) in a year. Cruise tracks are designed systematically in accordance with mathematical and scientific calculation in the research area. The sighting survey is conducted using (1) Closing mode and (2) Passing with Independent Observer mode. Both survey modes follow the protocol already endorsed for the IDCR/SOWER surveys. Primary search effort is conducted only when weather conditions are acceptable (see details in Matsuoka *et al.*, 2003) (Figure 3).

Biopsy and photo-ID (Figures 4 and 5)

Biopsy and photo-ID experiments are conducted routinely for large whales such as blue, fin, humpback, southern right and killer whales. Feasibility studies of biopsy sampling and telemetry for Antarctic minke whales are also conducted. Photo-ID and DNA data obtained from biopsy samples are useful for studies of distribution, movement and stock structure of whales.



Figure 3. Sighting activity by the dedicated sighting vessels.



Figure 4. Humpback whale biopsy sampling by the dedicated sighting vessel.

(a) Head callosities



(b) Pigmentation pattern in ventral flukes



Figure 5. Morphological characteristics used for individual identification in southern right (a) and humpback (b) whales.

Krill survey (Figure 6)

The main objective of the krill surveys is to estimate the relative abundance of Antarctic krill acoustically, and to obtain the length frequency distribution and maturity stage of Antarctic krill in the survey area. Information on krill distribution and abundance is important as input data for the development of ecosystem modelling. Acoustic data using quantitative echosounders EK80 and EK60 are recorded continuously. Net samplings using a small ring net and an Isaacs-Kidd Midwater Trawl (IKMT) are carried out to identify species and size compositions of plankton echo signs. See details in GOJ (2015).

Oceanographic observations (Figure 7)

The conductivity-temperature-depth profilers (CTD) are used to obtain water temperature and salinity data. Oceanographic data are important to understand the physical environment in which whales live. Changes in oceanographic conditions determine the distribution of krill, which is the main prey species of large baleen whales.

Marine debris (Figure 8)

The observation and collection of debris, both in the environment and in the stomachs of the whales sampled provide valuable information on the surrounding environment of whales. Such information is collected systematically during the sampling and sighting surveys.

Data and analyses

Table 1 shows the kinds of data and samples being collected by NEWREP-A. These data are analyzed in the context of the objectives and sub-objectives of the program, with some of the analyses of temporal trends incorporating data from the previous research programs JARPA and JARPAII (see details of the data and samples collected by JARPA and JARPAII in IWC, 2008 and IWC, 2015, respectively).



Figure 6. Small ring net (left) and an Isacs-Kidd Midwater Trawl (IKMT) net (right).



Figure 7. Oceanographic survey by CTD.



Figure 8. Rope found in the stomach contents of an Antarctic minke whale.

Research outputs

As in the cases of JARPA and JARPAIL, the scientific outputs from NEWREP-A will be summarized as a) scientific reports to be presented to international meetings, mainly to the IWC SC meetings; and b) as peer-reviewed publications. In the case of the previous JARPA, results were presented to the IWC SC review workshops conducted in 1997 (mid-term review) (IWC, 1998) and 2006 (final review) (IWC, 2008). The results of the JARPAIL were presented to the IWC SC review workshops conducted in 2014 (IWC, 2015). Peer-reviewed publications based on JARPA and JARPAIL are presented in this issue of TERE-ICR.

Western North Pacific

The first program was the Japanese Whale Research Program under Special Permit in the western North Pacific (JARPN), which was conducted in the spring-autumn seasons between 1994 and 1999. The main objective was to elucidate the stock structure of common minke whales in the Pacific side of Japan. A second objective, 'the feasibility study on the feeding ecology of minke whales in the research ground,' was added in 1996. The second phase of JARPN, JARPNI, started with two feasibility surveys in 2000 and 2001. The first full survey started in 2002. The objectives of the JARPNI were the following: a) feeding ecology and ecosystem studies; b) monitoring environmental pollutants in cetaceans and the marine ecosystem; and c) stock structure of large whales. See details of the objectives, methodologies and outputs from JARPNI in Tamura *et al.* (2016).

The current New Scientific Whale Research Program in the western North Pacific (NEWREP-NP) started in 2017 as a 12-year long research program. NEWREP-NP has two main objectives: a) contribution to optimizing the establishment of a sustainable catch limit for common minke whales in the coastal waters of Japan; and b) contribution to the RMP/IST for North Pacific sei whales. In order to attain the first objective, four secondary objectives are set: (i) investigate the spatial and temporal occurrence of J stock common minke whales around Japan, by sex, age and reproductive status; (ii) estimate the abundance of the J and O stocks in coastal waters of Japan; (iii) verify that there is no structure in the O stock common minke whales in the Pacific side of Japan; and (iv) improve RMP trials by incorporating age data in their conditioning. In order to attain the second objective, four secondary objectives are set: (i) abundance estimates for North Pacific sei whales taking account additional variance; (ii) estimation of biological and ecological parameters in North Pacific sei whales for RMP *Implementation*; (iii) study of the pattern of movement of whales of the 'pelagic stock' within the feeding grounds and between feeding and

Table 1
Data and samples collected under the NEWREP-A (from GOJ, 2015).

Data	Sample
Abundance estimate	
*# Weather data	
*# Effort data	
*# Sighting record of whales	
*# Angle and distance experiments	
*# Ice edge line	
Environmental data	
# Temp. Salin. (CTD)	
# Echo sound (krill distribution/abundance)	
Marine debris (sea surface)	
Antarctic minke whale	
*# Catching date and location	# Prey species in stomach for feeding ecology
Photographic record of external character	# Feces and colon contents for feeding ecology
Record of internal and external parasites	*# Testis for reproductive study
*# Sex and body length	*# Ovary for corpora counting and reproductive study
* Body proportion for stock structure	Mammary gland and endometrium for reproductive study
* Skull measurements (length and breadth) for stock structure	*# Earplug for age determination
* Satellite tracking for stock structure	*# Ocular lens for age determination
# Body weight for feeding ecology	*# Baleen plates for age determination
# Organ weight including fat weight for feeding ecology	*# Tissue samples for genetic study
# Diatom film record for feeding ecology	*# Tissue and organ samples for chemical study
# Blubber thickness for feeding ecology	# Tissue and plasma samples for physiological study
# Stomach content : freshness and weight for feeding ecology	Vertebral epiphyses for physical maturity
# Diving behaviour for feeding ecology	
*# Testis weight for reproductive study	
Mammary gland : lactation status and measurement for reproductive study	
Fetal number, sex, length and weight for reproductive study	
Marine debris (stomach)	
Gross pathological observation and sampling	
Other large whales	
Photo-ID	Skin sample (biopsy)

*: Data or samples to be used for Main Objective I; # : Data or samples to be used for Main Objective II (other items will be used for other research purposes)

breeding grounds; and (iv) specification of RMP *ISTs* for North Pacific sei whales. See details of the objectives and methodology of NEWREP-NP in GOJ (2017).

An outline of the research area, survey methodologies and data being obtained by NEWREP-NP are given below.

Research area

The research area of NEWREP-NP is shown in Figures 9a and 9b. The research area for lethal sampling comprises the Pacific side and the Okhotsk Sea side of Japan, which involves IWC Management Sub-areas 7, 8, 9 and 11 (Figure 10). The research area for the sighting surveys

comprises the Pacific side, the Sea of Japan side and the Okhotsk Sea side of Japan, which involves IWC Management Sub-areas 6, 7, 8, 9, 10 and 11.

Research activities in the field based on lethal techniques

The lethal part involves the sampling of a limited number of common minke whales (n=80 whales annually in sub areas 7CS and 7CN; n=47 whales annually in sub area 11; and n=43 whales annually in sub areas 7WR, 7E, 8 and 9), and of sei whales (n=134 whales annually in sub areas 7WR, 7E, 8 and 9).

Sampling methodology (offshore component)

Survey courses are established in the research area by the Line Transect Method. Two sampling and sighting vessels advance along parallel track-lines 7n. miles apart, at a standard speed of 10.5 knots. All common minke and sei whales sighted as primary and secondary sightings, excluding cow and calf pairs, are targeted for sampling. When a sighting consists of more than one animal, the first targeted animal is selected using tables of random sampling numbers (TRS).

All whales are taken using explosive grenades to attain instantaneous death in line with existing norms of whale killing methods. If instantaneous death is not achieved by the primary killing method, a suitable secondary method, such as a large caliber rifle or another grenade is chosen, depending on the condition of the whale.

Sampling methodology (coastal component)

A land-based operation system which takes into account

operational capacity, ability, and arrangements of the small boats is applied, and thus is different from the sampling procedures adopted by the offshore component. A predetermined course (direction from the port) at an angle of regular intervals (usually 10–15 degree intervals) is set up by the head office, and allocated to the respective boats. The boats depart the port with their respective course, and start searching at a survey speed of 10–11 knots. All common minke whales sighted as primary and secondary sightings, excluding cow and calf pairs, are targeted for sampling. When a sighting consists of more than one animal, the first targeted animal is selected using tables of random sampling numbers (TRS). All whales are taken using explosive grenades to attain instantaneous death in line with existing norms of whale killing methods. If instantaneous death is not achieved by the primary killing method, another grenade is chosen as a suitable secondary method.

Biological surveys

Sampled whales are immediately transported to the research base vessel (offshore component) or land base (coastal component), where biological measurements and sampling are carried out in a systematic manner by researchers (Figure 11). The kind of data and biological sampling is similar to those in NEWREP-A.

Research activities in the field based on non-lethal techniques

Dedicated sighting surveys

Sighting surveys are conducted by the Line Transect Method and the survey protocols are the same as in the Antarctic dedicated sighting surveys.

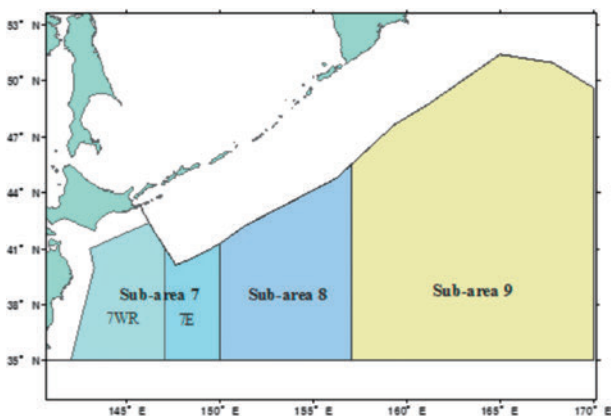


Figure 9a. Research area of NEWREP-NP for lethal sampling (offshore component).

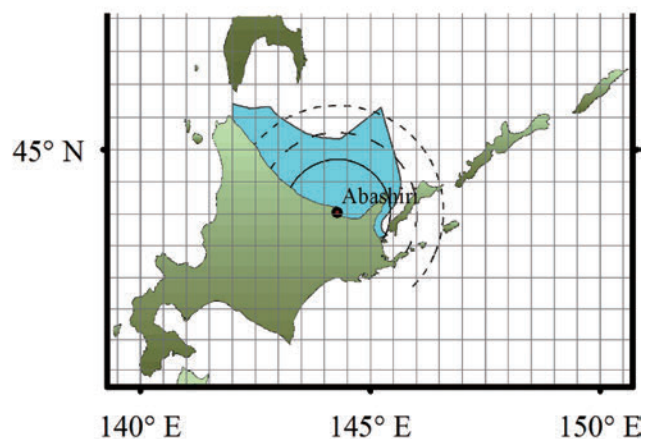
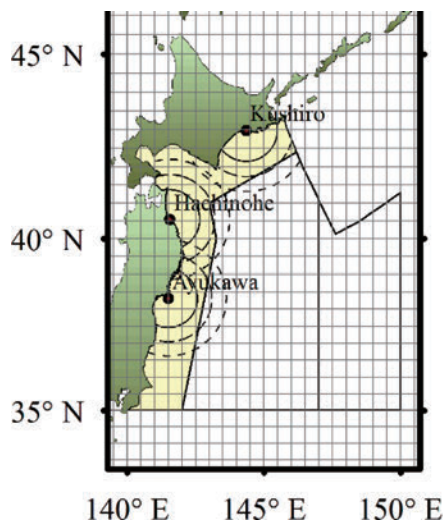


Figure 9b. Research area of NEWREP-NP for lethal sampling (coastal component).

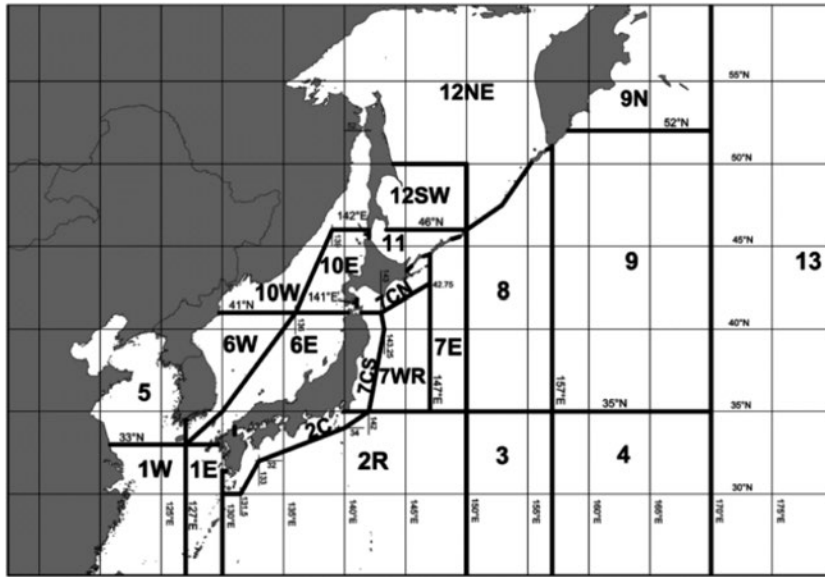


Figure 10. Sub-areas to be covered by the dedicated sighting surveys under the NEWREP-NP (Sub-areas 6E, 10E, 11, 7CS, 7CN, 7WR, 7E, 8 and 9).

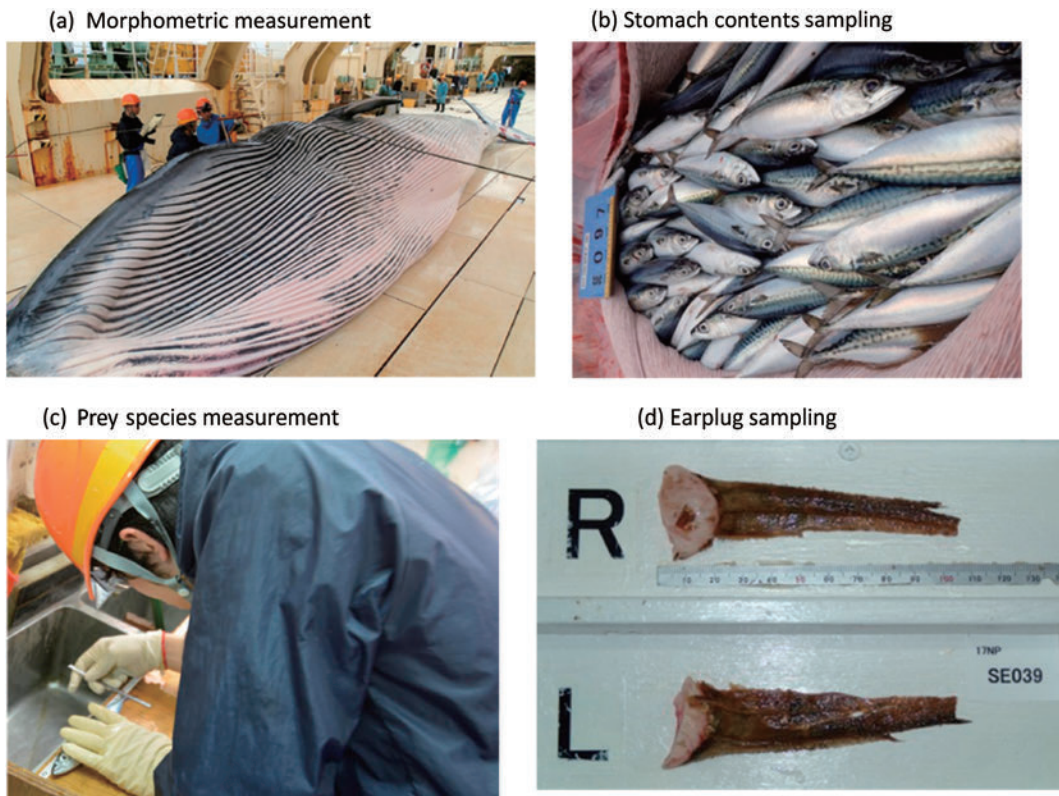


Figure 11. Biological surveys of sei whales under the NEWREP-NP.

Provided that two dedicated sighting vessels are available, surveys are planned to cover sub-areas 7, 8 and 9 in summer once in three years. Sub-areas 6E, 10E and 11 would be surveyed once in the other three years (Figure 10).

Biopsy and photo-ID

Biopsy and photo-ID experiments are conducted routinely for large whales such as blue, fin, humpback, North

Pacific right, gray and killer whales. Feasibility studies of biopsy sampling for common minke whales are also conducted. As in the Antarctic, photo-ID and DNA data obtained from biopsy samples are useful for studies of distribution, movement and stock structure of these whales (Figure 12).



Figure 12. Biopsy sampling of a sei whale under the NEWREP-NP.



Figure 13. Satellite tagging of sei whales under the NEWREP-NP.

Satellite tagging

Satellite tagging experiments are conducted for common minke and sei whales to elucidate movement within the feeding grounds and the location of breeding grounds (Figure 13).

Oceanographic observations

In the offshore component of NEWREP-NP, oceanographic conditions are investigated using data collected by ocean circulation models such as FRA-ROMS (Okazaki *et al.*, 2016). In the Sanriku region, the oceanographic surveys are conducted using a trawler-type R/V, 'Miyashio' (199 GT) by the Miyagi Prefecture Fisheries Technology Institute. Vertical oceanographic observations are conducted using CTD. Subsurface (approximately 5 m water depth) temperature, salinity and chlorophyll-*a* index are recorded every minute along the track-lines.

Marine debris

As noted above, the observation and collection of debris, both in the environment and in the stomachs of the whales sampled, provide valuable information on the surrounding environment of whales. Such information is collected systematically during the sampling and sighting surveys of NEWREP-NP (Figure 14).

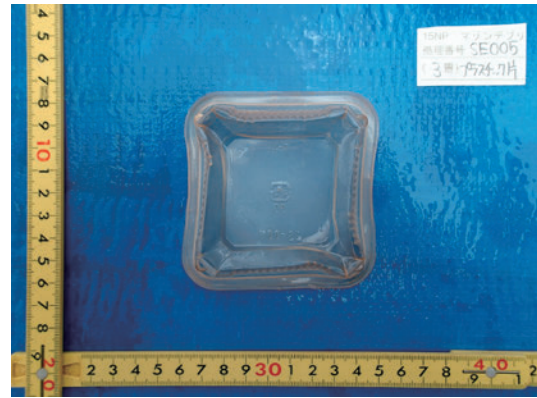


Figure 14. Plastic bowl found in the stomach of a sei whale.

Data and analyses

Table 2 shows the kinds of data and samples being collected by NEWREP-NP. These data are analyzed in the context of the objectives and sub-objectives of the program, with some of the analyses on temporal trends incorporating data from the previous research programs JARPN and JARPNII (see details of the data and samples collected by JARPN and JARPNII in IWC, 2001 and IWC, 2016a, respectively).

Research outputs

As in the cases of JARPN and JARPNII, the scientific output from NEWREP-NP is summarized as a) scientific reports to be presented to international meetings, mainly to the IWC SC meetings; and b) as peer-reviewed publications. The results of the JARPN were presented to the IWC SC review workshop conducted in 1999 (IWC, 2001). In the case of the JARPNII, results were presented to the IWC SC review workshops conducted in 2009 (mid-term review) (IWC, 2010) and 2016 (final review) (IWC, 2016a). Peer-reviewed publications based on JARPN and JARPNII are presented in this issue of TERE-ICR.

International dedicated sighting surveys

One example of international sighting surveys in which ICR scientists participate is the IWC-Pacific Ocean Whale and Ecosystem Research (POWER) program. The IWC-POWER program is an international effort coordinated by the IWC and designed by the IWC SC with special partnership of the Japanese Government. The vessel is provided by the Government of Japan. The IWC-POWER surveys in the North Pacific follow the series of IWC IDCR/SOWER surveys that were conducted in the Antarctic since 1978. The IWC POWER program has the following main objectives: (a) provide information for the proposed future *in-depth assessment* of sei whales in terms of both abundance and stock structure; (b) provide information relevant to the

Table 2
Data and samples collected under NEWREP-NP, by research objective (from GOJ, 2017).

Data		Sample	
Abundance estimate			
1,2,3	Weather data		
1,2,3	Effort data		
1,2,3	Sighting record of whales		
1,2,3	Angle and distance experiments		
Common minke whale/sei whale			
1,2,3,4	Catching date and location	1,2,3,4	Testis
1	Photographic record of external character	1,2,3,4	Ovary
1,2,3,4	Sex and body length	1,2,4	Earplug
1,2,3	Satellite tracking	1,2,4	Ocular lens
3,4	Body weight	1,2,3,4	Baleen plates
3,4	Blubber thickness and nutrition condition	1,2,4	Tissue samples for genetic study (including fetus)
3,4	Stomach content: freshness and weight	1,2,3,4	Tissue and organ samples for chemical study
1,2,3,4	Testis weight	4	Tissue and plasma samples for physiological study
1,2	Fetal number, sex, length and weight	3,4	Prey species in stomach
4	Marine debris (in stomach)		
Other large whales			
5	Photo ID	3,5	Skin sample (biopsy)

¹: Data or samples to be used for Primary Objective I. ²: Data or samples to be used for Primary Objective II. ³: Data or samples to be used for Ancillary Objective I. ⁴: Data or samples to be used for Ancillary Objective II. ⁵: Data or samples to be used for Ancillary Objective III

Implementation Reviews of whales in terms of both abundance and stock structure; (c) provide baseline information on distribution and abundance for a poorly known area for several large whale species/populations (including those that were known to have been depleted in the past, but whose status is unclear); (d) provide biopsy samples and photo-identification data to contribute to discussions of stock structure for several large whale species/populations and (e) provide essential information for the intersessional workshop to plan for a medium to long term international research programme in the North Pacific (IWC, 2016b).

Research area

The research area was set north of 20°N, south of the Aleutian Islands, between 170°E and 135°W in the period 2010–2016 (Figure 15). The research area for the 2017–19 period and agreed by the IWC SC is shown in Figure 16.

Research activities

Dedicated sighting surveys

Survey methodology is similar to that described above for the dedicated sighting surveys in the Antarctic.

Other surveys

Photo-ID and biopsy experiments are conducted for North Pacific right, blue, fin, sei, Bryde’s, gray, humpback, sperm and killer whales. Marine debris information is collected systematically. Acoustic studies using sonobuoys

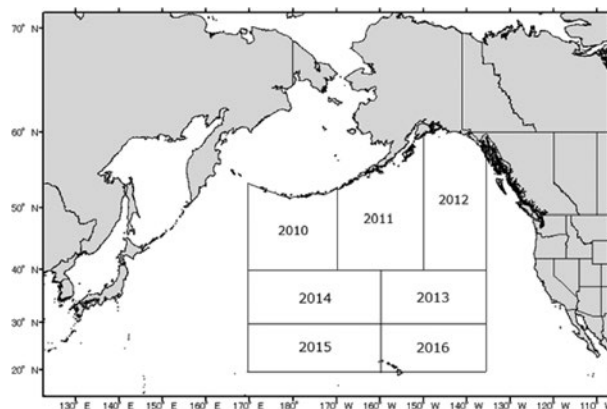


Figure 15. Research area of the IWC POWER program in 2010–2016.

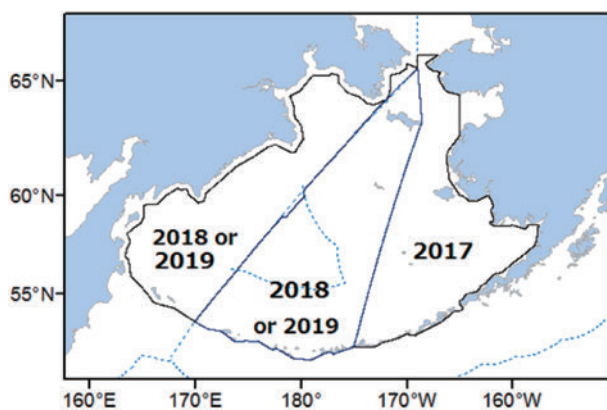


Figure 16. Research area of the IWC POWER program in 2017–2019.

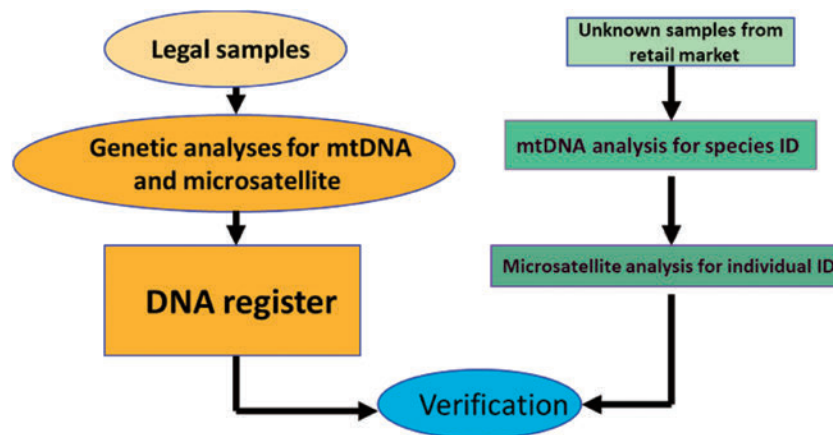


Figure 17. Flow chart of the DNA monitoring system for regulation of whale products in the market in Japan.

have also been conducted since 2017.

Data and analyses

The data and samples collected are analyzed in the context of the objectives of the program (see details of the data and samples collected by IWC-POWER in Matsuoka *et al.*, 2011, 2012, 2013, 2014, 2015, 2016 and 2017). Results of analyses are presented to the annual meetings of the IWC SC and/or published in peer-reviewed journals (e.g. Kanda *et al.*, 2013; 2015; Hakamada *et al.*, 2017).

DNA register and market monitoring

Background

The basic position of the Government of Japan is that matters related to regulation of the market for whale products are outside the jurisdiction of the IWC. Therefore management and regulation of whale products in Japan is carried out by the Government.

As in the case of Norway and Iceland, Japan is using a DNA-based system for tracking whale products in the market, and this task has been assigned to the ICR. The system involves two main components: establishment and maintenance of a diagnostic DNA register for large whales and molecular market monitoring through systematic surveys in the retail market (Figure 17).

Technical details of the DNA register for large whales

Source of tissue samples

In Japan there are two main sources of tissue samples of large whales: 1) special permit scientific whaling in the North Pacific (JARPN/JARPNII and NEWREP-NP) and the Antarctic (JARPA/JARPAII and NEWREP-A), and 2) coastal by-catches in set nets.

In the case of special permit scientific whaling, samples for genetic analysis are collected from each whale by researchers. These involve skin samples (two or three pieces

of 5×5×5 mm kept in 99% ethanol). A large amount of information of each whale sampled is collected using established protocols including species name, catch date and location (longitude, latitude), body length and sex.

The Japanese regulation on by-catches (established from 1 July 2001) requires that all animals should be DNA-registered before whale meat can be sold in the market. Details of the regulation and procedure can be found in the following web page: <http://www.icrwhale.org/pdf/higekujira.pdf>.

Skin or muscle samples (5×5×5 cm) are taken by the fisherman and sent to the laboratory at the ICR (as frozen samples). Together with these samples, the fishermen should provide information following an established protocol including: species, date and location of the by-catch, type of set net, body length and sex.

Genetic markers

The Japanese DNA register for large whales is composed of three parts: a) an approximately 500 bp fragment of the 5'-end of the mitochondrial DNA control region, which is used for the purpose of species identification of unknown whale products, based on phylogenetic analyses; b) a set of nuclear DNA markers (microsatellites: 13–17 loci in each species), which is used for the purpose of individual identification; and c) data from Y chromosome DNA, which is used for the purpose of sex identification. See details in Pastene and Goto (2006).

Technical details of the market monitoring

Sampling procedure

One or two technicians, who are familiar with market operations, are appointed to carry out the sampling of whale products in the retail market. In each year, a total of 350 samples have been collected between September and December, involving around 19 cities or towns. Table 3 shows

Table 3

The number of whale products by sampling localities and tissue type purchased in the 2016 survey.

City/Town	Meat	Blubber	Ventral grooves	Total
Sapporo	15	2	13	30
Sendai	25	3	7	35
Niigata	4	12	4	20
Kanazawa	4	2	1	7
Noto	4	5	1	10
Anamizu	1	0	0	1
Nanao	1	1	0	2
Toyama	4	0	2	6
Himi	2	6	1	9
Nagoya	13	0	4	17
Kochi	8	9	8	25
Kobe	13	5	6	24
Osaka	14	7	7	28
Taiji	3	6	1	10
Nachikatsuura	4	4	3	11
Hiroshima	18	0	3	21
Shimonoseki	15	8	9	32
Fukuoka	13	6	11	30
Nagasaki	12	7	13	32
Total	173	83	94	350

an example of cities and whales products sampled in the 2016 survey. The sampling attempts to cover the entire Japanese archipelago but the method of sampling is not a random procedure. The following information is collected for each whale product sample: kind of whale product, sampling locality, date, and price. Once sampled, the products are sent to the laboratory at the ICR for genetic analysis.

Genetic markers

The laboratory work for the whale product samples involves the same genetic markers used for the DNA register, which were explained above.

Outputs

Results of the comparisons between ‘test’ (sequences and genotype of whale’s products) and ‘type’ (sequences and genotype in the register) samples are summarized in an annual report to the Fishery Agency of the Government of Japan. An example of analyses is available in Goto and Pastene (2000).

Stranding record

Background

The ICR has been recording strandings of marine mam-

mals on the Japanese coast since 1986, with the purpose of obtaining information on their migration and distribution through the record and analysis of samples collected from the stranded animal.

Record procedure

The ICR compiles information on stranding received from researchers, government officials or the general public who send the information following a protocol and record sheet developed and distributed by the ICR among Japanese coastal prefectures, or from other sources such as newspapers. See details on the protocol and data sheet in the following link: (<http://www.icrwhale.org/pdf/stranding.pdf>).

Together with the stranding record sheet, people are requested to send skin or muscle samples for genetic analysis in pieces of 5×5×5 mm kept frozen or in 99% ethanol. The ICR corroborates species identity by DNA analysis.

Outputs

Information on stranding of large cetacean has been reported to the IWC SC annual meetings on a voluntary base as Progress Reports on Cetacean Research. Stranding records since 1996 are available in the following link: (<http://www.icrwhale.org/zasho2.html>).

Data and samples from strandings are available to interested scientists under the data access protocol of the ICR.

RESEARCH COLLABORATION

Several aspects of the research programs described above are conducted as research collaboration between the ICR scientists and several Japanese and international research organizations. Within Japan in particular the ICR conducts research collaboration with the National Research Institute of Far Seas Fisheries and with the Tokyo University of Marine Science and Technology. A list of peer-reviewed publications derived from research collaborations is included in the relevant section of this issue of the TEREP-ICR. Protocols for data access and research proposals are available at the ICR Home Page.

FINAL REMARKS

The ICR has been heavily involved in the design and implementation of several research programs on large whales and the ecosystem. The ICR will maintain this effort by continuing the ongoing surveys. A unique and valuable data base of whale biology and whale’s environment has become available. Analyses of these data have contributed and will continue contributing to the understanding of whale biology and ecology, and to the

management of whaling on a sustainable basis.

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Technical Report (not peer reviewed)

What do we know about the stock structure of Antarctic minke whales in the Indo-Pacific region of the Antarctic? A brief review of methodologies and research outputs

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ABSTRACT

This paper presents a brief review of the studies on stock structure of the Antarctic minke whale conducted under the Japanese Whale Research Program under Special Permit in the Antarctic (JARPA, austral summer seasons 1987/88–2004/05) and the Second Phase of JARPA (JARP-II, 2005/06–2013/14), and summarizes the main research findings. The stock structure of the Antarctic minke whale was examined using genetic and non-genetic approaches. Both research programs were conducted in the International Whaling Commission (IWC) Management Areas III E (35°–70°E), IV (70°–130°E), V (130°E–170°W) and VI W (170°–145°W). Both genetics, *i.e.*, RFLP of the whole mitochondrial DNA (mtDNA), sequencing of mtDNA control region, microsatellite DNA, and non-genetic, *i.e.*, morphometric and mean body length at physical maturity analyses were used. These studies provided evidence for the occurrence of at least two stocks in the research area, which were called ‘eastern Indian Ocean stock’ (I-stock) and ‘western South Pacific stock’ (P-stock), which overlap geographically in the central part of the research area. The results of a modelling approach incorporating genetic and morphometric data suggested that the two stocks have a soft boundary (or area of mixing) mainly in the western part of Area V (130°–165°E), which changes by year and sex.

INTRODUCTION

The Antarctic minke whale *Balaenoptera bonaerensis* (Figure 1), like all the other Southern Hemisphere baleen whales species apart from the Bryde’s whale (*B. edeni*), was managed by the International Whaling Commission (IWC) on the basis of six geographical ‘Areas’ (Figure 2). The IWC established these Areas from the 1974/75 austral summer season, based mainly upon information from Mackintosh (1942; 1966) on distribution of catches of blue, fin and humpback whales (see review by Donovan, 1991). These Areas were used by the IWC for the implementation of the New Management Procedure (NMP) on baleen whale species.

However, biological evidence for the particular bound-

aries are weak, especially for those species such as the Antarctic minke whale, whose data were not considered when the original management Areas were established. In this regard, important questions were formulated originally by Hoelzel and Dover (1989): ‘Are the Antarctic minke whales found in two geographically distinct management Areas from two different genetic stocks?’ or ‘Are individuals from more than one genetic stock present in a particular management Area? If so, what level of interchange may have occurred between different genetic stocks?’ Several approaches were used in the past to identify genetic stocks of this species in the Antarctic feeding grounds and to determine to what extent genetic stocks and IWC management Areas coincide.

Studies on stock structure of the Antarctic minke whale



Figure 1. Antarctic minke whale (*Balaenoptera bonaerensis*).

started at the end of the decade of the 1970's, and results of genetic and non-genetic analyses were revised by the IWC Scientific Committee (IWC SC) during the comprehensive assessment (CA) of the species in 1990. All the analyses presented at the CA were based on samples and data from commercial pelagic whaling in the Antarctic. The genetic studies were based mainly on allozyme at that time, although studies based on mitochondrial and nuclear DNA were also conducted, most of the analyses involved small sample sizes from only Areas IV and V. Non-genetic studies revised in the 1990's CA involved morphology, catch and sighting distribution pattern, analysis of Discovery marks and ecological markers. Results from the different approaches failed to unambiguously identify any isolated stock in the Antarctic (IWC, 1991).

Studies on stock structure under the Japanese Whale Research Program under Special Permit in the Antarctic (JARPA) started after the CA. It was considered that JARPA samples were more useful for studies on stock structure than the commercial samples, given the wider geographi-

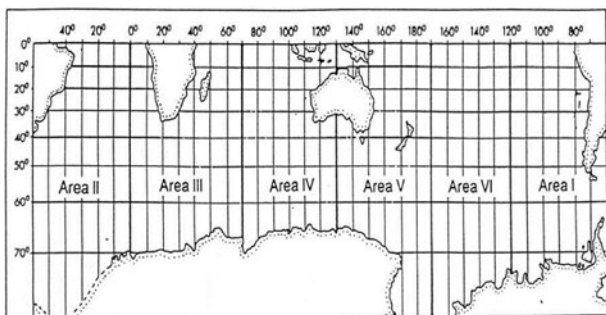


Figure 2. IWC Antarctic Areas for the management of baleen whale species (except Bryde's whale) (Donovan, 1991).

cal coverage of the surveys and that samples were taken along track-lines in a random mode design. Studies on stock structure of Antarctic minke whale continued under the second phase of JARPA (JARPAII).

This paper reviews the studies on stock structure conducted under the JARPA and JARPAII, emphasizing the technical aspects and main research outputs.

SAMPLING AND ANALYTICAL APPROACHES

JARPA

JARPA was conducted during the austral summer seasons 1987/88–2004/05 and the research area was composed of Areas III E (35°–70°E), IV (70°–130°E), V (130°E–170°W) and VI W (170°–145°W) (Figure 2). The main analytical procedure used in the genetics and non-genetic studies was hypothesis testing under the null hypothesis of panmixia.

Genetic approaches

Mitochondrial DNA (mtDNA)

In JARPA, restriction fragment length polymorphisms (RFLP; Box 1.1) was applied for the whole mtDNA (Pastene *et al.*, 1993; 1996). The mtDNA is an extra-chromosomal genome in the cell mitochondria and is inherited only from the mother. This marker is widely used in constructing intra- and related species phylogenies and inferring evolutionary history.

The mtDNA extracted from a total of 6,256 Antarctic minke whales was digested with six-base sequence recognition endonucleases (*AccI*, *BanI*, *EcoRV*, *HincII*, *HpaI* and *SspI*). Restriction fragments were separated by submarine electrophoresis in 1% agarose gels. After electrophoresis, the gels were stained with ethidium bromide and were

Box 1.1 Restriction Fragment Length Polymorphisms

This method can use for total DNA, mitochondrial DNA or specific sequences first amplified from total DNA by PCR. The DNA is digested with restriction endonucleases, which are called 'restriction enzymes', to generate a series of DNA fragments. These restriction enzymes only cut DNA at specific sequences, which yields a consistent set of fragments that can be separated according to the size by agarose gel electrophoresis (Figure 1.1). The RFLP pattern is different between individuals which have different haplotypes because mutations destroy or generate new cutting sites, which enable to measure genetic variations.

Digestion by restricted enzymes

Population 1

Ind. 1 + *Hae III* → ...CTA GGT G CC CTT GAG ATT CCA TAA GGC CCA TAC TCC TGG GGT... (digested)

Ind. 2 + *Hae III* → ...CTA GGT G CC CTT GAG ATT CCA TAA C C CCA TAC TCC TGG GGT... (undigested)

Population 2

Ind. 3 + *Hae III* → ...CTA GGT G CC CTT GAG ATT CCA TAA C C CCA TAC TCC TGG GGT... (undigested)

Ind. 4 + *Hae III* → ...CTA GGT G CC CTT GAG ATT CCA TAA GGC CCA TAC TCC TGG GGT... (digested)

Ind. 5 + *Hae III* → ...CTA GGT G CC CTT GAG ATT CCA TAA GGC CCA TAC TCC TGG GGT... (digested)

Agarose gel electrophoresis

Haplotype	Population 1	Population 2
A	1	2
B	1	1

Figure 1.1 Measurement of genetic variations by RFLP analysis
 Restriction sites shown by arrows from single restriction enzyme (*Hae III* recognizes sequence of 'GGCC' and cuts the site into 'GG' and 'CC') are mapped in five individuals from two populations, and the resulting pattern of restriction fragments on an agarose gel is shown in the right-hand diagram. The right-bottom of the diagram shows a fundamental data for the stock structure analyses generated by RFLP analysis.

This box was written based on Beebee and Rowe (2008) and Lowe *et al.* (2004)

photographed using Polaroid film under an UV irradiation. Distinctive restriction fragment patterns produced by each enzyme were assigned letters. Individuals were assigned haplotypes consisting of a list of the letters designating the fragment profiles produced by each of the six restriction enzymes. Then, the composite haplotype for each individual comprises a string of six letters.

Haplotype frequencies were employed to determine genetic relationships between the samples of the designed strata. Genetic relationships were first quantified using the chi-square statistics for heterogeneity of mtDNA haplotype frequencies (Roff and Bentzen, 1989). The level of significance obtained by this method is referred to in this paper as the *P*-value. A *P*-value smaller than 0.05 was used as a criterion to reject the null hypothesis of panmixia. Additionally, the quantification of the geographical differentiation of mtDNA was carried out using the analysis of molecular variance (AMOVA) of Excoffier *et al.* (1992).

More detailed descriptions of laboratory and analytical procedures were provided in Pastene *et al.* (1993; 1996).

Microsatellite DNA

In addition to the mitochondrial RFLP analysis, microsatellite analyses (Box 1.2) was also applied on JARPA samples. Microsatellites are codominant and become a popular marker for many aspects of molecular ecology, in particular for intraspecific studies, because of its high

mutation rate and polymorphisms compared to other markers.

Microsatellite polymorphisms were examined at six loci in samples from a total of 6,260 minke whales: EV1, EV104 (Valsecchi and Amos, 1996), GT023, GT211, GT4195 (Bérubé *et al.*, 2000), and DlrFCB14 (Buchanan *et al.*, 1996). Polymerase chain reaction (PCR) amplifications at these loci were performed, which were run with an internal size standard (GENESCAN400HD, Applied Biosystems Japan) using BaseStation100 DNA fragment analyzer. Allelic sizes were determined manually in relation to the internal size standard and Antarctic minke whale DNA of known size that were re-run on each gel.

The allele frequencies at the six microsatellite loci were calculated and the departure from expected Hardy-Weinberg genotypic proportions was tested at each locus as well as overall loci. The number of alleles per locus, allelic richness and heterozygosity were also computed. A conventional hypothesis testing procedure was performed using a heterogeneity test in allele frequencies. The probability test or Fisher's exact test with the Markov chain method was used for the heterogeneity tests among minke whales in the designed strata. Statistical significance of the heterogeneity tests was determined using the chi-square value obtained from summing the negative logarithm of *P*-values over the six microsatellite loci (Sokal and Rohlf, 1995).

More detailed descriptions of laboratory and analytical procedures were provided in Pastene *et al.* (2006).

Non-genetic approaches

Morphometric analyses

The relationship between body length and longitudinal strata for ten body measurements of Antarctic minke whale was investigated using ANCOVA. A cluster analysis was also applied for an estimated average length of measurements to examine the degree of relationships among whales in six longitudinal strata. A more detailed description of the analytical procedure was provided in Hakamada (2006).

Mean body length at physical maturity

Mean body length at physical maturity was compared among whales in Areas III, IV, V and VIW for males and females. Physically matured individuals were defined as those with epiphysis fusion occurring in the 6th thoracic vertebrae. The t-test and ANOVA were used for testing differences in mean body length among strata of the JARPA survey. A more detailed description of the analytical procedure was provided in Bando *et al.* (2006).

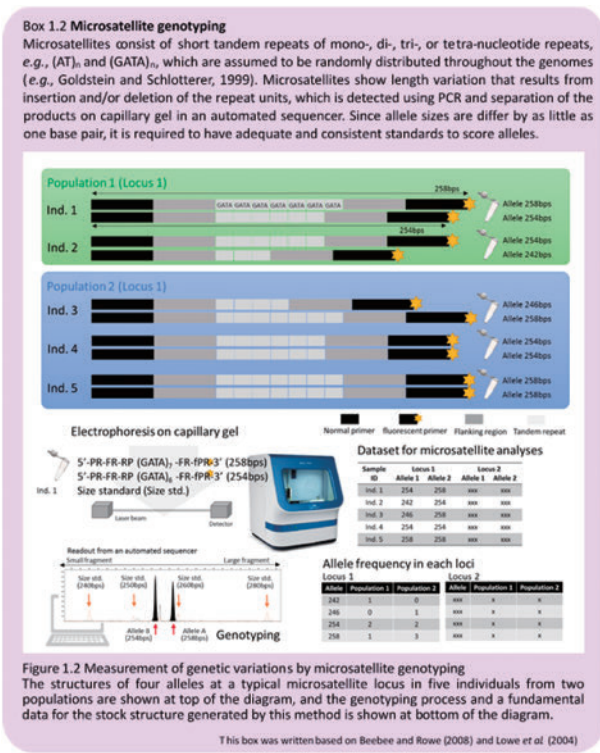


Figure 1.2 Measurement of genetic variations by microsatellite genotyping. The structures of four alleles at a typical microsatellite locus in five individuals from two populations are shown at top of the diagram, and the genotyping process and a fundamental data for the stock structure generated by this method is shown at bottom of the diagram.

This box was written based on Beebe and Rowe (2008) and Lowe *et al.* (2004)

JARPAII

JARPAII was conducted during the austral summer seasons 2005/06–2013/14 in the same research area covered by JARPA. The analyses on stock structure were refined in two ways: by using additional genetic markers and by applying new analytical approaches.

Genetic approaches

Mitochondrial DNA control region sequencing

In JARPAII, sequencing analysis (Box 1.3) was performed instead of the mitochondrial RFLP used in JARPA, which provides higher resolution.

A 338 bp-segment of the mtDNA control region was sequenced for a total of 2,278 samples using the primers MT4 (Arnason *et al.*, 1993) and Dlp 5R or P2 developed by the ICR. PCR and subsequent cycle sequencing reaction for each sample were performed following the manufacturer’s protocol. The nucleotide sequence of each cycle sequencing product was determined using Applied Biosystems 3500 Genetic Analyzer (Life Technology) under standard conditions. Both strand samples were sequenced in their entirety for all samples.

The statistical approaches used for the RFLP analyses in JARPA (hypothesis testing) were also adopted for the mitochondrial control region sequence data analyses. In addition to this, constructing haplotype network and mismatch distribution analysis were conducted.

More detailed descriptions of laboratory and analytical procedures were provided in Pastene and Goto (2016).

Microsatellite DNA

Genetic variation was analyzed at 12 microsatellite loci

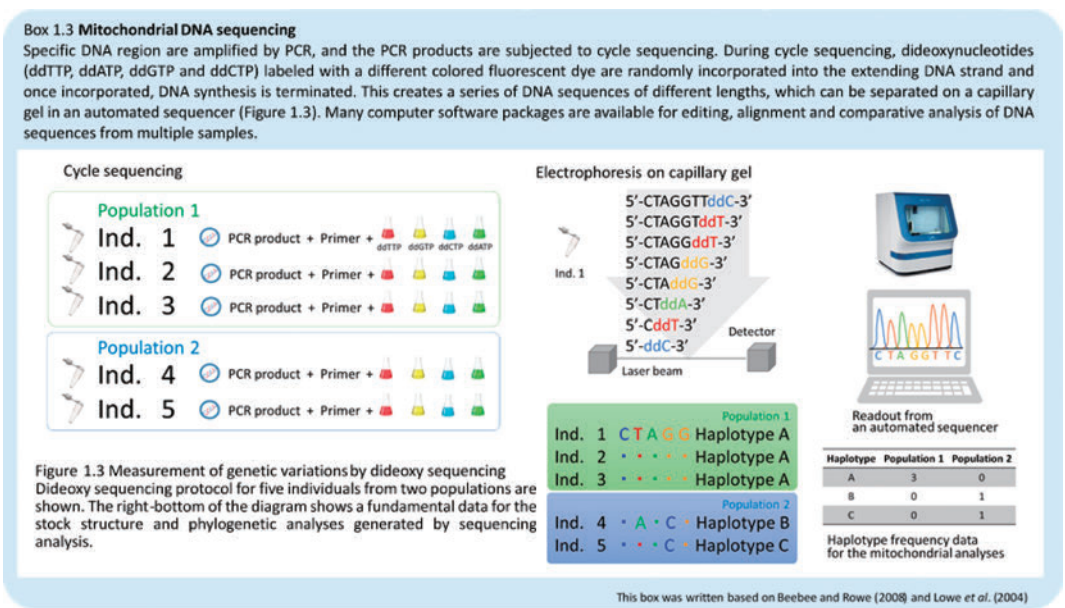
(instead of the six used in JARPA) in a total of 2,551 samples: AC045, AC082, AC087, AC137, CA234, GT129 (Bérubé *et al.*, 2005), DlrFCB14 (Buchanan *et al.*, 1996), EV1, EV104 (Valsecchi and Amos, 1996), GT023, GT195, and GT211 (Bérubé *et al.*, 2000). Laboratory protocols were the same as in JARPA.

The statistical approaches used for microsatellite analyses in JARPA were adopted for the JARPAII microsatellite analyses. In addition to this, the Bayesian clustering approach using STRUCTURE (Pritchard *et al.*, 2000) was used to determine the number of genetically distinct populations present in the samples based on information on the individual genotypes.

More detailed descriptions of laboratory and analytical procedures were provided in Pastene and Goto (2016).

Modelling approach

Schweder *et al.* (2011) developed an integrated approach for estimating longitudinal segregation of two stocks using different sources of data: morphometric, microsatellite and mtDNA data. Under this approach, areas of overlap between stocks were allowed to vary by year and sex. A joint likelihood function was defined for the estimation of mixing proportions and 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. More detailed analytical procedure was described in Kitakado *et al.* (2014).



SUMMARY OF RESULTS

JARPA

A summary of the results of the analyses on stock structure under the JARPA is shown in Table 1.

Taking all results together, Pastene (2006) suggested that whales in the eastern part of Area III and western part of Area VI were more differentiated than they were to whales in the Areas IV and V. Therefore, the author concluded that the single stock scenario cannot be applied to Antarctic minke whales in the feeding grounds of Areas IIIE–VIW, and proposed the occurrence of at least two genetic stocks in the research area. The author also suggested that this observation is probably related to the breeding areas in the eastern Indian Ocean and western South Pacific proposed by Kasamatsu *et al.* (1995). The following names were proposed for these stocks by Pastene (2006): ‘Eastern Indian Ocean Stock’ (I-stock) and ‘Western South Pacific Ocean Stock’ (P-stock).

While the microsatellite and morphometric analyses

were unable to identify any boundary between them, the mtDNA RFLP analyses suggested that the western part of Area V was more related to the I-stock than the P-stock, and a boundary in the sector 150°–160°E was proposed. This was consistent with the results of mark-recapture that showed movement of whales through 130°E (division between Areas IV and V). No western boundary of the I-stock and eastern boundary of the P-stock were proposed.

JARPAII

The main objective here was to test the stock structure hypothesis derived from JARPA analyses by using a set of new genetic samples obtained by JARPAII, and mitochondrial control region sequencing and microsatellite DNA genotyping.

Results of the heterogeneity test for both markers showed significant genetic differences between whales in two sectors, the western (35°–130°E) and eastern (165°E–145°W) research area, confirming that different

Table 1
Summary of the results on stock structure in JARPA.

Analytical approaches	Sex	Period of sampling	Pattern of geographical variation	References
mtDNA	F+M	1987/88–2004/2005	III E=IVW=IV E VIW=VE III E, IVW, IV E≠VE, VIW Possible boundary in the sector 150°–160°E	Pastene <i>et al.</i> (2006)
			IVWN, IVWS≠VE, VIW Some degree of difference in haplotype frequency between Areas III E and IVW	
Microsatellites	F	1989/90–2003/2004	No significant difference in haplotype frequency	Pastene <i>et al.</i> (2006)
	M	1989/90–2004/2005	IVW≠VE	
	F+M	1989/90–2004/2005	III E=IVW=IV E=VW VIW=VE III E, IVW, IV E, VW≠VE, VIW VEN≠VES	
MBLM	F	1987/88–2004/2005	III E=IVW=IV E=VW VIW=VE III E, IVW, IV E, VW≠VE, VIW VEN≠VES	Bando <i>et al.</i> (2006)
	M	1987/88–2004/2005	III E=IVW=IV E=VW VIW=VEN, VES III E, IVW, IV E, VW≠VEN, VES, VIW	
Morphometrics	F	1987/88–2004/2005	III E=IVW=IV E=VW VIW=VE III E, IVW, IV E, VW≠VE, VIW	Hakamada (2006)
	M	1987/88–2004/2005	III E=IVW=IV E VIW=VE III E, IVW, IV E≠VW, VE, VIW	

MBLM indicates mean body length of physically matured whales. A sign ‘=’ means that the statistical test found no significant difference at $\alpha=0.05$

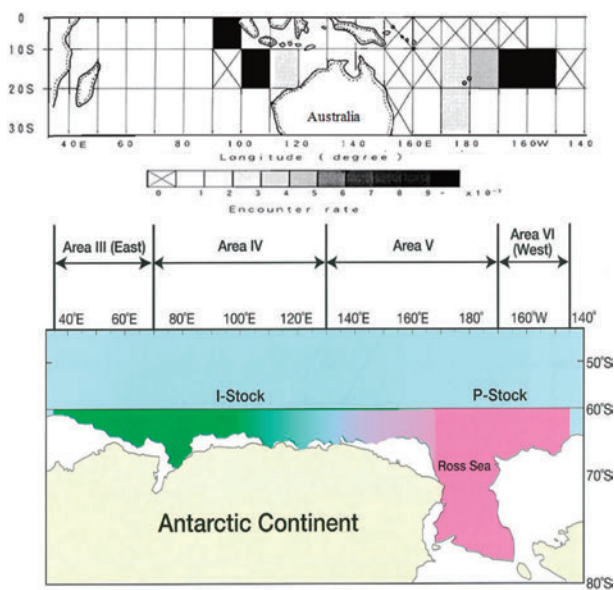


Figure 3. Hypothesis on stock structure of the Antarctic minke whale. The upper figure shows the encounter rates of Antarctic minke whales in 10° squares of latitude and longitude in waters 0–30°S during October (Kasamatsu *et al.*, 1995). The high sighting densities in the eastern Indian Ocean and western 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).

stocks inhabit the Indian and Pacific sectors of the Antarctic (I- and P-stocks) (Pastene and Goto, 2016). On the other hand, the STRUCTURE analysis for the microsatellites did not show genetic structuring of this species. This observation is probably due to a very low level of genetic differentiation. Microsatellite DNA analyses also showed more dispersal in males than females, and also some degree of annual variation.

The results of the modelling approach confirmed the occurrence of at least two stocks (I- and P-stocks) in the JARPA/JARPAII research area, which could be related to the suggested breeding areas in the eastern Indian Ocean and western South Pacific Ocean (Figure 3). Furthermore, the results indicated that the spatial distribution of the two stocks has a soft boundary (or area of mixing) mainly in Area VW (130°–165°E) (Figure 3), which changes by year. Results also suggested possible sex differences in the pattern of distribution of the two stocks (Kitakado *et al.*, 2014).

CONCLUSION

In conclusion, stock structure analyses for the Antarctic

minke whale conducted under the JARPA and JARPAII program indicated that the structure of Antarctic minke whale in Areas IIIE–VIW is more complex than originally thought: there are at least two stocks (I- and P-stocks), which overlap geographically in a wide area located mainly at 130°–165°E, which changes with year and sex.

Pastene and Goto (2016) postulated that Antarctic minke whales originating from breeding grounds in the western South Pacific (P-stock) have some degree of fidelity to the krill concentration associated with the Ross Sea Gyre (120°W–180°) ('home' sector). On the other hand, whales originating in the Indian Ocean (I-stock) have some fidelity to the krill concentration associated with the gyre located between 90°E and 120°E ('home' sector). They also postulated that both stocks could expand longitudinally (depending on the particular oceanographic conditions in a given year) and interact in the middle sectors and that therefore any boundary (or proportion of the populations in areas with mixing) in the Antarctic should be considered 'soft' probably changing annually according to changes in the oceanographic conditions.

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Technical Report (not peer reviewed)

Laboratory and analytical approaches to estimate biological parameters in the Antarctic minke whale and summary of results

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ABSTRACT

Life history-related biological parameters in marine mammals such as age at sexual maturity and pregnancy rate are known to change in response to changes in abundance, food availability or competition among species. Therefore, monitoring of biological parameters and investigations of how these parameters change with time are important information for whale stock assessment and management. This paper presents an outline of the laboratory and analytical approaches used to estimate biological parameters in large whales and a summary of biological parameters estimated for the Antarctic minke whales based on JARPA and JARPAII biological samples.

INTRODUCTION

Biological parameters such as age at sexual maturity and pregnancy rate are directly correlated to reproduction of whale stocks. Information on sex ratio and age at sexual maturity is necessary to assess the proportion of whales which contribute to reproduction; fetus sex ratio and litter size is information necessary to estimate the composition of young whales which recruit into the stock. Some of these parameters are known to change in response to changes in abundance, food availability or competition among whale species (Gambell, 1973; Kato, 1986a, 1986b, 1987; Kato and Sakuramoto, 1991; Masaki, 1979; Lockyer, 1972, 1979, 1984a). Therefore, monitoring of biological parameters is indispensable for assessment and sustainable management of whale stocks (Ohsumi *et al.* 1997).

The Antarctic minke whale *Balaenoptera bonaerensis* is one of the smallest Balaenopterid species with body length generally less than 10m. Antarctic minke whales are widely distributed in the Southern Hemisphere, migrating seasonally between feeding grounds in the Antarctic in summer (south of 60°S) and breeding grounds in the tropical or temperate regions in winter (Perrin and Brownell, 2009). The Antarctic minke whale was exploited by commercial whaling in the decades of the 1970's and 1980's, until the moratorium on commercial whaling was implemented in the 1987/88 austral summer season. Given their large abundance, this species is a target of possible future commercial whaling operations

in the Antarctic.

Previous estimates of biological parameters of Antarctic minke whale were based on samples collected during the commercial whaling period (Ohsumi *et al.*, 1970; Ohsumi and Masaki, 1975; Best, 1982; Kato, 1982, 1983, 1987; Masaki, 1979). However samples from commercial whaling are not representative of the stock as whaling operations targeted large individuals concentrated around the ice edge. Therefore biological parameters such as the age at sexual maturity estimated from those samples were thought to be biased (Kato, 1982, 1987).

The Japanese Whale Research Program under Special Permit in the Antarctic (JARPA) was conducted between the seasons 1987/88 and 2004/05. The main objective of JARPA was the estimation of biological parameters to improve the management of Antarctic minke whales. Relevant samples and data to estimate several biological parameters were collected systematically throughout the 18-year research period. Under the JARPA, track lines were designed in each stratum established in the research area independently and random sampling was conducted to ensure representativeness of samples to accurately estimate biological parameters. Similar procedures were used in the second phase of JARPA (JARPAII).

The International Whaling Commission's Scientific Committee (IWC SC) had recommended previously a recalculation of biological parameters of Antarctic minke whale by biological stock (IWC, 1998). Since then, stock structure analyses of Antarctic minke whale were refined, and a new stock structure hypothesis proposing an

'Eastern Indian Ocean Stock (I-stock)' (distributed mainly between 35°–130°E) and a 'Western South Pacific Stock (P-stock)' (distributed mainly between 165°E–145°W) in the Antarctic Ocean, was proposed. Pastene and Goto (2016) presented the latest genetic analyses in support of this hypothesis.

This paper presents an outline of the laboratory and analytical approaches to estimate biological parameters of large whales and a summary of biological parameters estimated for the Antarctic minke whales.

MATERIALS AND METHODS

This section summarizes the sampling and analytical procedures used by different authors for estimating several biological parameters in the Antarctic minke whale.

Age determination based on earplugs

One of the most important information obtained by JARPA and JARPAIL was the age of the whales, which was obtained by examining the internal earplugs of whales.

Sampling at the field

The left and right earplugs were collected carefully by researchers at the field, which were immediately fixed in 10% formalin.

Laboratory work

At the laboratory, individual age was determined by counting growth layers appearing on the bisected surface of the earplug using a stereoscopic microscope, assuming an annual deposition of growth layers (i.e. one pair of dark and pale laminae accumulated per year) (Figure 1) (Lockyer, 1984b).

Calibration work

The inter-reader variability has been evaluated under suggestions and guidance from the IWC SC. After such evaluation, the estimated ages with this variability was in-

corporated into the Statistical Catch-At-Age (SCAA) analysis (IWC, 2012; Kitakado *et al.*, 2013; Punt *et al.*, 2014), which was conducted to estimate the historical and current population trajectories of the stocks as well the natural mortality rates by age classes in the Antarctic minke whale (see below).

The ages of whales with unreadable earplugs have been estimated by applying an age-length-key for the purposes of the SCAA. In more recent years the racemization technique was developed for the Antarctic minke whale (Yasunaga *et al.*, 2017) and therefore this technique can provide age estimation for unreadable earplugs, which in turn will improve the SCAA performance.

Sexual maturity determination

Sampling at the field

Testes samples of about 1.5cm square were collected from testes from the right side and immediately fixed in 10% formalin. Ovaries from both sides were collected and preserved at -20°C for later analyses.

Laboratory work

Sexual maturity of males was determined by examination of histological samples of testes stained by hematoxylin-eosin. Males with seminiferous tubules over 100 µm diameter, spermatid or open lumen in the tubules were determined as sexually mature (Figure 2) (Kato, 1986a; Kato *et al.*, 1990, 1991). Sexual maturity for females was



Figure 1. Earplug of Antarctic minke whale.

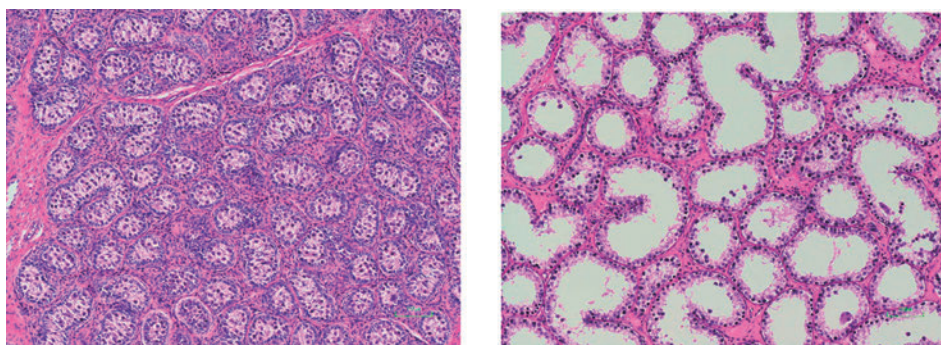


Figure 2. Histological testis sample of immature (left) and mature (right) Antarctic minke whales.

determined by the presence of corpora lutea (CL) or albicans (CA) in both ovaries (Lockyer, 1984a) (Figure 3).

Physical maturity

Sampling at the field

Physical maturity status was identified by examination of vertebrae. The fusion of the vertebral epiphysis to the centrum was known to start at the anterior cervical, then at the posterior caudal vertebrae, and is completed on the middle or posterior dorsal vertebrae (Kato, 1988). A part of the 6th dorsal vertebra which contains cartilage between centrum and anterior epiphysis was collected and fixed in the 10% formalin solution.

Laboratory work

Physical maturity was determined by examination of the vertebrae stained by 0.25% toluidine blue-O solution. Cartilage between epiphyses and centrum was observed by naked eye or stereoscopic microscope. Whales with the epiphyses fused to the centrum were defined as physically mature (Figure 4) (Kato, 1988).

Age/length at sexual maturity

Body length and age at sexual maturity was estimated by two methods. The first is the body length and age at first ovulation (*L_{mov}*, *t_{mov}*) (Kato, 1987). Under this method the mean body length and age were calculated for the whales with one corpus luteum and no corpus albicans in both ovaries.

The second method is the body length and age at 50% sexual maturity (*L_{m50%}*, *t_{m50%}*) (Kato, 1987). Under this method the body length and age at 50% maturity was estimated by logistic regression analysis based on maximum likelihood. The logistic model was fitted to age/body length and sexual maturity rate as:

$$Y = [1 + e^{(aX+b)}]^{-1}$$

where *Y* is the proportion mature at age/body length *X*, and *a* and *b* are empirical parameters. Age/body length at 50% maturity was calculated as $-ba^{-1}$.

Age at physical maturity

Age at 50% physical maturity was estimated by logistic regression analysis based on maximum likelihood. This is the same as the method for estimation of the age at 50%

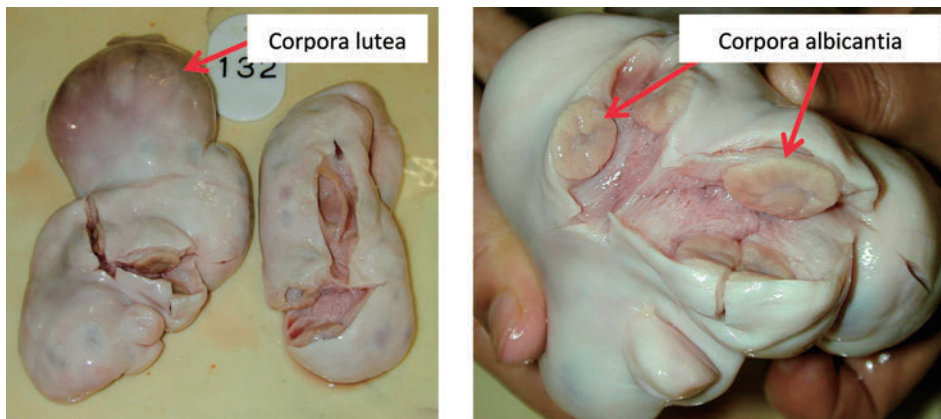


Figure 3. Ovary of Antarctic minke whales with corpora lutea (left) and corpora albicantia (right).

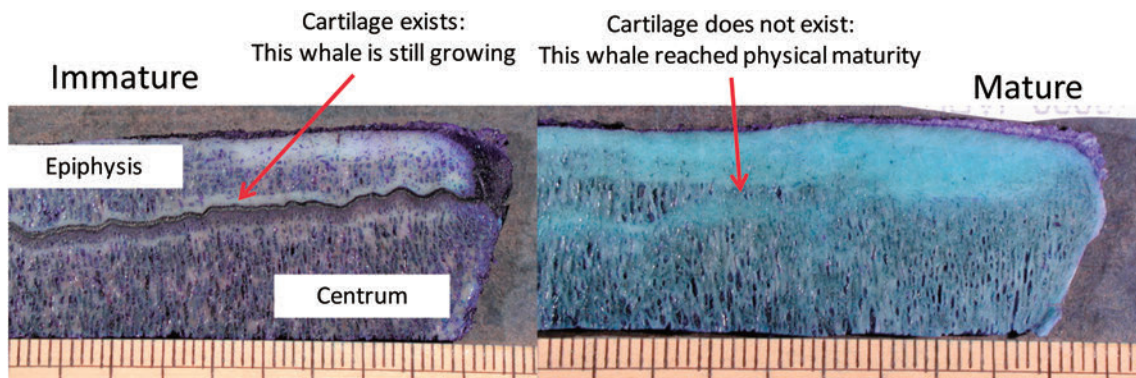


Figure 4. Vertebra of physically immature (left) and mature (right) Antarctic minke whale.

sexual maturity (*tm50%*).

Growth curve

The von Bertalanffy growth model was fitted to the body length and age as:

$$L_t = L_\infty (1 - e^{-K(t-t_0)})$$

where L_t is the body length at age t , L_∞ is asymptotic length, K is the growth rate coefficient and t_0 is the theoretical time at zero length.

Proportion of pregnant whales in matured females (PPF)

PPF is defined as the proportion of pregnant females within the sexually matured females.

Annual ovulation rate

Annual ovulation rate was estimated by applying linear regression analysis between age and total number of corpora (CL and CA). Only individuals after the 1970 cohort were used because of the reported decline of age at sexual maturity (determined from transition phase in ear plug) from the 1940s to the 1960s cohorts (Kato and Sakuramoto, 1991; Thompson *et al.*, 1999). The regression line was fitted to age 10 and 30 because almost all animals are mature at the age of 10 and sample size was not large enough for ages over 30.

Fetal sex ratio and litter size

Presence or absence of fetus was identified by cutting both sides of the uterus horn. Fetus sex was identified

by the appearance of the genital organ. Small fetuses of which sex could not be identified were excluded from the analysis.

Natural mortality via SCAA

Natural mortality was estimated by the Statistical Catch-at-Age (SCAA) model (Punt *et al.*, 2014). Individual age, abundance and catch history information were incorporated in the SCAA model and age dependent natural mortality was estimated (Punt *et al.*, 2014).

SUMMARY OF RESULTS

Biological parameters of Antarctic minke whales on stock basis (Pastene and Goto, 2016) are presented in Table 1. Both stocks presented similar values for age at sexual and physical maturity. In both stocks the values were smaller in males than females.

Remarkable differences between stocks were found in body length at sexual maturity (*Lmov*, *Lm50%*) and in the asymptotic length calculated from growth curves, which was about 20 cm larger in I-stock than P-stock for both sexes. The PPF of both stocks was as high as about 0.9 and annual ovulation rate supported the high pregnancy rate in both stocks. The fetus sex ratio is almost parity for both stocks and multiplets are rare in this species.

Age dependent natural mortality was estimated from SCAA. The pattern was similar between the two 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-stock and 0.046 (for age=15) to 0.103

Table 1
Summary of biological parameters of Antarctic minke whales estimated from 1987/88 to 2004/05 JARPA samples.

		I-stock (35°E–130°E)		P-stock (165°E–145°W)	
		Male	Female	Male	Female
Growth					
Growth curve		$L_t=8.60(1-e^{-0.272(t+1.97)})$	$L_t=9.16(1-e^{-0.228(t+2.19)})$	$L_t=8.44(1-e^{-0.269(t+2.08)})$	$L_t=8.97(1-e^{-0.203(t+2.90)})$
Age at physical maturity	50% mature	16.9	20.8	16.9	20.6
Sexual maturity					
Age at sexual maturity	<i>tmov</i>		7.6		8.5
	<i>tm50%</i>	5.3	7.6	5.4	8.1
Body length at sexual maturity (m)	<i>Lmov</i>		8.39 m		8.32 m
	<i>Lm50%</i>	7.28 m	8.20 m	7.14 m	7.99 m
Reproductive characteristics					
Proportion of pregnant in matured female (%)			92.1%		87.7%
Annual ovulation rate			0.989/year		1.005/year
Foetal sex ratio		52.1%	47.9%	46.8%	53.2%
Litter size			1.002		1.013

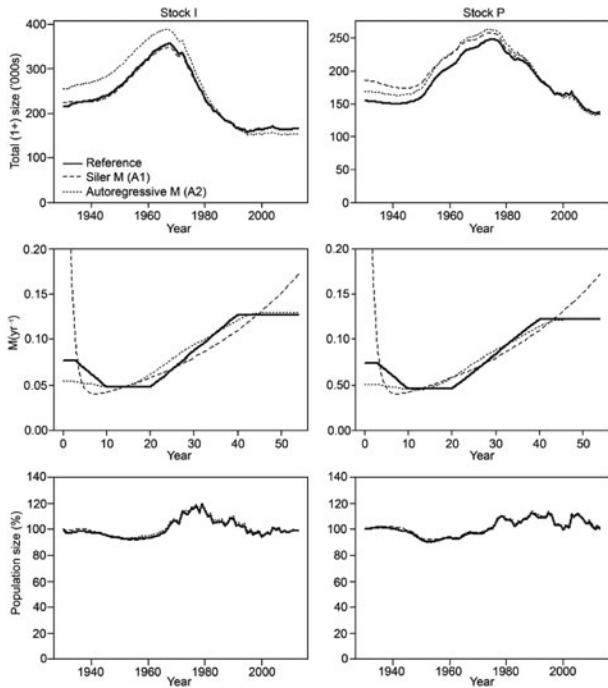


Figure 5. Time-trajectories of total (1+) population size (upper panels), age-specific natural mortality (center panels), and total (1+) population size relative to carrying capacity (lower panels) for three ways to model natural mortality (the Siler model, autoregressive and piecewise linear) of two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

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

DISCUSSION

At present examination of earplugs is the only practicable means to obtain age data at the annual scale. The earplug has proved to be a valid and useful tool for age determination (Lockyer, 1984b), and is the only method providing age data accurate enough for population-level analyses such as the SCAA of Antarctic minke whales. Because there are a number of whales sampled with unreadable earplugs, the feasibility of other ageing techniques is being investigated by ICR scientists such as that based on enantiomers of aspartic acid in eye lens, which are measured using high performance liquid chromatography (Yasunaga *et al.*, 2017) and the DNA methylation approach which was applied to humpback whale (Polanowski *et al.*, 2014). Potentially these techniques could complement the age information obtained from earplugs.

Age and other reproductive data obtained by JARPA and JARPAIL enabled the estimation of important biological parameters in the Antarctic minke whale, which are significant for assessment and management of this species in the Antarctic. Of particular importance was the estimate of age-specific natural mortality rate (Punt *et al.*, 2014) in this species, which was one of the main research objectives of JARPA.

Another important aspect was that the estimation of biological parameters was made on the basis of biological stocks and not on the basis of politically-based geographical boundaries. It was found that some biological parameters differed between stocks.

Some of the biological parameters are known to change in response to changes in abundance, food availability or competition with other species (Gambell, 1973; Lockyer, 1979; Kato, 1986b, 1987). Monitoring of biological parameters is useful for understanding of the present status of stocks and to predict future trends, which are essential for sustainable management of whale stocks. Moreover, information on biological parameters is useful to improve the performance of the Revised Management Procedure (RMP).

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Technical Report (not peer reviewed)

Sighting survey procedures for abundance estimates of large whales in JARPA and JARPAL, and results for Antarctic minke whales

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ABSTRACT

This paper provides an overview of the sighting surveys and analytical procedures for abundance estimates of large baleen whales under the non-lethal component of JARPA/JARPAL. The paper also summarizes the abundance estimates and abundance trends for Antarctic minke whales based on sighting data collected by JARPA and JARPAL surveys between 1989/90 and 2008/09.

INTRODUCTION

There are two main sources of sighting data for abundance estimates of large whales in the Antarctic. One is the International Decade for Cetacean Research (IDCR)/Southern Ocean Whale and Ecosystem Research (SOWER) program (Matsuoka *et al.*, 2003), and the other is the sighting surveys conducted as part of the non-lethal component of the Japanese Whale Research Programs in the Antarctic (JARPA, JARPAL and NEWREP-A).

One of the features of the sighting surveys under the Japanese whale research programs is that, unlike the IDCR/SOWER program, surveys are repeated in the same area and in the same months every second austral summer season, over a long period. Therefore, those sighting surveys facilitate both estimations of abundance trends and the extent of inter-year variability in local abundance in a particular area.

The objective of this paper was to outline the sighting procedures adopted during JARPA/JARPAL and the analytical procedures used to estimate whale abundance and abundance trends from sighting data. A summary of the results for Antarctic minke whales based on the JARPA and JARPAL sighting data is also presented. Surveys and analytical procedures used took into consideration the recommendations from the Scientific Committee (SC) of the International Whaling Commission (IWC) (see IWC 2008, pp. 349).

MATERIALS AND METHODS

Survey area and geographical stratification

The main research areas were IWC Management Areas IV (70°E–130°E) and V (130°E–170°W) until 1994/95.

Since 1995/96 the sighting surveys also covered Areas III E (35°E–70°E) and VI W (170°W–145°W), south of 60°S. Each of these Areas was divided into smaller strata as shown in Figure 1. The surveys were conducted from the end of December to March in each austral summer season, with the surveys in Areas IV and V concentrated in January and February in most years, which coincides with the peak period for migration of Antarctic minke whales to their Antarctic feeding grounds (Kasamatsu *et al.*, 1996). The starting and ending dates in JARPA/JARPAL surveys are shown in Figure 2.

Research vessels

JARPA/JARPAL comprised a combination of sighting and sampling surveys, and several specialized vessels participated in the surveys as Sighting Sampling Vessels (SSVs) and Sighting Vessels (SVs). *Kyo-Maru No. 1*, *Toshi-Maru No. 25* and *Toshi-Maru No. 18* operated from 1989/90. *Kyoshin-Maru No. 2* operated since 1995/96. *Yushin-Maru* operated since the 1998/1999 survey as the replacement of *Toshi-Maru No. 18*. *Yushin-Maru No. 2* operated since the 2001/2002 survey as the replacement of *Toshi-Maru No. 25*. *Yushin-Maru No. 3* operated since the 2007/2008 survey as the replacement of *Kyo-Maru No. 1*. *Kaiko-Maru* operated from 2005/06 to 2008/09 surveys. Details of the vessels were provided in Matsuoka *et al.* (2011) and Hakamada *et al.* (2013).

Line transect method

Tracklines are designed randomly or systematically so that they are independent from distribution of the objects (e.g., whales). Tracklines consist of parallel lines or zigzag lines as shown in Figure 3. In shipboard or aerial

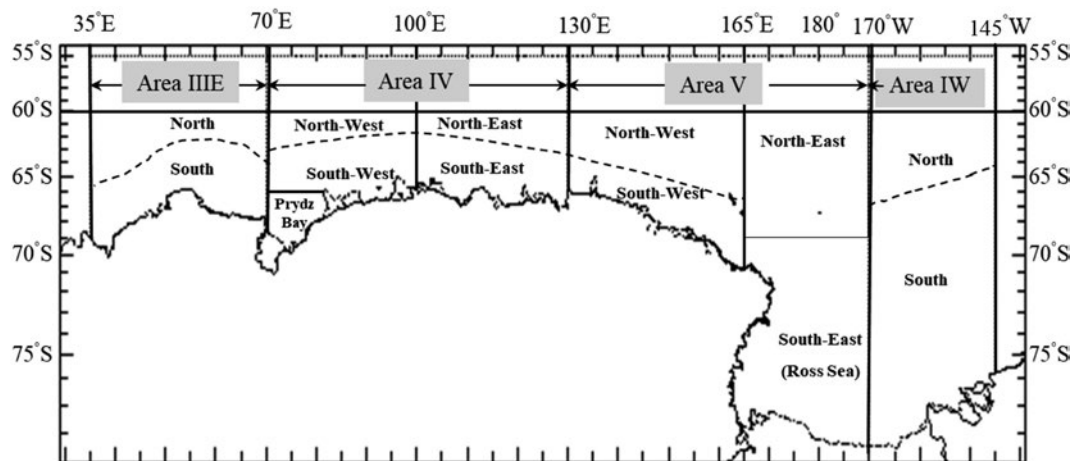


Figure 1. Research area for the sighting surveys under the JARPA/JARPAII. Dotted lines indicate 45n.mile lines from the ice edge lines.

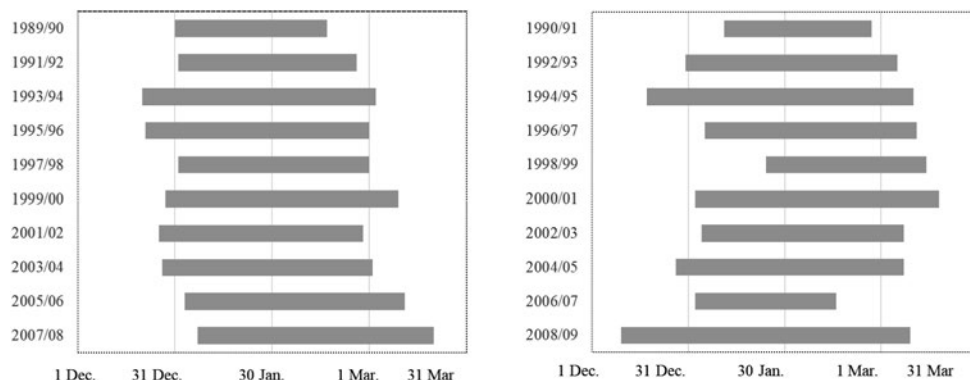


Figure 2. Starting and ending dates of JARPA/JARPAII sighting surveys for abundance estimation of large whales in Areas IV and V.

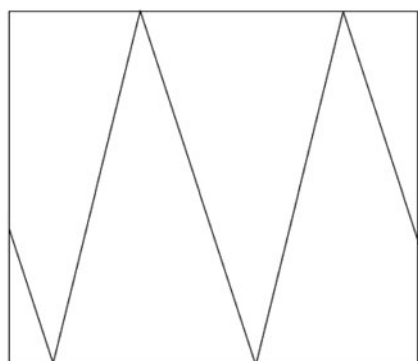


Figure 3. Example of zigzag lines used during a line transect survey.

surveys, there is significant cost for travelling from one transect to another. In such circumstance, zigzag lines (or saw-tooth line) are used to save costs (Buckland *et al.*, 2015).

During the line transect survey, observers on the research vessels search for whale schools from the platforms on the research vessels (Figure 4). When a whale school is detected, the distance between the school and

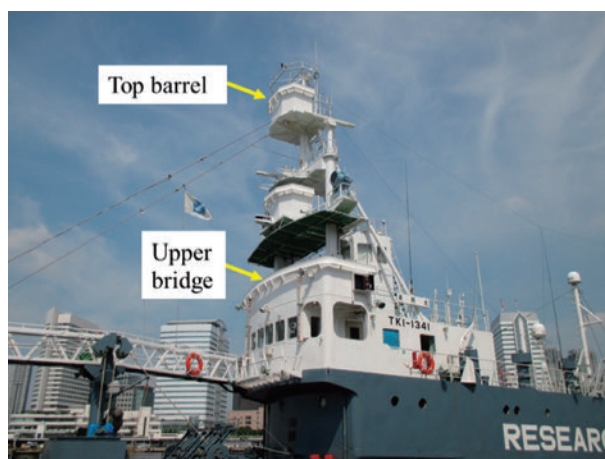


Figure 4. Platforms of observation of whale schools in specialized vessels used during JARPA and JARPAII.

the observer (r), and the angle from the track line (θ) are recorded (Figure 5). Position of the research vessel and time of the detection are also recorded.

After the detection of a whale school, in principle, the vessels approach the school to confirm the species and

school size. Activities of the research vessel are recorded to calculate distance and time on-effort. Weather (e.g., air temperature, sea surface temperature, Beaufort scale, visibility etc.) are recorded every hour during sighting surveys to record the weather condition that could potentially affect the detectability.

Trackline design and sighting procedures in JARPA/JARPAII

Survey design and procedures used under JARPA and JARPAII fundamentally followed the ‘Requirements and Guidelines for Conducting Surveys and Analyzing Data within the Revised Management Scheme (RMS)’ (IWC, 2012). More details on trackline design and sighting procedures in JARPA and JARPAII are found in Hakamada *et al.* (2013) and Hakamada and Matsuoka (2014a). The Sighting Vessels (SVs) conducted the surveys in closing

mode (SVC) up to and including 1996/97. Surveys in passing mode (SVP) started in 1997/98 because previous studies (Butterworth and McQuaid, 1986; Haw, 1991) had shown potential effect on abundance estimate of the survey mode.

The trackline was designed to cover the whole research area and the design was consistent throughout the JARPA and JARPAII surveys. The starting points were selected at random from 1n.mile intervals on lines of longitude. Trackline way points (where the trackline changes direction) were systematically allocated on the ice edge and on the locus of points 45n.miles from that edge in southern strata, and on this locus and the 60°S latitude line in the northern strata.

There were two modifications in trackline design in JARPAII surveys in relation to the previous JARPA, which responded to recommendations from the IWC SC to improve abundance estimation. One is that a saw-tooth type trackline for the southern strata was not used. The other is that the northern and southern strata were surveyed in the same period (Nishiwaki *et al.*, 2014). As noted above, JARPA/JARPAII comprised a combination of sighting and sampling surveys using SSVs and SVs. SSVs and SVs surveyed the area independently. An example plot of sightings on tracklines actually surveyed is shown in Figure 6.

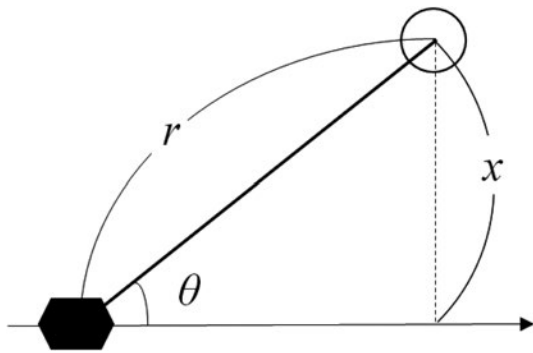


Figure 5. Illustration of the detection of whales from observers on research vessels. Black hexagon indicates a research vessel, white circle indicates a whale school, horizontal line indicates a trackline, r is distance to the whale school from the research vessel, θ is an angle from the track line and x is perpendicular distance from the trackline.

Smearing parameters and truncation distance

The data recorded for radial distance and angle are smeared using the Method II of Buckland and Anganuzzi (1988). The smearing parameter values were estimated for Antarctic minke (Hakamada *et al.*, 2013; Hakamada and Matsuoka, 2014a) whales. After the smearing, the perpendicular distances are truncated at 1.5n.miles for Antarctic minke whales. This treatment is the same as

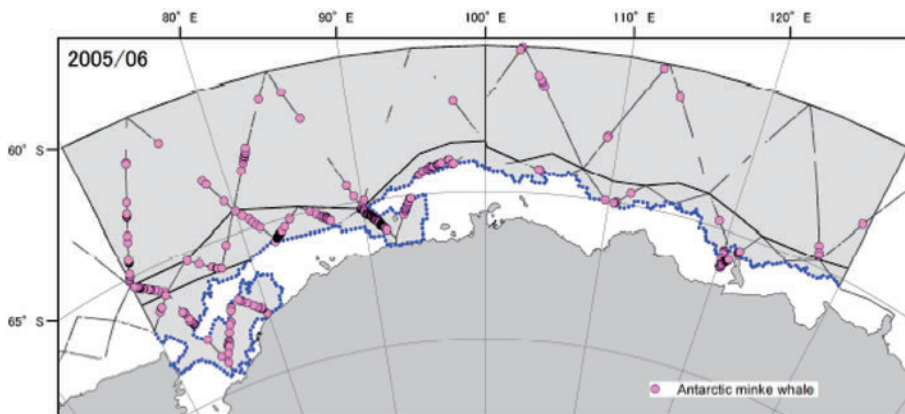


Figure 6. Primary searching effort (thin lines) and associated primary sightings of Antarctic minke whales (pink circles) in Area IV together with the ice edge (dotted blue line) in the 2005/06 JARPAII survey.

the one employed in abundance estimation from the IWC IDCR/SOWER surveys (Branch and Butterworth, 2001a; Branch, 2006). The number of sightings remaining after smearing and truncation includes sightings with both confirmed and unconfirmed school sizes.

Correction of observed angle and distance

To be able to correct for bias in distance and angle estimation, a distance and angle estimation experiment was conducted on each vessel each year (Nishiwaki *et al.*, 2014). The correction factors estimated in Matsuoka *et al.* (2011) and Hakamada and Matsuoka (2014b) were used for these analyses. More details of the methodology for the estimation of the correction factors are found in Burt and Stahl (2000).

Abundance estimation

The methodology for abundance estimation used in this study is described by Branch and Butterworth (2001a; 2001b) and Branch (2006), and has been termed the 'standard methodology' in the IWC SC terminology. The program DISTANCE ver. 6.0 (Thomas *et al.*, 2010) was used to provide abundance estimates corresponding to each trackline. The following equation was used for abundance estimation in each stratum:

$$P_i = \frac{AE(s)n_i}{2wL_i} \quad (1)$$

where P_i is the abundance in numbers as estimated from the i th trackline, A is the open ocean area of the stratum, $E(s)$ is the estimated mean school size, n_i is the numbers of primary sightings of schools on the i th trackline, w is the effective search half-width for schools and L_i is the primary search effort on the i th trackline.

For SSVs, the total abundance in each stratum is calculated as:

$$P = \frac{\sum_i L_i P_i}{L} \quad (2)$$

where L is sum of the L_i for each of the SSVs in the stratum.

The CV of the total abundance estimate P , is then calculated for each stratum using the equation:

$$CV(P) = \sqrt{\left\{ CV\left(\frac{n}{L}\right) \right\}^2 + \{CV(E(s))\}^2 + \{CV(w)\}^2} \quad (3)$$

where n is the sum of n_i for all the SSVs. Estimation of the CV of n/L is as specified in equation (5) below.

Detection function

A hazard rate model with no adjustment terms was used for the detection function:

$$g(y) = 1 - \exp\left\{-\left(\frac{y}{a}\right)^{-b}\right\} \quad (4)$$

where y is perpendicular distance, and $a > 0$ and $b \geq 1$ are parameters of the model to be estimated. It is assumed here that $g(0) = 1$ (i.e. the detection probability of a school on the track line is 1). Detections with perpendicular distances of more than 1.5 n.miles were truncated when estimating effective search half-width (ESW) w . More details of this detection function are given in Buckland *et al.* (1993; 2001).

Stratification of data to estimate ESW

In line with an IWC (2008) recommendation, ESWs were estimated by stratum. In cases where the sample size was smaller than 15, the sighting data were pooled among strata to estimate the detection function in line with other IWC (2008) recommendations. In such cases, data were pooled across West-East strata because sighting conditions and school size distributions are expected to be more similar than for North-South strata. In instances where there were less than 15 detections in southern/northern strata, data were aggregated over the whole of each Area.

Estimated mean school size

In line with an IWC (2008) recommendation, mean school sizes were estimated by stratum. Only the primary sightings for which the school size was confirmed were used for the estimation. The method for estimation of the mean school size described in Buckland *et al.* (1993; 2001) was used. More specifically, regressions of the log of observed school size against $f(y)$ was conducted for this purpose. If the regression coefficient was not significant at the 15% level, the observed mean school size for sightings within the truncated distance was substituted instead in the equation (1). If the consequent mean school size estimated was less than 1, then the observed mean school size was substituted instead in the equation (1) even if the regression coefficient was statistically significant at this 15% level. Similarly to the analyses for the IDCR/SOWER data (Branch and Butterworth, 2001a; Branch 2006), for SVP the mean school size estimated from SVC data was used instead of estimating this from SVP data, for which school size estimates are known to be negatively biased as a result of not approaching all

schools closely (Butterworth and McQuaid, 1986).

Combined encounter rate taking account of correlation among two or three SSV track lines

The survey by the SSVs comprised two or three parallel tracklines. There may be a positive correlation in the encounter rates along these lines, which would cause a negative bias in the estimate of the CV of the overall encounter rate if the results from each vessel were assumed to be independent. To take this possible covariance into account, the CV of the encounter rate when combined over the two or three SSVs with their parallel tracklines was estimated as:

$$CV\left(\frac{n}{L}\right) = \frac{\sqrt{\text{Var}\left(\frac{n}{L}\right)}}{\frac{n}{L}} \quad (5)$$

where

$$n_i = \sum_j n_{i,j}, L_i = \sum_j L_{i,j}$$

with $n_{i,j}$ and $L_{i,j}$ being the number of primary sightings of minke whale schools and the primary effort on the i th transect as surveyed on the j th tracklines. The variance of (n/L) is calculated as:

$$\text{Var}\left(\frac{n}{L}\right) = \sum_{i=1}^k \frac{1}{(k-1)} \left(\frac{L_i}{L}\right)^2 \left(\frac{n_i}{L_i} - \frac{n}{L}\right)^2 \quad (6)$$

where k is the number of transects on each trackline.

Estimating abundance trend

In order to examine the potential effect of survey timing and that of survey mode, the four models shown below were considered.

$$\begin{aligned} \text{Model i)} \quad & \log\{P_{obs}(y, a)\} = \\ & \log\{P_{true}(0, a)\} + \alpha y + \varepsilon_{y,a} + \eta_{y,a} \end{aligned} \quad (7)$$

$$\begin{aligned} \text{Model ii)} \quad & \log\{P_{obs}(y, a)\} = \\ & \log\{P_{true}(0, a)\} + \alpha y + M + \varepsilon_{y,a} + \eta_{y,a} \end{aligned} \quad (8)$$

$$\begin{aligned} \text{Model iii)} \quad & \log\{P_{obs}(y, a)\} = \\ & \log\{P_{true}(0, a)\} + \alpha y + M + T + \varepsilon_{y,a} + \eta_{y,a} \end{aligned} \quad (9)$$

$$\begin{aligned} \text{Model iv)} \quad & \log\{P_{obs}(y, a)\} = \\ & \log\{P_{true}(0, a)\} + \alpha y + M + T + a * T + \varepsilon_{y,a} + \eta_{y,a} \end{aligned} \quad (10)$$

where y is the year, a is the stratum, $P_{obs}(y, a)$ is the observed abundance estimate in stratum a and in year y as obtained from the line transect analyses, $P_{true}(y, a)$ is the

underlying abundance (i.e. free from the effect of survey mode) which is to be estimated in year y and in stratum a , M is the survey mode factor, T is the categorical variable related to survey time as defined below, $a * T$ is an interaction between strata and survey timing, $\varepsilon_{y,a}$ is an error reflecting the sampling error of the survey abundance estimate in year y and stratum a and $\eta_{y,a}$ is a normally distributed error with mean of 0 and variance of σ^2 associated with "model error."

The middle day of the survey period in each stratum was calculated and categorized into groups as a basis to specify T . Because the estimate of trend α might be sensitive to the definition of T , four grouping were considered:

- 1) **T=1:** Dec 1–Jan 15, **T=2: Jan 16–31**, T=3: Feb 1–15, T=4 Feb 16–Mar15 (Grouping T1)
- 2) T=1: Dec 1–Jan 15, **T=2: Jan 16–Feb 15** and T=3: Feb 16–Mar 15 (Grouping T2)
- 3) T=1: Dec, **T=2: Jan**, T=3: Feb and T=4: Mar (Grouping T3)
- 4) **T=1: Dec and Jan** and T=2: Feb and Mar (Grouping T4)

The groups in bold letters were included in the intercept of the alternative models considered (i.e. the effect of those groups is set to zero in the calculations). T1–T4 were used as categorical covariates in Models iii) and iv) (equations (9) and (10)) above. The best grouping was selected by comparing the corrected AIC (AIC_c) (Sugiura, 1978; Hurvich and Tsai, 1989; 1991), which can be applied to linear models with normal errors, and is used instead of Akaike Information Criterion (AIC) (Akaike, 1973) for each model.

Correction of nominal abundance estimates and their variance-covariance matrices

Using the estimated coefficients of models i)–iv), nominal abundance estimates for each survey mode can be corrected, and the weighted average of the corrected abundance estimates for each mode were calculated. Among the weighted average of the abundance estimates, that using correction factors based on the best model is treated as 'Base case.' Further details are provided in Hakamada *et al.* (2013).

Sensitivity analyses

There are various possible sources of bias in abundance estimates and their trends, the more important of which were discussed at the IWC SC meeting in 2007 (IWC, 2008; Table 1). In order to examine their possible magnitudes, sensitivity analyses to examine robustness of abundance estimates and trend were conducted.

Table 1

List of the factors for which the sensitivity of abundance estimates and/or trends is examined. Specifications are given for both the base case and the sensitivities, with more details provided in Hakamada *et al.* (2013) and Hakamada and Matsuoka (2014a).

Sensitivity factors	Specification for the base case	Specification for sensitivities
Shoulder of detection function	Estimation by stratum, except that when sample size is less than 15, strata are pooled.	For SSVs, ESW averaged over vessels concerned was used. For SVs the detection function estimation takes account covariates.
Trackline following ice edge contours	Complete tracklines used.	(1) Exclude trackline segments along the ice edge (Option B). (2) Use only transects parallel to lines of longitude (Option C).
Abundance in gaps between northern and southern strata	Assume same density as in stratum to the north.	(1) Assume the density is 0. (2) Assume the same density as in the stratum south.
Interpolation of density in the unsurveyed area within a stratum	Estimated density assumed to apply to complete stratum for JARPA. Interpolation using GLM was used for JARPAII.	Extrapolate based on average ratio of density in the unsurveyed to surveyed area as estimated in other years with complete coverage for JARPA and JARPA II.
'Skipping'	Assumed not to introduce bias.	Exclude the abundance estimates for years when 'Skipping' occurred when estimating trends.
$g(0)$	Assumed to equal 1.	Adjust for $g(0)$ estimates provided by the regression model.

RESULTS

This section presents the results of abundance estimate for Antarctic minke whale.

Abundance estimate

Nominal abundance estimates by strata in Areas III E, IV, V and VIW in each survey mode (SSV, SVC and SVP) during JARPA/JARPAII were provided in Hakamada *et al.* (2013) and Hakamada and Matsuoka (2014a). Detection functions to estimate ESW for abundance estimation were also provided by these authors. Hakamada and Matsuoka (2014a) shows interpolated abundance estimate for strata where survey coverage was incomplete. Abundance estimates for Areas IV and V for SSV, SVC and SVP survey modes during the JARPA period to apply log-linear models (7)–(10) were referred from Hakamada *et al.* (2013).

Log-linear models to estimate abundance trend

Model i) was selected for both Areas III E+IV and V+VIW. These rates of increase (ROI) are 1.1% with a 95% CI of [−2.3%, 4.5%] for Area III E+IV and 0.6% with a 95% CI of [−2.2%, 3.3%] for Area V+VIW (Hakamada and Matsuoka, 2014a). The point estimates from the other models range from 1.1% to 4.4% for Area III E+IV and from −2.1% to 0.6% for Area V+VIW, so that all lie within the 95% CI for the abundance trend estimate for the model selected (Hakamada and Matsuoka, 2014a).

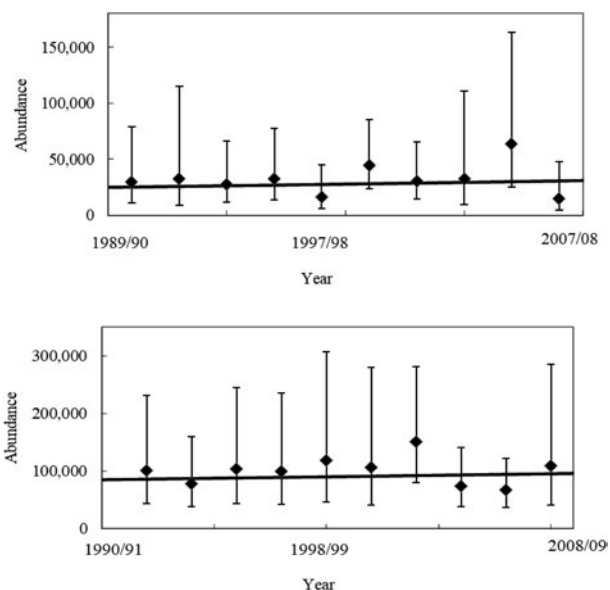


Figure 7. The base case estimates of annual abundance together with their 95% CIs are compared to exponential trend estimated by the AICc-selected model i) of equation (7) for Areas IV (upper panel) and V (lower panel) (from Hakamada and Matsuoka, 2014a).

Abundance estimates averaged over survey modes

The inverse variance weighted averages of abundance estimates over survey modes for Areas IV and V are shown in Figure 7 with their 95% CIs. The point estimates of the weighted average abundance and their CVs (taking into account model error) are shown in Table 2 for Areas IV and V. The CVs for these abundance estimates are all higher than

Table 2

The abundance estimates for the base case scenario for Area IV (left) and Area V (right) (from Hakamada and Matsuoka, 2014a).

Year	P_{WA}	$CV(P_{WA})$	Year	P_{WA}	$CV(P_{WA})$
1989/90	29,993	0.527	1990/91	100,745	0.445
1991/92	32,418	0.720	1992/93	78,919	0.371
1993/94	27,598	0.473	1994/95	104,013	0.458
1995/96	32,970	0.458	1996/97	99,680	0.461
1997/98	16,562	0.542	1998/99	118,779	0.515
1999/00	44,945	0.338	2000/01	106,769	0.524
2001/02	30,807	0.402	2002/03	151,072	0.326
2003/04	32,970	0.682	2004/05	74,030	0.336
2005/06	63,794	0.509	2006/07	67,661	0.308
2007/08	15,088	0.645	2008/09	109,173	0.523

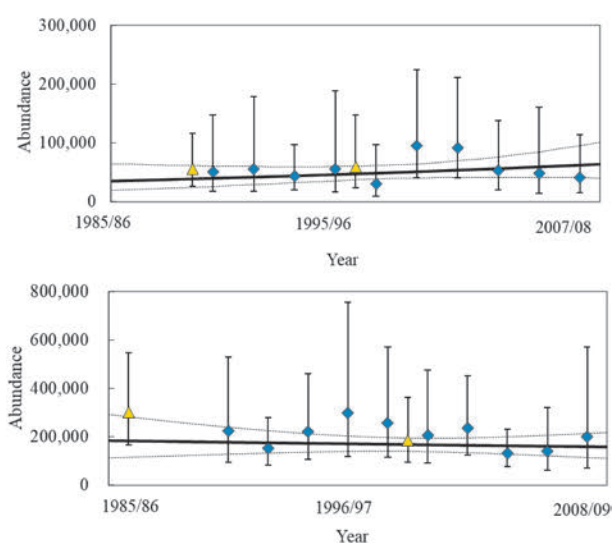


Figure 8. Same plots as for Figure 7, but with the abundance estimates and associated exponential model for the base case replaced by the corresponding $g(0)$ -adjusted results. The IDCR/SOWER estimates for a common northern boundary for CPII and CPIII as agreed by the 2012 IWC SC meeting are shown by the yellow triangles (IWC, 2013); their confidence intervals include allowance for additional variance, as do those for the JARPA and JARPAII surveys. The dashed curves indicate the 95% CIs for the exponential model (from Hakamada and Matsuoka, 2014a).

those from a previous analysis (Hakamada *et al.*, 2006) because model error is now taken into account.

Abundance estimates and trends for the sensitivity tests

Besides the $g(0)$ adjusted scenario, abundance estimates for Areas IV and V do not change substantially for the sensitivities excluding scenarios models other than the model i) (i.e. the best model in the base case) was selected as the best model (Hakamada *et al.*, 2013; Hakamada and Matsuoka, 2014a). These annual abundance rate

of increase estimates range over [0.1%, 3.7%] for Area IIIE+IV and [-1.7%, 0.6%] for Area V+VIW for the various sensitivity tests.

When the abundance estimates are $g(0)$ -adjusted, as would be expected the estimates increase by an average of 23,984 (88%) for Area IV and 105,906 (109%) for Area V (Figure 8). The estimates of annual rates of increase and their 95% CIs change to 2.5% [-1.3%,6.3%] for Area IV and -0.6% [-3.9%,2.6%] for Areas V, reflecting 1.5% increase for the former and 1% decrease for the latter, and slightly less precision (an increase in standard error of about 0.02) than when $g(0)$ is assumed to be 1 because of the further variance introduced in estimating the $g(0)$ values (Hakamada and Matsuoka, 2014a).

DISCUSSION

Comparison of the abundance estimates from JARPA and JARPAII with those based on IDCR/SOWER

As shown in Figure 8, abundance estimates for Antarctic minke whales for Areas IV and V based on JARPA and JARPAII agree with the estimates of Okamura and Kitakado (2012) based on IDCR/SOWER (Hakamada *et al.*, 2013; Hakamada and Matsuoka, 2014a).

Application of JARPA and JARPAII abundance trends

As noted earlier, one of the features of JARPA and JARPA II is that, unlike for the IDCR/SOWER program, surveys have been repeated in the same area and in the same months every second year over a long period. Therefore, the JARPA and JARPAII surveys facilitate both estimation of trends and the extent of inter-year variability in local abundance. These abundance series as well as those from IDCR/SOWER can be used to estimate abundance trends using population dynamics models which incorporate catch-at-age data and so integrate information from a number of different sources (Punt *et al.*, 2014; Mori

et al., 2006).

Through their use in such population models, the abundance estimates and trends derived from JARPA and JARPAII which are reported in this paper provide information to complement that available to estimate sustainable catch levels for minke whales in Areas III-E, IV, V and VI-W.

It should be noted that the abundance estimates in this study were made on the basis of geographical Areas. These estimates should be made on the basis of biological stocks definition in the future, as such information is already available (Pastene and Goto, 2016).

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Technical Report (not peer reviewed)

Distribution and movement of 'O' and 'J' stock common minke whales in waters around Japan based on genetic assignment methods

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ABSTRACT

A total of 4,275 western North Pacific common minke whales were examined with a set of 16 microsatellite DNA loci and the program STRUCTURE to assign individuals to either O or J stocks. Samples were available from JARP/JARPNII (1994–2014; n=2,637), and by-catches (2001–2014; n=1,638), from waters around Japan. Results of the Bayesian clustering analysis confirmed that the whales came from two genetically differentiated stocks, O and J stocks. By using 16 loci, more than 90% of the individual whales were assigned to either stock. Almost all of the individuals collected from the Sea of Japan side belonged to the J stock, whereas almost all of the individuals from the offshore North Pacific belonged to the O stock. Intermediate areas contained individuals from both stocks. The southern part of the Pacific side of Japan was mainly occupied by the J stock, which predominated (around 80% in proportion) throughout the year. In the north part of the Pacific side of Japan the proportion of the J stock animals increased in autumn/winter and decreased in spring/summer, and the O stock showed a reverse pattern.

INTRODUCTION

In the western North Pacific at least two biological stocks of common minke whales *Balaenoptera acutorostrata* are known to exist: the Okhotsk Sea-West Pacific (O stock) and the Sea of Japan-Yellow Sea-East China Sea (J stock) (Omura and Sakiura, 1956; Ohsumi, 1977; 1983). The two stocks are differentiated in morphological and reproductive characters (Omura and Sakiura, 1956; Ohsumi, 1977; Kato, 1992), as well in genetics (Wada and Numachi, 1991 for allozymes; Goto and Pastene, 1997 for mtDNA; and Kanda *et al.*, 2009a; b for microsatellites), suggesting their reproductive isolation. The International Whaling Commission (IWC) had proposed some boundaries for these stocks (Donovan, 1991, Figure 1).

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

There are several analytical approaches for estimating

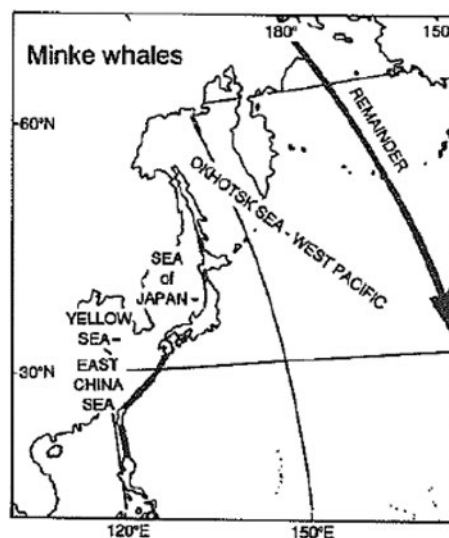


Figure 1. Historical IWC stock boundaries of western North Pacific common minke whale (Donovan, 1991).

the mixing proportion in areas where two or more stocks overlap, based on genetic data. One of those is the frequency-based approach (e.g. Pastene *et al.*, 1998). Under this method the allele or haplotype frequency distribution for regions where only a single stock is considered to be present is determined first ('pure stock' frequencies). Then the proportion of each stock is estimated in overlap

areas by Maximum Likelihood Methods. The other is the individual assignment-based approach, which does not need 'pure stock' assumptions and is based on minimizing departures from Hardy-Weinberg equilibrium (e.g. Pritchard *et al.*, 2000).

The objective of this study was to gain further understanding of the spatial and temporal distribution of the O and J stocks around Japan by using an individual assignment-based approach on the large number of genetic samples of this species collected in waters around Japan.

MATERIALS AND METHODS

Sample collections

To describe the distribution and movement of stocks a total of six Areas were defined for waters around Japan: Areas A=southern part of the Okhotsk Sea; Ba=coastal in the Pacific side off Hokkaido; Bb=coastal in the northern part of the Pacific side of Japan; C=offshore Area in the Pacific side; D=coastal in the southern part of the Pacific side of Japan; and E=coastal Area in the Sea of Japan side (Figure 2). Sample sizes used in this study are shown in Table 1, by Area.

Offshore samples were from the Japanese Whale Research Program under Special Permit in the western North Pacific (JARP/JARPNII) surveys from 1994 to 2013 in Areas A, Ba, Bb and C. Common minke whale samples obtained from the coastal JARPNII survey between 2002 and 2014 were also used in this study, Kushiro in Area Ba and Sanriku in Area Bb. Samples from bycaught whales in set net fisheries along the Japanese coast from 2001 to 2014 were also used. The bycatches were from Areas A, Ba, Bb, D and E year-round.

DNA extraction

The IWC guidelines for DNA data quality (IWC, 2009) were followed as much as possible (see Kanda *et al.*, 2014). Genomic DNA was extracted from 0.05 g of skin or muscle tissues using standard proteinase K, phenol-chloroform procedure described by Sambrook *et al.* (1989) or using Genra Puregene kits (QIAGEN). Extracted DNA was stored in the TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Microsatellites

Microsatellite polymorphisms were analyzed using 16 loci: EV1, EV14, EV21, EV37, EV94, (Valsecchi and Amos, 1996), GT23, GT195, GT211, GT310, GT509, GT575 (Bérubé *et al.*, 2000), GATA28, GATA98, GATA417, TAA31 (Palsbøll *et al.*, 1997), DlrFCB14 (Buchanan *et al.*, 1996). EV1, EV14, EV21 were developed from sperm whale, EV37, EV94, GT23, GT310, GT575, GATA28, GATA98, GATA417, TAA31 from humpback whale, and DlrFCB14 from beluga whale. All GT, EV and DlrFCB primers are

Table 1
Sample size used in this study, by Area.

Area	Number of samples
A	128
Ba	1066
Bb	921
C	942
D	535
E	683
Total	4275

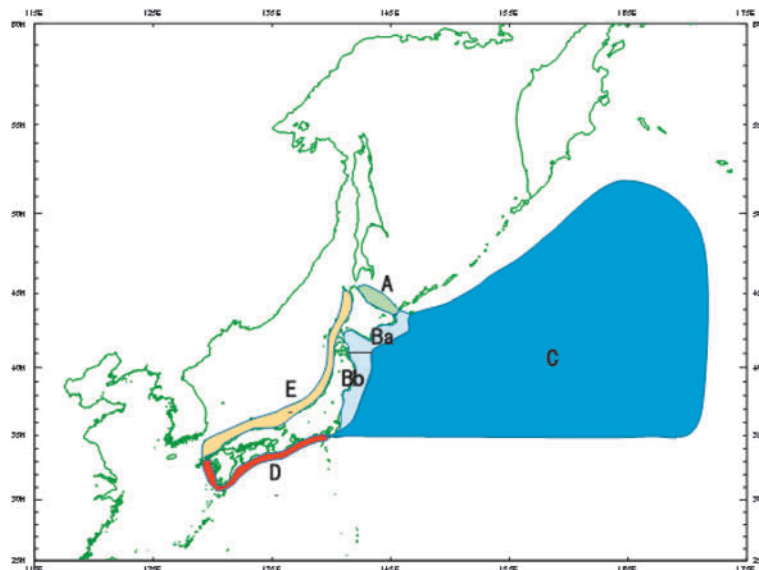


Figure 2. Geographical location of each Area defined for this study.

dinucleotide repeats, TAA31 trinucleotide repeats, and all GATA primers tetranucleotide repeats. Primer sequences and PCR profiles follows those of the original authors with slight modifications.

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), and 2 mM of each dNTP, and 10x reaction buffer containing 20 mM MgCl₂ (Takara Shuzo). PCR amplifications followed the manufacturer's instructions for the use of Ex Taq DNA polymerase (Takara Shuzo). Amplified products with internal size standard (GENESCAN400HD, Applied Biosystems Japan) were run on a 6% polyacrylamide denaturing gel (Long Ranger™) using a BaseStation TM100 DNA fragment analyzer (Bio-Rad) or were electrophoresed on an Applied Biosystems 3500 Genetic Analyzer. Allele sizes were determined using a 600 LIZ size standard and GeneMapper v. 5.0 (ABI).

Data analysis

The Bayesian clustering approach was implemented with the microsatellite data in the program STRUCTURE version 2.0 (Pritchard *et al.*, 2000) to determine the most likely number of genetically distinct stocks present in our samples. The program is a model-based clustering method for inferring stock structure (K, the number of stocks in the model) using multilocus genotype data with and without information on sampling locations. STRUCTURE allowed for the analyses of the samples without choosing sample units that did not necessarily correspond to real biological stock boundaries. A conceptual diagram of individual assignment under STRUCTURE is shown in Figure 3.

Posterior probabilities for K were estimated from ten independent runs for each value of K from one to five with only genetic information. These data were calculated based on burn-in period of 10,000 iterations and runs of 100,000 iterations. Individual assignment was then conducted for the most plausible K using the estimated individual proportion of membership probability (90%). The admixture model used for the simulation was the admixture model, which assumes individuals may have mixed ancestry. The allele frequency model used was the correlated allele frequencies model, which assumes frequencies in the different stocks are likely to be similar due to migration or shared ancestry.

RESULTS

Genetic assignment

Bayesian clustering analyses conducted on the total samples (4,275 individuals) without information on their

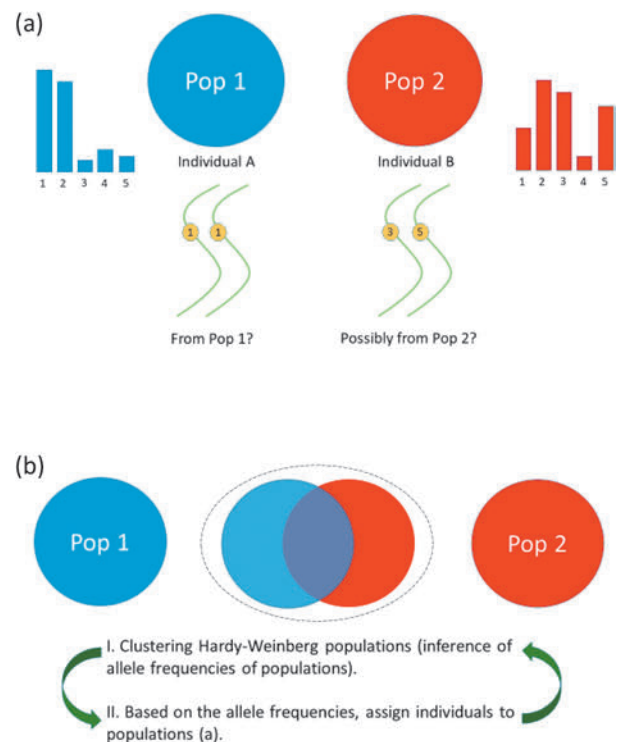


Figure 3. Basic concept of individual assignment: (a) with allele frequency at single loci (bar plot in this figure) in each source population. In this case, it is highly possible that individual A originates from population 1 since it has two alleles that are major in population 1. In contrast, individual B is likely to come from population 2 since it has two alleles that are minor in population 1; (b) with no allele frequency in each source population. In this case, genotypes at multiple loci in each individual enable estimation of allele frequency for the source population and an assignment probability for each individual by repeating the following steps: (I) estimation of allele frequency from tentative clustering in Hardy-Weinberg equilibrium, and (II) individual assignment based on the tentative allele frequency according to the concept of Figure 3a.

geographic origins presented the highest likelihood probability at K=2 (Table 2). These results confirmed that the samples came from two genetically distinct stocks of common minke whales (O and J stocks). In this study, the individuals with the membership probability of over 90% for either of the two stocks at each of the runs were assigned as pure individuals. All other individuals with the membership probability of less than 90% to the either groups were 'unassigned'.

Spatial distribution of O and J stocks along the Japanese coast

Both the stock-assigned and unassigned individuals were

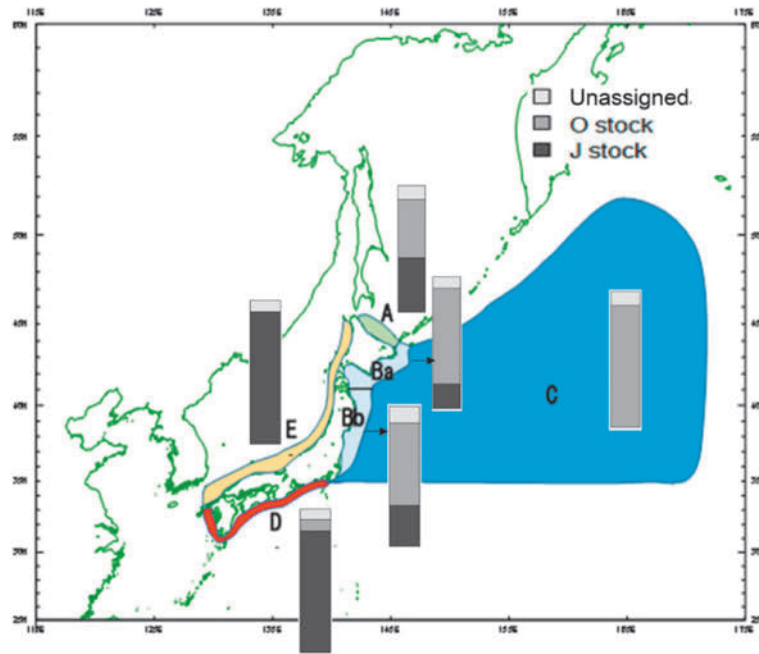


Figure 4. Spatial occurrence of O and J stocks common minke whale in waters around Japan.

Table 2

Results of the Bayesian clustering method analyzed for overall samples.

K	Log P (k/x)	Variance	Pr (k/x)
1	-210753.0	85.2	~0.0
2	-202085.7	873.1	~1.0
3	-203026.1	3693.0	~0.0
4	-202960.7	4761.0	~0.0
5	-204283.6	8075.9	~0.0

grouped based on the defined Areas (Figure 4). Almost all of the individuals collected from the Sea of Japan side (Area E) belong to the J stock, whereas almost all of the individuals from the offshore North Pacific (Area C) belong to the O stock. Area D (southern part of the Pacific side of Japan) was mainly occupied by the J stock. Areas A (northern Hokkaido) and Ba and Bb (northern part of the Pacific side of Japan) represent areas where both stocks overlap geographically.

Temporal distribution of O and J stocks along the Pacific coast of Japan

Figure 5 shows the temporal distribution of the O and J stocks in Areas Ba, Bb and D in the Pacific side of Japan, expressed as three months moving average. In Area D, the J stock was predominant (around 80% in proportion) throughout the year. In Areas Ba and Bb the proportion of the J stock increased in autumn/winter and decreased in spring/summer. Conversely the proportion of O stock decreased in autumn/winter and increased in spring/summer.

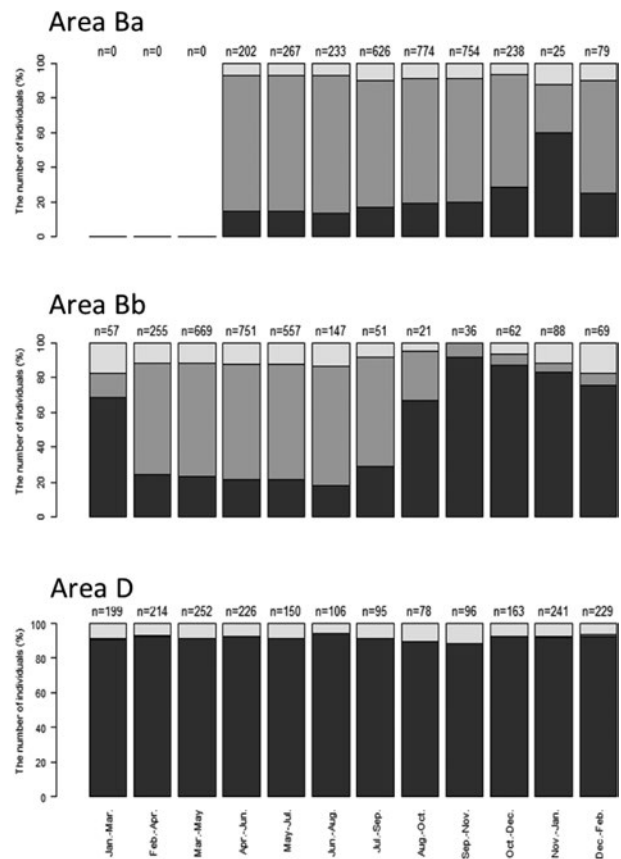


Figure 5. Monthly occurrence of O and J stocks in Areas Ba, Bb and D in the Pacific side of Japan. Each bar is expressed as three months moving average. Sample size is on the top of each bar. The sampling years in Areas Ba and Bb were 1994–2014. In Area D sampling years were 2001–2014.

DISCUSSION

Assignment methods based on genetic data

There are several methods for assigning individuals to source populations. Dawson and Belkhir (2001) developed a maximum likelihood method for assigning the individuals in a sample to source populations, on the basis of their genotypes at co-dominant marker loci. Cornuet *et al.* (1999) used a partially Bayesian exclusion test that uses an exclusion simulation method in the GeneClass software. Mantel *et al.* (2002) showed that the fully Bayesian assessment test of Pritchard *et al.* (2000) performed better than the partially Bayesian exclusion test of Cornuet *et al.* (1999). However they recognized that the fully Bayesian method required the assumption that the true population origin was sampled. Given this background the fully Bayesian clustering approach of Pritchard *et al.* (2000), implemented in the STRUCTURE program, was used in this study to assign individuals to either O and J stocks, which are highly differentiated genetically.

Temporal and spatial distribution of O and J stocks

Results of the Bayesian clustering analysis confirmed that the whales came from two genetically differentiated stocks, O and J stocks. By using 16 loci, more than 90% of the individual whales were assigned to either stock. Almost all of the individuals collected from the Sea of Japan side (Area E) belonged to the J stock, whereas almost all of the individuals from the offshore North Pacific (Area C) belonged to the O stock. Intermediate areas (Areas A, Ba and Bb) contained individuals from both stocks. In Area D the J stock was predominant (around 80% in proportion) throughout the year. In Areas Ba and Bb the proportion of the J stock increased in autumn/winter and decreased in spring/summer.

The fact that the J stock is distributed in Area D throughout the year suggests that the Kuroshio Current, which is one of the strongest west-boundary currents of the subtropical gyre, is working as the stock boundary between O and J stocks.

It is important to note that the individuals from the JARPN/JARPNII and those from the bycatch samples differ in their body length. Average body length of the JARPN/JARPNII samples including both the offshore and coastal components was 6.67 m (SD=1.13) and that of the all bycatch sample was 4.94 m (SD=0.99). Kato (1992) estimated mean body length at sexual maturity of North Pacific minke whales to be 6.3 m for males and 7.1 m for females, so that the bycatch sample in the present study

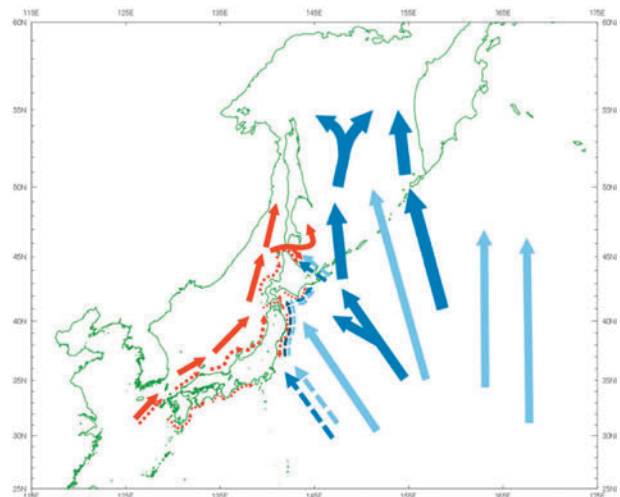


Figure 6. Assumed feeding migration route of O stock and J stock animals (modified after Hatanaka and Miyashita, 1997 and Goto *et al.*, 2010). Dark blue: female of O stock animals, light blue: male of O stock animals, red: J stock animals. Solid line: mature, dotted line: immature.

consisted mostly, if not all, of immature whales. The observed difference in the maturity status between the individuals from the bycatch and JARPN/JARPNII samples, however, could indicate that the patterns of the temporal and spatial distributions illustrated with the bycatches for the Areas Ba, Bb and D in this study may be different to some extent from those of adults. In regard to Area D, common minke whales from the offshore Area are not available. A related concern can be also seen in Area A. The number of J stock individuals in Area A differed between the bycatch and JARPN samples. This difference could be due to the immature/mature, temporal, or both factors, but we were not able to distinguish among these possibilities. Although we substantially increased our understanding of common minke whale distribution in the waters around Japan from this study, our sample set is still missing samples from some areas to depict the whole picture of distribution and movement of the two stocks. Genetic samples from the central and northern Okhotsk Sea are therefore highly desirable.

The pattern described above confirms the migration pattern proposed by Hatanaka and Miyashita (1997) for the O stock and that proposed by Goto *et al.* (2010) for the J stock (see a summary of movements in Figure 6).

Possibility of additional structure with the O and J stocks

STRUCTURE has generated considerable discussion in the IWC Scientific Committee (SC) (e.g. IWC, 2007; 2010). One major concern is the well-documented difficulty that STRUCTURE had in detecting weakly differentiated

populations/stocks, e.g. the approach could not detect additional structure within the O and J stocks.

In this regard one of the issues discussed at the IWC SC was the significance of the unassigned individuals that could not reliably be assigned to either O or J stocks (IWC, 2010). Some IWC SC members have argued that some if not all of the unassigned individuals, might belong to a different stock. Alternatively, these unassigned individuals could be the product of low statistical power of the analysis. Taguchi *et al.* (2017) showed that the proportion of unassigned individuals decreased with an increase of the number of loci used. Therefore the most likely explanation for the unassigned individuals is the low power of the analysis due to a small number of loci used.

On the other hand, Pastene *et al.* (2016b) conducted hypothesis testing to examine the genetic population structure of O stock common minke whales assigned by STRUCTURE, and found no significant heterogeneity in the sample providing support for a single O stock in the western North Pacific. A simulation exercise showed that the statistical power of the test was high.

More recently Tiedemann *et al.* (2017) investigated the spatial distribution of parent/offspring pairs based on microsatellite genotype profiles. Several pairs were found in which the parents were distributed in offshore waters and the offspring in the coastal waters in the Pacific side of Japan, a pattern difficult to reconcile with a multiple O stocks scenario in the western North Pacific.

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Technical Report (not peer reviewed)

Overview of stomach content analyses for sei, Bryde's and common minke whales under the offshore component of JARPNII, and temporal changes in feeding habits

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ABSTRACT

This study presents an overview of the stomach content analyses for sei, Bryde's and common minke whales off the Pacific coast of Japan based on data and samples collected by the offshore component of JARPNII during 2000–2016. The three species were highly dependent on small pelagic fish, in addition to planktonic crustaceans. The prey species composition in sei whales drastically changed from Japanese anchovy in the early 2000s to Japanese sardine in 2014 to 2016, while copepods (*Neocalanus* spp.) steadily occurred throughout the years in offshore waters. Bryde's whale had a simple prey composition involving mainly Japanese anchovy, but a lesser amount of this prey species was observed during the last three years. Prey composition in common minke whales in offshore waters showed that Japanese anchovy and Pacific saury are the main prey species, while in the vicinity of northern Japan, Japanese anchovy and walleye pollock were the dominant prey species. These three whale species showed diversities in their feeding habits reflecting changes in prey species populations and availability through the years.

INTRODUCTION

The Japanese Whale Research Program in the western North Pacific, Phase II (JARPNII) started in 2000 with the primary purpose to study the interactions between fisheries and cetaceans through ecosystem modeling of the Pacific side of Japan, an area well known as a large fisheries ground (GOJ, 2002; 2004). The goal of JARPNII was to assist in the formulation of effective ecosystem-based fisheries management in this research area.

This paper focuses on the study of the feeding ecology of three baleen whale species, sei whale (*Balaenoptera borealis*), Bryde's whale (*B.edeni*) and common minke whale (*B. acutorostrata*) based on surveys under the offshore component of JARPNII (whales sampled by the pelagic research vessels). The baleen whales in offshore waters are highly dependent on abundant pelagic fishes and zooplankton which are important components of the food web in the subarctic region of the western North Pacific.

Eggs and larvae of pelagic fish, such as Japanese anchovy *Engraulis japonicus* and Japanese sardine *Sardinops melanostictus* are transported by the Kuroshio Current to offshore waters (Itoh *et al.*, 2009; 2011; Okunishi *et al.*, 2011), and these species are important prey items for baleen whales, in addition to crustaceans such as krill.

In fact, the Japanese anchovy was found in the stomach of sei, Bryde's and common minke whales sampled in the early half of the JARPNII (Konishi *et al.*, 2009), in addition to mackerel (genus *Scomber*). Acoustic and trawling surveys during JARPNII (Murase *et al.*, 2012) showed that Japanese anchovy was distributed widely in the survey area between 2004 and 2007. Because these pelagic fish species are also commercially important, there are many studies on the catch history (Yatsu *et al.*, 2005; Yonezaki *et al.*, 2015), optimal environment (Takasuka *et al.*, 2007) and transportation to offshore areas (Itoh and Kimura, 2007; Itoh *et al.*, 2009; 2011). These studies suggested synchronized exchange of favorable environmental conditions between Japanese sardine and Japanese anchovy caused by climate change, which can be defined by Pacific Decadal Oscillation (PDO) (Mantua and Hare, 2002).

In the early 2000s, when sampling of sei and Bryde's whales started in the JARPNII, the sardine population had already collapsed and the anchovy catch had increased (Yatsu *et al.*, 2005; Takasuka *et al.*, 2008; Itoh *et al.*, 2009). Since PDO fluctuations occur in scale from 15–25 years and 50–70 years (Mantua and Hare, 2002), the continuous sampling under the JARPNII for more than a decade is useful to see changes in the feeding habits of sei, Bryde's and common minke whales.

The main purpose of this study was to present an overview of the studies of feeding ecology of sei, Bryde's and common minke whales based on stomach content analyses, and to examine decadal changes in the feeding habits of these three species.

MATERIALS AND METHODS

Study area

The JARPNII research area has a unique environment under the effect of both the cold Oyashio Current and the warm Kuroshio Current and this transition region covers most of this research area (see Favorite *et al.*, 1976). High productivity is an important factor related to spring blooming in the lower trophic level (Liu *et al.*, 2004). Copepods (*Neocalanus* spp.) are the dominant zooplankton in the Oyashio region during spring to summer in the surface layer, which depend on phytoplankton (see Kobari and Ikeda, 1999; Tsuda *et al.*, 2001).

The occurrence of subarctic gyres near the Kuroshio Current is also important to marine mammals (Springer *et al.*, 1999). Basic oceanic features of oceanic fronts, Kuroshio-current and eddies in July are illustrated as an

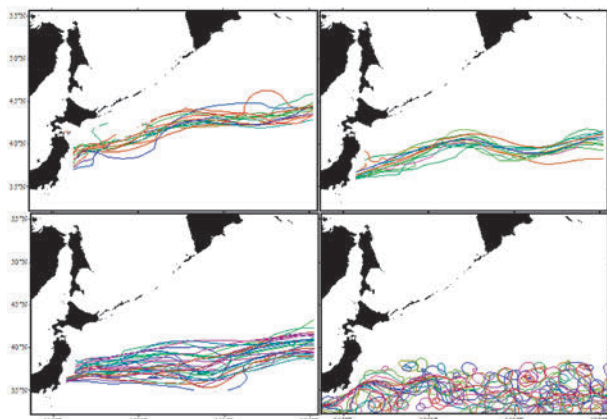


Figure 1. Geographical locations of oceanic fronts at western North Pacific in July during 2001–2013. Original data from Argo float (JAMSTEC). Upper left: Subarctic Front: 4°C isotherm at 100m depth (Argo data from JAMSTEC). Upper right: Subarctic boundary. Salinity of 34 psu at near surface (Argo data from JAMSTEC). Bottom left: Kuroshio extension northern branch. Bottom right: Kuroshio extension from sea surface height (AVISO absolute dynamic topography derived from sea surface height measured by several satellites, AVISO, France, <http://www.aviso.altimetry.fr>); estimated current eddies area also drawn. Detail of oceanographic features in the JARPNII area during 2000 to 2013 is well described in Okazaki *et al.* (2016). Definition of boundary followed description in Kida *et al.* (2015).

example (Figure 1). To examine positions of oceanic fronts, Argo data were used for salinity and temperature profiles (<http://www.argo.ucsd.edu>, <http://argo.jcommops.org>). To draw the Kuroshio-current and eddies, sea surface height data were obtained from the AVISO webpage (<http://www.aviso.altimetry.fr/duacs/>). Another topographic feature associated with prey distribution in this area is the Emperor Seamounts and the Shatsky Rise which cause upwelling and changing current stream (Figure 2). The northward branch of the Kuroshio Extension Front near the Shatsky Rise is located around 160°E (Mizuno and White, 1983).

Whale sampling

Data and samples used in this study were obtained by the JARPNII offshore component in the period 2002–2016 for sei whales; 2000–2016 for Bryde's whales; and 2000–2013 for common minke whales. Sample sizes of the whales examined are shown in Table 1. Details of the survey procedure under JARPNII are available in Bando *et al.* (2016) and Tamura *et al.* (2016). During the sampling survey, information on catch date and location for each whale sampled as well surface temperature where the whales were first sighted, was recorded.

Prey information

Prey information was obtained by examining stomach content of the whales sampled. Most of the stomachs were occupied by a single prey species. The methodology for stomach content analysis was described in previous

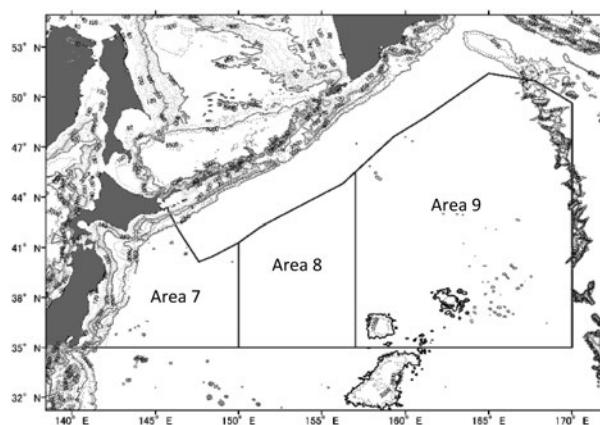


Figure 2. The locations of major geographic features at the JARPNII research area with isobath to 4000 m depth. The contours are from the GEBCO bathymetric atlas (Amante and Eakins, 2009). Survey Areas are shown with thick black line. Shatsky Rise and Emperor Seamounts are also drawn by 3000–4000 m isobath in depth in the eastern part of the research area at 155°E–160°E and around 170°E, respectively.

Table 1
 Number of sei, Bryde's and common minke whales caught under the JARPNII, by area, month and year.

Sei	Area 7					Area 8						Area 9					Total	
	May	Jun.	Jul.	Aug.	Sep.	May	Jun.	Jul.	Aug.	Sep.	Oct.	May	Jun.	Jul.	Aug.	Sep.		Oct.
2002								4		3				32				39
2003		1	4			3	16						11	12	3			50
2004							2						9	36	27	26		100
2005						12	3	16				5	41	17	6			100
2006		1	4				19	28					19	6	23			100
2007		2	4			16	2	6				22	23	16	9			100
2008							24	9					35	15	17			100
2009						11	1	19				18	38	13				100
2010		10					9	6					18	29	28			100
2011				1			5	11	13				26	11	28			95
2012							31	3					21	45				100
2013									11						35	54		100
2014						3	10	8				13	49	7				90
2015							7	10						44	29			90
2016	4					6	6	16				12	46					90

Bryde's	May	Jun.	Jul.	Aug.	Sep.	May	Jun.	Jul.	Aug.	Sep.	Oct.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Total
2000				24	19													43
2001	6	33	11															50
2002				13				7	23					7				50
2003	1	19	7				14							9				50
2004							13						26	5		6		50
2005		3	36				8						3					50
2006			5					11					16	5	13			50
2007		4	1	6				20	9					8	2			50
2008			26					9						15				50
2009							27						23					50
2010		1												49				50
2011			32	5				4					5		4			50
2012							3	14						17				34
2013								1	14		3			1	4	4	1	28
2014			13					10						2				25
2015		21					4											25
2016								9						16				25

Minke	May	Jun.	Jul.	Aug.	Sep.	May	Jun.	Jul.	Aug.	Sep.	Oct.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Total
2000				6	18										16			40
2001	28	22						22						23	5			100
2002				6	54			1	7	3				20	5	4		100
2003	22		2			19	18						11		28			100
2004					16									24	60			100
2005		14	18			10		4				10	3	3	38			100
2006	16	11	11				26	12					10	14				100
2007		79				1	14					1	5					100
2008							5						3	29	22			59
2009	4	11	4			1		16				3	4					43
2010														12	2			14
2011			47										1		1			49
2012	57	14					3											74
2013															3			3

reports (e.g. Tamura *et al.*, 1998; Konishi *et al.*, 2009), and it involves the steps shown in Figure 3. Prey length distributions for pelagic fish in the stomachs of the sampled whales are shown in the Appendix.

RESULTS

Sei whale

Figure 4 shows the geographical position (based on the first sighting) of sei whales sampled including information of the prey species found in the stomachs. Whales were widely distributed in the survey area, but mostly from east of 150°E and south of 46°N between north of the Kuroshio extension and just north of the Subarctic Front (see also Figure 1).

The main prey species were copepod (*Neocalanus spp.*), euphausiid (*Euphausia spp.*), Japanese anchovy, two mackerel species (*Scomber japonicus* and *S. aus-*

tralicus) and Japanese sardine. Pacific saury (*Cololabis saira*) was found in the eastern side of the research area in every year. Most of the copepods found in the stomachs were 5th copepodite stage of *Neocalanus cristatus* and *N. plumchrus* (also see Konishi *et al.*, 2009). Minimal armhook squid (*Berryteuthis anonychus*) was also found mainly in far eastern areas near the Emperor Seamounts. The locations of whales feeding on Japanese sardine was the most eastern in the survey area, overlapping with whales feeding on Japanese anchovy and copepods.

Prey composition in the stomach of sei whales sampled during 2002–2016 is shown for each Area in Figure 5. Copepod and Pacific saury were dominant preys in Area 9 than in western Area 8, while Japanese anchovy and euphausiid were found widely in both Areas 8 and 9. In the years 2002–2012, Japanese anchovy, copepod and



Figure 3. A schematic diagram of the steps in the analyses on stomach contents.

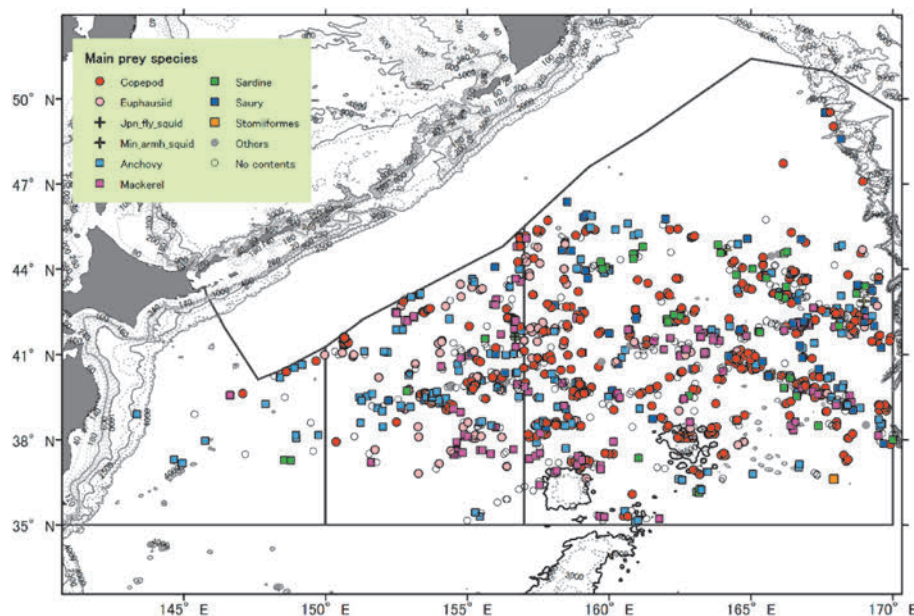


Figure 4. The distribution of sampled sei whales (based on first sighting), and information of prey species in their stomachs (period 2002–2016). The contours are from the GEBCO bathymetric atlas.

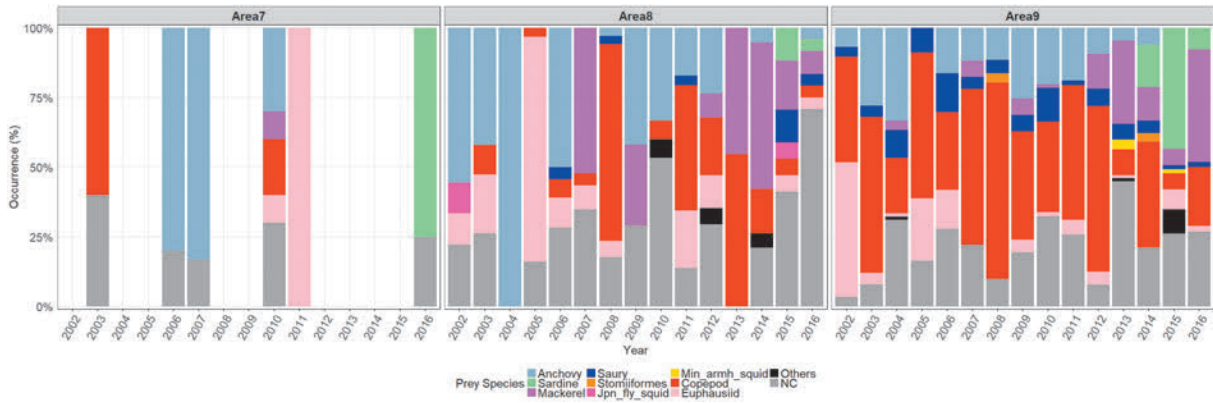


Figure 5. Prey composition in the stomach of sei whales sampled in the period 2002–2016, by Area and year.

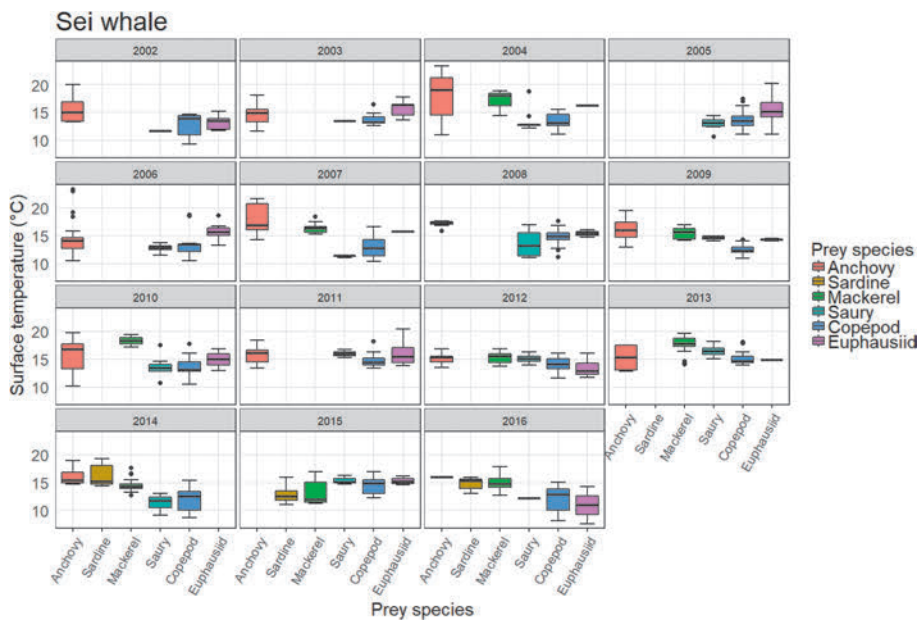


Figure 6. Surface temperature in which sei whales were sampled, and prey species in their stomachs (2002–2016).

euphausiid were major components with the exception of years 2005 and 2008. After 2007, mackerel became one of the major preys which coincided with a decreasing occurrence of anchovy and copepod. In the years 2014–2016, Japanese sardine was the dominant prey while some Japanese anchovy was found only in 2016. Stomachs with no contents (NC) were also found widely throughout the survey years. Japanese sardine was not the main prey species until recently.

Figure 6 shows the surface temperature in which sei whales were found, including information on prey species in their stomachs. Surface temperature in which sei whales were found varied according the prey species. Japanese anchovy was found in a wider range of surface temperatures. Copepods and Pacific saury tend to be found at lower temperatures (*c.a.* <15°C) than mackerel. Although a variation of surface temperature is observed within years, these are not consistent among years. Length dis-

tribution of main fish prey species in the stomachs of sei whales had different modes among years (see Appendix).

Bryde’s whale

Figure 7 shows the geographical position (based on the first sighting) of Bryde’s whale sampled including information of the prey species found in the stomach. Their distribution is mainly south and west of the distribution of sei whales sampled. They were concentrated around 145°E–150°E and 155°E–160°E, south of 41°N. Japanese anchovy and euphausiids were found in a wide longitudinal range. Mackerels and stomiiforms (*Vinciguerria nimbaria* and *Maurolicus muelleri*) occurred offshore.

Prey composition in the stomachs of Bryde’s whales sampled during 2000–2016 is shown for each Area in Figure 8. Japanese anchovy, stomiiforms and euphausiid were the dominant prey species. Mackerels were occasionally found in some years. The composition of main

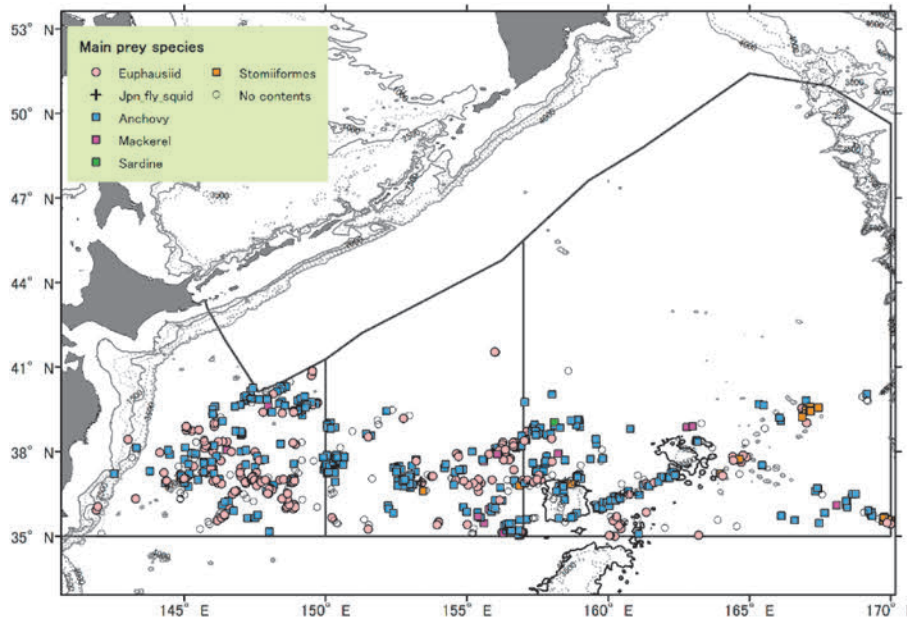


Figure 7. The distribution of sampled Bryde’s whales (based on first sighting), and information of prey species in their stomachs (period 2000–2016). The contours are from the GEBCO bathymetric atlas.

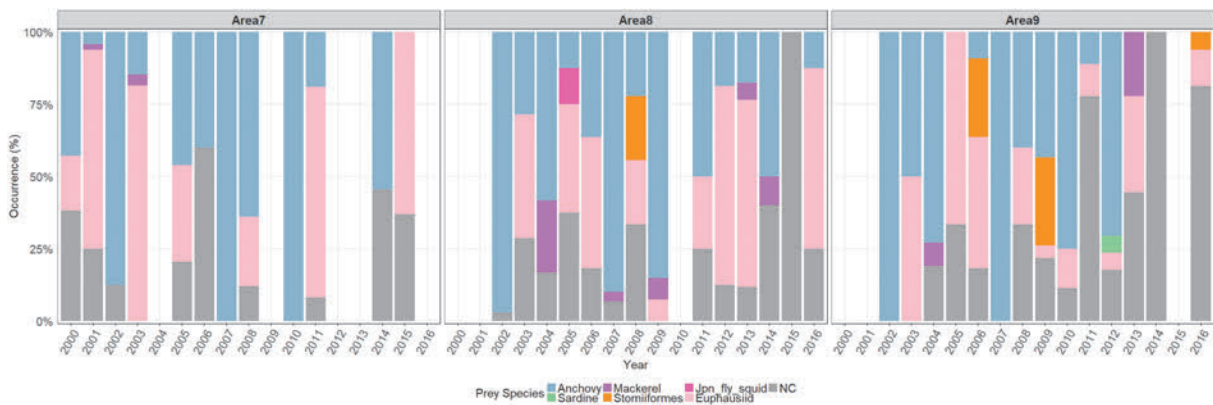


Figure 8. Prey composition in the stomach of Bryde’s whales sampled in the period 2000–2016, by Area and year.

prey species is highly variable among years, however there was no obvious variation among Areas and decadal change in the prey composition. However, it should be noted that lower occurrence of anchovy and high occurrence of ‘NC’ stomachs were observed in the last two years (2015–2016). Japanese sardine was not a main prey species and was just found occasionally.

Figure 9 shows the surface temperature in which Bryde’s whales were found, including information on prey species in their stomachs. Bryde’s whales fed on prey in warmer surface temperature areas compared to sei and common minke whales in JARPNII. No obvious variation of surface temperature among the prey species was observed. Length distribution of pelagic fish prey species showed variation of mode among years (see Appendix).

Common minke whale

Figure 10 shows the geographical position (based on the first sighting) of common minke whales sampled including information of the prey species found in the stomach. Figure 11 shows details of the coastal area in the western side of the research area.

Pacific saury is the key prey species in offshore waters and common minke whales occurred mainly north compared to the sei whales sampled. Japanese anchovy was found widely through longitudinal sectors, and also was intensively fed on by common minke whales in the coastal area (Figure 11). Minimal armhook squid and Pacific pomfret (*Brama japonica*) were found in the far northeastern regions near and above the Emperor Seamounts (Figure 10). In coastal waters, walleye pollock (*Theragra chalcogramma*), which occurs at bottom or midwater depth, was commonly found above shelf edge and slope off eastern

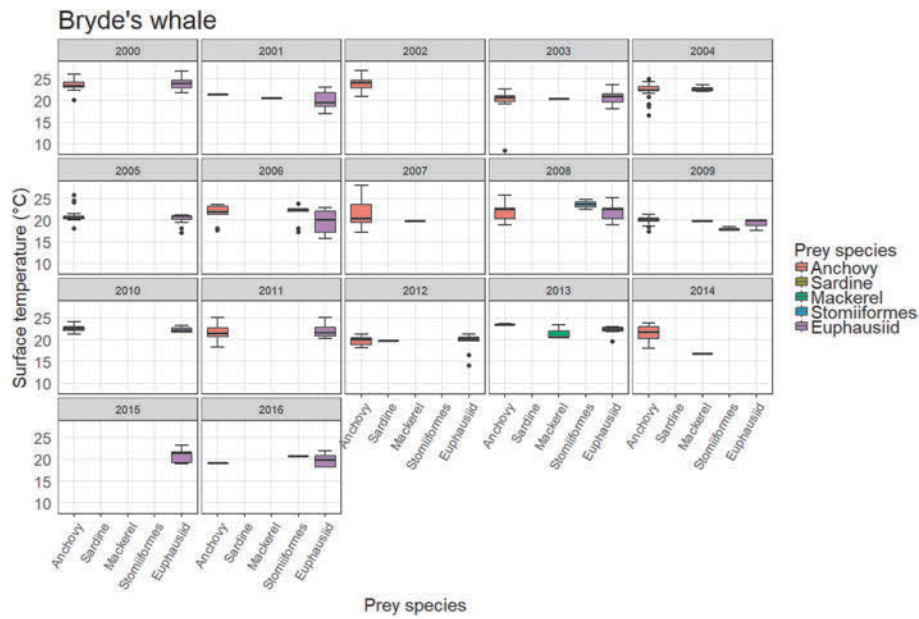


Figure 9. Surface temperature in which Bryde’s whales were sampled, and prey species in their stomachs (2000–2016).

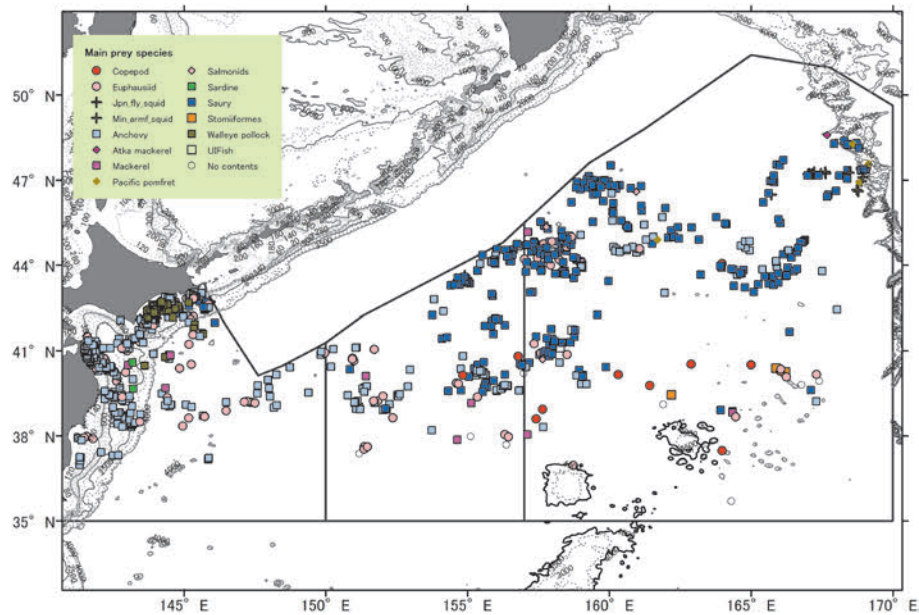


Figure 10. The distribution of sampled common minke whales (based on first sighting), and information of prey species in their stomachs (period 2000–2013). The contours are from the GEBCO bathymetric atlas.

Hokkaido (Figure 11). Japanese anchovy, Pacific saury and euphausiids were also common prey species in this area. The Japanese flying squid (*Todarodes pacificus*) was also found just on the continental slope but only in early years of JARPNI (Figures 10 and 11). Feeding on Japanese anchovy and euphausiid by common minke whales was also found above the continental shelf off Hachinohe (Figure 11).

Prey composition in common minke whales differs among years, but not to the extent as in the case of the sei whale. Recent transition of pelagic fish species was not found probably due to the absence of common minke whale samples

during 2014–2016 (see Table 1 and Figure 12).

Surface temperature varied in the range of 10°C–20°C, and the range was small in the offshore area (Figure 13). Length distribution of Japanese anchovy, mackerel and Pacific saury in the stomach of common minke whales differs within and among years (see Appendix).

DISCUSSION

This study showed a general overview of stomach content analyses and decadal changes in feeding habits for sei, Bryde’s and common minke whales in the western

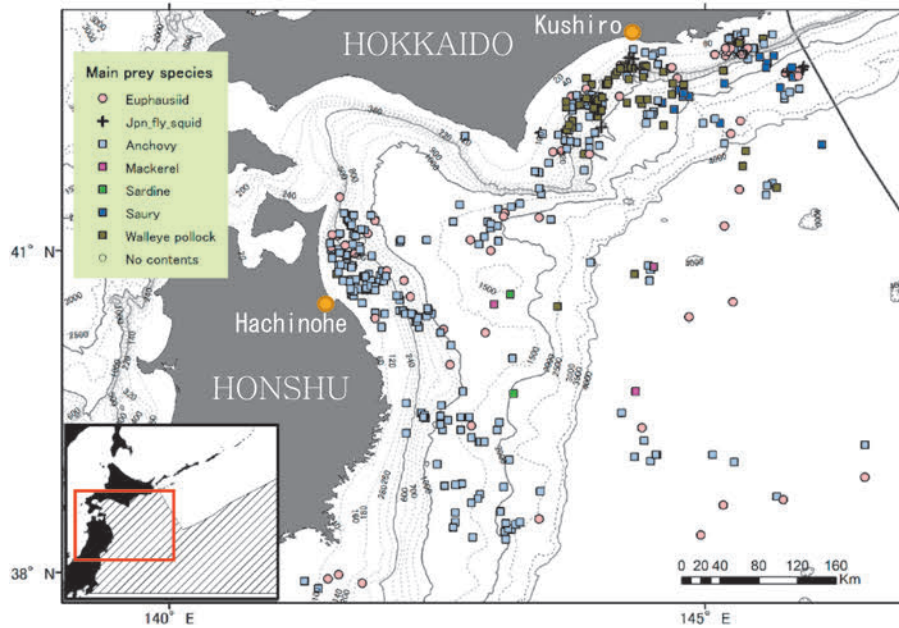


Figure 11. Amplified plots of Figure 10 to show details of the situation in areas near to the coast of the Pacific side of Japan and northern Honshu and Hokkaido.

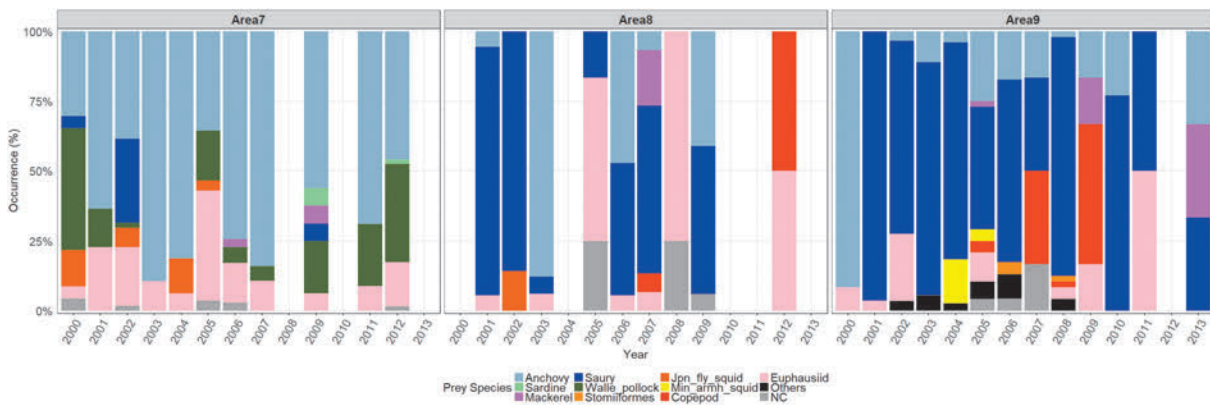


Figure 12. Prey composition in the stomachs of common minke whales sampled in the period 2000–2013, by Area and year.

North Pacific in terms of rather qualitative information based on data collected by JARPNII in the period 2000–2016. The Japanese anchovy was an important prey for the three whale species across the entire survey area, and it was also important in the coastal area for common minke whales. The results showed a remarkable shift in prey species in the case of the sei whales late in the JARPNII period (2015–2016), *i.e.* a limited occurrence of Japanese anchovy and higher incidence of Japanese sardine as main prey in the last three years (2014–2016). A similar decrease of Japanese anchovy as prey species of Bryde's whales also supported the prey shift.

There is no doubt that prey composition in the baleen whales reflected a pelagic fish shift from Japanese anchovy to Japanese sardine and mackerel in the western North Pacific. Mechanisms of these decadal scale shifts of pe-

lagic fish composition in the western North Pacific were influenced by large-scale SST variability by the Aleutian Low (Miller *et al.*, 2004; Sasaki *et al.*, 2012). The responses to the SST in terms of growth rate in sardine, saury and anchovy differ. For example, the patterns of spawning temperature clearly show 'warm' and 'eurythermal' Japanese anchovy and 'cool' and 'stenothermal' Japanese sardine in the western North Pacific (e.g. Takasuka *et al.*, 2007; 2008; Oozeki *et al.*, 2009; Watanabe, 2009; Itoh *et al.*, 2011).

Locations where whales were found in JARPNII elucidated the site-specific feeding behaviors of the whales. Sei whales occurred where both pelagic fish and copepod were available which covers the offshore research area to mainly feed on copepod and small pelagic fish. Bryde's whales occurred from the near coastal area in sub-area 7 to around the Shatsky Rise in sub-area 9, where both

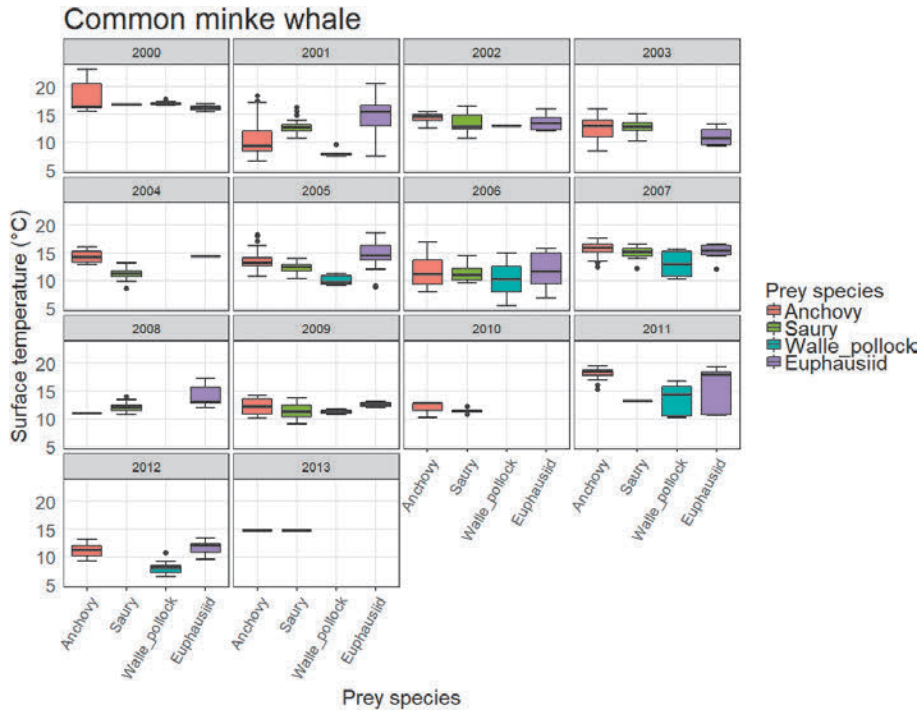


Figure 13. Surface temperature in which common minke whales were sampled, and prey species in their stomachs (2000–2013).

pelagic fish from the Kuroshio Current and euphausiid are both common. They possibly can feed on euphausiids even if pelagic fish is not available. Common minke whales showed unique feeding habits with occurrences at higher latitude to ca. 48°N and high dependency on Pacific saury in the offshore area and prey related to the continental shelf in coastal areas. They feed on minimal armhook squid near the Emperor Seamounts where the mature minimal armhook squid occurs in summer (Konishi and Tamura, 2007). Minke whales also feed on larger prey, such as walleye pollock, Pacific pomfret and salmonids.

This study highlighted the fact that feeding ecology studies of sei, Bryde’s and common minke whales in the western North Pacific are benefited by long-term systematic surveys, which allow decadal-level variability be studied.

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Appendix

Length distribution of main pelagic fish in the stomachs of sei, Bryde's and common minke whales during JARPNII period (2000–2016)

Length distributions of pelagic fishes in the stomach of sei, Bryde's and common minke whales are shown in Figs. A1–A4. Mackerels include both *Scomber japonicus* and *S. australasicus* because these species were sometimes found mingled in half digested stomach contents. The length distributions differ among years and whale species, suggesting that prey length, as also defined as age class, reflects the relative abundance and availability in the feeding area for the baleen whales.

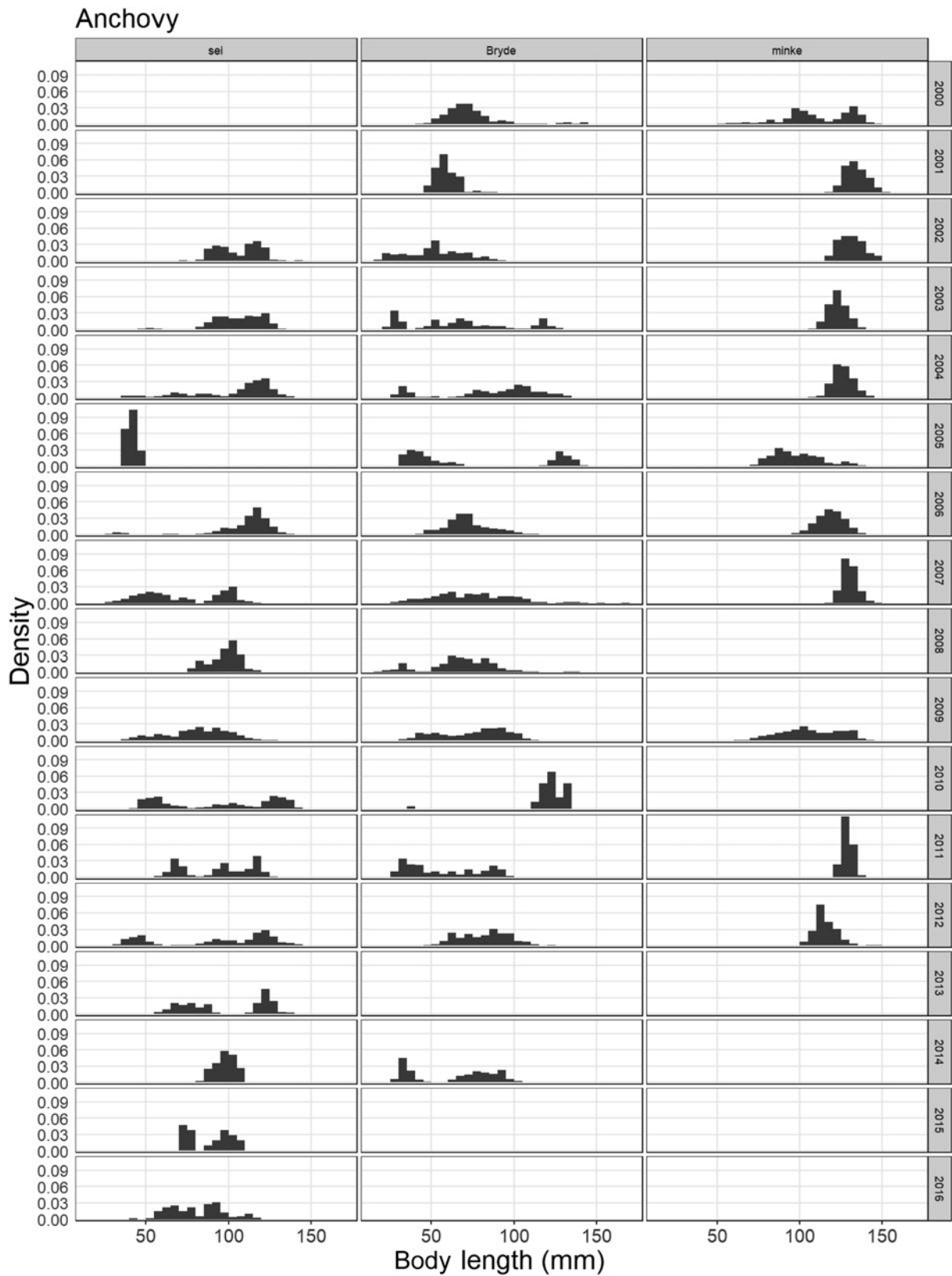


Figure A1. Length distribution of Japanese anchovy in the stomach of sei, Bryde's and common minke whales during JARPNII (2000–2016).

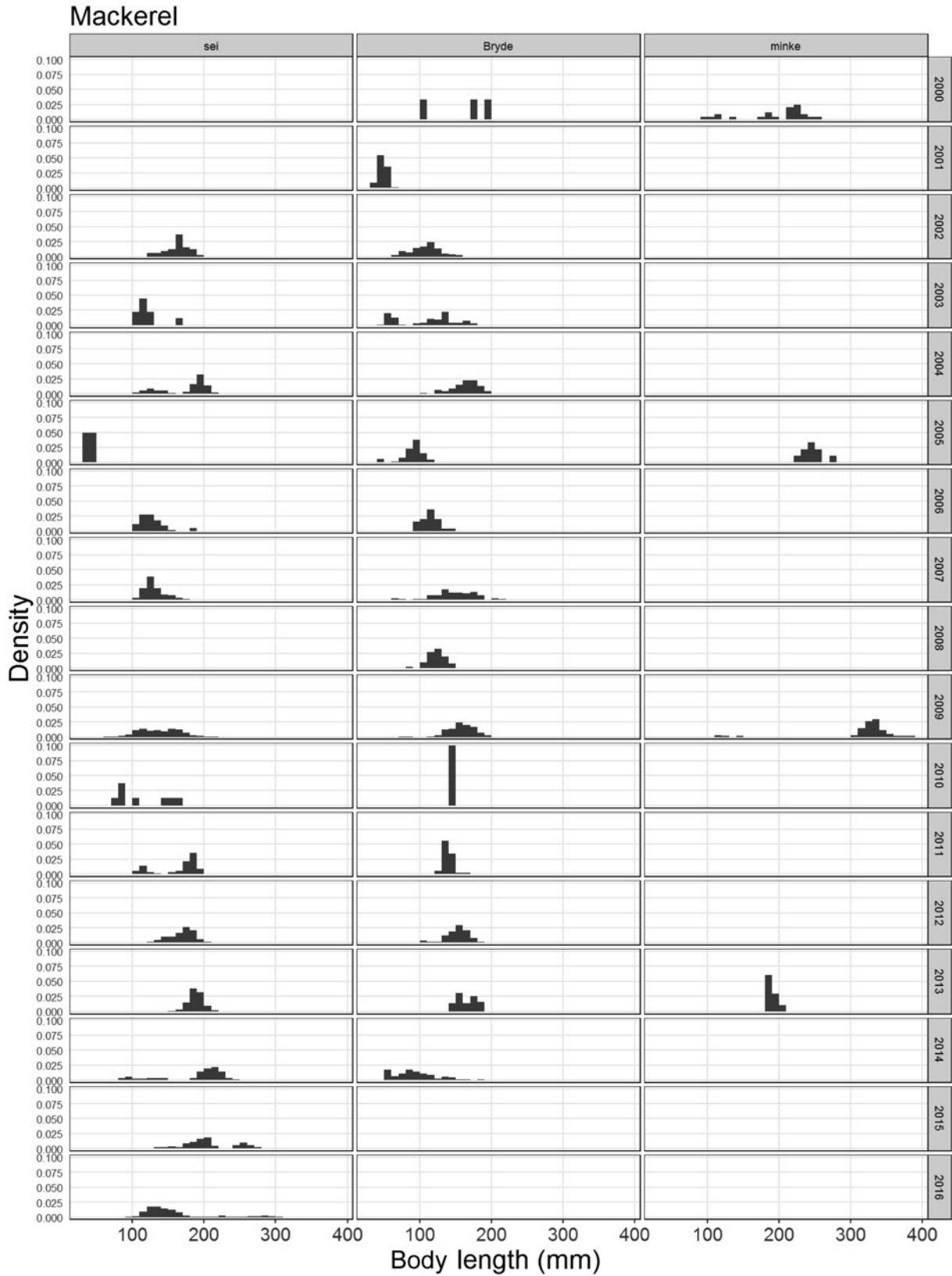


Figure A2. Fork length distribution of mackerels (including *Scomber japonicus* and *S. australasicus*) in the stomach of sei, Bryde's and common minke whales during JARPNII (2000–2016).

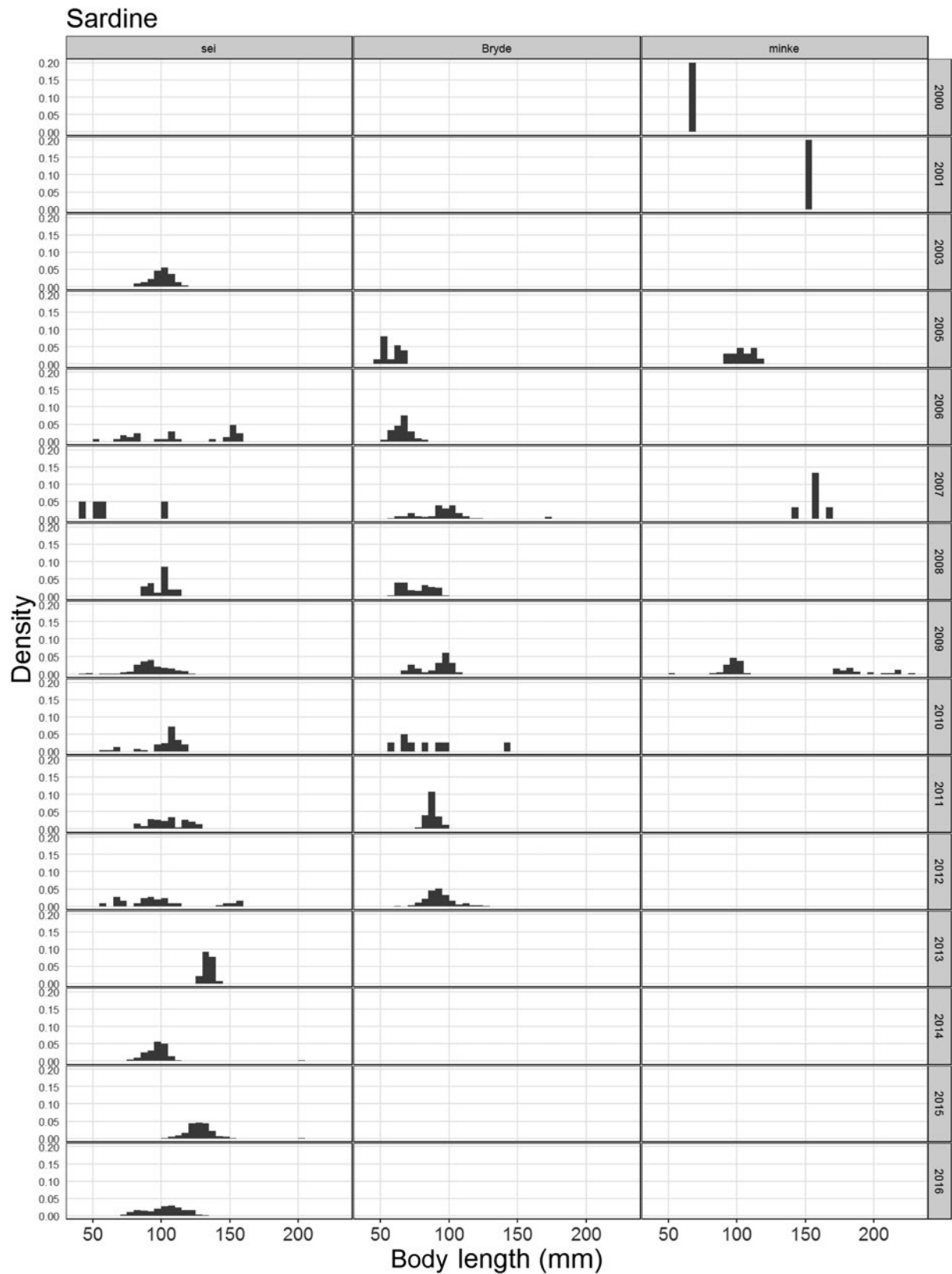


Figure A3. Length distribution of Japanese sardine in the stomach of sei, Bryde's and common minke whales during JARPNII (2000–2016).

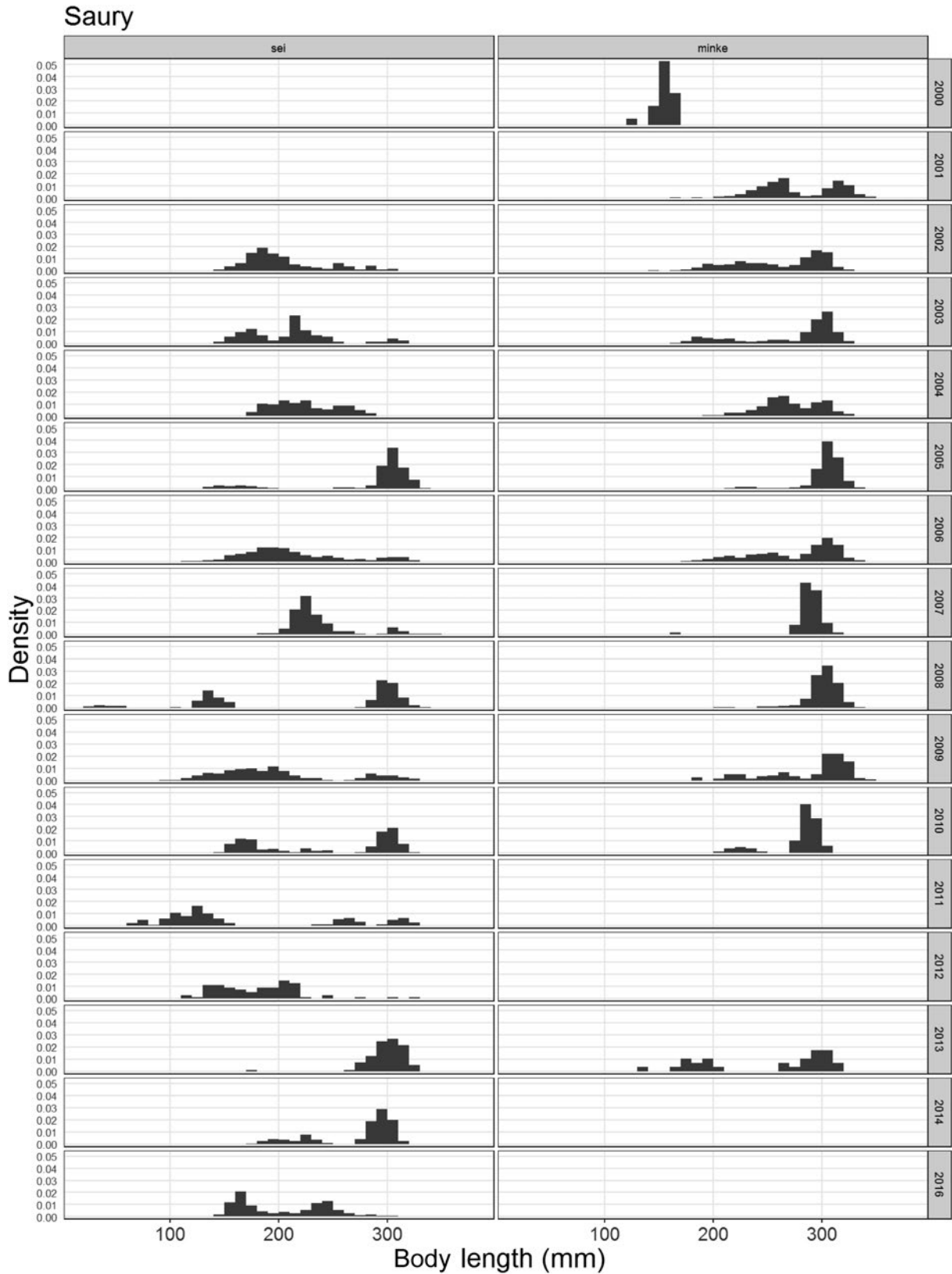


Figure A4. Knob length distribution of Pacific saury in the stomach of sei, and common minke whales during JARPNII (2000–2016).

Technical Report (not peer reviewed)

Temporal trend of total mercury levels in common minke, sei and Bryde's whales in the western North Pacific in the period 1994–2014

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ABSTRACT

To examine yearly changes of total mercury (Hg) concentrations in common minke (*Balaenoptera acutorostrata*), sei (*B. borealis*) and Bryde's (*B. edeni*) whales in the western North Pacific, total Hg concentrations were measured in muscle samples from whales collected between 1994 and 2014 by JARPN/JARPNII. Averages and standard deviations of total Hg concentrations in the samples of mature male common minke whales from coastal area off Kushiro and Sanriku were 0.22 ± 0.05 , 0.22 ± 0.06 , ppm wet wt, and from off-shore sub-areas 7, 8 and 9 were 0.22 ± 0.05 , 0.22 ± 0.06 and 0.24 ± 0.10 ppm, respectively. The concentration in sei whales from sub-area 9 was 0.044 ± 0.013 ppm, and that in Bryde's whales from sub-areas 8 and 9 was 0.044 ± 0.013 ppm. Multiple robust linear regression analysis was carried out considering several variables: sampling year, sampling location (longitude and latitude), sampling date, body length, blubber thickness and main prey item observed in their stomach. Results of the analyses can be summarized as follow: no significant yearly changes of total Hg concentrations in muscle of common minke whales off Kushiro, Sanriku and sub-area 8; no significant yearly changes of total Hg concentrations in muscle of Bryde's whales in sub-areas 8 and 9; significant yearly changes of total Hg concentrations in muscle of minke whales from sub-areas 7 and 9, and significant yearly changes of total Hg concentrations in muscle of sei whales from sub-area 9. Temporal trends of total Hg concentrations have not been observed in environmental samples, lower trophic organisms and baleen whales, except for those cases mentioned above. Temporal changes in total Hg concentrations in common minke whales (sub-areas 7 and 9) and sei whales (sub-area 9) may reflect changes in their food habits rather than changes of background levels of total Hg in the marine environment. Consequently, it is concluded that the temporal trend of total Hg concentrations in the marine habitat of baleen whales in the western North Pacific remained stable in the research period.

INTRODUCTION

Mercury (Hg) is one of the most neurotoxic chemicals to human and wildlife in the environment. It is released into the ocean from a variety of sources such as volcanic emission, degassing from soil and combustion of coal from power plants. It is released in the form of elemental mercury, and is accumulated into top predators through the food chain in the marine ecosystem. There is a need to monitor Hg in the oceans.

Cetaceans have been used to monitor spatial and temporal trends of Hg in the oceans (Sanpera *et al.*, 1993; Borrell and Reijnders, 1999). Pollutants can be monitored in wide areas and integrated in some way over time by using cetacean as indicators because they are located at the top of the food chain in the marine ecosystem, and are mobile and long-lived animals.

The present study examines temporal changes of total Hg concentrations in common minke, sei and Bryde's whales in the western North Pacific. To understand the pattern of accumulation of total Hg in whales it is important to consider some biological information such as sex and sexual maturity of the animals, and ecological information such as feeding habits of whales and indicators of body condition. The present study, which is based on samples collected by the Japanese Whale Research Program under Special Permit in the western North Pacific (JARPN) and its Second Phase, JARPNII in the period 1994–2014, considers such biological and ecological information to understand and interpret the pattern of accumulation of Hg in whales.

MATERIALS AND METHODS

Whale sampling

Common minke (1994–2014), sei (2002–2014) and Bryde's (2002–2013) whales were sampled in sub-areas 7, 8 and 9 under the JARPN and JARPNII. In addition common minke whales were sampled in coastal areas off Sanriku (2003–2014) and off Kushiro (2002–2014) (Figure 1). Details of the sampling procedures are given in Tamura *et al.* (this TEREP-ICR issue).

For all sampled whales, biological sampling and measurements were conducted on the research base vessel, *Nisshin-maru* in the offshore component (JARPN/JARPNII), and at the land research stations in the case of the coastal component (JARPNII).

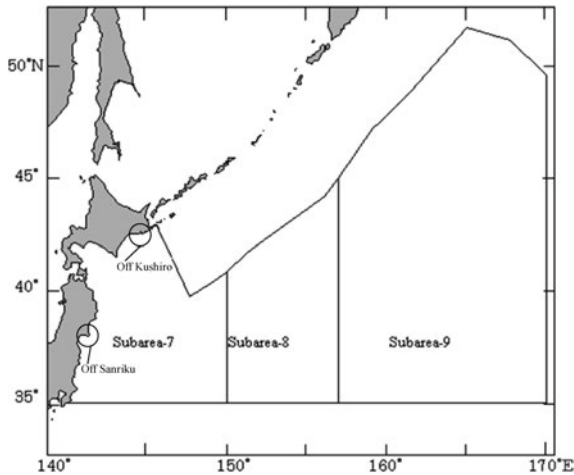


Figure 1. Sub-areas surveyed by the JARPN and the JARPNII surveys, excluding the EEZ of foreign countries.

Tissue sampling for Hg analysis

Figure 2 shows the overall process for Hg analysis from the tissue sampling at the field to the laboratory work. Muscle tissues were collected for total Hg analysis.

Biological information

The kind of biological information obtained from each sampled whale was described by Bando (this TEREP-ICR issue). In this study information on body length, blubber thickness and reproductive status of whales was used. Only mature male animals were used. Males of common minke, Bryde's and sei whales were defined as sexually mature by testis weight of more than 290g, 560g and 1,090g (heavier side), respectively. Only common minke whales from the Okhotsk Sea-West Pacific stock (O-stock) as identified by the microsatellite DNA analysis (Goto *et al.*, this TEREP-ICR issue), were used.

The forestomach contents have proved sufficient for determination of the common minke whale diet (see Konishi *et al.*, this TEREP-ICR issue). Data on prey composition and prey's Hg concentrations used in the present study were based on contents from forestomach and analyses conducted by Yasunaga and Fujise (2009) and MHLW (2005).

Tables 1–3 show the number of samples by sub-area and year, and the biological information used for common minke, sei and Bryde's whales in this study.

Measurement of total mercury concentration in muscle of whales

Total Hg analysis was conducted at the laboratory of the Institute of Cetacean Research (ICR) in Tokyo and also at the



Figure 2. Schematic diagram of total Hg analysis in muscle tissue of whales.

Table 1

Biological data and total mercury concentrations in muscle of common minke whales (mature males) from the western North Pacific during 1994 and 2014.

Sub area	Year	<i>n</i>	Body length (m)	Blubber thickness (cm)	Muscular Hg (ppm wet wt.)
7	1996	18	(7.36±0.28)	(3.1±0.4)	(0.23±0.05)
	1998	35	(7.50±0.27)	(2.6±0.4)	(0.20±0.05)
	1999	31	(7.41±0.29)	(3.6±0.8)	(0.20±0.04)
	2000	6	(7.30±0.25)	(3.6±1.0)	(0.24±0.04)
	2001	27	(7.45±0.31)	(2.4±0.5)	(0.21±0.04)
	2002	33	(7.52±0.27)	(3.7±0.8)	(0.22±0.03)
	2003	11	(7.42±0.40)	(2.4±0.5)	(0.20±0.07)
	2004	6	(7.56±0.21)	(4.1±0.6)	(0.22±0.02)
	2005	17	(7.51±0.23)	(3.3±0.9)	(0.27±0.04)
	2006	16	(7.39±0.39)	(2.4±0.7)	(0.22±0.05)
	2007	41	(7.47±0.26)	(3.0±0.7)	(0.23±0.04)
	2009	11	(7.71±0.26)	(3.2±0.7)	(0.21±0.06)
	2011	23	(7.47±0.34)	(3.5±0.6)	(0.25±0.05)
	2012	19	(7.37±0.34)	(2.6±0.4)	(0.24±0.08)
	Total	294	(7.46±0.30)	(3.1±0.8)	(0.22±0.05)
Kushiro	2002	14	(7.22±0.34)	(4.3±1.0)	(0.21±0.06)
	2004	23	(7.43±0.24)	(4.2±0.6)	(0.20±0.03)
	2005	25	(7.44±0.32)	(3.5±0.6)	(0.25±0.05)
	2006	10	(7.52±0.29)	(4.4±0.8)	(0.22±0.03)
	2007	14	(7.33±0.59)	(4.1±0.8)	(0.23±0.06)
	2008	5	(7.26±0.21)	(4.3±1.0)	(0.25±0.01)
	2009	6	(7.54±0.37)	(4.3±0.4)	(0.21±0.07)
	2010	3	(7.49±0.21)	(3.8±0.4)	(0.23±0.02)
	2011 spring	4	(7.40±0.41)	(3.2±0.6)	(0.24±0.03)
	2011 autumn	10	(7.26±0.37)	(4.4±1.0)	(0.23±0.02)
	2012	5	(7.48±0.21)	(5.0±0.7)	(0.21±0.06)
	2013	23	(7.33±0.35)	(5.0±0.9)	(0.19±0.04)
	2014	10	(7.33±0.28)	(5.0±0.8)	(0.21±0.09)
	Total	152	(7.38±0.35)	(4.3±0.9)	(0.22±0.05)
	Sanriku	2003	8	(7.10±0.35)	(2.9±0.5)
2005		3	(7.27±0.13)	(2.5±0.3)	(0.29±0.02)
2006		6	(7.24±0.32)	(2.8±0.2)	(0.19±0.05)
2007		10	(7.50±0.30)	(2.5±0.4)	(0.25±0.08)
2008		3	(7.49±0.32)	(3.2±0.3)	(0.19±0.03)
2009		1	(6.85±)	(2.8±)	(0.21±)
2010		5	(7.32±0.24)	(3.1±0.6)	(0.20±0.05)
2012		2	(7.31±)	(3.4±)	(0.18±)
2013		2	(7.52±)	(3.5±)	(0.30±)
2014		2	(7.38±0.39)	(2.7±0.3)	(0.31±0.04)
Total		42	(7.32±0.32)	(2.8±0.5)	(0.22±0.06)

field research stations of the coastal and offshore components. The muscle sample was stripped externally to avoid contamination before analysis, approximately 100mg of tissue was weighed, and set on the total Hg analyser (Nippon Instruments Co., MA-3000) which applies a thermal

decomposition system (Figure 2). To increase the accuracy of the determination, triplicate analyses were performed on each sample. Accuracy and precision of the present system were confirmed using standard reference materials, DORM-3 (NRCC, muscle of dogfish).

Table 1
Continued.

Sub area	Year	<i>n</i>	Body length (m)	Blubber thickness (cm)	Muscular Hg (ppm wet wt.)
8	1996	11	(7.44±0.33)	(2.6±0.5)	(0.25±0.04)
	1997	26	(7.44±0.36)	(3.5±0.6)	(0.27±0.06)
	1998	28	(7.54±0.25)	(2.6±0.4)	(0.23±0.05)
	2001	13	(7.78±0.34)	(2.3±0.4)	(0.21±0.05)
	2002	5	(7.71±0.21)	(3.2±0.6)	(0.20±0.11)
	2003	27	(7.50±0.24)	(2.4±0.4)	(0.18±0.06)
	2005	2	(7.54±)	(2.2±)	(0.16±)
	2006	24	(7.51±0.26)	(2.9±0.6)	(0.21±0.05)
	2007	10	(7.55±0.26)	(2.3±0.4)	(0.22±0.06)
	2008	3	(7.52±0.05)	(2.7±0.4)	(0.23±0.02)
	2009	6	(7.23±0.34)	(3.2±0.6)	(0.20±0.06)
	Total		155	(7.52±0.30)	(2.8±0.6)
9	1994	16	(7.42±0.27)	(3.5±0.7)	(0.36±0.16)
	1995	68	(7.45±0.31)	(3.1±0.5)	(0.27±0.06)
	1997	39	(7.41±0.32)	(3.1±0.5)	(0.25±0.11)
	2000	12	(7.51±0.23)	(2.6±0.5)	(0.16±0.03)
	2001	19	(7.69±0.29)	(2.5±0.4)	(0.19±0.05)
	2002	21	(7.55±0.21)	(2.9±0.6)	(0.20±0.05)
	2003	28	(7.50±0.32)	(2.9±0.6)	(0.18±0.05)
	2004	50	(7.47±0.21)	(3.9±0.7)	(0.20±0.07)
	2005	25	(7.49±0.29)	(3.5±0.6)	(0.17±0.05)
	2006	16	(7.56±0.30)	(3.4±0.6)	(0.28±0.08)
	2007	4	(7.56±0.20)	(2.2±0.3)	(0.18±0.09)
	2008	36	(7.45±0.26)	(3.1±0.7)	(0.30±0.15)
	2009	5	(7.41±0.41)	(3.0±0.8)	(0.19±0.06)
	2010	9	(7.66±0.23)	(3.2±0.5)	(0.22±0.03)
	2013	3	(7.59±0.22)	(4.0±1.3)	(0.19±0.06)
	Total		351	(7.49±0.28)	(3.2±0.7)

Table 2

Biological data and mercury concentrations in muscle of sei whales (mature males) from the western North Pacific during 2002 and 2014.

Sub area	Year	<i>n</i>	Body length (m)	Blubber thickness (cm)	Muscular Hg (ppm wet wt.)
9	2002	5	(13.60±0.16)	(4.7±0.5)	(0.050±0.012)
	2003	5	(13.80±0.43)	(4.7±0.7)	(0.057±0.007)
	2004	5	(13.61±0.04)	(4.1±0.6)	(0.050±0.009)
	2005	5	(13.54±0.38)	(4.8±0.6)	(0.049±0.011)
	2006	5	(13.84±0.51)	(4.9±0.6)	(0.052±0.006)
	2007	5	(13.92±0.08)	(5.8±0.7)	(0.058±0.010)
	2011	15	(13.82±0.42)	(4.8±0.8)	(0.039±0.009)
	2012	21	(13.74±0.45)	(5.0±0.5)	(0.030±0.011)
	2013	26	(13.76±0.50)	(5.6±0.8)	(0.040±0.009)
	2014	21	(13.80±0.36)	(4.8±0.5)	(0.053±0.009)
Total	2002–2014	113	(13.76±0.41)	(5.0±0.8)	(0.044±0.013)

Table 3

Biological data and mercury concentrations in muscle of Bryde's whales (mature males) from the western North Pacific during 2002 and 2013.

Sub area	Year	<i>n</i>	Body length (m)	Blubber thickness (cm)	Muscular Hg (ppm wet wt.)
8, 9	2002	5	(12.67±0.43)	(4.5±0.6)	(0.051±0.008)
	2004	5	(12.59±0.36)	(4.4±0.7)	(0.045±0.008)
	2006	5	(12.36±0.47)	(4.5±0.5)	(0.040±0.011)
	2007	5	(12.63±0.35)	(3.9±0.7)	(0.049±0.008)
	2011	3	(12.31±0.27)	(3.6±0.5)	(0.022±0.005)
	2012	6	(12.85±0.48)	(4.8±1.0)	(0.046±0.019)
	2013	9	(12.79±0.38)	(4.7±0.8)	(0.047±0.010)
Total	2002–2013	38	(12.64±0.41)	(4.4±0.8)	(0.044±0.013)

Statistical analysis

The yearly changes of total Hg concentrations in muscle of whales were assessed by multiple robust linear regression in the context of several variables (R Development Core Team, 2006). The following independent variables were considered: 'Year,' 'Date,' 'Latitude,' 'Longitude,' 'Body length,' 'Blubber thickness' and 'Main prey item'. All variables except the main prey item, were logarithmically transformed.

Categorical parameters of main prey items used in the analyses were the following: minke whale and sub-area 7 (Japanese anchovy: *Engraulis japonicus*, Euphausiids, Japanese flying squid: *Todarodes pacificus*, mackerel: *Scomber japonicus*, Japanese sardine: *Sardinops melanostictus*, Pacific saury: *Cololabis saira* and walleye pollock: *Theragra chalcogramma*); minke whale and sub-area 8 (anchovy, copepods, Euphausiids, Japanese flying squid, mackerel and Pacific saury); minke whale and sub-area 9 (anchovy, Atka mackerel: *Pleurogrammus monopterygius*, Copepods, Euphausiids, mackerel, minimal armhook squid: *Berryteuthis anonychus*, oceanic lightfish: *Vinciguerria nimbaria*, Pacific pomfret: *Brama japonica*, salmonids, Pacific saury); sei whale and sub-area 9 (Japanese anchovy, Copepods, Euphausiids, mackerel, minimal armhook squid, Japanese sardine and Pacific saury); Bryde's whale and sub-areas 8 and 9 (Japanese anchovy, Euphausiids and mackerel). Anchovy is the baseline prey item in sub-areas 7–9 so the effects of anchovy being the main prey item are included in the model intercepts. Categorical parameters of main prey items were not used in the case of Sanriku and Kushiro. In Sanriku the prey items of common minke whales were almost all sand lance. In Kushiro the diversity of prey items was too high in relation to the number of whale samples.

The cases of whales with empty stomach and damage by harpoon were excluded from the analysis. Furthermore, a generalized additive model (GAM) was used to

examine the flexion point in the yearly changes of total Hg concentrations in muscle of whales. A *p* value of less than 0.05 was considered to indicate statistical significance in all tests. These statistical analyses were performed using the free software R, version 3.3.0.

RESULTS

Tables 1–3 show the total Hg concentrations in muscle of common minke, sei and Bryde's whales, respectively.

The results of multiple robust linear regression to examine yearly changes in the context of several variables are given in Tables 4–10. F statistics showed that the overall regression was statistically significant in all cases ($p < 0.05$). Only in the cases of minke whales from sub-areas 7 and 9 (Tables 4, 8), and sei whales from sub-area 9 (Table 9), the total Hg concentrations were significantly associated with sampling year. In these cases the simple and GAM plots against research year are shown in Figures 3 and 4 for minke whales in sub-areas 7 and 9, respectively, and in Figure 5 for sei whale in sub-area 9.

Slight flexion points of yearly trends of muscular total Hg were observed in minke whales from sub-area 9 in 2008 and in sei whales from sub-area 9 in 2012.

DISCUSSION

Results of the analyses can be summarized as follows: no significant yearly changes of total Hg concentrations in muscle of common minke whales off Kushiro, Sanriku and sub-area 8; no significant yearly changes of total Hg concentrations in muscle of Bryde's whales in sub-areas 8 and 9; significant yearly changes of total Hg concentrations in muscle of minke whales from sub-areas 7 and 9, and significant yearly changes of total Hg concentrations in muscle of sei whales from sub-area 9.

It should be noted here that no significant trends of total Hg concentrations were found in prey species such as the krill, Japanese anchovy and Pacific saury from 1994

Table 4

Results of multiple robust linear regression analysis with 'total Hg levels in muscle of common minke whales from subarea 7' as the dependent variable.

a) Analysis of Variance				
Source				
Robust residual SE	0.20			
R ²	0.135			
Adjusted R ²	0.0969			
b) Variables				
Model	B	SE	T	p value
Intercept	-198.59771	53.16048	-3.736	p<0.05
Year	26.054	6.788	3.84	p<0.05
Body length	-0.138	0.237	-0.58	0.559
Blubber thickness	0.059	0.060	0.98	0.328
Latitude	-0.156	0.541	-0.29	0.773
Longitude	-0.064	0.568	-0.11	0.911
Date	0.038	0.017	2.21	p<0.05
MainPrey_Euphausiids	0.076	0.045	1.70	0.089
MainPrey_JFSquid	0.062	0.054	1.15	0.250
MainPrey_Mackerel	0.372	0.026	14.53	p<0.05
MainPrey_Sardine	-0.294	0.036	-8.25	p<0.05
MainPrey_Saury	0.097	0.047	2.07	p<0.05
MainPrey_WalleyePollock	0.138	0.049	2.83	p<0.05

Table 5

Results of multiple robust linear regression analysis with 'total Hg levels in muscle of common minke whales from off Kushiro' as the dependent variable.

a) Analysis of Variance				
Source				
Robust residual SE	0.2834			
R ²	0.3472			
Adjusted R ²	0.3176			
b) Variables				
Model	B	SE	T	p value
Intercept	63.6	98.2	0.648	0.518
Year	-2.14	10.61	-0.20	0.840
Body length	0.904	0.843	1.07	0.285
Blubber thickness	-0.322	0.077	-4.15	p<0.05
Latitude	5.384	6.941	0.78	0.439
Longitude	-14.215	13.618	-1.04	0.298
Date	0.119	0.149	0.80	0.424

to 2008 (Yasunaga and Fujise, 2009), and that total Hg concentrations in surface water of the North Pacific did not change from the 1980's to 2000's (Laurier *et al.*, 2004; Sunderland *et al.*, 2009).

Firstly those cases where significant yearly trend was observed, are discussed.

Table 6

Results of multiple robust linear regression analysis with 'total Hg levels in muscle of common minke whales from off Sanriku' as the dependent variable.

a) Analysis of Variance				
Source				
Robust residual SE	0.2543			
R ²	0.2374			
Adjusted R ²	0.1067			
b) Variables				
Model	B	SE	T	p value
Intercept	-50.2	286.8	-0.175	0.862
Year	31.50	44.65	0.71	0.485
Body length	-0.029	0.025	-1.15	0.257
Blubber thickness	-0.400	0.274	-1.46	0.153
Latitude	23.792	17.674	1.35	0.187
Longitude	-56.288	43.990	-1.28	0.209
Date	1.079	0.569	1.90	0.066

Table 7

Results of multiple robust linear regression analysis with 'total Hg levels in muscle of common minke whales from subarea 8' as the dependent variable.

a) Analysis of Variance				
Source				
Robust residual SE	0.2439			
R ²	0.3021			
Adjusted R ²	0.2473			
b) Variables				
Model	B	SE	T	p value
Intercept	163.35009	95.210	1.716	0.088
Year	-17.288	13.636	-1.27	0.207
Body length	1.219	0.534	2.29	p<0.05
Blubber thickness	0.288	0.108	2.67	p<0.05
Latitude	-0.281	1.628	-0.17	0.863
Longitude	-7.181	2.778	-2.59	p<0.05
Date	0.495	0.699	0.71	0.481
MainPrey_Copepods	-0.020	0.080	-0.25	0.805
MainPrey_Euphausiids	-0.104	0.144	-0.72	0.471
MainPrey_JFSquid	-0.137	0.101	-1.36	0.175
MainPrey_Mackerel	0.309	0.170	1.82	0.072
MainPrey_Saury	0.180	0.063	2.84	p<0.05

In sub-area 7, total Hg concentrations in muscle of common minke whales (period 1996–2012) were significantly associated with Intercept (-), Year (+), Date (+), Main prey items (mackerel, sardine, saury and Walleye pollock) (Table 4). A comparison of total Hg concentrations in muscle of common minke whale and in the whole

Table 8

Results of multiple robust linear regression analysis with 'total Hg levels in muscle of common minke whales from subarea 9' as the dependent variable.

a) Analysis of Variance				
Source				
Robust residual SE	0.2834			
R ²	0.3472			
Adjusted R ²	0.3176			
b) Variables				
Model	B	SE	T	p value
Intercept	330.3	55.7	5.932	p<0.05
Year	-43.74	7.31	-5.99	p<0.05
Body length	0.150	0.441	0.34	0.735
Blubber thickness	0.164	0.092	1.78	0.076
Latitude	1.809	0.672	2.69	p<0.05
Longitude	-0.784	0.899	-0.87	0.384
Date	-1.424	0.301	-4.73	p<0.05
MainPrey_AtkaMackerel	-0.368	0.076	-4.86	p<0.05
MainPrey_Copepods	0.044	0.224	0.20	0.843
MainPrey_Euphausiids	-0.177	0.159	-1.12	0.266
MainPrey_Mackerel	0.050	0.139	0.36	0.722
MainPrey_MAFSquid	-0.386	0.209	-1.84	0.066
MainPrey_OceanicLightfish	-0.439	0.060	-7.30	p<0.05
MainPrey_PacificPomfret	0.930	0.335	2.78	p<0.05
MainPrey_Salmonids	0.213	0.132	1.61	0.108
MainPrey_Saury	0.188	0.050	3.73	p<0.05

body of the main prey species in the stomachs is shown in Table 11. Total Hg concentrations in minke whales having mackerel in the stomach were the highest while those having sardine in stomach was the lowest. The number of minke whales having mackerel and sardine in their stomach were only one and two whales, respectively. These observations indicate that total Hg concentrations of common minke whales from sub-area 7 may be less affected by total Hg in the prey species.

In sub-area 9, total Hg concentrations in muscle of common minke whales (period 1996–2012) were significantly associated with Intercept (+), Year (-), Latitude (+), Date (-) and Main prey items (Atka mackerel, oceanic lightfish, Pacific pomfret and saury) (Table 8). A comparison of total Hg concentrations in muscle of minke whale and in the whole body of the main prey species in the stomachs is shown in Table 12. Total Hg concentrations in common minke whales having Pacific pomfret in their stomach were the highest, and total Hg concentrations in Pacific pomfret were one or two orders of magnitude higher than those in the other prey items. Also total Hg concentrations in common minke whales having zooplankton such as copepods and euphausiids were

Table 9

Results of multiple linear regression analysis with 'total Hg levels in muscle of sei whales from subarea 9' as the dependent variable.

a) Analysis of Variance				
Source				
Robust residual SE	0.2526			
R ²	0.3344			
Adjusted R ²	0.2293			
b) Variables				
Model	B	SE	T	p value
Intercept	405.9	137.4	2.954	p<0.05
Year	-54.46	18.35	-2.97	p<0.05
Body length	3.579	1.029	3.48	p<0.05
Blubber thickness	0.024	0.262	0.09	0.927
Latitude	0.910	1.188	0.77	0.446
Longitude	-1.233	1.431	-0.86	0.392
Date	-0.602	0.481	-1.25	0.215
MainPrey_Copepods	-0.182	0.096	-1.90	0.061
MainPrey_Euphausiids	-0.259	0.159	-1.63	0.107
MainPrey_Mackerel	-0.064	0.097	-0.66	0.510
MainPrey_Min.arm squid	-0.012	0.105	-0.12	0.906
MainPrey_Sardine	0.193	0.104	1.85	0.069
MainPrey_Saury	-0.136	0.096	-1.41	0.164

Table 10

Results of multiple robust linear regression analysis with 'total Hg levels in muscle of Bryde's whales from subareas 8 and 9' as the dependent variable.

a) Analysis of Variance				
Source				
Robust residual SE	0.2025			
R ²	0.3592			
Adjusted R ²	0.1029			
b) Variables				
Model	B	SE	T	p value
Intercept	-101.1	162.8	-0.621	0.541
Year	9.93	22.02	0.45	0.657
Body length	2.431	1.453	1.67	0.110
Blubber thickness	-0.320	0.317	-1.01	0.325
Latitude	1.693	1.534	1.10	0.283
Longitude	1.933	2.810	0.69	0.499
Date	0.503	0.376	1.34	0.196
MainPrey_Euphausiids	-0.106	0.071	-1.51	0.148
MainPrey_Mackerel	-0.240	0.274	-0.88	0.391

lower than the others. Total Hg concentrations in the zooplankton were one or two orders of magnitude lower than those in the other prey items. Furthermore, yearly changes were observed in food items of the common minke whales in the same period (Konishi *et al.*, 2016).

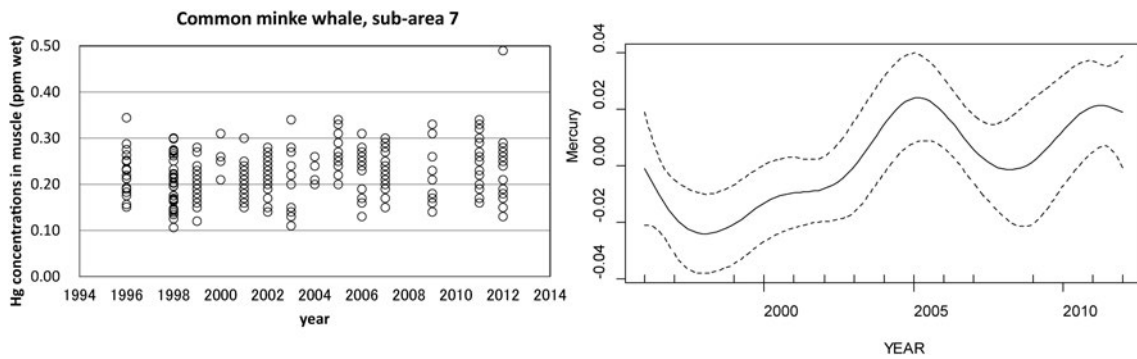


Figure 3. Simple plots and smoothing plots using the generalized additive model of total Hg concentrations in muscle of common minke whales (mature males, O-stock) in sub-area 7 against research years during the period 1996–2012.

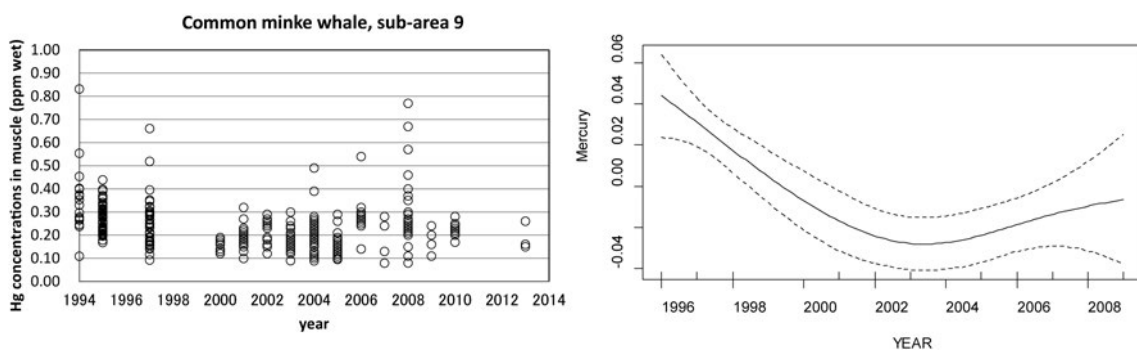


Figure 4. Simple plots and smoothing plots using the generalized additive model of total Hg concentrations in muscle of common minke whales (mature males, O-stock) in sub-area 9 against research years during the period 1994–2013.

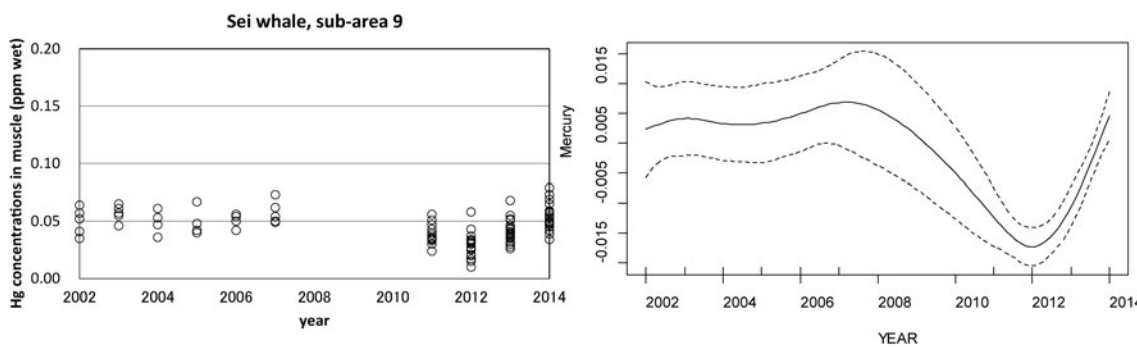


Figure 5. Simple plots and smoothing plots using the generalized additive model of total Hg concentrations in muscle of sei whales (mature males) in sub-area 9 against research years during the period 2002–2014.

A peak of total Hg concentration was observed in 2008 (Figure 4), and Pacific pomfret was observed in the stomach contents of common minke whales from sub-area 9 in that year (Tamura *et al.*, 2009). These results indicate that changes of total Hg concentrations in common minke whales in sub-area 9 reflect changes in food habits of this whale species rather than changes of background levels of total Hg in the marine environment.

In sub-area 9, total Hg concentrations in sei whales (period 2002–2014) were significantly associated with Intercept (+), Year (–) and Body length (+) (Table 9), whereas

those in 2012 were lower than the others in the GAM plots (Figure 5). A comparison of total Hg concentrations in muscle of sei whale and in the whole body of the main prey species in the stomachs are shown in Table 13. Total Hg concentrations in sei whales having anchovy and sardine in the stomach were slightly higher than the other two whale species from sub-area 9. These results indicate that total Hg concentrations of sei whales from sub-area 9 may be less affected by total Hg in the food items.

Temporal trends of total Hg concentrations have not been observed in environmental samples, lower trophic

Table 11
Comparison between Hg concentrations in muscle of common minke whales and in the whole body of prey items in sub-area 7.

	Anchovy	Euphausiids	JFSquid	Mackerel	Sardine	Saury	Walleye Pollock
Muscle of whales	Ave.±SD (0.21±0.051)	(0.22±0.049)	(0.22±0.03)	(0.33±)	(0.17±)	(0.23±0.043)	(0.24±0.056)
<i>n</i>	238	33	8	1	2	26	28
Whole of prey spp.	Ave.±SD (0.037±0.025)	(0.005±0.003)	(0.058±)	(0.020±0.002)	(0.018±)	(0.038±0.015)	(0.045±)
<i>n</i>	20*	19*	57**	5*	66**	41*	2*

*: Yasunaga and Fujise (2009); **: Ministry of Health, Labour and Welfare (2005)

Table 12
Comparison between Hg concentrations in muscle of common minke whales and in the whole body of prey species in sub-area 9.

	Anchovy	Atka Mackerel	Copepods	Euphausiids	Mackerel	MAFSquid	Oceanic Lightfish	Pacific Pomfret	Salmonids	Saury
Muscle of whales	Ave.±SD (0.22±0.10)	(0.13±)	(0.17±0.07)	(0.18±0.09)	(0.19±0.04)	(0.19±0.12)	(0.11±)	(0.44±0.26)	(0.32±0.08)	(0.24±0.09)
<i>n</i>	83	1	6	15	3	5	1	6	5	257
Whole of prey spp.	Ave.±SD (0.037±0.025)	(0.086±)	(0.005±0.003)	(0.005±0.003)	(0.020±0.002)			(0.23±0.03)	(0.027±)	(0.038±0.015)
<i>n</i>	20*	61**	5*	19*	5*			3*	41**	41*

*: Yasunaga and Fujise (2009); **: Ministry of Health, Labour and Welfare (2005)

Table 13
Comparison between Hg concentrations in muscle of sei whales and in the whole body of prey species in sub-area 9.

	Anchovy	Copepods	Euphausiids	Mackerel	Min.arm squid	Sardine	Saury
Muscle of whales	Ave.±SD (0.049±0.013)	(0.043±0.014)	(0.045±0.016)	(0.045±0.016)	(0.040±0.005)	(0.052±)	(0.041±0.015)
<i>n</i>	11	48	9	9	3	2	7
Whole of prey spp.	Ave.±SD (0.037±0.025)	(0.005±0.003)	(0.005±0.003)	(0.020±0.002)		(0.018±)	(0.038±0.015)
<i>n</i>	20*	5*	19*	5*		66**	41*

*: Yasunaga and Fujise (2009); **: Ministry of Health, Labour and Welfare (2005)

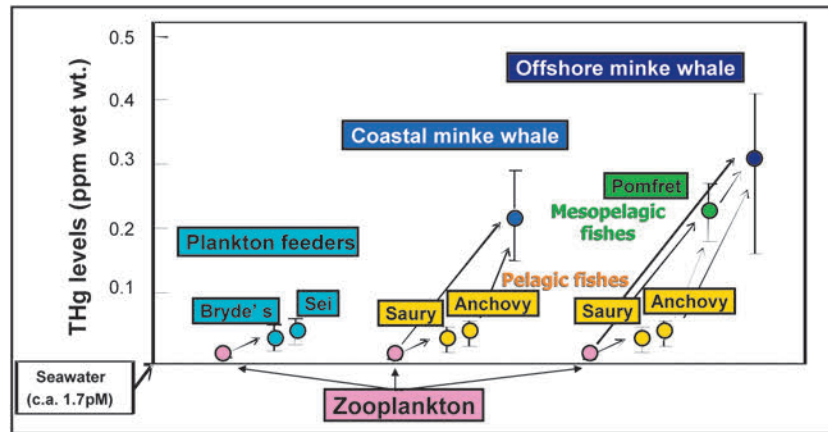


Figure 6. Schematic diagram showing total Hg flow in the western North Pacific food web.

organisms and baleen whales, except for common minke whales collected from sub-areas 7 and 9, and sei whales from sub-area 9 from 1990's to 2000's. Total Hg concentrations in these exceptions may reflect changes in food habits rather than changes of background levels of total Hg in the marine environment. This phenomenon is illustrated in Figure 6, which shows that differences in food habitat explain the pattern of Hg accumulation of baleen whales.

Consequently, it is concluded that temporal trend of total Hg concentrations in the marine habitat of baleen whales in the western North Pacific remained stable in the research period. In future, other variables such as age of the animals, should be included in the analyses to investigate yearly trend of Hg concentrations in baleen whales.

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Technical Report-Note (not peer reviewed)

A summary of the genetic samples of large whales collected by the Institute of Cetacean Research

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Biological samples of whales for genetic analyses are collected through different kinds of surveys conducted by the Institute of Cetacean Research (ICR) as well as from by-caught and stranded animals and from past commercial whaling samples. The objective of this note is to summarize the number of samples/data collected to date.

The sources of samples are the following:

- Skin samples from whales taken under special permit scientific whaling. See details in GOJ (2015; 2017).
- Skin samples obtained by biopsy sampling under the non-lethal component of the special permit scientific programs. See details in Mogoe *et al.* (2017).
- Skin samples obtained by biopsy sampling during dedicated sighting surveys (national and international). See details in Isoda *et al.* (2017) and Matsuoka *et al.* (2017).
- Skin and muscle samples obtained from by-caught whales (such samples are provided to ICR from 1 July 2001 following a national-established protocol). See details in the link: http://www.jfa.maff.go.jp/j/whale/w_document/pdf/041012tsuuchi.pdf.
- Skin and muscle samples obtained from stranded whales. Systematic record and sampling from stranding are conducted by ICR since 2004. See details in the link: http://www.jfa.maff.go.jp/j/whale/w_document/pdf/manyuaru2012kaisei.pdf.
- Historical samples from past commercial and special permit scientific whaling (e.g., baleen and blood samples).

Table 1 summarizes the genetic samples available in ICR, which were collected from the above described sources. This data set is one of the largest and most comprehensive in the world. Samples are used mainly for genetic analyses on population structure and phylogeny of baleen whales, and to a lesser extent, for analyses on pollutants and feeding ecology.

Several genetic studies based on these samples have

been presented to international meetings (e.g., Pastene *et al.* 2016; Taguchi *et al.* 2017), and others have been published in peer-reviewed journals (e.g., Pastene and Goto, 2016; Malde *et al.* 2017).

Samples and data in Table 1 are available for cooperative studies under established ICR protocol for data access and collaboration. See details in the following link: <http://www.icrwhale.org/pdf/appendix2.pdf>.

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Table 1
Number of genetic samples of several cetacean species available at the ICR. The numbers on the left and right sides in parenthesis indicate the available microsatellite and mtDNA data, respectively.

Species	A	B	C	D	E	F	G	Period
Antarctic								
Antarctic minke whale	11,011 (9,777: 4,096)				—	—		1987/88–2015/16
Blue whale		35 (0: 35)	10 (0: 10)		—	—		1994/95–2015/16
Fin whale	18 (18: 18)	54 (39: 42)	16 (16: 16)		—	—		1998/99–2015/16
Sei whale		3 (3: 3)			—	—		2000/2001
Humpback whale		488 (483: 488)	20 (20: 20)	115 (115: 113)	—	—		1993/94–2015/16
Southern right whale		73 (73: 73)	40 (40: 40)	61 (61: 61)	—	—		1993/94–2015/16
North Pacific								
Common minke whale	2,680 (2,672: 2,672)	9 (5: 9)			2,008 (1,980: 2,008)			1994–2016
Blue whale		17 (0: 17)	4 (0: 4)	9 (0: 1)				2001–2015
Fin whale		10 (10: 10)	5 (5: 5)	27 (2: 2)	11 (11: 11)	5 (3: 5)		1997–2016
Sei whale	1,354 (1,354: 1,350)	55 (55: 55)		82 (82: 82)			303 (302: 303)	1972–2016
Bryde's whale	730 (730: 727)	87 (87: 87)	58 (58: 58)	84 (84: 84)	1 (1: 1)		323 (323: 287)	1977–2016
Humpback whale		15 (9: 15)	9 (9: 9)	1 (0: 0)	63 (63: 63)	18 (0: 18)		1997–2016
Right whale		8 (3: 8)	14 (14: 14)		3 (2: 3)	4 (0: 4)	5 (0: 5)	1958–2016
Sperm whale	56 (56: 56)	20 (18: 20)			2 (2: 2)			2000–2010
Killer whale		2 (0: 2)						2016

A: Samples from recent special permit scientific whaling. Tissue samples collected before the 2011 tsunami in Japan are not available. B: Biopsy samples obtained from sighting/sampling vessels of the surveys under recent special permit scientific whaling. C: Biopsy samples obtained from Japanese dedicated sighting surveys (conducted under or outside the special permit scientific whaling). D: Biopsy samples obtained from international dedicated sighting surveys e.g., IWC IDCR/SOWER in the Antarctic and IWC POWER in the North Pacific. E: Samples from by-catches. Tissue samples collected before the 2011 tsunami in Japan are not available. F: Samples from stranding. G: Historical samples from past commercial whaling in the North Pacific (sei whale), past special permit scientific whaling in the North Pacific (right whales); past commercial whaling in the western North Pacific and eastern South Pacific, and past special permit scientific whaling in other oceanic basins (Bryde's whale).

Commentary

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

Evolution of the IWC Scientific Committee

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INTRODUCTION

The Scientific Committee is one of four Committees established by the International Whaling Commission (hereafter, the Commission), the others being the Finance and Administration Committee, the Technical Committee and the Conservation Committee. The Scientific Committee was established by the IWC in 1950. This in part is a reflection of Article IV of the International Convention for the Regulation of Whaling (hereafter, the Convention) that refers to scientific research and the publication of results, statistics and reports² and in part a reflection of Article V2 of the Convention that states inter alia that Schedule amendments ‘...shall be based on scientific findings...’³. The Scientific Committee has met each year since its establishment (Donovan and Hammond, 2017).

The Scientific Committee was established in accordance with the IWC’s Rules of Procedure M1 and its terms of reference are given in Rule M4. The duties of the Scientific Committee are further elaborated in the Rules of Procedure for the Scientific Committee which were significantly amended by the Commission at its meeting in 2016 (see details below). Scientific Committee priorities and the initial agenda for the next meeting together with

work plans for the intersessional period are approved at meetings of the Commission.

Just as the Commission has moved away from its core responsibilities related to the management of whaling in a manner prescribed by Article IV of the Convention (see footnote 2), the Scientific Committee has, to a significant degree evolved away from providing advice on sustainable catch levels for large whales to that of providing advice on non-direct catch related threats to all cetacean stocks. This paper describes some of the major factors and events related to or responsible for this evolution.

The Committee of Three (Four)

Evolution of the Scientific Committee essentially began in 1961 with the establishment by the Commission of the Committee of Three, later Four, scientists who were experts in population dynamics. They were tasked with assessing the whale stocks, reporting on the sustainable yield of these stocks and advising on any conservation measures that would increase yield. This new focus which developed and extended the mathematical techniques as used in fisheries assessments to the science of managing whale stocks led to recommendations for reduced catches and that the regulation of catches should be on an

¹ The author is a Councillor at the Institute of Cetacean Research in Tokyo and a former Visiting Researcher at the Government of Japan’s Fisheries Agency. The author has been involved in all aspects of the work of the International Whaling Commission for more than 30 years. He is a former Senior Advisor in the Government of Canada’s Department of Fisheries and Oceans and represented the Government of Canada at meetings of the IWC from 1979 to 1996. He is a member of the IWC Scientific Committee and has been a member of the Government of Japan’s delegation to the IWC since 1998.

² Article IV

The Commission may either in collaboration with or through independent agencies of the Contracting Governments or other public or private agencies, establishments, or organizations, or independently

(a) encourage, recommend, or if necessary, organize studies and investigations relating to whales and whaling;

(b) collect and analyze statistical information concerning the current condition and trend of the whale stocks and the effects of whaling activities thereon;

(c) study, appraise, and disseminate information concerning methods of maintaining and increasing the populations of whale stocks.

³ Article V(2)

These amendments of the Schedule (a) shall be such as are necessary to carry out the objectives and purposes of this Convention and to provide for the conservation, development, and optimum utilization of the whale resources; (b) shall be based on scientific findings; ...

individual species basis rather than in the combined Blue Whale Unit (BWU) system (Gambell, 1977; Allen, 1980).

The New Management Procedure (NMP)

The next major development to impact the work of the Scientific Committee was the Commission's adoption of the New Management Procedure (NMP) in 1974. This followed: (i) the adoption of a resolution at the 1972 United Nations Conference on the Human Environment that called for a 10-year moratorium on all commercial whaling; a demand for greatly increased scientific research into the status of the world's whale stocks; and a call for the strengthening of the IWC secretariat and its capabilities (Gambell, 1977) and, (ii) the consensus agreement by the Scientific Committee that a blanket moratorium could not be scientifically justified (IWC, 1973). With the adoption of the NMP the focus of the Scientific Committee became classifying whale stocks into three categories according to their relative abundance (IWC, 2016a). Also in response to the 1972 United Nations Conference on the Human Environment resolution, the IWC Scientific Committee compiled a large-scale program for an International Decade of Cetacean Research (Gambell, 1977).

The Moratorium and the Revised Management Procedure (RMP)

Between 1972 and 1982, a total of 29 proposals for a moratorium on commercial whaling were proposed. The Commission rejected all of them except 1 of the 5 proposals proposed in 1982.⁴ The proposal that was adopted is paragraph 10(e) of the Schedule (IWC, 2016a)—commonly referred to as 'the Moratorium'. It was (at least on its face) intended as a temporary measure (Morishita, 2013) based on the view that there was too much uncertainty in the scientific knowledge to ensure safe harvest levels (Morishita and Goodman, 2005; IWC, 2017a). There was however, no advice from the Scientific Committee that such a measure was required for conservation (Morishita and Goodman, 2005).

Following the moratorium decision, the Commission asked the Scientific Committee to develop a new approach to providing advice on the setting of catch limits that was both safe and practical. This was a complex task and formed a major part of the work of the Scientific Committee during the eight years it took to complete.

⁴Details of the proposals and the manner in which they were dealt with by the Commission are recorded in the Chair's reports of the Annual Meetings. Available at: <https://archive.iwc.int/pages/search.php?search=%21collection49&k=>

The new process was called the Revised Management Procedure (RMP). It was adopted by the Commission in 1994 and set a new standard in scientific management advice for marine and other living resources (IWC, 2017a).

Scientific Committee membership and agenda

Referencing IWC reports, Morishita and Goodman (2005) recorded that in 1976, 29 scientists representing eleven countries and one intergovernmental organization participated in the Annual Meeting of the Commission's Scientific Committee. They note that the agenda for this meeting consisted of 21 items primarily focused on the status of stocks and providing advice to the Commission on quotas for whaling. They further note that in contrast to this, the 2004 meeting of the Scientific Committee was attended by 202 scientists from 30 member countries and eight international organizations, and included 41 'invited participants' and one representative from a non-governmental organization and that the 26-item agenda included numerous items which are regarded by approximately half of the IWC member countries as outside of the Commission's mandate such as small cetaceans, DNA testing, environmental concerns, whalewatching, by-catch in fisheries and ship strikes.

This drastic change in the Scientific Committee took place over a number of years beginning in the late 1970's with the recruitment of additional Commission members with an anti-whaling position in order to obtain the 3/4 majority vote required to adopt the moratorium. Importantly, the additional membership also provided anti-whaling members with the means to change to focus of the Scientific Committee's work away from the provision of management advice for the regulation of commercial whaling through the adoption of resolutions and changes to the Rules of Procedure of the Scientific Committee that only require a simple majority for adoption. The following examples elaborate this point.

The Berlin Initiative

At its 55th Annual Meeting the Commission adopted resolution 2003-1 titled 'The Berlin Initiative on Strengthening the Conservation Agenda of the International Whaling Commission'. This resolution references what are referred to as 'more than 100 conservation-oriented resolutions' and notes that 'the Commission has gradually developed an extensive conservation-oriented agenda'. The resolution established the 'Conservation Committee' and, inter alia 'requests the Scientific Committee to advise the Conservation Committee in the performance

of the tasks entrusted to it in this Resolution, and to ensure that the appropriate scientific research items, including inter alia, whalewatching, environmental issues and behavioural research, under the responsibility of the Scientific Committee, are incorporated in the Conservation Agenda' (IWC, 2004). This major shift in the focus of the Scientific Committee agenda has been enhanced by additional resolutions since 2003⁵ and, approval of the Scientific Committee agenda is a specific agenda item at each meeting of the Commission.

Resolution 2014-4: Resolution on the Scientific Committee

Resolution 2014-4 that was adopted by consensus recalls 'more than 50 resolutions of the International Whaling Commission addressing the work of the Scientific Committee, particularly regarding the increase and evolving work over decades on conservation aspects, including small cetaceans'. Further, the resolution notes 'that the work of the Scientific Committee oriented towards issues related to other threats than direct takes, has increased over the last decades...', 'consolidates the mandate of the Small Cetaceans Standing Sub-Committee' and establishes 'a working group between the Conservation Committee and the Scientific Committee in order to propose a procedure to facilitate the implementation and follow-up of conservation recommendations'. Annex 1 of Resolution 2014-4 is a 'Compiled list of IWC resolutions addressing the work of the Scientific Committee 1976–2012' (IWC, 2016b).

As with the Berlin Initiative described above, this resolution adds emphasis to those aspects of the Scientific Committee's work that are unrelated to its core responsibility of providing advice on the regulation of commercial whaling as provided for by the Convention.

Amendments to the Commission's Rules of Procedure and Financial Regulations and to the Rules of Procedure of the Scientific Committee.

Resolution 2014-4 proposed a number of amendments to the Commission's Rules of Procedure and Financial Regulations and to the Rules of Procedure of the Scientific Committee. The Resolution including proposed amendments to the Commission's Rules of Procedure and Financial Regulations (Annex II of the Resolution) were adopted by consensus while, in accordance with

the Resolution, proposed amendments to the Rules of Procedure of the Scientific Committee (Annex III of the Resolution) were referred to the Scientific Committee for their advice.

Changes to the Commission's Rules of Procedure included references to 'cetaceans' rather than 'whales' and the addition of the words 'shall review current and potential threats and methods to mitigate them in order to maintain cetacean populations at viable levels...' to the duties of the Scientific Committee contained in Rule of Procedure M. 4 (a). Changes to the Financial Regulations established a Research Fund and prescribed that the Research Fund 'shall have a balanced distribution among activities ... including small cetaceans...'

Proposed amendments to the Rules of Procedure of the Scientific Committee also referred to 'cetaceans' and 'small cetaceans' rather than 'whales' and proposed significant changes to the items listed under the heading 'SPECIFIC TOPICS of current concern to the Commission' (IWC, 2016b).

Proposed amendments to the Rules of Procedure for the Scientific Committee included in resolution 2014-4 were considered by the Scientific Committee at its meeting in 2015. Their recommendations together with some additional proposed amendments were incorporated in its Annex R (IWC, 2016c). These additional proposed amendments included deletion of the section titled 'Specific Topics of current concern to the Commission'. The Scientific Committee proposed that this section would be more effectively located in the introduction to its work plan'. Annex R of the 2015 Scientific Committee report was then considered by the Finance and Administration Committee and adopted by the Commission at its 2016 meeting (IWC, 2016d).

In summary, amendments to the Commission's Rules of Procedure and Financial Regulations and to the Rules of Procedure of the Scientific Committee that resulted from the adoption of Resolution 2014-4 provide strong support for the view of anti-whaling Commission members that the Scientific Committee and the Commission that approves the work plan and agenda of its Scientific Committee have a mandate concerning the management of small cetaceans as well as a broad mandate related to threats to cetacean populations.

Whalewatching

The IWC adopted its first resolution on whalewatching in 1993 at IWC45, and the following year, at IWC46, a further resolution requested advice from the Scientific Committee on whalewatching and established what has

⁵See for example Resolution 2009-1, 2012-1, 2016-3, and 2016-4. Available at: <https://archive.iwc.int/pages/search.php?search=%21collection72&k=>

in practice now become an ongoing program of work. In 1998, a standing Whale Watching Sub-Committee was set up under the Scientific Committee (IWC, 2011).

Others

Other records that document or demonstrate the shift in the activities of the Commission and its Scientific Committee away from their core responsibilities provided by the Convention to a focus on threats to cetaceans from issues other than direct takes include:

- (i) The list of issues on the IWC website under the tab titled 'Conservation and Management'. Items on this list include 3 items related to whaling and 11 non-whaling issues including animal welfare issue, bycatch, entanglement of large whales, strandings, ship strikes, environmental concerns, conservation management plans, sanctuaries and MPAs and, whalewatching (IWC, 2017b).
- (ii) The 60 correspondence groups established by the Scientific Committee (IWC, 2017c) of which almost one half are not related to Commission's primary responsibility.
- (iii) The agenda for the 2017 meeting of the Scientific Committee that includes: cooperation with other organizations, bycatch, ship strikes, environmental concerns, small cetaceans, whalewatching and whale sanctuaries (IWC, 2017d). At the meeting, a number of Sub-committees and Working Groups were established to address some of these issues even though many members of the Commission view these as outside of the Commission's mandate.

CONCLUSIONS

While the above has clearly documented the increase in the work of the Scientific Committee oriented towards issues related to other threats than direct takes, it should be noted that the Scientific Committee has continued work related to the management of whale stocks. In this regard, the agenda for the 2017 meeting of the Scientific Committee includes: General assessment issues related to the Revised Management Procedure (RMP); RMP implementation matters related to North Atlantic common minke whales, Western North Pacific common minke whales; Aboriginal Subsistence whaling; In-depth assessments of whale stocks not subject to directed takes; and, cetacean abundance estimates and stock status (IWC, 2017d). However, three aspects of the context of this continuing work need emphasis:

- (i) Changes to the Financial Regulations adopted as part of Resolution 2014-4 added the following:

'The Research Fund shall have a balanced distribution among activities, defined according to conservation priorities and the work of the Commission, including small cetaceans (IWC, 2016b)'⁶.

- (ii) Resolution 1997-5 that 'Instructs the Scientific Committee not to consider Southern Hemisphere minke whales in the context of implementation of the RMP unless advised to do so by the Commission' remains in effect (IWC, 1998).
- (iii) The current politicized nature of the Scientific Committee means that it is highly unlikely that its work on RMP related matters would result in recommendations to the Commission for the setting of quotas for the resumption of commercial whaling (Morishita and Goodman, 2005).

These aspects of the context of the Scientific Committee's work lend strong support for the following conclusions reached by Morishita and Goodman (2005).

- (i) 'The deep philosophical and political divisions between the International Whaling Commission (IWC) member countries that support managed whaling activities and those opposed to any harvesting of whales has caused a seriously dysfunctional situation in the IWC'.
- (ii) 'Strong personal positions on the issues related to whaling, the influence of national government positions on scientists and advocacy have polarized the debates within the Scientific Committee'.
- (iii) 'Unless the Commission and its member governments change their institutionalized discourse and procedures, it is naïve to expect outputs from the Scientific Committee that are useful for the sustainable use and management of whale resources in accordance with the objectives of the ICRW'.

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⁶This is a not so subtle attempt to reduce funding for the Commission and Scientific Committee work related to the provision of management advice.

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International meetings

Participation of Scientists of the Institute of Cetacean Research in International Meetings in 2017

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 2017 meeting of the IWC SC was held at the Golf Hotel, Bled, Slovenia, from 9–21 May. A total of ten scientists from the Institute of Cetacean Research (ICR) participated in the meeting (Yoshihiro Fujise, Luis A. Pastene, Tsutomu Tamura, Koji Matsuoka, Mutsuo Goto, Takashi Hakamada, Genta Yasunaga, Kenji Konishi, Tatsuya Isoda and Mioko Taguchi), who presented eight documents at plenary sessions, one document at the Sub-Committee on Revised Management Procedure, 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 Stock Definition and DNA Testing, one document at the Sub-Committee on In-Depth Assessments, and two at the Sub-Committee on Conservation Management Plans.



Hotel Golf, Bled, Slovenia.

In 2017 ICR scientists also participated in several intersessional meetings of the IWC SC: a) Review Workshop for the New Scientific Whale Research Program in the western North Pacific (NEWREP-NP), held at the Toyomi Center Building, Tokyo, Japan, from 30 January–3 February (Seiji Ohsumi, Yoshihiro Fujise, Luis A. Pastene, Tsutomu Tamura, Koji Matsuoka, Mutsuo Goto,

Takeharu Bando, Takashi Hakamada, Genta Yasunaga, Satoko Inoue, Mioko Taguchi and Megumi Takahashi); b) Workshop on the *Implementation Review* of western North Pacific Bryde's Whales held at the Fisheries Agency of Japan (FAJ)'s Sanban-cho, Tokyo, Japan from 21–24 March (Luis A. Pastene, Tsutomu Tamura, Mutsuo Goto, Mioko Taguchi, Takashi Hakamada and Koji Matsuoka); c) Workshop on the Comprehensive Assessment of North Pacific Humpback Whales held at the Marine Mammal Laboratory, Seattle, USA from 18–21 April (Koji Matsuoka); d) Planning Meeting for the 2018 and 2019 IWC-POWER Cruises in the North Pacific held at the FAJ's Crew House, Tokyo, Japan from 15–18 October (Koji Matsuoka and Takashi Hakamada).

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 2017 meeting of the CCAMLR-EMM was held at the Palacio San Martin, Buenos Aires, Argentina from 10–14 July. One scientist from ICR participated in the meeting (Tsutomu Tamura) presenting a document titled 'Feeding habits and prey consumption of Antarctic minke whale *Balaenoptera bonaerensis* in the Indo-Pacific region of the Southern Ocean' relevant to the meeting agenda item 'Ecological interactions: predators.'



Palacio San Martin, Buenos Aires, Argentina.

XIIIth Scientific Committee for Antarctic Research (SCAR) Biology Symposium

The Scientific Committee on Antarctic Research (SCAR) is an interdisciplinary body of the International Council for Science (ICSU). It was established in February 1958. SCAR is charged with initiating, developing and coordinating scientific research in the Antarctic region. The scientific business of SCAR is conducted by its Standing Scientific Groups. SCAR also provides scientific advice to the Antarctic Treaty Consultative Meetings and other organizations on issues of science and conservation affecting the management of Antarctica and the Southern Ocean. The XIIIth SCAR Biology Symposium was held at the University of Leuven, Leuven, Belgium from 10–14 July 2017. One scientist from ICR participated in the meeting (Luis A. Pastene) who presented the study titled 'Cetacean as indicators of historical and current changes in the Antarctic ecosystem' as an oral presentation at the session 'Distribution and trends of top predators.' He was also co-author of the study titled 'Changes in circumpolar spatial distribution of baleen whales in the Antarctic from 1980s to 2000s,' which was presented as a poster at the session 'Large scale analyses of spatial diversity pattern.'



Leuven University, Leuven, Belgium.

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

The North Pacific Marine Science Organization (PICES) is an intergovernmental organization that promotes and coordinates marine scientific research in the North Pacific Ocean and provides a mechanism for information and data exchange among scientists in its member countries. The 2017 meeting of the PICES was held at the Far Eastern Federal University, Vladivostok, Russia from 22 September-1 October. One scientist from ICR participated in the meeting (Tsutomu Tamura) presenting the study titled 'Estimation of prey consumption by cetaceans in the western North Pacific-Update of Hunt *et al.* (2000)' as an oral presentation at the session 'Seasonal and climatic influences on prey consumption by marine birds, mammals and predatory fishes.' He was also co-author of another study titled 'Spatial estimation of prey consumption by sei whales in the western North Pacific during the summers of 2008–2009: Density surface model approach,' also presented to the meeting.



Far Eastern Federal University, Vladivostok, Russia.

22nd Biennial Meeting of the Society for Marine Mammalogy (SMM)

The Society for Marine Mammalogy (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 22nd Biennial meeting of the SMM was held at the Halifax World Trade and Convention Centre, Halifax, Nova Scotia, Canada from 23–27 October 2017. One scientist from ICR participated in the meeting (Kenji Konishi) presenting the study 'Movements of satellite monitored Antarctic minke whales inside and along the ice edge at the feeding area in the Pacific sector of the Antarctic Circle,' as a poster at

the session 'Habitat and Distribution.' He and other ICR members were co-authors of another study titled 'Estimation of feeding records in pregnant Antarctic minke whales using stable isotope analysis of carbon and nitrogen of baleens,' which was also presented as a poster.



Halifax World Trade and Convention Centre, Halifax, Nova Scotia, 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 Agree-

ment was signed in Nuuk, Greenland on 9 April 1992 by Norway, Iceland, Greenland and the Faroe Islands, and entered into force 90 days later 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 2017 NAMMCO SC meeting was held at the Marine Research Institute, Reykjavik, Iceland from 14–17 November. One scientist from ICR participated in the meeting (Genta Yasunaga) as an observer for Japan and presented the Japan progress report on cetacean research in 2016.



Marine Research Institute, Reykjavik, Iceland.

Peer-reviewed publications

List of peer-reviewed publications based on the Institute of Cetacean Research (ICR)'s surveys up to 2017

This section presents a list of peer-reviewed publications based on data collected by surveys conducted under special scientific permit (JARPA/JARPAII and JARPNI/JARPNII), 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 surveys

1989 (2)

Kato, H., Hiroshima, 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 Hiroshima, 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.

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