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Cover photo: Stereoscopic microscope used for age determination based on counting of earplug growth laminae (top); capillary sequencer ABI SeqStudio used for DNA sequencing analysis (middle); ultra performance liquid chromatography-tandem mass spectrometer used for age determination based on racemization analysis (bottom).

TECHNICAL REPORTS OF THE INSTITUTE OF CETACEAN RESEARCH

TEREP-ICR
No. 4

The Institute of Cetacean Research (ICR)
Tokyo, 2020

Foreword

It is a pleasure for me to introduce the fourth issue of the Technical Reports of the Institute of Cetacean Research (TEREP-ICR-4). Following the change in Japan's whaling policy last year, the ICR has been designing and implementing whale research programs based on non-lethal methods. In the North Pacific, ICR scientists have contributed to the design and implementation of national dedicated sighting surveys and of the IWC-sponsored Pacific Ocean Whale and Ecosystem Research (POWER) surveys. In the Antarctic, ICR scientists designed the new program called 'Japanese Abundance and Stock structure Surveys in the Antarctic' (JASS-A), and the first survey under this program was completed successfully during the 2019/20 austral summer season. In addition, ICR scientists commenced the collection of biological samples and data based on whales caught for commercial purposes in Japan's Exclusive Economic Zone since 1 July 2019.

Consistent with its stated objectives, TEREP-ICR describes and reports on the process, progress, and results of technical or scientific research, or the state of technical or scientific research programs conducted by the ICR, including those commenced recently.

ICR scientists were preparing for their participation in several national and international meetings planned for 2020. Due to the current Covid-19 pandemic affecting the world, these meetings were either cancelled or replaced by virtual meetings (or e-mail discussions), and our scientists participated in those virtual meetings and discussions as much as possible, given the circumstances.

Similar to previous TEREP-ICR issues, TEREP-ICR-3 was widely distributed to approximately 120 individual scientists from Japan and 30 foreign countries. It was also distributed to approximately 190 research institutions (including universities, research institutes, public libraries, museums and aquariums), both in Japan and foreign countries. As a result of disseminating information on ICR's research activities through the TEREP-ICR, an increasing number of international scientists are showing interest in conducting research in collaboration with the ICR. Based on this, I believe that TEREP-ICR is making contribution toward achieving its stated objectives. Furthermore, TEREP has been a good opportunity for our scientists to compile and summarize their research, conducted over the years, as a prior step before submitting their works for peer-review publications.

Finally, I am pleased to announce that the reports published in TEREP are now indexed in the Japan Science and Technology Agency's J-GLOBAL, which aims to link, expand and spark scientific information in Japan and the world.

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

Dr. Yoshihiro Fujise
Director General ICR
Tokyo, December 2020

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Editorial

Welcome to the fourth issue of the Technical Reports of the Institute of Cetacean Research (TEREP-ICR-4).

This issue contains eight technical reports and one commentary article. We continue with the series of reports summarizing the research findings on whales and the ecosystem in the Indo-Pacific sector of the Antarctic. This time, Tamura and colleagues focus on the findings of the ecological research on whales and their environment. Outlines of two important whale research programs, including results of the latest surveys, are included in this issue. Isoda and colleagues explain the program 'Japanese Abundance and Stock structure Surveys in the Antarctic (JASS-A)', while Matsuoka explains the IWC-sponsored program 'Pacific Ocean Whale and Ecosystem Research (IWC-POWER)'. The basic concept of genetic tagging, and its application for abundance estimates in whales, are explained in the reports by Taguchi and Takahashi, respectively. Works on earplug-based age determination and the estimation of biological parameters have continued, based on the samples and data collected during the former whale research programs under special scientific permit. This time, Bando and Maeda show the results of age determination and biological parameter estimates of North Pacific sei whales. The first Note by Goto and Oikawa describes Japan's system for monitoring of whale products sold in its domestic market through DNA registration, and the second Note by Konishi and colleagues focuses on the preliminary results of satellite-monitored tracking of Antarctic minke and fin whales during the first JASS-A survey.

In the commentary article, Yasunaga presents his views on the use of lethal and nonlethal techniques for the research of large whales.

This fourth TEREP-ICR issue also includes sections to outline the contribution of ICR scientists to international meetings in 2020, as well as their contribution in terms of peer-reviewed publications up to December 2020.

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

Dr. Luis A. Pastene
Editor TEREP-ICR
Tokyo, December 2020

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Technical Report (not peer reviewed)

What do we know about whales and ecosystem in the Indo-Pacific region of the Antarctic? Part 2: summary of ecological studies

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ABSTRACT

The Institute of Cetacean Research conducted whale research under special scientific permit in the Antarctic starting from the austral summer season 1987/88. The research was conducted systematically under different research programs such as JARPA and JARPAII, and more recently, under NEWREP-A. These research programs employed both lethal and non-lethal methods. NEWREP-A ceased after the 2018/19 austral summer season as a consequence of Japanʹs decision to withdraw from the International Convention for the Regulation of Whaling. Japanʹs whale research continues in the Antarctic, using non-lethal methods only. This paper summarizes the most relevant ecological outputs from Japanʹs whale research under special scientific permit in the Indo-Pacific region of the Antarctic.

INTRODUCTION

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

As a consequence of Japanʹs change in whaling policy, the NEWREP-A ceased on 30 June 2019, the date of Japanʹs withdrawal from the International Convention for the Regulation of Whaling (ICRW). From the 2019/20 austral summer season, Japan started whale research in the Antarctic using non-lethal methods. The new research program is called the Japanese Abundance and Stock-structure Surveys in the Antarctic program (JASS-A) (see Isoda *et al.*, this issue).

At this point, it was considered important to summarize the knowledge on whales and the Antarctic ecosystem accumulated so far by Japanʹs whale research in the Antarctic. The objective of this paper is to summarize the most relevant ecological outputs from Japanʹs whale research under special scientific permit in the Indo-Pacific region of the Antarctic.

SURVEYS, DATA AND SAMPLES

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

MAIN RESEARCH OUTPUTS ON WHALE ECOLOGY

Abundance and abundance trends of baleen whales

JARPA and JARPAII conducted vessel-based sighting surveys under the Line Transect Method with the aim of estimating abundance and abundance trends of large whales in the Indo-Pacific sector of the Antarctic. Details of the methodology used are available in Tamura *et al.* (2017) and Hakamada and Matsuoka (2017). The latter authors summarized the information on abundance and abundance trend of Antarctic minke whales, which is not repeated here. Figure 2 shows pictures of the relevant baleen whale species.

Baleen whale species other than the Antarctic minke whale were depleted by unregulated commercial whaling

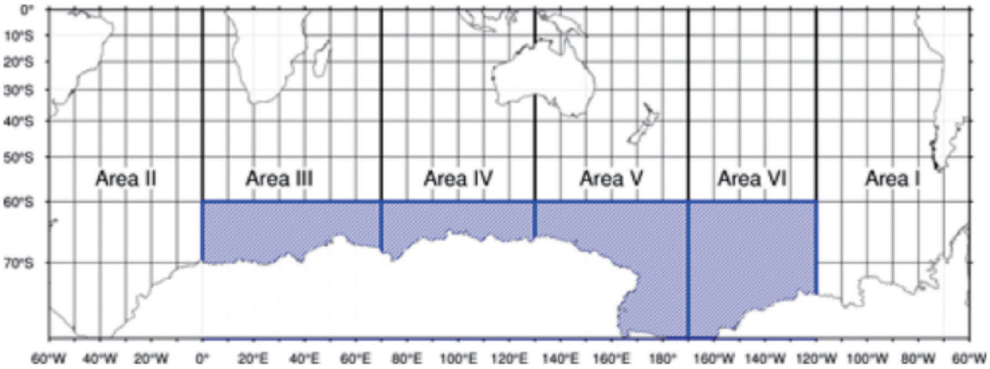

Figure 1. Research area of JARPA, JARPAII and NEWREP-A in the Indo-Pacific region of the Antarctic.

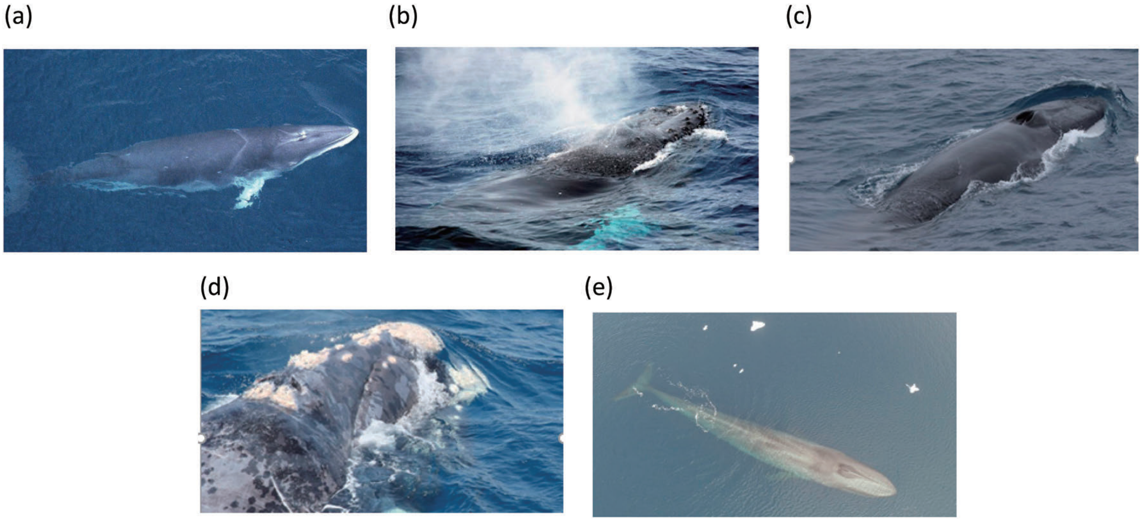

Figure 2. Baleen whale species in the Indo-Pacific region of the Antarctic: (a) Antarctic minke whale; (b) humpback whale; (c) fin whale; (d) southern right whale; (e) Antarctic blue whale.

in the past, and there is an interest to investigate the recovery of those species after protection. Fortunately, the abundance of large whale species such as humpback, fin, southern right and blue whales has been increasing, albeit at different rates over the past decades. An increase in the abundance of large baleen whales has implications for the Antarctic ecosystem as a whole (Fujise and Pastene, 2018).

The scientific evidences for the recovery of baleen whale species are shown below.

Humpback whales

Abundance and abundance trend should be ideally estimated on a biological stock basis. The IWC SC has identified seven breeding stocks of humpback whales in the Southern Hemisphere, which are denominated with alphabetic letters from 'A' to 'G' (IWC, 2011). The breeding stocks occurring in the Indo-Pacific sector of the Antarctic are Stock 'C' (mainly in Area III), 'D' (mainly in Area IV), 'E' (mainly in Area V), and 'F' (mainly in Area VI) (Kanda *et al.*, 2014). There are some geographical overlaps between adjacent stocks in the Antarctic.

Abundance and abundance trend estimates based on Japanese surveys have focused mainly on Areas IV (Breeding Stock D) and V (Breeding Stock E). In Area IV the abundance was estimated at 29,067 whales (CV=0.255) based on sighting data collected in 2007/08; in Area V the abundance was estimated at 13,894 whales (CV=0.338) based on sighting data collected in 2008/09 (Hakamada and Matsuoka, 2014a).

Figure 3 shows the abundance trend of Breeding Stocks D and E based on JARPA and JARPAII data. For comparison purposes, the figures include data from the International Decade for Cetacean Research/Southern Ocean Whale and Ecosystem Research (IDCR/SOWER) programs. The figures show a clear increasing trend which is consistent for JARPA, JARPAII and IDCR/SOWER survey data. Annual rate of increase was estimated at 13.6% (95% CI=8.4–18.7%) and 14.5% (95% CI=7.6–21.5%) for Areas IV and V, respectively. The rate of increase in Areas IV and V were statistically significant (Hakamada and Matsuoka, 2014a).

The current abundance of Breeding Stock D in Area

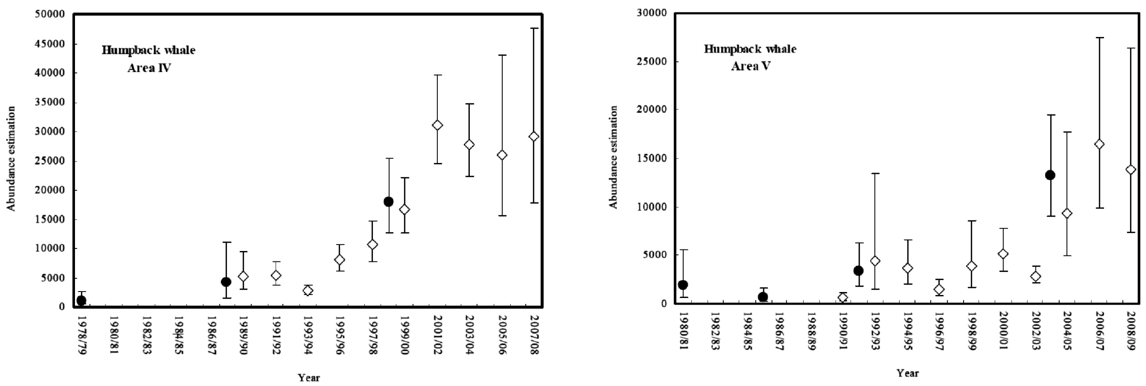

Figure 3. Abundance estimates for humpback whales in Areas IV and V (south of 60°S). Estimates were based on sighting data collected by JARPA and JARPAII between 1989/90 and 2008/09 primarily during January to February. Estimates from the IDCR-SOWER surveys (Branch, 2011) are shown for comparative purposes (filled circles). Vertical lines show 95% confidence intervals (after Hakamada and Matsuoka, 2014a).

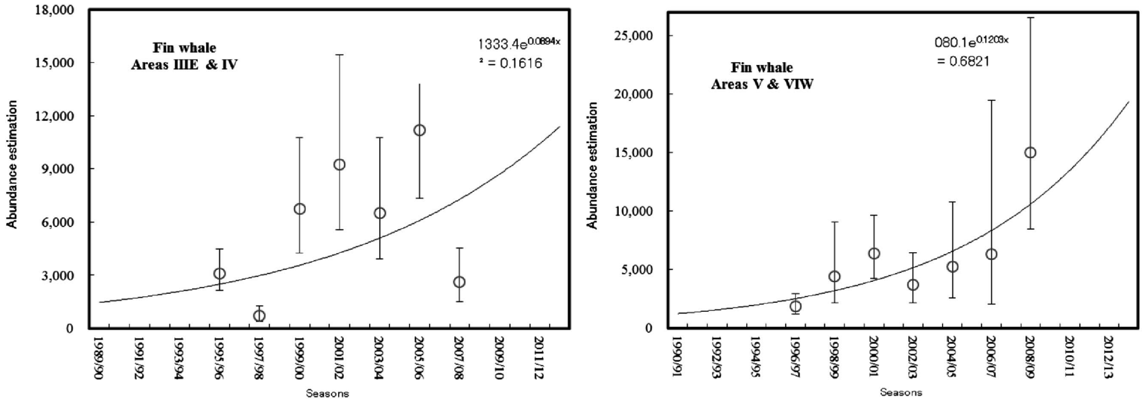

Figure 4. Abundance trend of fin whales in the Indo-Pacific region of the Antarctic, south of 60°S. Vertical lines show 95% confidence intervals (after Matsuoka and Hakamada, 2014).

IV is close to that of its pre-exploitation level (IWC HP: https://iwc.int/status).

Fin whales

There is limited information on stock structure of fin whales in the Indo-Pacific sector of the Antarctic. For the purpose of the abundance estimates based on JARPA and JARPAII surveys, the whole area was divided into western area (assuming an Indian Ocean stock in Area IIIE+IV) and eastern area (assuming a Pacific Ocean stock in Area V+VIW). Figure 4 shows the abundance estimates of fin whales in Areas IIIE+IV and V+VIW, plotted against the survey seasons. An increasing trend in abundance is evident for these two areas. For the western area the abundance south of 60°S was estimated at 3,087 (CV=0.191) in 1995/96, and 2,610 (CV=0.285) in 2007/08. For the eastern area the abundance south of 60°S was estimated at 1,879 (CV=0.226) in 1996/97, and 14,981 (CV=0.298) in 2008/09.

For the western area the increasing trend between 1995/96 and 2007/08 seasons was estimated at 8.9% (95% CI: −0.145%, 32.4%), while the trend in the eastern area between 1996/97 and 2008/09 was estimated at 12.0% (95% CI: 2.6%, 21.5%). The estimate for the eastern area was statistically significant (Matsuoka and Hakamada, 2014).

Southern right whales

During the JARPA and JARPAII surveys in Areas III–VI, this species was mainly sighted in Area IV. In this Area, abundance estimates (south of 60°S) ranged from 6 (CV=0.761) in the 2003/04 season to 1,557 (CV=0.303) in the 2007/08 season.

Figure 5 shows the abundance estimates in Area IV plotted against the survey seasons. The abundance trend was estimated at 5.9% (95% CI: −16.4%, 28.1%) between 1989/90 and 2007/08, which was not statistically significant (Matsuoka and Hakamada, 2014).

Blue whales

There is limited information on stock structure of blue whales in the Antarctic. Abundance of this species for the

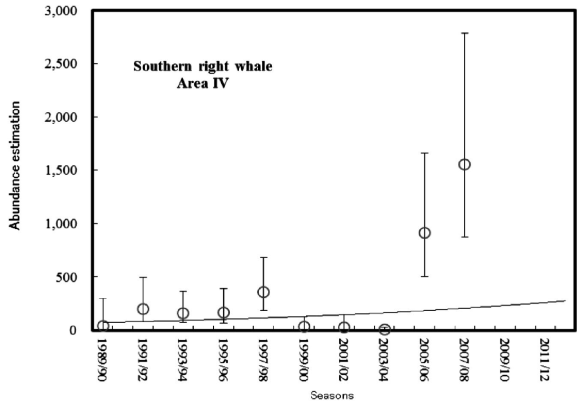

Figure 5. Abundance estimates of southern right whales in Area IV (south of 60°S). Vertical lines show 95% confidence intervals (after Matsuoka and Hakamada, 2014).

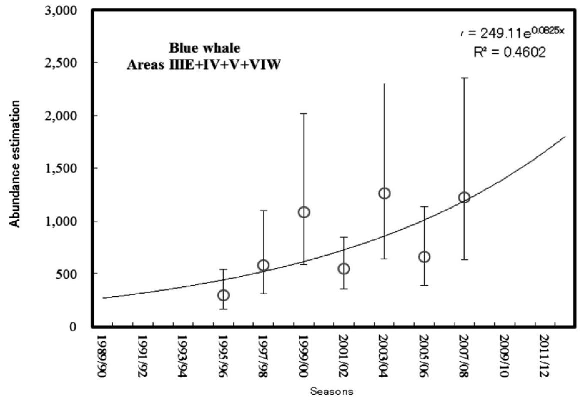

Figure 6. Abundance trend of blue whales in the Indo-Pacific region, south of 60°S. Vertical lines show 95% confidential intervals (after Matsuoka and Hakamada, 2014).

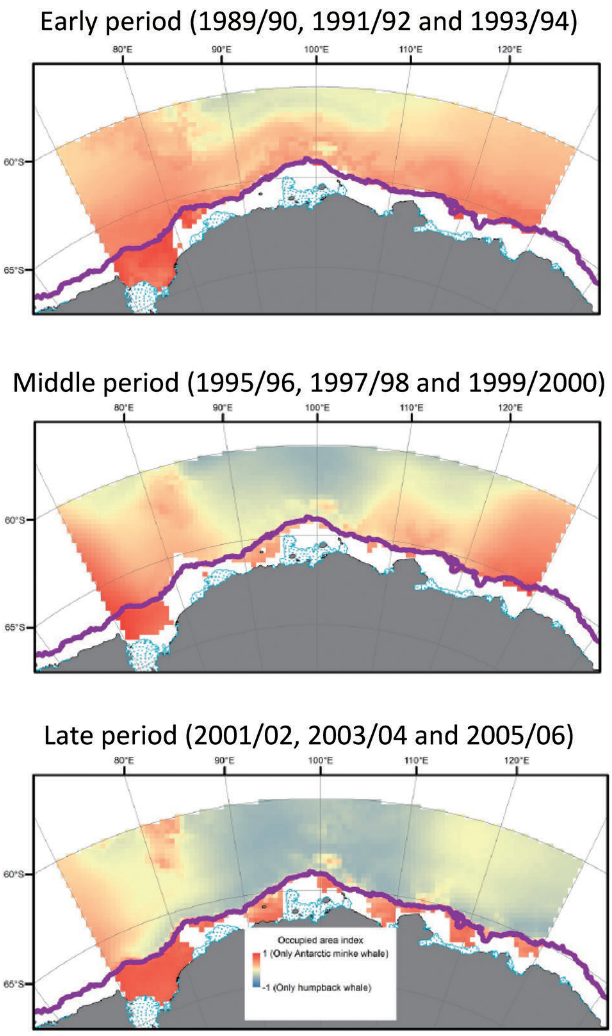

Figure 7. Occupied area indices of Antarctic minke and humpback whales in Area IV in three periods of JARPA and JARPAII (Murase *et al.*, 2014).

Indo-Pacific region of the Antarctic (35°E–145°W), south of 60°S was 664 (CV=0.328) in 2005/06 and 2006/07 seasons. The abundance was estimated at 1,223 whales (CV=0.345) in the 2007/08 and 2008/09 seasons.

Figure 6 shows the abundance estimates for the whole Indo-Pacific region plotted against the survey seasons. The abundance trend was estimated at 8.2% (95% CI: 3.9%, 12.5%) between 1995/96 and 2008/09. This estimate was not statistically significant.

Future surveys and analyses

Dedicated sighting surveys were planned for the seasons 2009/10–2013/14 under JARPAII. However, those surveys could not be conducted due to external interferences by an NGO (Nishiwaki *et al.*, 2014). Subsequently, dedicated sighting surveys were conducted under the NEWREP-A between 2015/16 and 2018/19 and data collected during these surveys are under analyses. From 2019/20 Japan started the JASS-A, which will be conducted for a tentative period of eight years. The main objective of JASS-A is to estimate the abundance of large whales in the Indo-Pacific region of the Antarctic (Government of Japan, 2019) (see Isoda *et al.*, this issue). Data collected during NEWREP-A and JASS-A will be analyzed by ICR scientists in conjunction with previous data in the same area.

Geographical distribution of baleen whales

Substantial increase in the abundance could have an implication in the pattern of distribution of the species. In the Indo sector of the Antarctic (Area IV), humpback whales have increased significantly over the past decades, while the abundance of Antarctic minke whales has been rather stable. Under this scenario, we should expect changes in the pattern of distribution of these two species.

Changes in the spatial and temporal distribution patterns of Antarctic minke and humpback whales in Area IV were examined for the period of JARPA and JARPAII. The spatial distribution was estimated using Generalized Additive Models (GAM) (Murase *et al.*, 2014). Presence or absence of whales was used as a response variable while seafloor depth, distance from shelf break and longitude were used as explanatory variables. Three time periods were defined: 'Early' (1989/90, 1991/92, 1993/94); 'Middle' (1995/96, 1997/98, 1999/00) and 'Late' (2001/02, 2003/04, 2005/06).

Figure 7 shows the results of the GAM analyses. In the early period, humpback whales were mainly distributed in the northern part of the survey area between longitudes 80°E and 100°E. In the middle period, humpback whales expanded their habitat closer to the shelf break between the longitudinal range of 80°E and 120°E. In the late period, humpback whales were distributed in the entire survey area along the shelf break. While the spatial distribution of humpback whales expanded during the period of JARPA and JARPAII, no strong change was observed in the distribution of Antarctic minke whales. These results indicated a possible competition for habitat between humpback and Antarctic minke whales as abundance of humpback whales increased.

The pattern of spatial distribution for other large whales was examined by Matsuoka and Hakamada (2018). Based on JARPA and JARPAII data, there was a common pattern for several whale species to concentrate mainly in the sector between 80°E and 110°E, south of 60°S. This could be related to krill availability, as a high density of euphausiids was reported between 100°E and 120°E in the 1999/00 JARPA survey (Murase *et al.*, 2002). Studies into the pattern of temporal (yearly) changes in relation to changes in environmental factors are underway.

Feeding ecology of Antarctic minke whale

An overview of the procedures for examining stomach content analyses by Japanese scientists was presented by Tamura and Konishi (2014). The type and amount of prey consumed by Antarctic minke whales in the feeding season is fundamental for understanding the impact of the consumption on the ecosystem. The analyses of stomach contents in Antarctic minke whales have benefited from useful suggestions and recommendations from the IWC SC.

Antarctic minke whales feed mainly on Antarctic krill (*Euphausia superba*) in offshore waters, and on ice krill (*E. crystallorophias*) (Figure 8) on the coastal shelf along the Ross Sea and Prydz Bay (Tamura and Konishi, 2009; 2014).

Figure 8. Ice krill (upper) and Antarctic krill (lower).

The maximum weight of stomach contents of Antarctic minke whales sampled by JARPA and JARPAII from 1987/88 to 2010/11 were 142.4 kg (2.4% of body weight) and 156.0 kg (3.4% of body weight) for immature males and females, respectively. The figures were 387.0 kg (3.8% of body weight) and 326.9 kg (3.6% of body weight) for mature males and females, respectively. The average weight and rate of stomach contents *per* body weight (RSC) were 33.2±28.3 kg (RSC: 0.8%) and 37.6±29.1 kg (RSC: 0.9%) for immature males and females, respectively and 63.5±47.1 kg (RSC: 0.9%) and 71.8±54.3 kg (RSC: 0.9%) for mature males and females, respectively (Tamura and Konishi, 2014).

The daily prey consumption by the whales in each reproductive status group was estimated using energy-requirement and energy deposition methods. The daily prey consumptions of Antarctic minke whales *per capita* during the feeding season based on these two methods were 95.1–127.0 and 182.6–250.3 kg for immature and mature males, 125.8–138.7 and 268.1–325.5 kg for immature and mature females, respectively. This is equivalent to 2.65–4.02% of their body weight. The total prey consumption of Antarctic minke whale *per capita* during the feeding season was 8.6–10.4 and 20.6–21.9 tons for immature and mature males, and 11.3–12.5 and 32.2–39.1 tons for immature and mature females, respectively (Tamura and Konishi, 2014).

Estimation of the uncertainties (e.g., allometric relationships, body weight of whales, energy values of prey species, assimilation efficiency and length of feeding period) in several components involved in estimating the amounts and types of prey consumed by North Pacific baleen whales was based on suggestions from the IWC SC.

Similar estimations of uncertainties are being conducted for Antarctic minke whales.

In Areas III east and IV in the 2007/2008 season, the abundance of Antarctic minke whales was estimated at 9,406 and 14,739, respectively. In Area V and VI west in the 2008/2009 season, the abundance was estimated at 108,097 and 26,364, respectively (Hakamada and Matsuoka, 2014b). These abundance estimates were used for investigating the total consumption of krill by Antarctic minke whales.

In Areas III east and IV the seasonal (120 days) prey consumption of krill by Antarctic minke whales was estimated at 0.17–0.19 and 0.33–0.37 million tons, respectively. In Areas V and VI west, the consumption was estimated at 2.51–2.88 and 0.50–0.54 million tons, respectively. The seasonal prey consumption by Antarctic minke whales in the total research area was estimated at 3.51–3.98 million tons, amounting to 7.6–8.6% of the krill biomass estimated by acoustic survey in the research area (Tamura and Konishi, 2014).

Energetics of Antarctic minke whale

Whales generally accumulate energy as lipid in the blubber during the summer feeding period at high latitudes, and they spend the energy in migration and reproduction in low latitude waters. The proxy of fat reserves in blubber have been used as an indication of body condition in whale studies (e.g., Lockyer *et al.*, 1985; 1986). The measurements of blubber thickness positively correlate with lipid content in the whole body fat and have proven to be a dependable proxy for energy storage in whales (Lockyer *et al.* 1985).

For the 18 years of JARPA period (1987/88–2004/05), the annual trend in energy storage in the Antarctic minke whales was examined using blubber thickness (Figure 9) at two lateral measurement points, in addition to total fat weight in the whale body and girth measured at two specified positions.

Blubber thickness measurements were available for 4,689 whales; fat weight was available for 740 whales; and half girth measurements were available for 4,681 animals. Regression analysis was carried out on blubber thickness in 4,689 whales. The explanatory variables were age, body weight, fetus length and total fat weight. A large number of linear mixed-effects statistical models were investigated for each of the dependent variables, and the Bayesian Information Criterion (BIC) was used to select the best model. All models examined had 'year' as a possible explanatory variable. This regression analyses clearly showed that blubber thickness had been decreasing in this period. The decrease per year in the JARPA period was estimated at approximately 0.02 cm for mid-lateral blubber thickness (Figure 10).

Similar analyses and results were found for fat weight, which decreased 17 kg in the 18-year period (Figure 10). Furthermore, the runs using 'half girth' as another dependent variable also gave similar results. The total magnitude of the decline over these 18 years was 4% for girth

Figure 9. Measurement of blubber thickness.

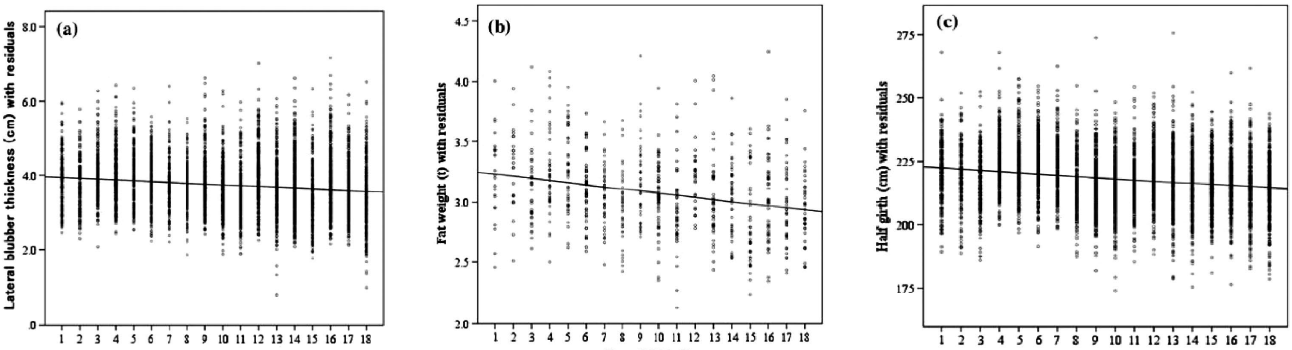

Figure 10. Temporal trends in body condition indicators of Antarctic minke whales in the feeding season (a: blubber thickness; b: fat weight; c: half girth) for the Antarctic minke whale in the feeding season (Konishi *et al.*, 2008).

measurements (Figure 10).

Similar analyses were conducted for stomach contents weight (sieved contents of the forestomach). Regression analyses using the weight of the stomach contents as the dependent variable, and explanatory variables such as year, date, local time, latitude, sex and body length, were conducted. A linear mixed-effects analysis showed a 31% (95% CI=12.6–45.3%) decrease in the weight of stomach contents over the 20 years since 1990/1991 (Konishi *et al.*, 2014).

The trend in stomach content was consistent with that of the blubber thickness (Konishi and Walløe, 2015). These results suggested that the food availability for Antarctic minke whales may have declined in recent decades.

Monitoring of environmental pollutants

Pollutant analyses by Japanese scientists started under the JARPA program for the objective of ʻElucidation of the effect of environmental changes on cetaceansʼ and continued under the JARPAII program. The objective involved the elucidation of the pattern of pollutant accumulation and the effects of pollutants on cetaceans. Different kinds of pollutants were monitored, which included trace elements and organochlorine compounds (OCs). Trace elements included the toxic ones Hg, Cd, Pb and Ni (non-essential elements), and Cu, Zn, Fe and Mn (essential elements). Toxic elements induce adverse effects in animals and humans, and are more persistent than the essential elements. Essential elements are important for survival and health of animals and humans.

Details of the analytical procedures for pollutants were given in Appendix 4 of Government of Japan (2005).

Regarding OCs, the levels of PCB and DDT in fin and Antarctic minke whales in the Antarctic Ocean could be the lowest among whale species in the world (Figure 11) (Yasunaga *et al.*, 2006). The results during the JARPA/JARPAII period suggested that levels of HCHs in the Antarctic Ocean have varied from decreasing slightly to being steady in the mid-1990s. HCB levels in large whales would be affected by trophic levels of its preys rather than by spatial differences.

Regarding trace elements, the hepatic Hg levels of Antarctic minke whales of all age groups in Area IV decreased significantly over the research years, whereas that of 15–26 years old whales in Area V increased significantly, possibly indicating that food availability of Antarctic minke whales in the 2000s may have differed from those in the 1980s and 1990s (Yasunaga *et al.*, 2014).

Oceanography

Information on oceanographic structure and its dynamics are important in interpreting changes in the Antarctic ecosystem. Changes in oceanographic conditions affect krill distribution and biomass and, in turn, the abundance and distribution of whales. Changes in oceanographic conditions might indicate an effect of climate change. Details of the oceanographic survey can be found in Wada (2019).

Watanabe *et al.* (2014) analyzed oceanographic observation data obtained by JARPA and JARPAII to clarify

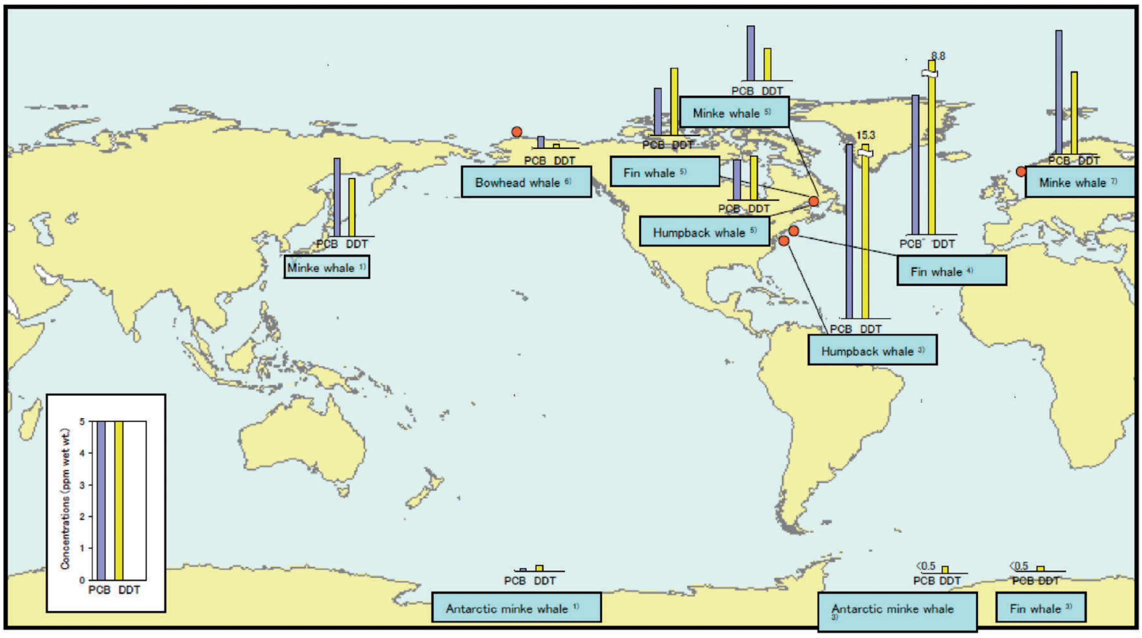

Figure 11. PCB and DDT levels in the blubber of baleen whales (Yasunaga *et al.*, 2006).

physical oceanographic conditions in the JARPA/JARPAII research area as a basis for understanding the habitat environment of whales. About 2,500 profiles were obtained by XBT, XCTD and CTD observations from 1990 to 2009. Based on this data set, the averaged feature of the oceanographic structure in the research area for two decades was described. In the area east of the Kerguelen Plateau, the position of the Southern Boundary (SB) changed on a decadal timescale. The SB is indicated by the 0°C contour on 27.6σ_0. The southward shift of the SB in the region was observed in the early 2000s, and the northward shift was observed in the later 2000s (Figure 12).

Unlike the Antarctic Peninsula region, the JARPA and JARPAII temperature data showed no statistically significant warming in the JARPA area for the two decades.

Investigation of the oceanographic conditions in the Indo-Pacific region of the Antarctic is one of the secondary objectives of JASS-A. Data will be analyzed in conjunction with the large data sets produced by JARPA/JARPAII and NEWREP-A in the Indo-Pacific region of the Antarctic.

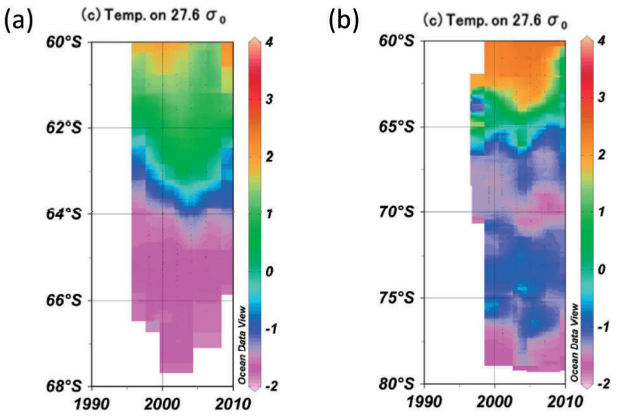

Figure 12. Temperature on the 27.6σ_0 isopycnal surface for the Area IV (a) and Area V (b) (Watanabe *et al.*, 2014).

Krill survey

Krill is a key prey species in the Antarctic ecosystem, supporting different species of baleen whales, pinnipeds, birds and fish. For this reason, the ICR has been conducting krill surveys alongside the whale sighting surveys. Sighting of whales and krill surveys were carried out concurrently in JARPAII and NEWREP-A. Details of the krill survey can be found in Wada (2019).

Wada and Tamura (2014) presented results of krill biomass estimations based on the data from a quantitative echo sounder (EK500; Simrad, Norway). Estimations were made based on data obtained in Area IV in the 2007/08 JARPAII survey and in Area V in the 2008/09 JARPAII survey. The biomass estimates obtained in Area IV was 12.5 million tons, and that in Area V was 24.0 million tons. These estimations were useful to evaluate the impact of the krill consumption by three baleen whale species.

Regarding krill demography, information on length frequency distribution and maturity stage of Antarctic krill are being obtained using samples from stomachs of Antarctic minke whales taken in JARPA, JARPAII and NEWREP-A (1989/90–2017/18). Figure 13 shows an example of outputs of such analyses.

Marine debris

In recent years marine debris has been recorded in the sub-Antarctic and Antarctic islands. Marine debris causes negative effects on whales through ingestion and by entanglement. JARPA, JARPAII and NEWREP-A conducted systematic monitoring of marine debris in whales and their environment in the Antarctic. Details of the results of marine debris during JARPA/JARPAII can be found in Isoda *et al.* (2018).

The density index (DI: number of marine debris per 100 nautical miles) of marine debris in the Antarctic is lower by two orders of magnitude in comparison to the North Pacific Ocean and its adjacent waters. Thus, the observa-

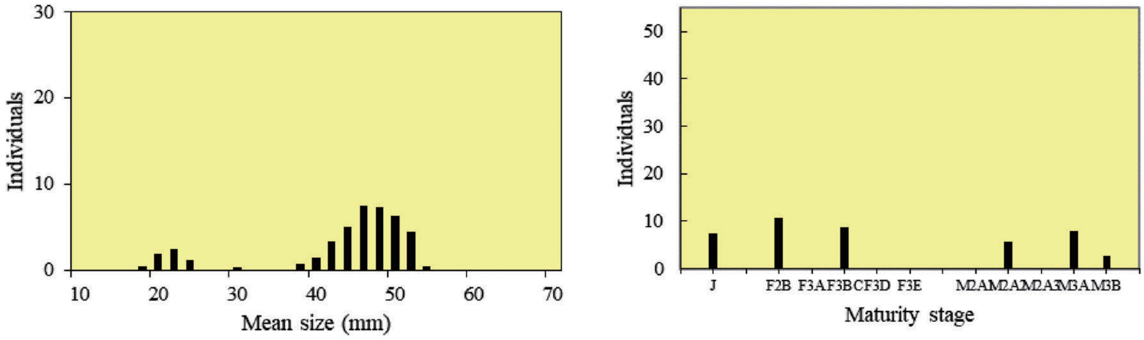

Figure 13. Length frequency distribution (left) and maturity stage (right) of Antarctic krill sampled from stomach contents of Antarctic minke whales in 2008/09 in the sector 130°E and 140°E.

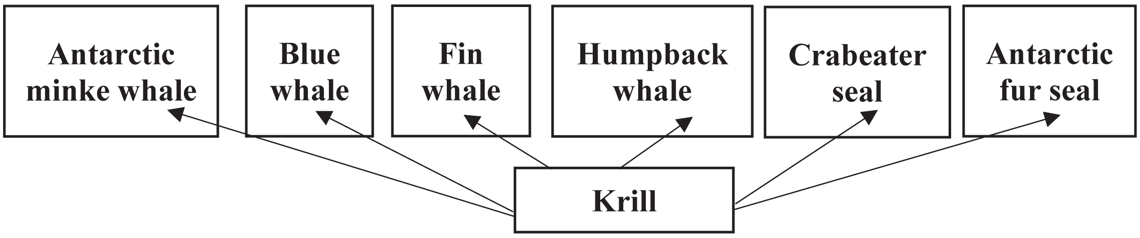

Figure 14. Food web in the Antarctic modeled in Mori and Butterworth (2006) and Moosa (2017).

tions proved that the Antarctic waters have a very low density of marine debris on the sea surface. The level of impact of entanglements on Antarctic minke whales was low in comparison with other oceanic basins. Given the low indices, the effect of marine debris on whales in the Antarctic was expected to be limited at the present time (Isoda *et al.*, 2018).

Modelling work

An overview of the ICR work on ecosystem modelling in the Antarctic was presented by Hakamada and Tamura (2018). The ecological background for the modelling exercise was provided by Fujise and Pastene (2018).

Mori and Butterworth (2006) constructed a multi-species modelling in the Antarctic Ocean using baleen whales and seals as predators and krill as prey (Figure 14). One of the main objectives of their modelling exercise was to determine whether predator-prey interactions alone can broadly explain observed population trends without the need for recourse to environmental change hypotheses. Preliminary results indicated that this is likely to be the case.

New ecosystem modelling studies are underway (Moosa, 2017).

CONCLUSIONS

Japan's whale research programs under special permit have provided important information on the ecology of Antarctic minke whales and other baleen whale species in the Indo-Pacific region of the Antarctic. The long time series of data produced by the research programs have been fundamental to the study and understanding of abundance trends of several large whale species, the changes in distribution of Antarctic minke and humpback whales, and the changes in nutritional conditions of the Antarctic minke whales. The research programs have produced new information in helping to understand the oceanographic structure of the Indo-Pacific region of the Antarctic.

Also, studies on the distribution, abundance and demography of the krill have commenced. Further, these programs have provided information on the level of pollutant and marine debris in the Antarctic, which fortunately are at low levels. Ecosystem models are being developed to understand the predator-prey interaction.

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Technical Report (not peer reviewed)

An outline of the Japanese Abundance and Stock structure Surveys in the Antarctic (JASS-A) including results of the first survey under this new research program

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ABSTRACT

This paper briefly outlines the objectives, survey and analytical procedures and work schedule of a new research program on whales and the ecosystem in the Indo-Pacific region of the Antarctic (JASS-A=Japanese Abundance and Stock structure Surveys in the Antarctic) and summarizes the first JASS-A survey conducted in the 2019/20 austral summer season. The main research objectives of JASS-A are i) the study of the abundance and abundance trends of large whale species, and ii) the study of the distribution, movement and stock structure of large whale species. JASS-A also has five secondary research objectives related to oceanography, marine debris and whale biology. JASS-A will be based on systematic sighting surveys utilizing the Line Transect Method, to be conducted alternatively in IWC Management Areas III, IV, V and VI by one or two specialized vessels, during a tentative period of eight austral summer seasons. Analyses related to main and secondary objectives will be conducted based on new as well previous data collected by JARPA/JARPAII and NEWREP-A in the same research area. The first JASS-A survey was conducted in the western part of Area III (000°–015°E) for 25 days, from 13 January to 6 February 2020. Systematic sighting and oceanographic data were obtained. Also biopsy, photo-identification and satellite tracking experiments were conducted successfully.

INTRODUCTION

Long-term research surveys in the Antarctic are scarce. Circumpolar whale sighting surveys were conducted during the International Decade for Cetacean Research/Southern Ocean Whale and Ecosystem Research (IDCR/SOWER) under the auspices of the International Whaling Commission Scientific Committee (IWC SC) between 1978/79 and 2009/2010 (Matsuoka *et al.*, 2003; IWC, 2013). These surveys produced important sighting data to study the abundance and abundance trends of large whales in different IWC Management Areas (Branch and Butterworth, 2001; IWC, 2013).

The Japanese Whale Research Programs under Special Permit in the Antarctic (JARPA/JARPAII) and the New Scientific Whale Research Program in the Antarctic Ocean (NEWREP-A) involved comprehensive long-term systematic surveys in the Indo-Pacific region of the Antarctic that collected biological information from whales and data from its environment, using lethal (biological sampling of Antarctic minke whale) and non-lethal (whale sighting surveys, oceanographic surveys, photo-identification, biopsy sampling and satellite tagging of large whales) approaches. A comprehensive biological and environmental data set, including abundance trends for several whale species, was obtained from these surveys conducted between 1987/88 and 2018/19 (IWC, 2015).

Japan considered it important to continue whale and ecosystem surveys in the Indo-Pacific region of the Antarctic through dedicated sighting surveys and other non-lethal techniques to investigate primarily abundance, abundance trends and stock structure of large whales. For such an aim a new research program was designed which is called JASS-A (Japanese Abundance and Stock-structure Surveys in the Antarctic). The JASS-A program was presented to the 2019 meeting of IWC SC (GOJ, 2019a), to the 2019 meeting of Commission for the Conservation of Antarctic Marine Living Resources-Working Group on Ecosystem Monitoring and Management (CCAMLR-EMM) (GOJ, 2019b), and to the meeting of the North Atlantic Marine Mammal Commission Scientific Committee (NAMMCO SC) (GOJ, 2019c). JASS-A was positively evaluated at these international forums.

The objectives of this paper are, i) to present a brief outline of the objectives, methodology and research schedule of JASS-A, and ii) to summarize the results of the first JASS-A survey in the Indo-Pacific region of the Antarctic in the 2019/20 austral summer season.

OUTLINE OF JASS-A

Objectives of the JASS-A

The main research objectives (MO) of the JASS-A are:

MO1: Study of the abundance and abundance trends of large whale species in the Indo-Pacific region of the Antarctic

Abundance and abundance trends of whales in the Antarctic is essential for conservation and management purposes. Many whale species were depleted in the past. Some of them have shown signs of recovery in recent years, and it is important to monitor their recovery process and how such recovery could affect other whale species in the ecosystem.

MO2: Study of the distribution, movement and stock structure of large whale species in the Indo-Pacific region of the Antarctic

Stock structure information is important to interpret distribution and abundance data. Genetic stocks are demographically independent units and therefore each stock will respond in a different way to changes that have occurred in the ecosystem. Ideally abundance estimates should be based on the geographical and temporal boundaries of genetic stocks.

JASS-A, in conjunction with the work already conducted under the previous research programs, will provide information to allow the determination of the status of the stocks of large whales that are found in waters of the Indo-Pacific region of the Antarctic in summer, and provide the necessary scientific background for future policies on conservation and sustainable utilization.

The secondary objectives (SO) of JASS-A are:

SO1: Investigation of the oceanographic conditions in the Indo-Pacific region of the Antarctic

Oceanographic structure and dynamics provide important information in interpreting changes in the Antarctic ecosystem. Changes in oceanographic conditions will affect krill distribution and biomass and, in turn, the abundance and distribution of whales. Changes in oceanographic conditions can be related to climate changes.

SO2: Investigation of the spatial and temporal trends of marine debris on sea surface

Studies on marine debris in the Antarctic are very scarce with only a few records made in sub-Antarctic, Antarctic islands and the Antarctic. It is important to continue with this kind of survey to monitor the future trends in the occurrence of marine debris.

SO3: To conduct feasibility studies to evaluate the utility of genetic data to estimate abundance

Systematic sighting surveys utilizing the Line Transect Method is the most used method to estimate abundance of whales. Basic line transect surveys however, are not always appropriate, especially for rare species/populations. Also in the case of the Antarctic it is not possible to utilize line transect methodology for areas inside the pack-ice, e.g., polynyas, where whales are also distributed. Genetic-based methods can assist in the abundance estimates in such cases.

SO4: To continue with feasibility studies to evaluate the utility of non-lethal techniques for whale biological research

During the NEWREP-A several studies were carried out to investigate the feasibility of novel non-lethal approaches to address some of the main objectives of the NEWREP-A on Antarctic minke whales, e.g., progesterone analysis in blubber to investigate reproductive status (Inoue *et al.*, 2019), and stable isotopes to investigate whales′ prey items. There is the need to continue with the investigation on the utility of such techniques for large whales.

SO5: Feasibility study on the utility of Unmanned Aerial Vehicle (UAV) for obtaining information relevant for abundance estimate of large whales

In particular this technique could be used to determine the number of individuals in the schools, which is information highly relevant for the abundance estimates.

Research area, season, vessels and period

The research area of JASS-A will be comprised by IWC Management Areas III, IV, V and VI, south of 60°S (Figure 1). This will allow for continuity and consistency with data already collected by JARPA/JARPAII, NEWREP-A and IDCR/SOWER surveys in those Management Areas.

The period of the annual surveys will be the austral summer season (January–February), which is the same as in the previous JARPA/JARPAII, NEWREP-A and IDCR/SOWER surveys.

Surveys will be carried out in principle by one or two spe-

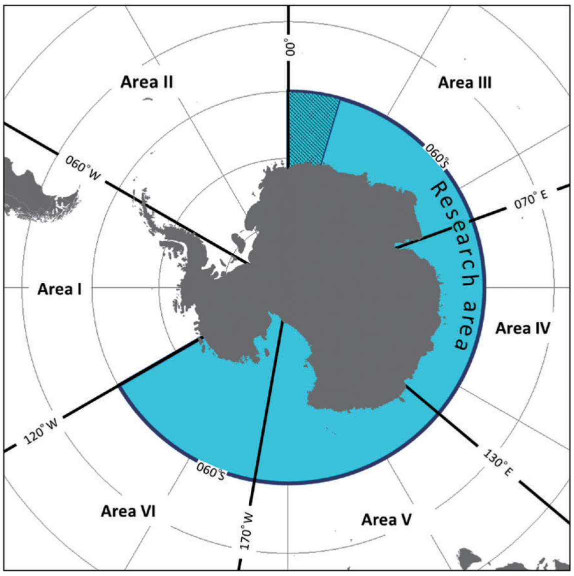

Figure 1. Research area of JASS-A. The shaded area (000°–015°E) indicates the surveyed area in the 2019/20 austral summer season.

cialized vessels, and the tentative length for JASS-A will be eight years (2019/20–2026/27), a period required to cover half of each Area once (Table 1). The final determination of the period and number of vessels will depend on funding availability and therefore some level of flexibility is required.

Survey and analytical procedures

Abundance and abundance trends (MO1)

The approach will be systematic vessel-based sighting surveys utilizing the Line Transect Method. Surveys will be designed and conducted following the protocols included in the 'Requirements and Guidelines for Conducting Surveys and analyzing data within the Revised Management Scheme' (IWC, 2012). Sighting protocols will be the same as those used in the former IDCR/SOWER surveys (Matsuoka *et al.*, 2003). Two sighting modes will be used for *g*(0) estimates, Passing with abeam Closing mode (NSP) and Passing with Independent Observer mode (IO). The OK model (Okamura and Kitakado, 2012) will be used to estimate abundance taking *g*(0) estimates into account. As stated above, the new data will be analyzed in conjunction with previous data in those Areas (Table 1). Log-linear models will be used to estimate abundance trends (see details in Hakamada *et al.*, 2013).

Distribution, movement and stock structure (MO2)

Distribution and movement of individual humpback, blue, southern right and fin whales will be investigated primarily by photo-id and genetic tagging. Photo-id pictures in conjunction with pictures obtained in previous programs will be used for the matching exercise to investigate distribution and movement of those large whales. Studies on stock structure in humpback, blue, southern right and fin whales will be based on genetic analyses of previous biopsy samples as well as additional biopsy samples collected during the new research program. Refinement of the stock structure of the Antarctic minke whales (e.g., Pastene and Goto, 2016) will continue under the new research program based on the large genetic sample set collected under JARPA/JARPAII and NEWREP-A.

Genetic markers such as mtDNA control region sequencing and microsatellite DNA will be used. Standard genetic analyses based on hypothesis testing, heterozygosity, diversity, and related measures will be used. Principal Component Analysis (PCA), Discriminant Analysis of Principal Component (DAPC) as well the Bayesian approach STRUCTURE will also be used. The final analytical procedure will be determined depending on the amount and nature of the data collected for each species.

Oceanographic surveys (SO1)

Oceanographic surveys will be conducted at one station per day, at least, using eXpendable Conductivity, Temperature and Depth (XCTD). Oceanographic stations will be deployed at equal intervals along the track-lines defined for the sighting surveys.

Spatial and temporal trend of marine debris (SO2)

Observation of marine debris on the sea surface will be made by visual observation along the track-lines of sighting surveys, the same as was done in JARPA/JARPAII and NEWREP-A. Details of the methodology can be found in Isoda *et al.* (2018).

Feasibility studies to evaluate the utility of genetic data to estimate abundance (SO3)

Approaches based on mark-recapture of genetically identified individuals will be used. Individual identification will be based on the genotype profile of a set of microsatellite loci. Studies have already started for southern right whales based on biopsy sampling (see details in Pastene *et al.*, 2018). Other potential target species are the blue and humpback whales as a considerable number of biopsy samples are already available.

Antarctic minke whales will also be a target species for paternity analyses based on the substantial amount of genetic and other biological data from JARPA/JARPAII and NEWREP-A.

Table 1
An overview of previous surveys with abundance estimates (AE) and tentative plans for future surveys in Areas III, IV, V and VI under the JASS-A (GOJ, 2019a).

No.	No.	Program	Season	Research area								Remarks
				IIIW	IIIE	IVW	IVE	VW	VE	VIW	VIE	
1	1	JARPA	1987/88	—	—	AE	—	—	—	—	—	—
2	2		1988/89	—	—	—	—	—	AE	—	—	—
3	3		1989/90	—	—	AE	AE	—	—	—	—	—
4	4		1990/91	—	—	—	—	AE	AE	—	—	—
5	5		1991/92	—	—	AE	AE	—	—	—	—	—
6	6		1992/93	—	—	—	—	AE	AE	—	—	—
7	7		1993/94	—	—	AE	AE	—	—	—	—	—
8	8		1994/95	—	—	—	—	AE	AE	—	—	—
9	9		1995/96	—	AE	AE	AE	—	—	—	—	—
10	10		1996/97	—	—	—	—	AE	AE	AE	—	—
11	11		1997/98	—	AE	AE	AE	—	—	—	—	—
12	12		1998/99	—	—	—	—	AE	AE	AE	—	—
13	13		1999/2000	—	AE	AE	AE	—	—	—	—	—
14	14		2000/01	—	—	—	—	AE	AE	AE	—	—
15	15		2001/02	—	AE	AE	AE	—	—	—	—	—
16	16		2002/03	—	—	—	—	AE	AE	AE	—	—
17	17		2003/04	—	AE	AE	AE	—	—	—	—	—
18	18		2004/05	—	—	—	—	AE	AE	AE	—	—
19	1	JARPAII	2005/06	—	AE	AE	AE	—	—	—	—	—
20	2		2006/07	—	—	—	—	AE	AE	AE	—	—
21	3		2007/08	—	AE	AE	AE	—	—	—	—	—
22	4		2008/09	—	—	—	—	AE	AE	AE	—	—
23	5		2009/10	—	—	—	—	—	—	—	—	Canceled
24	6		2010/11	—	—	—	—	—	—	—	—	Canceled
25	7		2011/12	—	—	—	—	—	—	—	—	Canceled
26	8		2012/13	—	—	—	—	—	—	—	—	Canceled
27	9		2013/14	—	—	—	—	—	—	—	—	Canceled
28	1	JASS	2014/15	—	—	AE	—	—	—	—	—	—
29	1	NEWREP-A	2015/16	—	—	—	AE	—	—	—	—	—
30	2		2016/17	—	—	—	—	AE	—	—	—	—
31	3		2017/18	—	—	—	—	—	AE	AE	—	—
32	4		2018/19	—	AE	—	—	—	—	—	—	—
33	1	JASS-A	2019/20	Plan	—	—	—	—	—	—	—	
34	2		2020/21	—	—	—	—	—	—	—	Plan	
35	3		2021/22	—	—	Plan	—	—	—	—	—	
36	4		2022/23	—	—	—	Plan	—	—	—	—	
37	5		2023/24	—	—	—	—	Plan	—	—	—	
38	6		2024/25	—	—	—	—	—	Plan	—	—	
39	7		2025/26	—	—	—	—	—	—	Plan	—	
40	8		2026/27	—	Plan	—	—	—	—	—	—	

Feasibility studies on the utility of non-lethal techniques to obtain biological and feeding ecology information from large whales (SO4)

Progesterone analyses of blubber (SO4-A)

Inoue *et al.* (2019) studied the feasibility of analyses of progesterone in blubber as an indicator of the reproduc-

Table 2

Tentative schedule for the work on main and secondary objectives of JASS-A.

Season	2019/20	2020/21	2021/22	2022/23	2023/24	2024/25	2025/26	2026/27
MO1 Abundance and trends								
Blue whale		○			○			○
Fin whale		○			○			○
Antarctic minke whale			○			○		○
Humpback whale			○			○		○
Southern right whale				○			○	○
Sperm whale				○			○	○
Southern bottlenose whale				○			○	○
MO2 Stock structure								
Blue whale								○
Fin whale								○
Antarctic minke whale		○						
Humpback whale		○						
Secondary Objectives								
Oceanography				○[1]				○[2]
Marine debris				○[3]				○[4]
Abundance genetics			○[5]					○[6]
Feasibility non-lethal (progesterone)				○[7]				○[8]
Feasibility non-lethal (stable isotope)				○[9]				○[10]
UAV experiments								○

[1]Preliminary results oceanography; [2]Complete the work, oceanography; [3]Preliminary results marine debris; [4]Complete the work, marine debris; [5]Complete the work, southern right whale; [6]Complete the work, humpback and Antarctic minke whale; [7]Preliminary results for humpback whale; [8]Complete the work, humpback whale and Antarctic minke whale; [9]Preliminary results for Antarctic minke, fin and southern right whales; [10]Complete the work, Antarctic minke, fin and southern right whales

tive status of Antarctic minke whales. The study was possible because information on the reproductive status of each sample used was available (from lethal sampling) for comparison purposes. Efforts will be made to collect biopsy samples on an opportunistic basis so that the progesterone study for this species can continue. Another candidate species for this study is the humpback whales for which a substantial number of biopsy samples are already available.

Stable isotopes analyses (SO4-B)

Prey items of large baleen whales will be investigated based on previous samples collected lethally, and biopsy samples (old and new samples) and stable isotope analyses (see details of the method in Mitani *et al.*, 2006). Priority species for this study will the Antarctic minke, fin and southern right whales.

Feasibility study on the utility of Unmanned Aerial Vehicle (UAV) for obtaining information relevant for abundance estimate of large whales (SO5)

Several types of UAV will be tested to investigate the utility of this technique for obtaining information relevant for abundance estimates of large whales including the number of whales in a school.

As noted above, MO and SO data will be analyzed in conjunction with the large data sets produced by JARPA/JARPAII and NEWREP-A in the Indo-Pacific region of the Antarctic.

Research schedule

Table 2 shows the research schedule of JASS-A with proposed dates for progressing and completing the work of MO and SO. Once again some degree of flexibility is required on the schedule proposed in this table.

Organization of JASS-A and opportunities for research collaboration

Scientists from the Institute of Cetacean Research (ICR) will play the leading role in order to pursue the research activities and achieve the research objectives of JASS-A, in collaboration with scientists from other domestic research organizations such as the National Research Institute of Far Seas Fisheries, and the Tokyo University of Marine Science and Technology. A domestic steering group has been formed to coordinate the research activities.

Qualified external scientists will be welcome to participate in the field and analytical works of JASS-A. Qualified external scientists can submit field or analytical research proposals for consideration of the domestic steering group. To facilitate the process, the steering group will prepare guidelines for the submission process.

SUMMARY OF THE FIRST JASS-A SURVEY

Research area

The research area covered by the survey was the western part of Area IIIW (000°–015°E), south of 60°S (Figure 1).

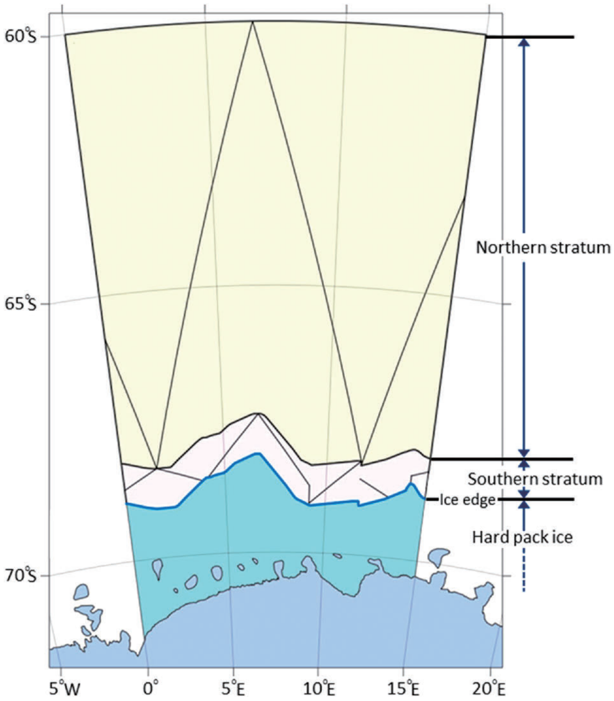

Figure 2. Research area (000°–015°E) and track-line of the JASS-A survey in 2019/20. The survey commenced at 68°40′S 000°00′ and ended at 63°06′S 15°00′E.

The area was divided into northern and southern strata. The boundary between southern and northern strata was defined by a line 45 n.miles from the ice edge (Figure 2). In the northern and southern strata, the survey tracklines consisted of a zigzag course changing direction at 5°00′ and 2°30′ longitudinal degree intervals in a 10 degrees longitudinal band respectively. A randomized start point for survey tracks was used.

Research vessel and sighting mode

The dedicated sighting vessel (SV) *Yushin-Maru* No. 2 (747GT, 69.6 m) was engaged in the survey. Three experienced Japanese researchers on line transect surveys, biopsy sampling, photo-id, satellite tagging experiments, and oceanographic survey, were aboard. The vessel was equipped with a top barrel platform (TOP, 19.5 m), Independent Observer Platform (IOP, 13.5 m) and an upper bridge platform (UBP, 11.5 m). For NSP mode, there were two primary observers on the TOP and there was open communication between the UBP and the TOP. For IO mode, there were two primary observers on the TOP and one primary observer on the IOP. The observers on the TOP or IOP communicated to the UBP independently. Two primary observers (captain and helmsman) and researchers were at the upper bridge, regardless of the research mode (Figure 3). These observers conducted searching for cetaceans by using angle board and binoculars with reticles (7x), which include the distance estimate scales.

Research effort in the research area

The dedicated sighting vessel was engaged in the survey for 25 days. The survey commenced on 13 January at position 68°40′S 000°00′ and ended on 6 February at position 63°06′S 15°00′E covering the predetermined transects. The total searching distance was 1,447.9 n. miles (2,681.5 km), including 650.3 n. miles covered in NSP mode and 797.6 n. miles in IO mode. The survey coverage

Figure 3. Sighting activity at the TOP (left); confirmation and tracking of whales including identification of duplicates at the UBP (middle); Researcher recording a sighting and inputting data at the UBP (right).

Table 3

Number of sightings in the research area, by stratum and species.

Species	Western part of Area IIIW: 000°–015°E								Sub-total				Total	
	Southern stratum				Northern stratum									
	Prim.		Second.		Prim.		Second.		Prim.		Second.			
	Sch.	Ind.	Sch.	Ind.	Sch.	Ind.	Sch.	Ind.	Sch.	Ind.	Sch.	Ind.	Sch.	Ind.
Blue whale	11	11	2	2	6	7	0	0	17	18	2	2	19	20
Fin whale	7	16	1	6	63	113	1	1	70	129	2	7	72	136
Like fin	0	0	1	3	2	3	0	0	2	3	1	3	3	6
Antarctic minke whale	51	106	14	15	48	73	6	9	99	179	20	24	119	203
Like minke	5	6	1	1	4	4	0	0	9	10	1	1	10	11
Humpback whale	13	22	12	16	62	123	3	7	75	145	15	23	90	168
Like humpback	0	0	0	0	2	2	0	0	2	2	0	0	2	2
Baleen whale	2	3	1	1	5	6	1	1	7	9	2	2	9	11
Sperm whale	0	0	0	0	5	5	0	0	5	5	0	0	5	5
Killer whale	1	1	0	0	0	0	0	0	1	1	0	0	1	1
Southern bottlenose whale	4	10	0	0	3	12	0	0	7	22	0	0	7	22
Ziphiidae	1	2	0	0	13	22	0	0	14	24	0	0	14	24
Unidentified whale	1	1	0	0	3	3	0	0	4	4	0	0	4	4

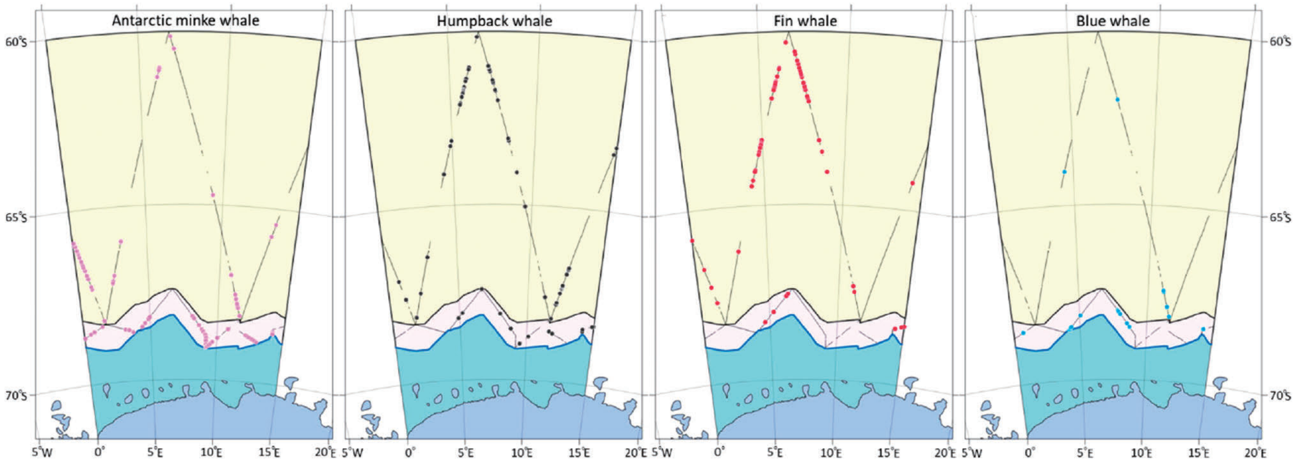

Figure 4. Position of primary sightings of Antarctic minke, humpback, fin, and blue whales with information of the surveyed transects.

was 71% in the northern stratum and 83% in the southern stratum. The total experimental time for photo-id, biopsy sampling, satellite tagging and distance and angle experiment was 26 hours and 49 minutes.

Whale sighting in the research area

Four baleen whale species, blue (19 schools/20 individuals), fin (72/136), Antarctic minke (119/203), humpback (90/168) whales and at least three toothed whale species, sperm (5/5), southern bottlenose (7/22) and killer (1/1) whales, were sighted (Table 3).

Antarctic minke whales

This species was the most frequently sighted. Density index (DI: schools sighted/100 n.miles searching distance) based on primary sightings was 0.04 in the northern stratum and 0.14 in the southern stratum. In this survey, the sightings of Antarctic minke whales were abundant near the ice edge. In addition, many sightings occurred in the western side of the northern stratum (Figure 4).

Humpback whales

This species was the second most frequently sighted. It was widely distributed in the research area (Figure 4). The DI based on primary sightings was 0.06 in the northern stratum and 0.03 in the southern stratum. The distribution was concentrated between 61°S and 62°S. The pattern of distribution and density of this species in this survey seems to be different from those in past surveys in the Area III.

Fin whales

This species was the third most frequently sighted. It was found mainly north of 64°S (Figure 4). The number of sightings were larger than in previous surveys in the same area.

Blue whales

This species was found mainly in the southern stratum of the research area (Figure 4). In this survey, 13 schools (13 individuals) were distributed in the southern stratum, similar to the situation in previous surveys. A total of 6 schools (7 individuals) was distributed in the northern stratum.

Duplicate sightings

Duplicates were recorded for a total of 75 sightings (involving several whale species) during the IO mode survey, and these data will be used to estimate whale abundance while taking estimated $g(0)$ into consideration.

Figure 5. Photo-id experiments on humpback whales from the bow deck of the vessel (left); details of the ventral fluke pigmentation (right).

Figure 6. Biopsy sampling of blue whale (left) and skin/blubber sample of blue whale obtained by biopsy sampling (right).

Figure 7. Satellite tagging and biopsy sampling of fin (left) and Antarctic minke (right) whales.

Sighting survey in the transit area

The sighting survey was conducted between 10°S and the research area, excluding the areas of foreign countries′ EEZs. Only NSP mode was used. The searching effort was 2,539.9 n.miles and total sightings included blue (3/4), fin (9/14), Antarctic minke (5/8), humpback (12/23), sperm (9/10), southern bottlenose (2/8) and killer whales Type A (1/17) whales.

Other research activities

Sighting distance and angle experiment

The sighting distance and angle experiment was conducted in order to evaluate the accuracy of sighting distance and angle provided by primary observers. The results of this experiment will be used for the calculation of abundance estimates. A training for this experiment was carried out on 15 January. The actual experiment was conducted on 30 January and 144 trials were completed successfully.

Photo-id (Figure 5)

A total of 20 blue, 14 humpback and 5 killer whales (Type A) were successfully photo-identified during the survey. These data will be registered into the ICR photo-id catalogue (e.g., Matsuoka and Pastene, 2014).

Biopsy sampling for large whales (Figure 6)

A total of 29 biopsy samples were collected, including 10 blue, 11 fin, and 8 Antarctic minke whales, using the Larsen system (Larsen, 1998). Satellite tags were attached on eight biopsied Antarctic minke whales and seven of the 11 biopsied fin whales. Biopsy samples were stored at −20°C. These samples will be used in genetic analyses and studies to evaluate the utility of non-lethal techniques for whale biological research.

Figure 8. Oceanographic survey by XCTD.

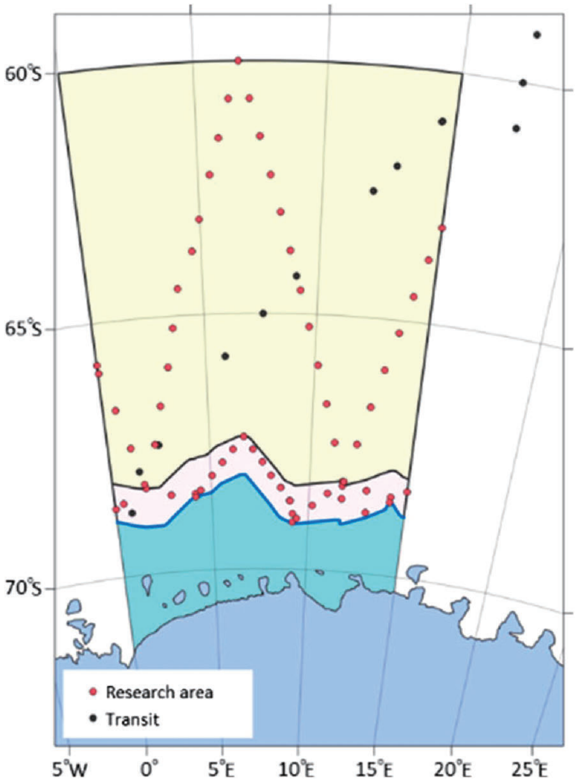

Figure 9. Oceanographic observation stations (XCTD casting points).

Figure 10. Image of two blue whales taken using a small UAV in the research area.

Satellite tagging (Figure 7)
The satellite-monitored tags (SPOT6, Wildlife Computers, Redmond, Washington, USA) were deployed with the Air Rocket Transmitter System (ARTS) (LK-ARTS, Skutvik, Norway). The details of the deployment system and protocols is described in Konishi *et al.* (2020). The tags were successfully deployed on 10 fin and 8 Antarctic minke whales (see Konishi *et al.*, this issue).

Oceanographic survey
Oceanographic survey was conducted by XCTD (Figure 8) at 75 stations on the survey track-lines, including transit survey (Figure 9). Oceanographic data will be analyzed to study the oceanographic structure of the research area and the relationship with whale distribution (Watanabe *et al.*, 2014). In addition, an Argo float, under the Argo oceanographic program (JAMSTEC, 2020), was successfully deployed on 4 January, during the transit to the Antarctic.

Marine debris observation
A total of three marine debris objects, comprising two plastic bottles and a fishing buoy, were observed during the survey in the research area. These data will be registered into the ICR database and reported in the future (e.g., Isoda *et al.*, 2018).

Feasibility study on the utility of UAV
A preliminary experiment was conducted for collecting aerial images of whale using a small UAV (Phantom 4 Pro; DJI, Shenzhen, China). A total of two blue (Figure 10) and three humpback whales were photographed using the UAV. These data will be registered into the photo-id catalogue of ICR.

Data and samples collected in this survey were validated and stored at the ICR, and will be analyzed in conjunction with data and samples obtained during past research programs in the Antarctic (JARPA, JARPAII, NEWREP-A), in the context of the primary and secondary objectives of the JASS-A. Data will be available to the national and international scientific community through established data access protocols of ICR.

ACKNOWLEDGEMENTS

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Technical Reports of the Institute of Cetacean Research (2020) pp. 23–34

Technical Report (not peer reviewed)

An outline of the IWC-Pacific Ocean Whale and Ecosystem Research (IWC-POWER) including results of the 2019 survey

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ABSTRACT

This paper outlines the objectives, research area and general methodology of the International Whaling Commission-Pacific Ocean Whale and Ecosystem Research Program (IWC-POWER). It also provides the main results of the 2019 survey conducted under this research program. The IWC-POWER is designed and implemented by the IWC Scientific Committee (SC) in special partnership with the Government of Japan. The long-term objective of the IWC-POWER is to ʻprovide information to allow determination of the status of populations (and thus stock structure is inherently important) of large whales that are found in the North Pacific waters and provide the necessary scientific background for appropriate conservation and management actionsʼ. To fulfill this objective, the IWC-POWER originally identified short and medium-term activities and priorities. The IWC-POWER is close to successfully completing its first-phase (2010–2021) related to the short-term priorities, and the IWC SC will start organizing the second phase (from 2022 onward). It has also updated the medium-term priorities for this second phase. The 2019 survey was conducted successfully between 3 July and 25 September 2019 in the Gulf of Alaska, within the U.S. Exclusive Economic Zone by the Japanese R/V *Yushin Maru* No. 2. Blue (17 schools/19 individuals), fin (266/458), sei (9/20), common minke (5/5), humpback (173/402), gray (6/15), sperm (20/20), Bairdʼs beaked (2/37), Cuvierʼs beaked (3/5) and killer (53/264) whales were observed during the survey. Photo-identification data were collected from 16 blue, 51 fin, 30 humpback, 6 gray and 19 killer whales. A total of 75 biopsy samples were collected from 12 blue, 45 fin, 4 sei, 12 humpback and 2 gray whales. A total of 229 sonobuoys were deployed, for a total of over 820 monitoring hours. The main species detected by the acoustic survey included fin, sperm, killer, blue, humpback, North Pacific right and sei whales. A total of 42 objects of marine debris were also observed.

INTRODUCTION

The International Whaling Commission-Pacific Ocean Whale and Ecosystem Research (IWC-POWER) program is an international research effort in the North Pacific coordinated by the IWC and designed by the IWC Scientific Committee (SC) in special partnership with the Government of Japan. Scientists from the Institute of Cetacean Research (ICR) participate regularly in the IWC-POWER program, both in designing and implementing the surveys. The IWC-POWER in the North Pacific follows the series of IWC International Decade for Cetacean Research/Southern Ocean Whale and Ecosystem Research (IDCR/SOWER) surveys that had been conducted in the Antarctic since 1978.

The long-term objective of the IWC-POWER is to ʻprovide information to allow determination of the status of populations (and thus stock structure is inherently important) of large whales that are found in the North Pacific waters and provide the necessary scientific background for appropriate conservation and management actionsʼ. The first survey of this program was conducted in 2010 and subsequent surveys have taken place on an annual basis.

The IWC SC is close to completing the first phase of the IWC-POWER, which is related to the short-term priorities, and now is preparing for the second phase related to medium term priorities, based on the results of the first phase.

The objectives of this document are: i) to present an outline of the IWC-POWER program including its objectives, research area, and general methodology; and ii) to present the results of the 2019 IWC-POWER survey based on Matsuoka *et al*. (2020).

OUTLINE OF THE IWC-POWER

Objectives

The IWC-POWER has the following long-term objective:

'Provide information to allow determination of the status of populations (and thus stock structure is inherently important) of large whales that are found in North Pacific waters and provide the necessary scientific background for appropriate conservation and management actions. The programme will primarily contribute information on abundance and trends in abundance of populations of large whales and try to identify the causes of any trends should these occur. The programme will learn from both the successes and weaknesses of past national and international programmes and cruises, including the IDCR/SOWER programme'. (IWC, 2011).

To attain this long-term objective, the IWC-POWER has utilized short and medium-term activities and priorities. The short-term priorities involved the definition and implementation of surveys in areas of the Central and Eastern North Pacific (east of 170°E) (see section on research area below). This region had not been surveyed since the time of commercial whaling in the 1970's (see Annex D of IWC, 2012). After reviewing current knowledge of the region, a list of medium-term priorities by species for the program was agreed to by the IWC SC (see Table 1 of IWC, 2013). This list was updated recently in view of the preparation of the second phase of IWC-POWER (see section 'WHAT NEXT?').

Research area for the short-term priorities

The research area was comprised between north of 20°N and south of the Bering Sea, and between 170°E and 135°W, including the Exclusive Economic Zone (EEZ) of the United States (U.S. EEZ) and Canada (Canadian EEZ). Figure 1 shows a map of the survey areas covered since 2010.

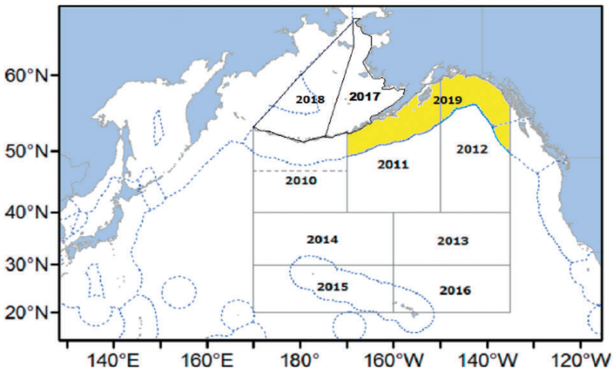

Figure 1. The research areas covered by IWC-POWER in 2010–2019 (EEZs are shown by blue dotted line). The yellow indicates the most recently surveyed area.

Survey season and research vessels

To collect consistent seasonal sighting data, the annual surveys were conducted in the middle of summer, during July to September (e.g., Matsuoka *et al.*, 2011). The following research vessels were engaged in the annual survey:

Kaiko Maru (860.25 GT) in 2010;
Yushin Maru No. 3 (742 GT) from 2011–2016; and
Yushin Maru No. 2 (747 GT) from 2017–2019.

These vessels were prepared with specialized equipment and personnel for visual survey of cetaceans.

Sighting survey procedure

Survey time

Research effort began 60 minutes after sunrise and ended 60 minutes before sunset, with a maximum of 12-hour research day (maximum 06:00–19:00, including 30 minutes for meal time for lunch and supper, when surveying in IO mode; see below).

Number of primary observers

Sighting effort was conducted by the boatswain and topmen from the TOP barrel and by two primary observers (the helmsman and captain) and at least four secondary observers (officer-on-watch, three researchers, and the chief engineer) from the upper bridge. Sighting activities aboard the ship were classified into two principal types: On-effort and Off-effort. On-effort activities were times when full search effort was executed and conditions (such as weather and sea state) were within acceptable parameters to conduct research. Off-effort activities were all activities that were not On-effort when no primary observers were in the TOP barrel (e.g., during drifting, Top Down (TD) or transiting along the trackline due to bad weather conditions). All sightings recorded during On-effort were classified as Primary sightings. All other sightings were considered as Secondary sightings.

Survey modes

Passing with abeam closing mode (NSP): Two topmen were on effort from the TOP barrel at all times. There was open communication between the upper bridge and the barrel. The observers on the upper bridge communicated with the topmen only to clarify sighting information. The upper bridge observers did not distract the topmen from their normal search procedure unless they were directed to do so by the cruise leader (IWC, 2020a).

Independent Observer Mode (IO): This is also in effect Passing Mode. Two topmen were observing from the TOP barrel and two from the IO barrel at all times. Communications were essentially one-way, with topmen from the TOP and IO platforms reporting information to the upper bridge in isolation from each other to ensure that no sighting information was exchanged between the TOP and IO barrel observers. The observers on the upper bridge would communicate with the topmen only to clarify sighting information and would not direct the topmen to disrupt their normal search procedure unless directed to do so by the cruise leader. Immediately after a sighting was detected from the barrel, the topman relayed information to observers on the upper bridge. After the sighting information was relayed to the upper bridge observers, the topman responsible for the sighting continued his normal searching pattern. Observers on the upper bridge located the sighting made by the topman and decided whether it would be possible to confirm species and conduct a school size count before the sighted whale(s) passed abeam of the vessel. The topmen gave no further information to the upper bridge unless the whale school resurfaced within their normal searching area. A designated researcher on the upper bridge recorded the species and estimated number of whales in the school when the sighting passed abeam of the vessel. This was done in consultation with other upper bridge observers/researchers (Anon, 2019a).

Approach to the whale schools

During both NSP or IO modes, and at the abeam time, the vessel altered the course to approach the whale(s), and the speed was increased to 15 knots to hasten the closure. Vessel speed was decreased when the school was close, usually within 0.2 to 0.4 n.miles from the initial sighting position. After the school was approached, the species, number of animals in the school, estimated body length(s), number of calves present, and behaviour were determined and recorded. Following this, other activities would normally be conducted (time allowing and at the discretion of the cruise leader), such as photography for natural marking (Photo-ID) studies and biopsy sampling. All sightings detected during these activities were classified as secondary sightings (Anon, 2019a).

Species identification

Species identification was based on agreed guidelines (IWC, 2020a). Positive identification of species was based on multiple cues and usually required clear observation of the whale′s body. Occasionally, repeated observations of the shape of the blow, surfacing and other behavioural patterns were sufficient to identify whales. This judgement was made only by the cruise leader or other designated researcher. Identification of species was recorded as ′probable′ where multiple cues were insufficient to be absolutely confident of identification (recorded as ′like′). This usually occurred when blows and surfacing patterns could be confirmed, but the whale′s body could not be clearly seen. Details of recording procedures during sightings can be found in Anon (2019a).

School size determination

Schools where the number of animals, or an accurate estimated range of the number of animals was determined, were classified as confirmed schools. Data from the confirmed schools can be used to determine a mean school size. Normally, schools believed to be confirmed for school size were approached to within 1 n.mile for large whales and to within 0.3 n.miles for common minke whales. Allowing for context-specific differences (i.e., environmental conditions and animal behaviour), every effort was made to be consistent with regard to the maximum time spent on identification of species and confirmation of numbers. Normally, if the sighting was thought to be common minke whales, no more than 20 minutes (after closure has been completed) was spent on confirmation. This reduces the potential for confusion with other whale sightings in the vicinity (IWC, 2020a).

Estimated Distance and angle experiments

This experiment has been conducted every year since 2010. The experiment is designed to calibrate and identify any biases in individual observers′ estimation of angle and distance. The experiment should be conducted during weather and sea conditions representative of the conditions encountered during the survey. In 2015, a major improvement was made increasing the number of target buoys to improve accuracy of the observer′s estimates (IWC, 2020b).

Photo-ID data collection

This experiment has been conducted since 2010. As appropriate and decided by the cruise leader, research time is allocated for photo-identification and/or video recording of large whales. The priority species were North Pacific right (highest priority), gray, blue and sei whales. Medium-priority species included fin, Bryde′s, common minke, sperm and killer whales. Generally, large whales were approached within 15–20 m. Adults, juveniles, and females accompanied by calves were approached for photo-identification. Photo-ID experiments involved a minimum of one photographer and a maximum of three on the bow, with additional photographers in the TOP barrel and IO barrel or upper bridge (Anon, 2019a).

Biopsy sampling

This experiment has been conducted since 2010 to analyze stock structure and movement for each species. The priority species for biopsy sampling were the same as for photo-identification (see above). The Larsen sampling system was used to collect samples within approximately 5 to 30 m of the bow of the vessel (Larsen, 1998).

Acoustic monitoring

Passive acoustics monitoring using sonobuoys were introduced to detect North Pacific right whales′ calls in the Bering Sea and a part of the Gulf of Alaska, and was conducted successfully from 2017 to 2019. A sonobuoy is a free-floating, expendable, short-term passive acoustic listening device that transmits signals in real time via VHF radio waves to a receiver on a vessel. Sonobuoys were deployed approximately every 2–2.5 hours, or approximately every 20–25 n.miles, from 06:00 to 18:00 (ship time) to obtain an evenly-sampled cross-survey census of marine mammal vocalizations. Sonobuoys were monitored in real-time by the acoustician, and presence of species-specific call types was noted (Crance *et al.*, 2017).

Marine debris observation

This observation has been conducted since 2010. Data on floating marine debris were observed and recorded. Observation of marine debris was limited to the first 15 minutes of each hour, as time permitted (so as not to interfere with marine mammal observations). In addition, marine debris were photographed if items were particularly large and/or could potentially lead to large whale entanglements. For all recorded marine debris items, observers recorded angle, distance and time of initial sighting, IWC code and a description. Photographs of items were archived and are available to interested scientists.

Data recording and format

Research data collected during the survey (weather, effort, sighting and distance experiment data) were entered by researchers using the ′onboard data collecting system′ developed by ICR (ICR, 2013). An English language version is now available.

RESULTS OF THE 2019 IWC-POWER SURVEY

In this section, the main results of the 2019 IWC POWER survey are summarized, based on Matsuoka *et al.* (2020).

Itinerary

The survey was conducted between 3 July and 25 September, 2019 in the Gulf of Alaska, within the U.S. Exclusive Economic Zone. The itinerary is shown in Table 1.

Table 1
The 2019 survey itinerary.

Date	Event
5-Jul	Pre-cruise meeting at Shiogama, Japan
6-Jul	Vessel departed Shiogama
6-Jul	Started transit survey to Dutch Harbor (D.H.)
11-Jul	Vessel enter U.S. EEZ (14:53, 48°08.3’N, 177°53.3’E)
11-Jul	Passed the dateline at 48-57.0N (23:03)
14-Jul	Vessel arrived at D.H., Alaska, USA
16-Jul	Pre-cruise meeting at D.H.
17-Jul	Vessel left D.H. and started transit survey to the start point of Western stratum
18-Jul	Vessel started surveying the Western stratum
12-Aug	Vessel finished surveying the Western stratum and moved to the starting point of the Eastern stratum
14-Aug	Vessel started the survey of Eastern stratum
8-Sep	Vessel completed the Eastern stratum and started transit survey back to Kodiak (via NPRW Critical Habitat)
12-Sep	Vessel completed transit survey
14-Sep	Vessel arrived Kodiak, Alaska, USA
16-Sep	Post-cruise meeting
16-Sep	Vessel left Kodiak and started the transit survey to Shiogama
26-Sep	Vessel completed the transit survey
28-Sep	Vessel arrived Shiogama, Japan

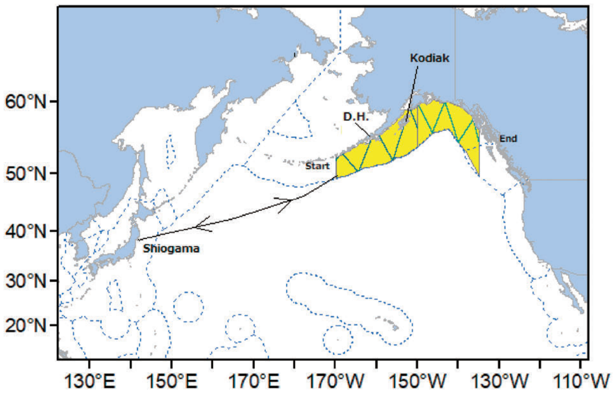

Figure 2. Research area, transit and survey track lines with start and end points for the 2019 IWC-POWER cruise. D.H.: Dutch Harbor.

Figure 3. Photography of the R/V *Yushin Maru* No. 2.

Table 2
Specifications of the R/V *Yushin Maru* No. 2.

Call sign	JPPV
Length overall [m]	69.61
Molded breadth [m]	11.5
Gross tonnage (GT)	747
Barrel height [m]	19.5
IO barrel height [m]	13.5
Upper bridge height [m]	11.5
Bow height [m]	6.5
Engine power [PS/kW]	5303/3900

Research area

The research area was the northern Gulf of Alaska, between 170°W and 135°W, and comprised the U.S. EEZ (Figure 2).

Research vessel and scientific personnel

The R/V *Yushin Maru* No. 2 was used for this survey. The vessel is shown in Figure 3 and its specifications in Table 2.

Four international researchers were nominated by the IWC SC for this survey:

Koji Matsuoka (Japan)–Cruise Leader/Chief Scientist, sighting, photo-ID;
Jessica Crance (USA)–acoustics, photo-ID;
James Gilpatrik (USA)–sighting, photo-ID data management, marine debris;
Isamu Yoshimura (Japan)–sighting data, marine debris and biopsy sample managements.

Searching effort

A total of 1,030.7 n.miles (NSP: 534.0 n.miles, IO: 496.7 n.miles) and 1,088.1 n.miles (NSP: 557.4 n.miles, IO: 530.7 n.miles) were surveyed in the western and eastern strata, respectively. A total of 420.0 n.miles was surveyed during transit surveys (e.g., transit from Dutch Harbor to the most western point and transit between the end and start points around Kodiak Island). The effort (time and distance) spent on sighting and several experiments are shown in Table 3.

Sighting summary

In the research area, blue (17 schools/19 individuals), fin (266/458), sei (9/20), common minke (5/5), humpback (173/402), gray (6/15), sperm (20/20), Baird's beaked (2/37), Cuvier's beaked (3/5) and killer (53/264) whales were sighted. Several dolphin species were also sighted (Table 4).

Gray whale (*Eschrichtius robustus*)

A total of 6 schools (15 individuals) were sighted in the Western stratum, south of Kodiak Island (5 to 10 n.miles off Dangerous Cape, south of Ugak Island, water depth between 60 m and 80 m) (Figure 4a, b). Several gray whales were feeding (mud trails were seen). No mother and calf pairs were seen. Sea temperatures ranged from 12.9°C to 15.7°C.

Blue whale (*Balaenoptera musculus*)

Blue whales were widely distributed in the offshore part of the Western and Eastern strata near EEZ border (Figure 5a, b). A total of 17 schools (19 individuals, including two mother and calf pairs) were sighted (Table 4). All blue whales were sighted in deep water of depths over 1,000 m. Sea surface temperatures of the sighting positions were between 10.9°C and 15.6°C.

Fin whale (*Balaenoptera physalus*)

Fin whales were the most frequently encountered baleen whale species in the research area, and they were widely distributed throughout both the Western and Eastern strata. Several high-density fin whale areas were observed, around Kodiak Island as well as the eastern part of the Eastern stratum (Figure 6). A total of 266

Table 3
Summary of the searching effort (time and distance) and experimental time (hours) by each survey with the area code conducted during 2019 survey. Dutch Harbor (D.H.), High Sea (H.S.), Research Area (R.A.).

Area	Area Code	Leg No.	Start	End	NSP		IO		NSP+IO		Photo-ID, Biopsy	Estimated angle and distance training/ experiment
		Start End	Date Time	Date Time	Time	Dist. (n.m.)	Time	Dist. (n.m.)	Time	Dist. (n.m.)	Time	Time
Shiogama to D.H.	1 H.S.	1 —	6 Jul. 6:00	11 Jul. 14:53	0:00:00	0	26:35:14	322.32	26:35:14	322.32	0:00:00	0:00:00
Shiogama to D.H.	11 U.S. EEZ	1 —	11 Jul. 14:53	13 Jul. 18:00	0:00:00	0	0:00:00	0	0:00:00	0	0:00:00	0:00:00
D.H. to R.A	78 U.S. EEZ	841 —	18-Jul. 7:20	18-Jul. 12:00	0:00:00	0	0:00:00	0	0:00:00	0	0:00:00	2:43:00
Western stratum	76 U.S. EEZ	101 114	18-Jul. 12:00	26-Jul. 13:13	20:02:43	228.61	19:35:18	224.71	39:38:01	453.32	10:08:11	0:00:00
Detour Shumagin Island	80 U.S. EEZ	844 —	26-Jul. 13:13	27-Jul. 8:13	1:19:22	15.3	0:00:00	0	1:19:22	15.3	0:00:00	0:00:00
Western stratum	76 U.S. EEZ	116 126	27-Jul. 8:13	8-Aug. 8:52	21:16:38	243.97	17:25:52	199.16	38:42:30	443.13	14:14:47	0:00:00
Detour Kodiak Island	80 U.S. EEZ	846 —	8-Aug. 8:52	8-Aug. 10:22	1:01:16	11.64	0:00:00	0	1:01:16	11.64	0:00:00	0:00:00
NPRW Critical Habitat	80 U.S. EEZ	847 —	8-Aug. 10:22	9-Aug. 10:38	9:24:46	106.28	0:00:00	0	9:24:46	106.28	0:36:39	0:00:00
Detour Kodiak Island	80 U.S. EEZ	846 —	9-Aug. 10:38	10 Aug. 8:59	7:02:32	80.2	0:00:00	0	7:02:32	80.2	0:33:50	0:00:00
Western stratum	76 U.S. EEZ	128 134	10 Aug. 8:59	12 Aug. 13:31	5:23:11	61.45	6:22:33	72.78	11:45:44	134.23	2:23:12	0:00:00
W. to E. Stratum	81 U.S. EEZ	849 —	12 Aug. 13:31	14 Aug. 7:42	8:57:11	100.98	0:00:00	0	8:57:11	100.98	1:45:04	0:00:00
Eastern stratum	77 U.S. EEZ	201 224	14 Aug. 7:42	8-Sep. 10:01	49:00:30	557.35	46:33:50	530.73	95:34:20	1,088.08	25:33:44	7:06:35
R.A to Kodiak	79 U.S. EEZ	854 —	8-Sep. 10:01	10-Sep. 13:22	0:00:00	0	0:00:00	0	0:00:00	0	0:00:00	0:00:00
NPRW Critical Habitat	79 U.S. EEZ	855 —	10-Sep. 13:22	12-Sep. 18:00	9:21:39	105.56	0:00:00	0	9:21:39	105.56	0:00:00	0:00:00
R.A to Kodiak	79 U.S. EEZ	854 —	12-Sep. 18:00	13-Sep. 18:00	0:00:00	0	0:00:00	0	0:00:00	0	0:00:00	0:00:00
Kodiak to Shiogama	12 U.S. EEZ	2 —	18-Sep. 7:55	22-Sep. 16:25	0:00:00	0	0:00:00	0	0:00:00	0	0:00:00	0:00:00
Kodiak to Shiogama	2 H.S.	2 —	23-Sep. 6:00	26-Sep. 12:00	0:00:00	0	9:23:32	115.03	9:23:32	115.03	0:00:00	0:00:00
Total			6-Jul. 6:00	26-Sep. 12:00	132:49:48	1,511.30	125:56:19	1,464.70	258:46:07	2,976.10	55:15:27	9:49:35

Table 4

Number of sightings for all species observed in the research area (Original trackline and transit tracklines) by effort mode. NSP: Normal Passing with abeam closing Mode; IO: Independent Observer Mode (IO), OE: Top down (TD) and drifting (DR). Numbers of Individuals includes the number of calves.

Species	NSP			IO			OE			Total		
	Sch.	Ind.	Calf	Sch.	Ind.	Calf	Sch.	Ind.	Calf	Sch.	Ind.	Calf
Blue whale	5	6	1	9	10	1	3	3	0	17	19	2
Fin whale	145	260	0	112	186	0	9	12	0	266	458	0
Like fin	6	11	0	13	17	0	1	2	0	20	30	0
Sei whale	3	4	0	6	16	0	0	0	0	9	20	0
Like sei	0	0	0	1	2	0	0	0	0	1	2	0
Common minke whale	3	3	0	2	2	0	0	0	0	5	5	0
Humpback whale	116	234	0	49	132	0	8	36	0	173	402	0
Like humpback	1	1	0	6	14	0	0	0	0	7	15	0
Gray whale	6	15	0	0	0	0	0	0	0	6	15	0
Sperm whale	8	8	0	12	12	0	0	0	0	20	20	0
Baird's beaked whale	2	37	2	0	0	0	0	0	0	2	37	2
Cuvier's beaked whale	1	1	0	2	4	0	0	0	0	3	5	0
Ziphiidae	2	4	0	3	3	0	0	0	0	5	7	0
Killer whale	36	156	9	17	108	5	0	0	0	53	264	14
Pacific white-sided dolphin	0	0	0	0	0	0	1	4	0	1	4	0
Habour porpoise	1	1	0	0	0	0	0	0	0	1	1	0
Dalli type Dall's porpoise	50	277	1	34	196	5	2	32	4	86	505	10
Unid. type Dall's porpoise	3	13	0	1	4	0	0	0	0	4	17	0
Unid. small cetacean	1	3	0	0	0	0	0	0	0	1	3	0

schools (458 individuals) of fin whales were observed in the research area. No mother and calf pairs were observed. A total of 20 schools and 30 individuals of "Like fin" were recorded; these looked like fin whale blows but could not be confirmed (Table 4). Most fin whales were sighted in deep water of depths over 1,000 m (Figure 6). Sea temperatures ranged from 9.9°C to 16.7°C.

Sei whale (*Balaenoptera borealis*)

A total of 9 schools (20 individuals) of sei whales were observed (Table 4). Observed mean school size was 2.22. Although sei whales were the third most frequently encountered baleen whale species through the surveys, almost all sei whale sightings were in the southern part of the Western stratum. There was a notable lack of sei whales in the Eastern stratum (Figure 7). This is similar to results seen during a previous POWER survey in 2012, where no sei whales were sighted in the U.S. EEZ (Eastern Stratum of current survey). Sea surface temperatures ranged from 11.2°C to 13.0°C, which was lower than the range for fin whales.

Common minke whale (*Balaenoptera acutorostrata*)

Common minke whales were the rarest baleen whale species in the research area, and were only sighted very close to shore (Figure 8). A total of 5 schools (5 individuals, all solitary schools) were observed (Table 4). Sea temperature at sighting locations ranged from 10.8°C to 16.9°C. Because of the difficulty in seeing their blow and small body, observations of this species were more difficult than in Antarctic waters. Common minke whale blows are very small and are difficult to spot in rough sea surface conditions (e.g., wind speed over 12 knots). During this survey, sea states averaged 4–5 on the Beaufort scale, which is assumed to be too rough for sighting common minke whales.

Humpback whale (*Megaptera novaeangliae*)

Humpback whales were the second most frequently encountered baleen whale species in the research area, primarily in the Western stratum. High-density areas of this species were observed in waters north-east and south-east of Kodiak Island, and along the most eastern trackline along the continental shelf (near 135°W). There was a lack of sightings between 150°W and 138°W in the Eastern stratum (Figure 9). A total of 173 schools (402 individuals) were observed in the research area. There were no mother and calf pairs. In some cases, red colored fecal matter was observed indicating that the whales were feeding on krill. Sea temperatures ranged from 10.0°C to 15.7°C.

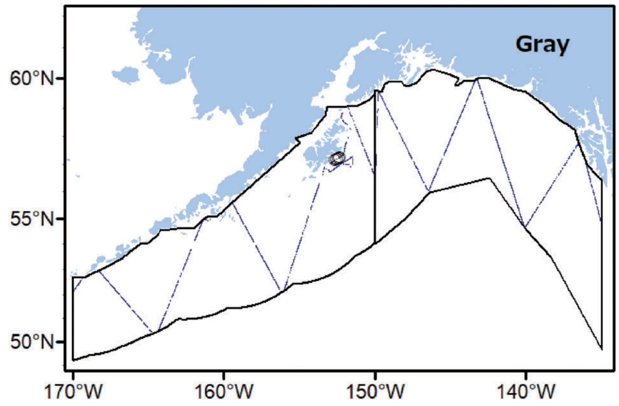

Figure 4a. The searching effort (thin line) and sighting positions (white circles) of gray whales during the 2019 IWC-POWER cruise.

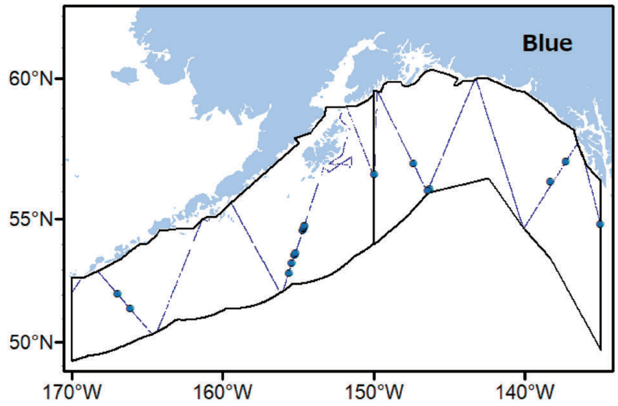

Figure 5a. The searching effort (thin line) and sighting positions (blue circles) of blue whales during the 2019 IWC-POWER cruise.

Figure 4b. Three gray whales observed during the 2019 IWC-POWER survey.

Figure 5b. Two blue whales observed during the 2019 IWC POWER survey.

Sperm whale (*Physeter macrocephalus*)

Sperm whales were widely distributed throughout the Western and Eastern strata where the water depth was over 1,000 m (Figure 10). A total of 20 schools (20 individuals) were sighted (Table 4). All schools observed were solitary individuals (probably large male). Sperm whales were recorded in waters with SST ranging from 10.9°C to 16.7°C.

Baird's beaked whale (*Berardius bairdii*)

A total of 2 schools (37 individuals) were sighted (Table 4). Both Baird's beaked whale schools were sighted along the most eastern trackline in the Eastern stratum along the continental slope off Prince of Wales Island (water depths over 1,000 m). Sea temperatures ranged from 13.9°C to 14.4°C.

Killer whale (*Orcinus Orca*)

Killer whales were mainly sighted in the Western stratum (Figure 11). A total of 50 schools (248 individuals, including 13 calves) were sighted (Table 4). They were sighted in waters with SST ranging from 10.9°C to 16.2°C.

Identification of duplicated sightings

A total of 154 resightings (duplicates) was made during IO Mode. Of these, for fin whales, there were 60 'Definite duplicates,' 1 'probable duplicate' and 13 'Not duplicates.' For sei whales, there were 12 'Definite duplicates' and 4 'Not duplicates.' For humpback whales, there were 34 'Definite duplicates' and 8 'Not duplicates.'

Photo-ID photographs

Photo-identified species included: blue (15 schools/16 individuals), fin (64/51), humpback (25/30), gray (2/6) and killer (8/19) whales (Table 5). Baird's beaked whales (20 individuals) and Dall's (24) porpoises were also photographed. Images collected during the cruise were uploaded to the IWC/ICR master photographic database in Adobe Lightroom (LR) (Anon, 2019b). Preliminary coding was completed for all cetacean images (7,762), including the allocation of species name, sighting number, school size and biopsy effort.

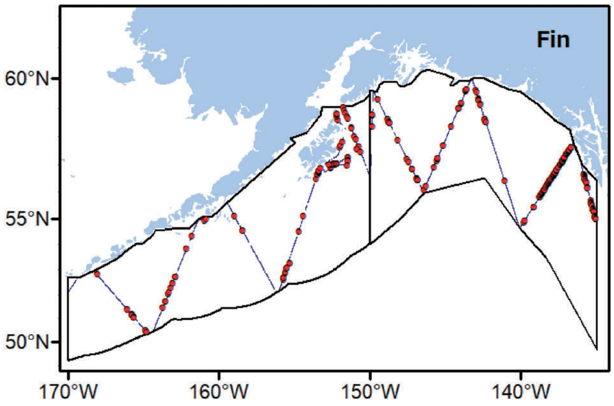

Figure 6. The searching effort (thin line) and sighting positions (red circles) of fin whales during the 2019 IWC-POWER cruise.

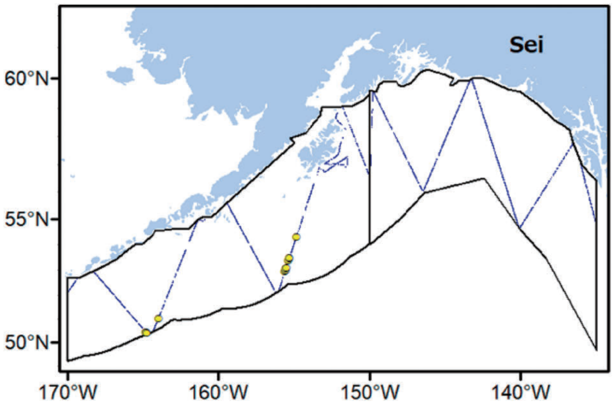

Figure 7. The searching effort (thin line) and sighting positions (yellow circles) of sei whales during the 2019 IWC-POWER cruise.

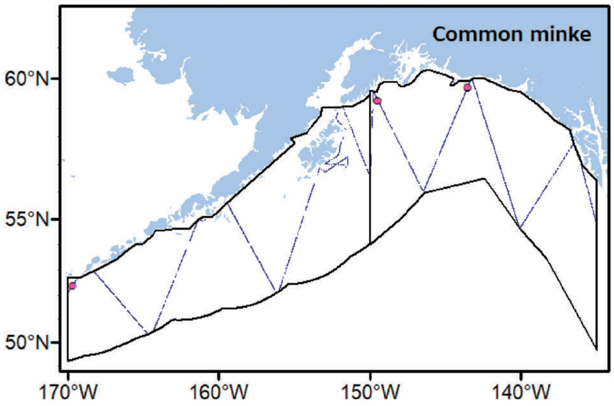

Figure 8. The searching effort (thin line) and sighting positions (pink circles) of common minke whales during the 2019 IWC-POWER cruise.

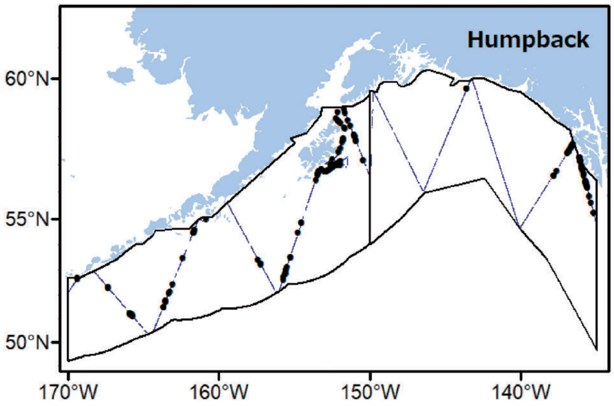

Figure 9. The searching effort (thin line) and sighting positions (black circles) of humpback whales during the 2019 IWC-POWER cruise.

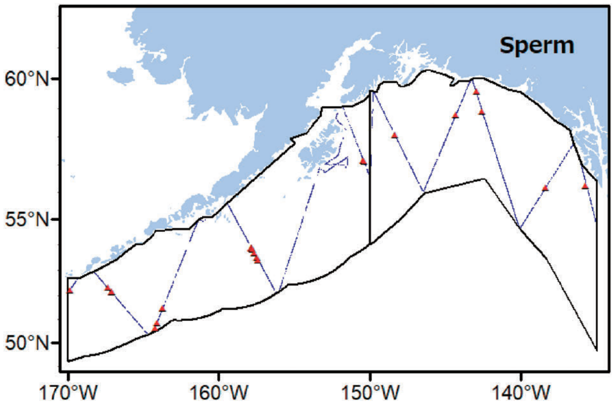

Figure 10. The searching effort (thin line) and sighting positions (red triangles) of sperm whales during the 2019 IWC-POWER cruise.

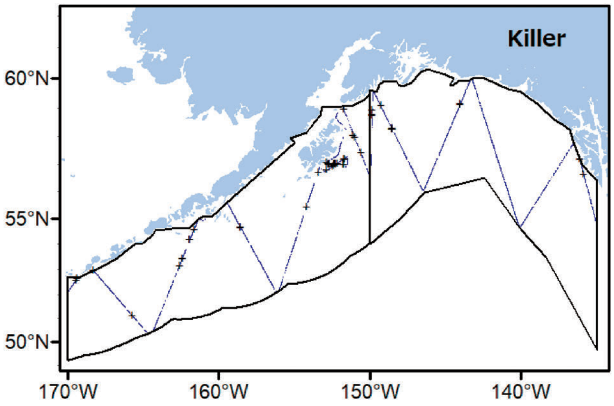

Figure 11. The searching effort (thin line) and sighting positions (black cross) of killer whales during the 2019 IWC-POWER cruise.

Biopsy sampling

Biopsy samples were collected from 75 individual whales: 12 blue, 45 fin, 4 sei, 12 humpback, 2 gray whales (Table 6). Every biopsy encounter was documented photographically. All biopsy samples were catalogued and stored in cryo-vials frozen at a temperature of −30°C on the vessel. These samples will be used for molecular genetics analyses. Figure 12 shows an event of biopsy sampling.

Table 5

Summary of the number of Photo-ID'd individuals by each species.

	Blue	Fin	Humpback	Gray	Killer	Total
Western Stratum	9	16	20	6	5	56
Eastern Stratum	7	35	10	0	14	66
Sub-total (US-EEZ)	**16**	**51**	**30**	**6**	**19**	**122**
Sub-total (High Sea)	**0**	**0**	**0**	**0**	**0**	**0**
Total	**16**	**51**	**30**	**6**	**19**	**122**

Table 6

Summary of the number of biopsy samples collected by each species.

	Blue*	Fin	Sei	Humpback	Gray	Total
Western Stratum	6	12	4	10	2	34
Eastern Stratum	6	33	0	2	0	41
Sub-total (U.S. EEZ)	**12**	**45**	**4**	**12**	**2**	**75**
Sub-total (High Sea)	**0**	**0**	**0**	**0**	**0**	**0**
Total	**12**	**45**	**4**	**12**	**2**	**75**

Blue* Including one mother and calf pair.

Figure 12. Biopsy sampling for sei whale during the IWC-POWER survey.

Acoustic monitoring

A total of 229 sonobuoys were deployed for a total of over 820 monitoring hours. Species detected included fin (119 buoys, 56.1% detection), sperm (112, 52.8%), killer (76, 35.8%), blue (54, 25.5%), humpback (47, 22.2%), North Pacific right (10, 4.7%), and sei (4, 1.9%) whales. Other species/events detected included Baird's beaked whales (3, 1.4%), Pacific white-sided dolphins (3, 1.4%), earthquakes (33, 15.6%), and unknown calls/signals (12, 5.7%).

Marine debris observation

A total of 42 marine debris objects were observed including 9 single fishing floats. A total of 41 items were recorded 'on effort' (i.e., during the first 15 minutes of each hour) and 1 item was recorded during 'off effort.'

WHAT NEXT?

The following tasks are identified for the immediate future of IWC-POWER.

Completion of the short-term priorities

To complete the short-term priorities, the IWC SC reiterated the importance of completing the Bering Sea survey areas. However, given the difficulties previously experienced, it was important to consider a backup plan for the 2020 cruise. If the Russian area cannot be covered in 2020, then every effort should be made to cover this in 2021, given its importance to meeting the objectives of the IWC-POWER program (IWC, 2020c). Also, several analytical tasks based on data collected in the first phase under the short-term priorities were recommended.

Design of the next phase

The IWC SC agreed on revised priorities for the medium-term (Table 7). To develop a detailed plan for the second phase of IWC-POWER, it is important to complete the analyses of the data collected during the first phase (short-term).

The IWC SC will hold a pre-meeting just before the 2021 Scientific Committee Meeting in order to develop a detailed proposal for a workshop to design the next

Table 7

Suggestions for updated medium-term priorities based upon results from Phase 1 for IWC-POWER (*refers to likelihood of obtaining an abundance estimate at least in some areas **refers to likelihood of obtaining biopsy and/or photo-ID data from encountered schools) (IWC, 2020c).

Initial priority/feasibility	Rationale/comments
Blue whale (High) Medium direct*, high opportunistic**	•Depletion level (i.e. highly depleted based on catch history) •Initial abundance estimates from IWC-POWER (still being finalised) suggest it remains heavily depleted. •Results of analyses of existing samples (27 IWC-POWER samples available) in conjunction with other samples (e.g. samples collected under Japanese national programmes) important in addressing population structure in context of idea/scope of occasional focussed cruises, especially samples from the west (see (4) below). •Potential for some blue whale focussed cruises in specific areas (including directional acoustics) should be considered (e.g. Gulf of Alaska) as well as continuing opportunistic studies. •Continued collaboration with existing photo-id work e.g. US and Japanese national programmes is important (42 individuals available from IWC-POWER)–and Japan west existing samples. •Consider telemetry studies
Fin whale (High) High direct*, high opportunistic**	•Depletion level (i.e. high based upon catch history) •Initial abundance estimates from IWC-POWER (still being finalised) suggest some recovery •Results of genetic analyses important to contribute to future survey strategy and future Comprehensive Assessment (e.g. is there evidence of more than one stock from the existing 124 biopsy samples that cover waters from 170°E to 135°W?). •Work in Russian Federation waters provided appropriate permits can be obtained is important •Co-ordination with national programmes in Japan, Korea and USA needed including existing samples
Right whale (High) Medium direct*, high opportunistic**	•Depletion level: (i.e. highly depleted based on catch history) •Still critically low numbers in east (from US studies and IWC-POWER) •Feasibility of collecting biopsy and photo-ID data high if targeted and using acoustics •Feasibility of obtaining abundance in east from line-transect low given such small numbers; may be much higher in west e.g. Sea of Okhotsk and southeast of Kamchatka Peninsula where population is at least 10X larger or more •Although new area, consideration should be given to a targeted survey in Sea of Okhotsk-high feasibility and priority to obtain good abundance, photo-id and biopsy data provided appropriate permits can be obtained from the Russian Federation.
Sei whale (Medium) High direct*, high opportunistic**	•Depletion level: (i.e. high based on catch history) •Initial abundance estimates from IWC-POWER (still being finalised) and Japan suggest some recovery •IWC-POWER has provided valuable information for the ongoing Comprehensive Assessment (the 2020 backup cruise will provide biopsy samples from a poorly covered area) •Results of that CA will help focus future IWC-POWER medium-term strategy and priority for this species-e.g. (a) possible focussed biopsy sampling in postulated coastal stock areas and (b) frequency and scope dedicated abundance surveys in ʻpelagicʼ area to examine trends
Humpback whale (Medium) High direct*, high opportunistic**	•Good information already available from SPLASH and national programmes suggests overall high abundance (genetic and photo-ID mark-recapture) hence medium priority •Continue to contribute to existing genetic and photo-ID databases. •Ongoing Comprehensive Assessment will assess status and potential depletion of [sub-] populations. •Abundance estimates from IWC-POWER (still being finalised) can provide interesting ʻsnapshotʼ estimates to compare with mark-recapture estimates by population/feeding aggregation •The results of the CA will assist in developing medium-term strategy and priority for this species within IWC-POWER
Sperm whale (Medium) Medium direct* and medium opportunistic**	•Depletion level: (unknown but possibly high given catch history) •Lack of good information on population structure and status at present although good distributional data from IWC-POWER •Obtaining abundance estimates from visual surveys can be problematic due to long dive times and other issues •Combined acoustic (towed array)/visual surveys have been successful for sperm whales however feasibility in the context of IWC-POWER depends on availability of equipment and practicality in light of other priorities •Possibility of using towed acoustic arrays in some years should be considered
Gray whale (Medium) Low direct*, high opportunistic**	•There are ASW hunts but that primary data sources to evaluate those are from other visual, genetic and photo-ID programmes (e.g. US, Mexico, Sakhalin Island)–hence medium priority •Main IWC-POWER contribution is in obtaining biopsy/photo-ID in areas outside those programmes for comparison and information on population structure •Sharing of data with other programmes should continue
Brydeʼs whale (Medium) High direct*, high opportunistic**	•Suggest low priority for first six or so years of next phase of POWER because: •Recently completed IR shows good population status and apparently low level of threats •Removing from target species allows a great reduction in size of priority research area to north of 40°N •If required, a targeted survey or surveys could be designed towards end of 10-year period (e.g. from 2027)
Common minke whale (Low) Suggest only opportunistic	•Depletion level (probably low east/central based upon catch history) and in west dealt with by national programmes •However, if Okhotsk Sea is able to be covered for high priority species (e.g. right whales) then would provide valuable information incl. biopsy samples •If permission granted by Russian Federation then consider modifying present ʻacceptableʼ conditions as at the present high range they are unsuitable for estimating abundance for this species

phase of the IWC-POWER program. The emphasis will be on participation from all range states and the availability of analyses/data. The workshop should include consideration of more methodologically focused cruises in designated years (e.g., use of towed acoustic arrays, telemetry work, use of SeaGlider, etc.).

The ICR scientists will participate actively in the discussions regarding the second phase of IWC-POWER. This research program has contributed and will continue to contribute important information relevant for the conservation and management of large whales in the North Pacific.

ACKNOWLEDGEMENTS

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Technical Reports of the Institute of Cetacean Research (2020) pp. 35–40

Technical Report (not peer reviewed)

Genetic tagging technique: basic concept and a case study by the Institute of Cetacean Research

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ABSTRACT

The studies on biological conservation require contemporary demographic estimates, e.g., migration rate, effective population size and stock structure. Although these estimates can be obtained through standard population genetics analyses, such studies could fail to infer them because of recent evolutionary and biogeographical events. Alternative approaches to obtain such parameters include individual identification and kinship inferences using genetic data, also called 'genetic tagging', which has become a common technique in the fields of ecology and conservation during the last several decades. This paper presents the basic concept of genetic individual identification and kinship inference. It subsequently outlines the parentage analysis for the western North Pacific common minke whale conducted by the Institute of Cetacean Research as a case study.

INTRODUCTION

The studies on biological conservation in animal species require information on contemporary population parameters, e.g., effective population size, migration rate and population structure. The estimates of these parameters can be obtained through traditional population genetics analyses. However, these analyses often fail to infer them in cases where the populations have diverged recently, or if changes in gene flow and effective population size have occurred. Therefore, such estimates have been mainly obtained by individual-based tagging research using conventional tagging or photo-identification (e.g., Calambokidis *et al.*, 2001; Johnson *et al.*, 2009; Mizroch and Rice, 2013; Urian *et al.*, 2015).

Individual animals can also be identified using genetic data, generally called 'genetic tagging' (Palsbøll, 1999). This has become a common technique to obtain the contemporary estimates in the ecological and conservation fields during the last several decades (e.g., humpback whales, *Megaptera novaeangliae*, Palsbøll *et al.*, 1997; North Pacific right whales, *Eubalaena japonica*, Wade *et al.*, 2011). In addition, in recent years, the extended genetic tagging incorporating information on kinship among individuals is being used to investigate contemporary population dynamics (e.g., Kanda *et al.*, 2014; Bravington *et al.*, 2016; Ohashi *et al.*, *in-press*). The methods for abundance estimates based on two types of genetic tags (individual identification and kinship) are summarized by Takahashi (this issue).

This paper presents the basic concept of genetic individual identification and kinship inference, with a brief summary of a case study by the Institute of Cetacean Research (ICR), which is the parentage analysis for the North Pacific common minke whale (*Balaenoptera acutorostrata*).

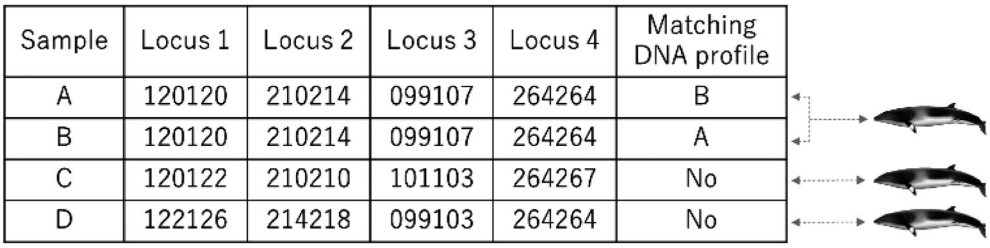
BASIC CONCEPT OF GENETIC TAGGING TECHNIQUES

Individual identification

Sexual reproduction guarantees that, in the main, each individual has a unique genotype. There are some exceptions, however, such as identical twins. This characteristic allows each individual to be tagged and, as a consequence, it can be determined if two genetic samples come from the same individual. Figure 1 shows an example of individual identification using microsatellite genotype data. In this example, the four samples are derived from three whales. Samples A and B have the same genotypes and therefore the two samples are assumed from the same whale, while samples C and D have different genotypes suggesting they are from different whales.

Probability of identity

The probability of identity (I) is the probability that two unrelated individuals in the population share the same genotype. This estimate requires information on allele frequencies in the population, because common alleles



Sample	Locus 1	Locus 2	Locus 3	Locus 4	Matching DNA profile
A	120120	210214	099107	264264	B
B	120120	210214	099107	264264	A
C	120122	210210	101103	264267	No
D	122126	214218	099103	264264	No

Figure 1. Example data involving four microsatellite loci genotyped for each of four whales. Genotypes are represented by 6-digit codes, and each allele is coded by 3 digits. The results of individual identification are shown in the column 'Matching DNA profile'.

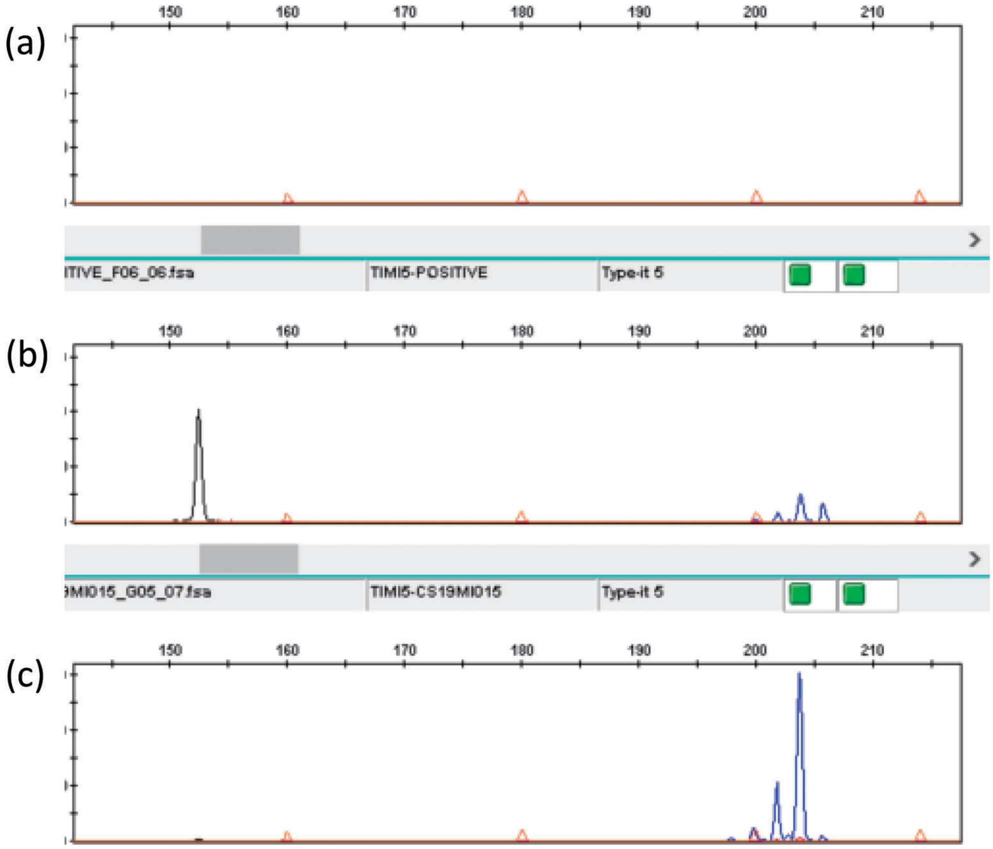


Figure 2. Example of sex determination in common minke whales through multiplex fluorescent PCR using the *SRY* locus (black) located on the Y chromosome and a microsatellite locus (blue) as the internal control: (a) failure to amplify PCR fragments; (b) male; (c) female.

are much more likely to be shared than rare ones. This can be estimated for a population with random mating according to the formula derived by Paetkau and Strobeck (1994):

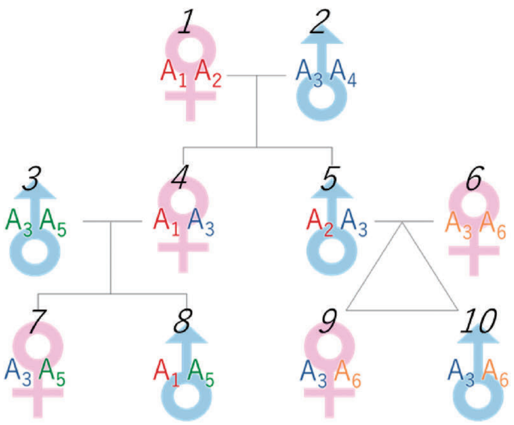
$$\hat{I}=\prod_{k}\left(\sum_{i}p_i^4+\sum_{i}\sum_{j>i}(2p_ip_j)^2\right)$$

where, p_i and p_j are the frequencies of the *i*th and *j*th alleles at the *k*th locus in the population. In practice, it is desirable to aim for a probability of shared genotype identity between any two individuals (I) of <0.05.

Molecular sex determination

Sex information is useful not only to assist in individual identification but also for a biological interpretation of the results of analyses.

The PCR-based technique has been proposed for sex determination using the presence or absence of genes on the sex chromosomes. The testis-determining *SRY* genes are male-specific in mammals and can be detected by PCR amplification using specific primers. The ICR genetic team developed the primer set that amplifies the *SRY* gene to determine the sex of cetaceans, using a microsatellite locus as the internal control (Abe *et al.*, 2001).



Kinship category	Pairs of individuals	Φ	κ_0	κ_1	κ_2
Identical / Monozygotic twins	9-10	0.50	0	0	1.00
Parent-offspring	1,2-4,5 / 3,4-7,8 / 5,6-9,10	0.25	0	1.00	0
Full siblings	4-5 / 7-8	0.25	0.25	0.50	0.25
Half siblings	1,2-7,8,9,10	0.125	0.50	0.50	0.00
First cousins	7,8-9,10	0.0625	0.75	0.25	0
Unrelated	3-6	0	1.00	0	0

Figure 3. Example of pedigree containing ten non-inbred individuals. The figure shows the kinship categories and the values for three IBD coefficients (κ_0, κ_1 and κ_2). The three IBD coefficients can be also summarized by the kinship coefficients (Φ) which is the probability that a random allele from one individual is IBD to a random allele from the other. The Φ is also equivalent to the inbreeding coefficient of their offspring.

Figure 2 shows an example of genetic sex determination using this method. In case of male, a PCR fragment of *SRY* gene is observed between 150 and 155bps (Figure 2b), while the fragment is absent in case of female or PCR failure. We can distinguish female (Figure 2c) from PCR failure (Figure 2a) by the presence of the PCR fragment of one microsatellite locus as an internal control.

Kinship inference

Individuals that are biologically related share genes that are identical-by-descent (IBD), i.e., identical copies of a gene segregating from a common ancestor within the defined pedigree. IBD is a fundamental concept that underlies kinship inference (Thompson, 2013). Here, the basic concept of the IBD coefficient is explained, which provides an introduction to the ideas of IBD. It is followed by the simple principle of parentage analysis which is a unique application of kinship inferences.

IBD coefficients

Two non-inbred diploid individuals can share none, one, or two alleles IBD. In case of the pedigree shown in Figure 3, full sibling individuals *4* and *5* share one allele IBD (A_3 which is inherited from their father) while unrelated individuals *3* and *6* share no allele IBD. The monozygotic twins *9* and *10* share two alleles IBD (A_3 and A_6, which are inherited from father and mother, respectively). The probabilities of each of the events are summarized by the IBD coefficients, i.e., κ_0, κ_1 and κ_2 (Cockerham, 1971), which are shown in Figure 3.

Table 1

Patterns of allele sharing IBD for a full sibling pair (individuals *4* and *5* in Figure 3).

Possible genotypes	A_1A_3	A_1A_4	A_2A_3	A_2A_4
A_1A_3	2	1	1	0
A_1A_4	1	2	0	1
A_2A_3	1	0	2	1
A_2A_4	0	1	1	2

Table 1 shows an example on how to determine the IBD coefficients for a category of full siblings. Consider that the genotypes of the parents at a locus are A_1A_2 and A_3A_4 (individuals *1* and *2* in Figure 3). Each offspring can have one of the four following genotypes: A_1A_3, A_1A_4, A_2A_3 or A_2A_4 (individuals *4* and *5* in Figure 3). Out of the sixteen ways to pair two offsprings, the dyad can share 2 alleles that are IBD in four ways, 1 allele in eight ways and 0 alleles in four ways. Thus, the IBD coefficients, κ_0, κ_1 and κ_2, for full siblings are 0.25 (4/16), 0.50 (8/16) and 0.25 (4/16), respectively. The IBD coefficients for other kinship categories can be calculated in the same manner.

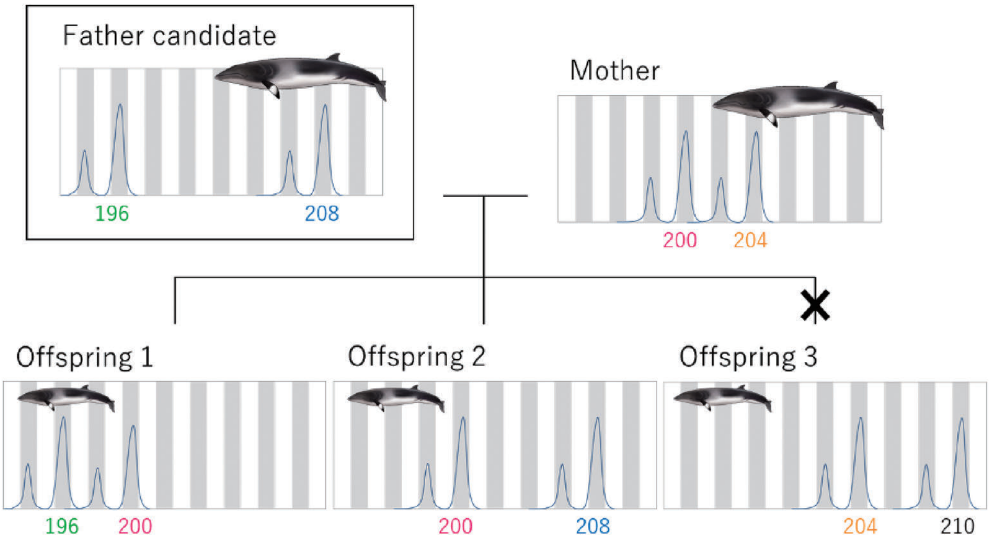


Figure 4. Principle of parentage analysis using the exclusion approach. The numbers under the peaks are the size of PCR fragment in base pairs which can be interpreted as alleles.

Parentage analyses

Parentage analysis is a unique application in which the most likely parents of a target offspring are sought amongst eligible candidates. The basic concepts of the parentage analysis are grouped into six categories according to Jones *et al.* (2010): (1) Exclusion, (2) Categorical Allocation, (3) Fractional Allocation, (4) Full Probability Parentage Analysis, (5) Parental Reconstruction, (6) Sibship Reconstruction. In this section, categories (1) (the conceptually simplest approach) and (2) (the most commonly used approach), are described.

Exclusion approach

This approach uses rules of Mendelian inheritance for diploid organisms wherein an offspring inherits one of two alleles at each locus from each of its parents. A simple exercise for the exclusion approach using a single microsatellite locus is shown in Figure 4.

In this exercise, the mother shows the genotype of 200/204, and the genotypes of her three offspring 1, 2 and 3 were 196/200, 200/208 and 204/210, respectively. Here, a male with genotype of 196/208 can be excluded as a father candidate for offspring 3 since no allele from the male is observed in offspring 3. On the other hand, the male is still a father candidate for offspring 1 because the alleles 196 and 200 can be found from the male and mother, respectively. The same is true for offspring 2.

This approach is powerful when there are few candidate parents and multiple highly polymorphic markers are available. In practice, most of the studies based on the exclusion approach actually require at least two mismatching loci between the candidate and the offspring to account for typing errors or mutations.

Categorical Allocation approach

As in the case of exclusion method, this approach requires at least one focal offspring and a set of candidate parents. The Categorical Allocation approach was developed to resolve situations in which complete exclusion may not be feasible (Meagher and Thompson, 1986). The main benefit of this approach is to choose the single most likely parent from a group of non-excluded putative parents. The logic stems from the observation that different genotypes of parent may differ in their probability of having produced the genotype of the focal offspring (Meagher and Thompson, 1986). This approach also has the advantage of handling genotyping errors or mutations.

Currently, most of the Categorical Allocation approaches use a likelihood approach (e.g., Marshall *et al.*, 1998). However, a Bayesian approach (e.g., Nielsen *et al.*, 2001) can also be used (see also Takahashi, this issue).

Genotyping errors and their effects

Microsatellite DNA marker is one of the most common genetic markers not only for individual identification and kinship analyses, but also for the standard analyses on population genetics. However, microsatellite DNA is known as the error-prone marker in its genotyping, which is recognized to have a serious impact on genetic individual identification as well as kinship inferences (reviewed in Hoffman and Amos, 2005).

The genotyping of a microsatellite allele depends on the microsatellite profile, and requires strict rules to be defined in advance. Typical microsatellite profiles are characterized by a succession of peaks with growing intensity due to stutter band. Researchers often suffer from several types of ambiguous microsatellite profiles in genotyping, e.g., null alleles, overlapping of stutter bands, split peak, large allele dropout or short allele dominance and false alleles. Some factors are known to be involved in the ambiguous profiles, e.g., variation in DNA sequences, low quality or quantity of template DNA, biochemical artifact and human error (Hoffman and Amos, 2005).

Figure 5 illustrates two types of genotyping error that are likely to induce false paternity and consequently bias the biological conclusions. Consider in the example of Figure 5 that the male is the real father of the offspring. The real genotype for male is 196/208 without error: the offspring inherited allele 200 from his mother and allele 208 from his father. In the cases with genotyping errors, the male would incorrectly be excluded as the father of the offspring because allele 208 is mistyped as allele 202.

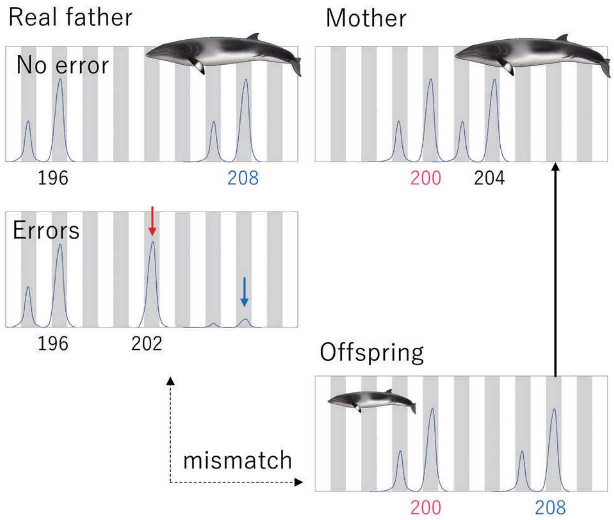


Figure 5. Simple example of parentage analysis by the Exclusion approach. Arrows show the genotyping errors due to false allele (red) and large allele dropout (blue).

In order to avoid this type of incorrect paternity exclusion, it is better to allow one or several genetic mismatches in the parentage analysis depending on the probability of identity and the calculated error rate.

A CASE STUDY ON PARENTAGE BY THE INSTITUTE OF CETACEAN RESEARCH

Parent-offspring inference in North Pacific common minke whales

The parent-offspring pair analysis was carried out based on a type of Categorical Allocation approach, using a total of 4,707 common minke whales in the western North Pacific (see Tiedemann *et al.* (2017) for the details of the analytical procedures). Each sample was genotyped at 16 microsatellite loci. The mitochondrial DNA control region haplotypes as well as biological information, e.g., sampling date and position, sex, sexual maturity and body length, were used, if available, to assist the interpretation of the results.

The parent-offspring analyses inferred a total of 40 and 13 parent-offspring pairs for the O and J stock (Goto *et al.*, 2017), respectively . The forty parent-offspring pairs of O stocks were widely distributed through the Pacific side of Japan (Figure 6). A total of 17 pairs of O stock animals were found between coastal and offshore waters. In most of the pairs, the offspring were found near the coast while their parents were in offshore waters. This is consistent with the pattern of migration with sexual segregation described previously for western North Pacific common minke whales (Hatanaka and Miyashita, 1997).

The thirteen parent-offspring pairs of J stocks were distributed within and between the Sea of Japan and the Pacific side of Japan (Figure 6). Four of them were found between the Sea of Japan side and the Pacific side. The

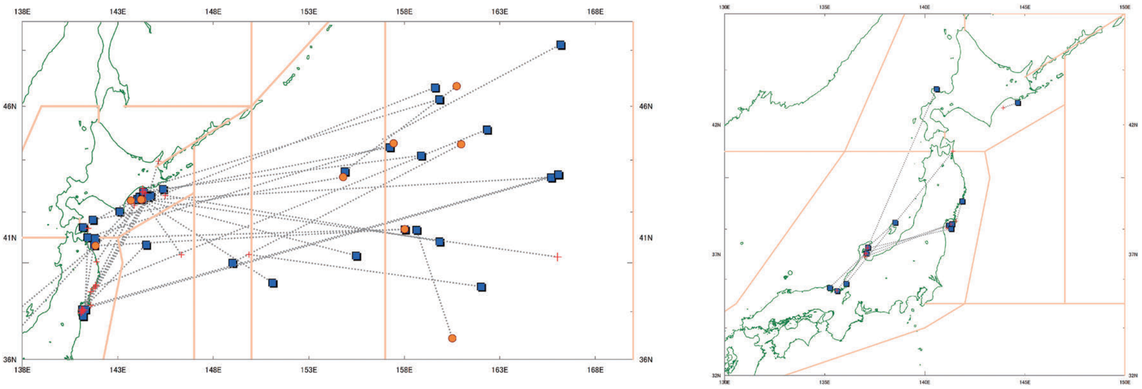


Figure 6. Distribution of parent-offspring pairs of O (left) and J (right) stocks in western North Pacific common minke whales. Blue square: parent; orange circle: mature offspring; red cross: immature or unknown maturity stage offspring.

results implied that the J stock individuals occurring in the Sea of Japan and the Pacific side of Japan were derived from the same stock.

Future work

The development of the new genetic marker which is less error-prone, that is, Single Nucleotide Polymorphisms (SNPs) marker is on-going at the ICR. The ICR genetic team will perform analyses using SNPs genotypes, not only for western North Pacific common minke whales, but also for other whale species such as Bryde′s, fin, right and blue whales. The results of these analyses will aid in obtaining the contemporary demographic estimates of these whale species using genetic data.

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Technical Report (not peer reviewed)

Use of genetic data for abundance estimate purposes: a brief review of methods and case studies by the Institute of Cetacean Research

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ABSTRACT

Sighting surveys by the line transect method is the most used approach to estimate abundance in whales. Sighting surveys have some limitations associated with bad weather and sea conditions, and with the difficulty to cover all areas of distribution of the stocks. Given these limitations, alternative methods are being investigated for estimating the abundance of whales. This paper presents an overview of the current genetic methods for abundance estimation in whales. These methods are based on the mark-recapture (MR) approach and can be divided into two groups: i) those based on the history of individual identification (genotyping based on biopsy samples), and ii) those based on kinship information including paternity analysis and close-kin MR (based on biopsy samples and/or catches). This paper also presents information on case studies conducted by the Institute of Cetacean Research.

INTRODUCTION

Abundance estimation is one of the most important sources of information required for the conservation and management of whales. The analysis of sighting data collected by the line transect (LT) method (Buckland *et al.*, 2001) is the most common approach for estimating the abundance of whales. However, the use of this method has some limitations. For example, bad weather and sea conditions can prevent the surveys, while some areas are inaccessible to the surveys (e.g., polynyas in the Antarctic or territorial waters of other countries).

The mark-recapture (MR) method (Hammond, 1986; Pollock *et al.*, 1990) based on Discovery-type tags is another method used for abundance estimation. However, this method depends on whaling operations for the recovery of the tags, and this has been limited in recent years. MR methods can be used for individual whales identified by natural marks (photo-identification), however not all species have distinctive marks for individual identification, and the method would not be practical for abundant populations.

Given these limitations, alternative methods for abundance estimation of whales are being explored. In recent years, several methods for abundance estimation based on genetic individual identification and kinship inference have been reported. This paper presents an overview of those methods and outlines some case studies conducted by the Institute of Cetacean Research (ICR).

BASIC CONCEPT OF MARK-RECAPTURE

The MR method is widely used in ecology, particularly to estimate population size and some biological parameters such as survival rates. The MR method relies on sampling and re-sampling individual animals. A capture history simply describes whether or not an animal was captured in a series of sampling occasions during a period of data collection. It is usually represented by a series of 1 (captured) and 0 (not captured). The key idea of the MR method is to determine the ratio of marked individuals to unmarked individuals in the target population, i.e., estimate p (capture probability) from the proportion of marked animals that are recaptured. Figure 1 shows the situation of marks and mark-recaptures in two sampling occasions (two-sample estimator).

In Figure 1, M is the number of individuals marked in a population in the first year; c is the total number captured in the second year (with and without a mark); R is the number of captured in the second year that are marked (recaptured); and N is the size of the population at the time of marking and release.

The simplest MR method is the 'Petersen method' also known as 'Lincoln method' (Seber, 1982):

$$\frac{R}{c} = \frac{M}{N}.$$

The proportion of marked animals in the second time R/c is an estimate of the probability of capturing individual ($\hat{p}$), so that:

$$\hat{N} = \frac{M}{\hat{p}}.$$

Chapman's modification of Petersen's two-sample estimator (Chapman, 1951) is more appropriate in situations of small sample sizes, and is expressed as follows:

$$N = \frac{(M+1)(c+1)}{R+1} - 1.$$

Here,

M=number of individuals captured in the first year;

c=number of individuals captured in the second year;

R=number of individuals captured in both years.

Multiple sampling occasions

In the case of the two-sample estimator in Figure 1, the assumption of 'closed population' is adopted (births, immigration, emigration and death are not considered). For multiple sampling occasions involving a long period of time, the assumption of 'open population' is more appropriate, and immigration, emigration, birth and death should be considered (Figure 2).

In the case of multiple sampling occasions, the Jolly-Seber (JS) model (Jolly, 1965; Seber, 1965), an open population model, is the most appropriate. This model is very flexible and, in addition, can provide estimates of survival, recruitment and population growth rates.

There are a number of formulations based on the JS for estimating abundance and related parameters, such as the 'POPAN,' Link-Barker and Pradel recruitment, and the Burnham JS and Pradel-λ formulations. In particular, the POPAN formulation (Schwarz and Arnason, 1996) can provide abundance and net births by using the full likelihood approach. Net birth refers to all animals that enter after sampling occasion *i* and survive until sampling occasion *i*+1.

ABUNDANCE ESTIMATES BASED ON GENETIC DATA

The main advantages of using genetic tags for individual identification and abundance estimates based on MR are that: i) they are permanent and ii) they exist in all individuals. It is important that the genetic approach for individual identification and kinship inferences are reliable. The genetic basis for individual identification and kinship inferences is shown in Taguchi (this issue).

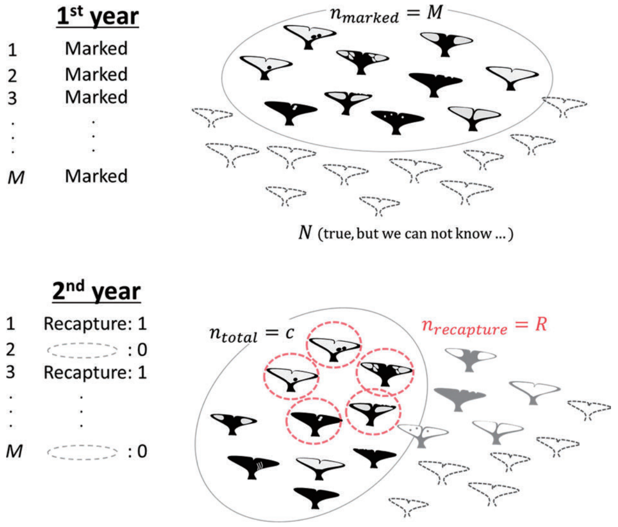

Figure 1. Diagram showing the basic idea of the MR method based on individual identification of humpback whales by photographic matching.

Methods based on the history of individual identification

The most common approach for genetic individual identification is based on biopsy sampling and genotyping, based on a number of nuclear marker loci. One of the most used markers is microsatellite DNA. If two biopsy samples, taken in different occasions/locations present the same genotype, then it is assumed that those biopsies were taken from the same individual (see Taguchi, this issue).

Some studies are presented here as examples of abundance estimates in whale populations based on biopsies, genetic individual identification and the MR methods. Palsbøll *et al.* (1997) conducted an analysis of six microsatellite loci for 3,060 skin samples collected from humpback whales throughout the North Atlantic. They detected 2,368 unique genotypes and, because the samples with identical genotypes were of the same sex, they considered that the 3,060 samples represented 2,368 individual whales. There was a total of 692 'recaptures.' The abundance and its confidence intervals (CI) were estimated based on the data collected in 1992 and 1993 and the two-sample estimators. The abundance was estimated at 4,894 (95% CI: 3,374–7,123) for males and 2,804 (95% CI: 1,776–4,463) for females.

Based on 354 biopsy samples collected from the New Zealand subantarctic southern right whales during the austral winter from 1995 to 1998, Carroll *et al.* (2011) were able to amplify 302 samples with 9–13 microsatellite loci. They identified 235 unique individuals during these four winter surveys. They estimated the superpopulation abundance using the POPAN JS model,

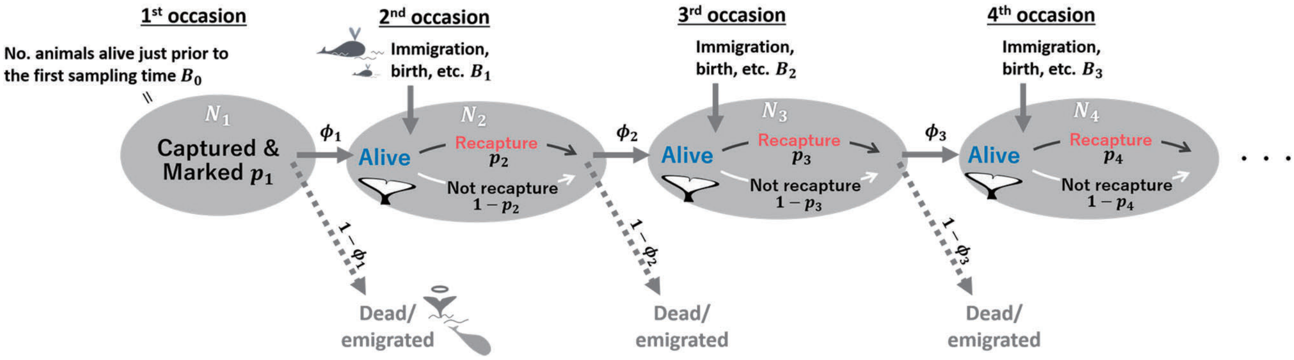

Figure 2. Diagram showing the situation of multiple sampling under the assumption of open population. A population changes in number and density due to births, deaths, immigration and emigration. Note that, φ_i represent the probability of (apparent) survival until the next sampling occasion; p_i is the probability of being captured and re-captured; B_i is the net number of new individuals joining the study area (modified from http://www.phidot.org/software/mark/docs/book/pdf/chap12.pdf).

which provided an estimate of 910 non-calf whales (95% CI: 641–1,354) in 1998.

Methods based on kinship information

An additional advantage of DNA tags is that they contain information about kinship among individuals. Hence, kinship relationship can be used for abundance estimates based on the classic MR using 'recapture of self' to 'recapture of closely-related kin'. Several studies used DNA profiles to detect instances of paternity or other kinship relationships in whale populations (see Taguchi, this issue). For this approach, samples obtained from biopsy sampling as well as from catches can be used.

Paternity analysis

For the aim of paternity inference, Marshall *et al.* (1998) derived likelihood ratios with codominant markers taking account of typing error, and defined a statistic Δ for resolving paternity. The likelihood ratio can be written as:

$$L(H_1,H_2|D)=\frac{P(D|H_1)}{P(D|H_2)},$$

In this formulation, $P(D|H_i)$ is the probability of obtaining data D, i.e., genotypes of offspring, mother and potential father at a particular locus, under hypothesis H_i (i=1, 2) . The hypothesis of $H_1$is that the potential father is the true father, and this is tested against hypothesis H_2 wherein the potential father is an unrelated individual selected at random from the population.

Nielsen *et al.* (2001) presented a new method for paternity analysis in natural populations based on genotypic data that can take the sampling fraction of putative parents into account. They developed an approach for estimating parentage probabilities for paternity assignment, which is a Bayesian alternative to the method developed by Marshall *et al.* (1998). They used the term 'parentage probability' to describe the posterior probability that a particular individual/putative father might be the actual father of a known offspring. They applied the method to genotypic data (six microsatellite DNA loci) collected from North Atlantic humpback whales.

The method of Nielsen *et al.* (2001) is briefly described here following the notations of the authors. They noted that the objective was to estimate the posterior probability that a particular individual might be the father of a known offspring. Let I_j (i) indicate the event that the jth potential father is the father of the ith offspring. The ith maternal genotype is $\boldsymbol{M}_i$, the associated genotype of the offspring is $\boldsymbol{O}_i$, the genotype of jth potential father is $\boldsymbol{F}_j$, and $\boldsymbol{A}$ is the matrix of allelic frequencies for all loci. If n of N males on the breeding ground were sampled, the posterior probability of paternity could be calculated as:

$$\Pr(I_j(i)|\boldsymbol{M}_i,\boldsymbol{F},\boldsymbol{A},N) = \frac{\Pr(\boldsymbol{O}_i|\boldsymbol{M}_i,\boldsymbol{F}_j)}{\sum_{j=1}^{n}\Pr(\boldsymbol{O}_i|\boldsymbol{M}_i,\boldsymbol{F}_j)+(N-n)\Pr(\boldsymbol{O}_i|\boldsymbol{M}_i,\boldsymbol{A})}.$$

In this formulation $\Pr(\boldsymbol{O}_i|\boldsymbol{M}_i, \boldsymbol{F}_j)$ is the shorthand notation for $\Pr(\boldsymbol{O}_i|\boldsymbol{M}_i, \boldsymbol{F}_j, I_j(i))$. $\sum_{j=1}^{n}\Pr(\boldsymbol{O}_i|\boldsymbol{M}_i, \boldsymbol{F}_j)$ refers to the sum of the probability that ith fetus has jth potential father as it's father when ith maternal genotype and jth potential father are given. Assuming Mendelian segregation and independence among loci, the probability of an observed offspring genotype, given the maternal genotype and the genotype of a particular potential father $\Pr(\boldsymbol{O}_i|\boldsymbol{M}_i, \boldsymbol{F}_j)$, could be calculated using standard methods (e.g., Thompson, 1975; 1976). Likewise, $\Pr(\boldsymbol{O}_i|\boldsymbol{M}_i, \boldsymbol{A})$ can be calculated assuming Hardy-Weinberg equilibrium and independence among loci (linkage equilibrium). $(N-n)\Pr(\boldsymbol{O}_i|\boldsymbol{M}_i, \boldsymbol{A})$ refers to the probability of the ith fetus's genotype given the *i*th mother's genotype. In estimating the posterior probability that a potential father is the father of an offspring, assumptions need to be made regarding the prior probability of a potential father being the father. In the absence of other information, it is assumed that the prior probability that a particular male is the father is 1/*N*, where *N* is the number of potentially breeding males in the breeding area (Nielsen *et al.*, 2001).

Here, assuming independence among offspring, the likelihood function for *N* can be calculated as:

$$\mathrm{L}(N) \propto \prod_i \Pr(\boldsymbol{O}_i|\boldsymbol{M}_i, \boldsymbol{F}, \boldsymbol{A}, N)$$

$$= \prod_i \left(\frac{(N-n)}{N} \Pr(\boldsymbol{O}_i|\boldsymbol{M}_i, \boldsymbol{A}) + \frac{1}{N} \sum_{j=1}^{n} \Pr(\boldsymbol{O}_i|\boldsymbol{M}_i, \boldsymbol{F}_j) \right),$$

$$N \geq n\,.$$

The maximum-likelihood estimate of *N* and the confidence interval is provided, for example, using parametric bootstrapping. Note that the estimate based on this approach such as parent-offspring genotypes is an estimate of the current (i.e., at the time of sampling) male population size. To estimate the total abundance of the target population/species, additional information is required. For example, the proportions of males and females, and the sexual maturity of whales from the sample, under the assumption that the sample was representative of the true population.

Based on the method above, the maximum-likelihood estimate of *N* was 6,540 breeding male humpback whales (95% CI: 3,700–17,000) using parametric bootstrapping (Nielsen *et al.*, 2001).

CKMR method

CKMR is a recent extension of the ordinary MR methods used to estimate animal abundance and other population parameters. The CKMR approach was first described by Skaug (2001) working on microsatellite data (10 loci) of North Atlantic common minke whales. He derived an estimator for the total population size based on the number of parent-offspring pairs (POPs).

Subsequently Bravington *et al.* (2016a) extended the classic MR framework (Lincoln-Petersen type estimator mainly) by incorporating close-kin information. Whereas ordinary MR methods only consider the subsequent identification of the same animal as a recapture, CKMR expands this by also viewing the genetic identification of a relative (e.g., parent-offspring, full-sibling, half-sibling) as a recapture.

Below is the simplest version of the CKMR by Bravington *et al.* (2016a). This is a two-sample estimator for the adult population size, which is the close-kin version of the Lincoln-Petersen population size estimator:

$$\widehat{N_A} = \frac{2n_J n_A}{H}\,. \tag{1}$$

Consider a sample of n_J juveniles and n_A adults from the same population. Each sampled juvenile can be considered a 'mark' of its two adult parents. The genotype of each of the n_J juvenile samples is compared to each of the n_A adult samples, to check if a 'mark' is recaptured. The probability that the adult happens to be one of the juvenile's two parents is $2/N_A$, where N_A is adult population size. The expected number of parent-offspring pairs across all $n_A \cdot n_J$ comparisons is then $2n_A\, n_J/N_A$. Equating this to the observed number of parent-offspring pairs gives the CKMR version of the Lincoln-Petersen estimator. Hence, if the entire set of $n_J \times n_A$ comparisons yields *H* which is the number of parent-offspring pairs, then adult abundance can be estimated as eq. (1).

In a more realistic setting, some types of kinship probabilities are likely to be present, and therefore, an explicit statistical MR model is required. That is, the prior probability that a pair is a POP is set by a population dynamics model, which accounts for pair-specific data, and which includes demographic parameters such as adult abundance, individual survival probability, age of maturity, per capita birth rate and probably age-specific fecundities.

For example, probability that individual *i* with covariates z_i (date of capture for example) is the mother of individual *j* with covariates z_i is represented as:

$$P(i \text{ is } j\text{'s mother}|z_i, z_j)$$

$$= E\left(\frac{i\text{'s reproductive output in } j\text{'s year of birth}}{\begin{array}{c}\text{Total female reproductive output}\\ \text{in } j\text{'s year of birth}|z_i, z_j\end{array}} \right)$$

where *E*() is the expected value and the absolute abundance (of females) now enters implicitly through the denominator.

Not only parent-offspring (PO) but also other kinship relationships such as half-sibling can be considered in the CKMR. A pairwise comparison between individuals *i* and *j* gives rise to a kinship category K_{ij} taking values from a discrete set *k*={*PO*, *HS*, ...} (see also Taguchi (this issue)). These probabilities are the building blocks of the pseudo-likelihood shown below.

Demographic parameters can be estimated from the log-likelihood summed over all pairwise comparisons. The joint distribution of $\{K_{ij};\ 1 \leq i < j \leq n\}$ is too complicated to permit the construction of a full likelihood. Instead, a pseudo-likelihood approach (Skaug, 2001; Bravington *et al.*, 2016a) which involves only the marginal probabilities of the K_{ij} in eq. (2) was used.

$$l_P(\theta) = \sum\nolimits_{1 \le i < j \le n} \log P(K_{ij} = k_{ij} | z_i, z_j ; \theta) . \qquad (2)$$

Let ϑ denote the vector of all parameters that govern the basic quantities, for example, total population size (males and females): N, individual survival probability: φ, per capita average birth rate: β and capture probability at a given sampling occasion: p).

The CK greatly expands the scope of MR because a variety of samples can be used, including biopsy samples and samples from catches. However, age information is essential for precise CKMR estimates because it identifies the time of 'marking', which is the year the sampled animal was born.

Bravington *et al.* (2016b) used the approach above for the case of the southern bluefin tuna, using microsatellite data at 20–25 loci.

CASE STUDIES BY THE ICR

Based on the histories of individual identification

Pastene *et al.* (2018) examined a total of 157 biopsy samples of southern right whales collected by IDCR/SOWER and JARPA/JARPAII surveys in austral summer between 1987/88 to 2013/14 in the longitudinal sector between 80°E and 135°E (Figure 3). These southern right whales are associated with the South West Australia population.

Biopsy samples were examined with 14 microsatellite DNA loci. After removing four duplicates, the sample sizes for the individual identification became 153. Eight incidences of individual matching ('mark-recapture') were detected (four males and four females). Individual matching by multilocus genotypes was supported by mitochondrial DNA analyses (same haplotype), sex determination (same sex), and in two cases where pictures were available, by photo-identification. Estimate of abundance was based on the POPAN model implemented in RMark (Laake, 2013). This preliminary analysis considered the assumption of constant apparent survival, constant probability of capture and constant probability of entry through the years.

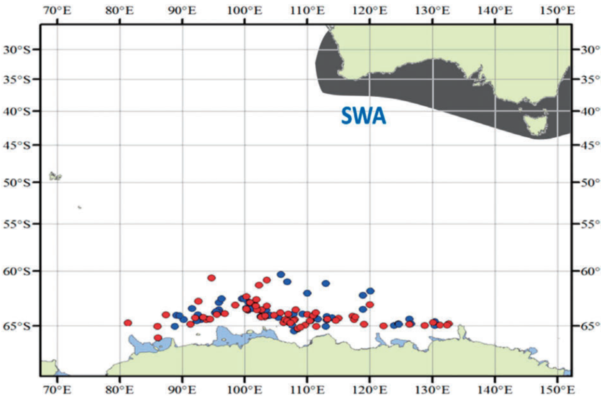

Figure 3. Geographical distribution of southern right whales in Area IV examined by Pastene *et al.* (2018). Red: females; Blue: males.

The results of the preliminary analyses showed similar annual abundance estimates to those obtained by sighting data in the same area and a similar period. For example, the estimate of abundance by the genetic 'mark-recapture' method was 1,619 (95% CI: 868–3,151) individuals for 2015/16, similar to the most recent (2007/08) sighting survey abundance estimate of 1,557 (95% CI: 871–2,783) individuals using the LT method in the same area.

Future analyses

The analysis conducted on southern right whales assumed constant apparent survival rate, constant capture probability, and constant probability of entry through the years. Some additional investigations are required to evaluate the effect of changing these assumptions on the abundance estimates. State-space models and hierarchical Bayesian approach have already been proposed as a convenient and flexible framework for specifying stochastic models for the dynamics of wild animal populations (Gimenez *et al.*, 2009; Kéry and Schaub, 2011; Hendrix *et al.*, 2012), and these models could be used for southern right whales in the future. Such models consider individual differences of the survival rate. Furthermore, the detection/capture probability, as a random effect can be evaluated by the state-space model.

Genetic databases for Antarctic blue and humpback whales are available at the ICR, and the abundance estimation approach for southern right whales can also be used for these other species.

One issue with mark-recapture analyses, regardless of which method of individual identification is used, is that the study area must provide adequate opportunities for 'capturing' all individuals within a population. However, this is hardly feasible because many populations are widely and unevenly distributed. Therefore, for obtaining more accurate abundance estimates, applying analytical techniques that take account of unsampled locations are also suggested for future analyses (e.g., Frasier *et al.*, 2020). Also, more information on the biological features of the species, such as migration and habitat use patterns, are required.

Paternity analyses

Kanda *et al.* (2014) conducted a study on paternity in

Antarctic minke whales based on samples collected by JARPA and JARPAII surveys. Specifically, genotypic data of a maximum of 12 microsatellite DNA loci were prepared from 137 fetuses collected from females captured during the 2003/04 JARPA survey, and their potential fathers were sought among 1,779 males collected from 2001/02 JARPA to 2010/11 JARPAII surveys.

One mother-fetus-father trio was detected using CERVUS (Marshall *et al.*, 1998), a computer program for assignment of parents to their offspring using genetic markers. This single match was used to tentatively estimate the mature male number in the Eastern Indian Ocean Stock (I stock) of Antarctic minke whales using the Petersen mark-recapture method modified by Chapman. Only mature males ($n=677$) (sexual maturity criteria of Kato, 1987) were used in the estimates.

The abundance estimate of the mature male component of the stock was 46,782 (CV=0.572) animals. Assuming the mature/immature ratio and the sex ratio were 1 : 1, respectively, the total stock size was estimated as 187,128 animals. This estimate was higher than the JARPA/JARPAII (51,474) and the IDCR (151,174) sighting surveys estimates in similar areas. Kanda *et al.* (2014) noted that their analysis estimated total stock size, while the JARPA/JARPAII and IDCR analyses estimated the number of individuals distributed in the research area based on sighting surveys, which would explain the differences.

Ohashi *et al.* (*in-press*) further examined the population size of mature males of Antarctic minke whales based on paternity analysis. Their analyses were based on biological and genetic (microsatellite DNA at 12 loci) data collected by JARPAII in the Indo-Pacific region of the Antarctic. A total of 2,126 Antarctic minke whales taken in the austral seasons 2006/07, 2008/09, 2009/10, 2010/11 and 2011/12 were used in the analyses. Two assumptions on stock structure were considered, a single stock in the research area and two stocks in the research area (the I stock and the Western South Pacific Stock (P stock)).

For abundance estimation, the approach of Nielsen *et al.* (2001) outlined above was used. For the hypothesis of a single stock, the abundance of mature males was estimated as 68,874 (90% CI: 42,625–122,779). Using the proportion of male and immature whales of 0.436 and 0.254, respectively, the estimate of total population was 211,600 (90% CI: 130,953–377,210). This estimate was lower than that from the IDCR/SOWER sighting surveys (413,202) and of the same order of JARPA/JARPAII estimates in Areas IV and V (263,711).

Future analyses

Ohashi *et al.* (*in-press*) reported that there are some technical issues in their analyses on Antarctic minke whales that should be improved in the future. Firstly, there is a need to optimize the use of the available genetic data and solve issues, for example, on genotyping errors and missing allele information for some loci. The second issue is related to the assumptions of the stock structure hypothesis. The current hypothesis on stock structure of Antarctic minke whales suggests a transition sector shared by the two stocks rather than a hard longitudinal boundary between them as assumed in the paternity analysis. This transition sector should be considered in future paternity analyses.

In addition, Ohashi *et al.* (*in-press*) noted that future analyses should consider not only the relationship between mother/fetus and father, but also some other kinship relationships (e.g., half-sibling) following the work of Bravington *et al.* (2016a) outlined above. Therefore, the next challenge for the ICR research on this topic is the application of the CKMR method for abundance estimation, which optimizes the use of the available genetic data.

Comparison of abundance between MR and LT methods

As noted by Kanda *et al.* (2014) and Pastene *et al.* (2018), estimates of abundance, using the ordinary MR approach based on genetic data, refer to the total population/stock size. The estimates based on sighting data under the LT approach, refer to the abundance of animals in a specific area and a specific period of a survey. Then, the difference in estimates will depend on the sighting survey coverage and on the movement of individuals into and out of the sighting survey area (Hammond, 2010).

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Technical Report (not peer reviewed)

Progress in the research on earplug-based age determination and biological parameters of North Pacific sei whales at the Institute of Cetacean Research

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ABSTRACT

This study summarizes the progress in the research on earplug-based age determination and estimation of biological parameters of the western North Pacific sei whales sampled by the JARPNII/NEWREP-NP surveys between 2002 and 2018. A total of 1,622 whales (744 males and 878 females) were sampled in the western North Pacific north of 35°N and between the Pacific coast of Japan and 170°E. Earplugs were read for whales collected up to 2016 (n=1,354). Age information was obtained for 887 whales (65.5%). Earplug readability was higher for mature (69.7%) than immature (52.1%) whales, and no difference between sexes was observed. The growth curves for males and females were L_t=14.14 $(1-e^{-0.174(t+6.650)})$ and L_t=15.17 $(1-e^{-0.150(t+7.407)})$, respectively. The age at sexual maturity for males and females were 6.7 (SE=0.29) and 6.9 (SE=0.27) years, respectively. The body length at sexual maturity was estimated as 12.72 m (SE=0.12) for males and 13.31 m (SE=0.12) for females. The annual ovulation rate was estimated as 0.744/year. Substantial biological information was obtained for the North Pacific sei whale during the 17 year of JARPNII and NEWREP-NP. The analyses of biological data will contribute to the management of this whale species in the North Pacific.

INTRODUCTION

Sei whale (*Balaenoptera borealis*) is widely distributed throughout the world. As with other baleen whale species, they undertake seasonal migration spending the summer feeding season in subpolar waters and returning to low latitude waters to calve in winter (Horwood, 2017). In the North Pacific, sei whales (Figure 1) distribute widely in the offshore area associated with the productive polar and subarctic fronts during the summer feeding season (Murase *et al.*, 2014).

The second phase of the Japanese Whale Research Program under Special Permit in the western North Pacific (JARPNII) started in 2000 (GOJ, 2000; 2002), and was completed in 2016. The sei whale was added as one of the target species for sampling in 2002. The New Scientific Whale Research Program in the western North Pacific (NEWREP-NP) started in 2017 (GOJ, 2017) and also had the sei whale as one of the target species. During the annual surveys, biological data and samples such as body length measurements and reproductive organs, were obtained systematically. A total of 1,622 sei whales were sampled during a 17 year survey period. The NEWREP-NP was completed in 2018.

Age data is one of the most important information for stock assessment and management of large whales. Age estimation based on counting of growth layers accumulated in the earplugs is considered the most reliable tool for age determination in baleen whales (Lockyer, 1984a;

Figure 1. A sei whale feeding on plankton near the sea surface in the western North Pacific.

Maeda *et al.*, 2016). During JARPNII and NEWREP-NP, earplugs were collected from each whale sampled by experienced biologists in order to increase the readability. Some age-related biological parameters are known to change in response to changes in abundance, food availability or competition among whale species (Gambell, 1973; Kato and Sakuramoto, 1991; Lockyer, 1984b). Therefore, monitoring of biological parameters is indispensable for the assessment and management of whale stocks.

This study summarized the progress in the research on earplug-based age determination and estimation of the biological parameters of the western North Pacific sei whales sampled by the JARPNII and NEWREP-NP surveys between 2002 and 2018.

MATERIALS AND METHODS

Whale sampling

A total of 1,622 (744 males and 878 females) sei whales were sampled by JARPNII and NEWREP-NP during 2002–2018. The geographical distribution of the sampled whales is shown in Figure 2. Table 1 shows the number of sampled whales by year and sexual maturity status.

Biological data

Body length was measured in a straight line to the nearest 1 cm, from the tip of the snout to the notch of the flukes using a stainless measure. Sexual maturity in males was determined by examining the histological samples from the testis. Males with seminiferous tubules over 100 µm diameter, spermatid or open lumen in the tubules were determined to be sexually mature (Masaki, 1976; Lockyer, 1984b). Maturity of females was preliminarily determined in the field by the presence or absence of at

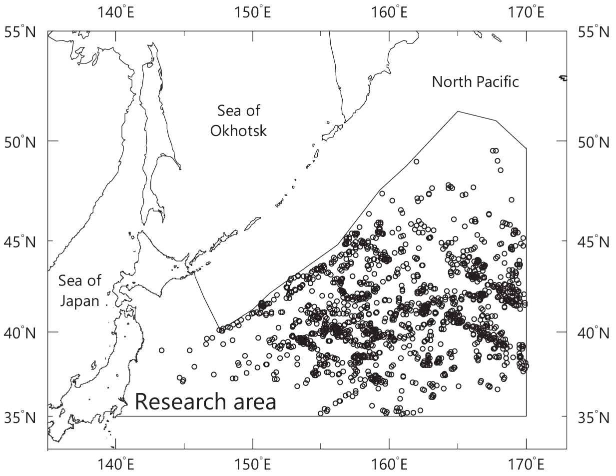

Figure 2. Research area of JARPNII and NEWREP-NP and sighting positions of sei whales sampled during the 2002–2018 surveys.

Table 1

Number of sei whales sampled by JARPNII and NEWREP-NP in the western North Pacific in the period 2002–2018, by year, sex and sexual maturity status.

Year	Male				Female			Total
	Immature	Mature	Unknown	Total	Immature	Mature	Total	
2002	3	12	0	15	4	20	24	39
2003	4	19	0	23	4	23	27	50
2004	11	36	0	47	14	39	53	100
2005	6	45	0	51	14	35	49	100
2006	18	30	0	48	14	38	52	100
2007	21	33	0	54	16	30	46	100
2008	6	38	0	44	17	39	56	100
2009	10	36	0	46	9	45	54	100
2010	12	31	0	43	10	47	57	100
2011	8	46	0	54	13	28	41	95
2012	12	32	0	44	11	45	56	100
2013	15	29	0	44	6	50	56	100
2014	7	31	0	38	11	41	52	90
2015	9	20	0	29	15	46	61	90
2016	10	28	0	38	16	36	52	90
2017	13	50	0	63	15	56	71	134
2018	12	50	1	63	17	54	71	134
Total	177	566	1	744	206	672	878	1622

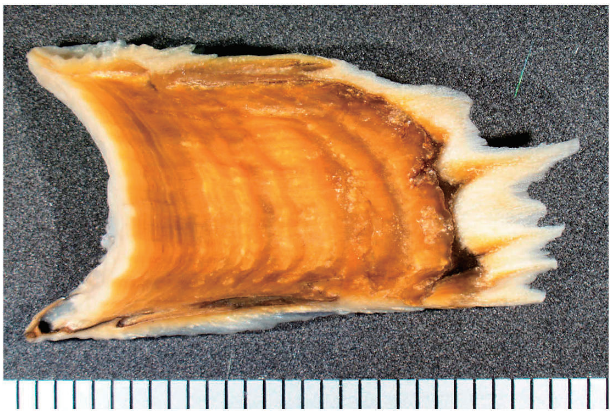

Figure 3. Bisected earplug surface of sei whale with light and dark laminae of growth layer groups (GLGs). The scale shows a 1 mm interval.

least one corpus luteum or corpus albicans in both sides of the ovaries, and confirmed later in the laboratory by counting number of corpora.

Earplug sampling and age determination

Earplugs were collected from all sampled animals, following the method developed for baleen whale (Omura, 1963; Maeda *et al.*, 2016). The left and right earplugs were collected and immediately fixed in 10% formalin until age determination. In the laboratory, the surface along the central axis of the earplug was cut using a sharp blade, then it was placed on a wet stone to expose the neonatal line and growth layers (Figure 3). Growth layers were counted under water using stereoscopic microscope. A growth layer group (GLG) was defined as one pair of light and dark laminae in the core and considered as one year of age.

Estimation of biological parameters

Growth curve

To estimate 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 the asymptotic length,

K is the growth rate coefficient, and

t_0 is the theoretical time at zero length.

Age at sexual maturity

Age at sexual maturity (t_m) was estimated by the following equation (Cooke, 1984).

$$T_m = g - 0.5 + \sum_{a=g}^{h} \left(\frac{I_a}{N_a} \right)$$

$$\mathrm{var}(t_m) = \sum_{a=g}^{h} \frac{M_a I_a}{N_a^2 (N_a - 1)}$$

where

M_a is the number of mature animals in age a,

I_a is the number of immature animals in age a,

N_a is the total number of animals in age a,

g is the age of the youngest mature animal in the sample, and

h is the age of the oldest immature animal in the sample.

Body length at sexual maturity

Body length at sexual maturity (L_m) was estimated by the following equation (Cooke, 1984; Kato, 1992). When calculating, body length data was rounded to the nearest 0.1 m.

$$L_m = j - 0.05 + 0.1 \sum_{b=j}^{k} \left(\frac{I_b}{N_b} \right)$$

$$\mathrm{var}(l_m) = q \sum_{b=j}^{k} \frac{M_b I_b}{N_b^2 (N_b - 1)}$$

where

M_b is the number of mature animals in body length b,

I_b is the number of immature animals in body length b,

N_b is the total number of animals in body length b,

j is the body length of the smallest mature animal in the sample, and

k is the body length of the largest immature animal in the sample.

Annual ovulation rate

The annual ovulation rate was estimated by applying linear regression analysis between age and the total number of corpora (corpora lutea and albicantia). The regression line was fitted to ages 14 years and older because all females were sexually mature at the age of 14 years in this study.

RESULTS AND DISCUSSIONS

Age readability

Earplugs were read for whales collected up to 2016 (n=1,354). Age information was obtained for 887 whales (65.5%). The readability of earplugs varied depending on the maturity status. Readability of sexually immature individuals was 55.9% for males and 48.9% for females (Table 2). Readability of earplugs in sexually mature animal was higher, 72.5% and 67.4% for males and females,

Table 2
Earplug-age readability of sei whales sampled during 2002–2016 surveys, by sex and sexual maturity status.

Maturity status	Male			Female			Total		
	Number of whales	Readable earplugs	Readability (%)	Number of whales	Readable earplugs	Readability (%)	Number of whales	Readable earplugs	Readability (%)
Immature	152	85	55.9	174	85	48.9	326	170	52.1
Mature	466	338	72.5	562	379	67.4	1028	717	69.7
Total	618	423	68.4	736	464	63.0	1354	887	65.5

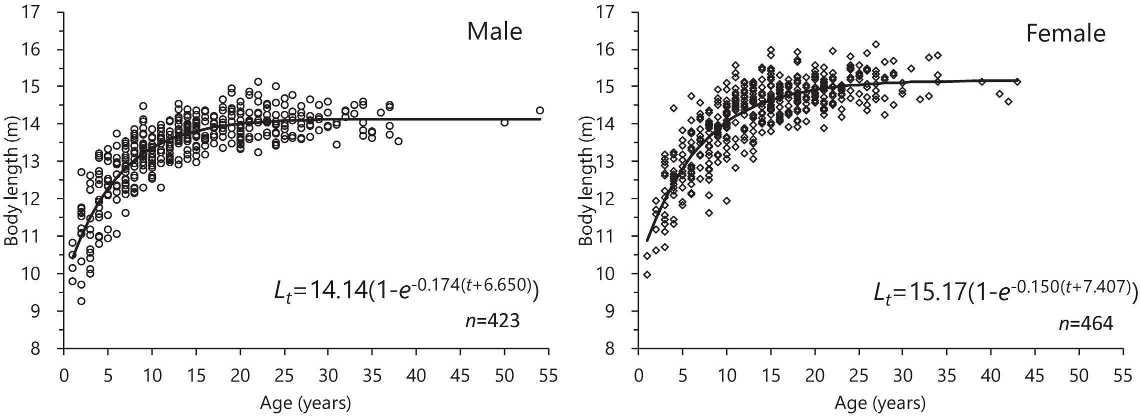

Figure 4. Relationship between body length and age in sei whales. The solid lines show the von Bertalanffy growth curves.

respectively (Table 2). Readability of all samples was 65.5%, which was higher than for western North Pacific common minke whales (44.1%) (Maeda *et al.*, 2016), and comparable to Bryde′s whales (65.2%) (Bando, 2018), which were based on samples collected by JARPN and JARPNII.

Growth curve

For both sexes, the growth rate was high at younger ages and stabilized after 15 years (Figure 4). The following von Bertalanffy growth curves were estimated:

$$\text{Male:} \quad L_t = 14.14\,(1 - e^{-0.174(t+6.650)})$$
$$\text{Female:} \quad L_t = 15.17\,(1 - e^{-0.150(t+7.407)})$$

Masaki (1976) estimated growth curves of sei whales based on samples collected from the commercial whaling in the North Pacific in the 1970s. However, the estimated curve was incomplete due to the lack of young individuals. During JARPNII and NEWREP-NP, samples from a wide range of ages, including young individuals, were collected, and for the first time a reasonable growth curve could be obtained for western North Pacific sei whales.

Age at sexual maturity

Sexually mature males first appeared at the age of 4 years, and from 13 years old onwards all animals were sexually mature (Figure 5). T_m for males was estimated as 6.7 years (SE=0.29). For females, sexually mature whales first appeared at the age of 3 years, and from 14 years old onwards all animals were sexually mature (Figure 5). T_m was estimated as 6.9 years (SE=0.27). Masaki (1976) reported the T_m of sei whales from the commercial whaling in the North Pacific in the 1970s as 2.5 and 6.5 years for males and females, respectively. However, these values were underestimated as the ages of younger animals are often estimated as less than their actual ages. This is because the softer and smaller earplug of young animals, together with a lack of neonatal layers, in some cases, make it difficult to recognize growth layers (Masaki, 1976).

Body length at sexual maturity

The relationship between sexual maturity and body length is shown in Figure 6. Sexually mature male first appeared at body length of 12.2 m, and from 13.6 m onwards all animals were sexually mature. L_m was estimated as 12.72 m (SE=0.12). For females, sexually mature whales first appeared at the body length of 12.6 m, and from 14.0 m all animals were sexually mature (Figure 6). L_m was estimated as 13.31 m (SE=0.12). Masaki (1976) estimated L_m of male sei whales as 12.9 m based on the

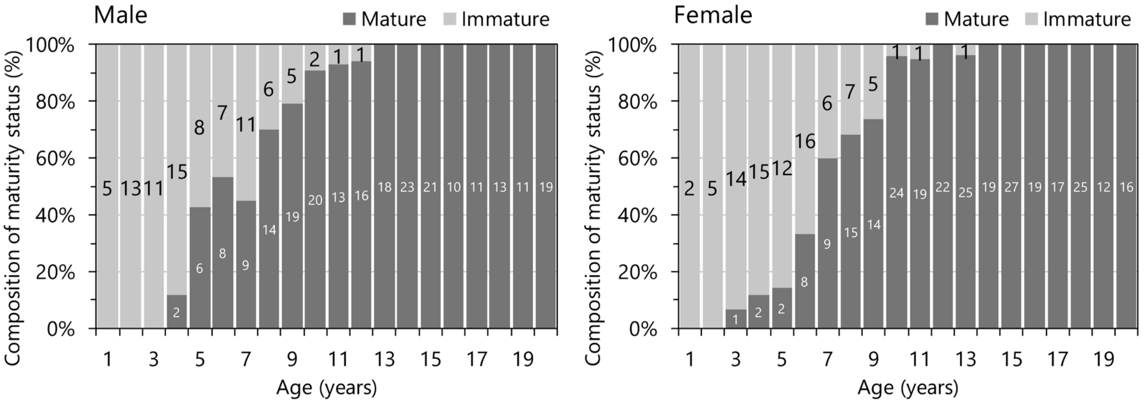

Figure 5. Sexual maturity status by age and sex in sei whales. Numbers in the bars show the numbers of samples examined.

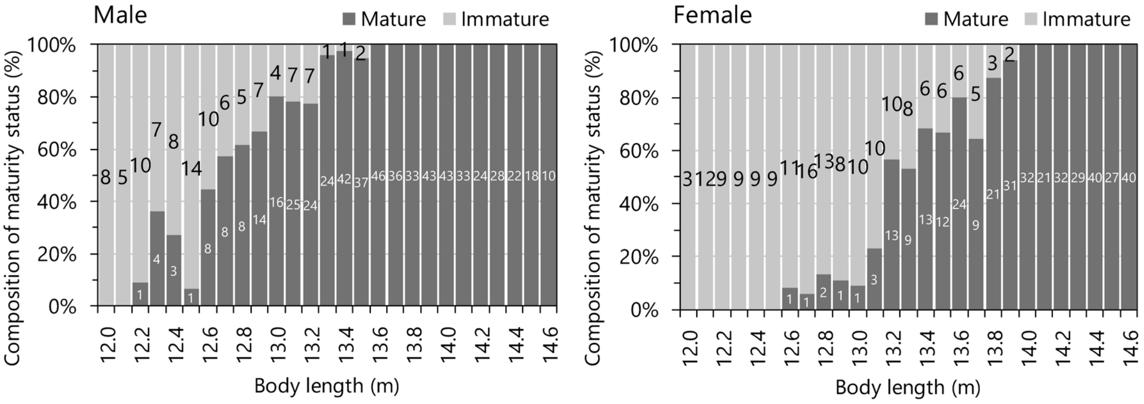

Figure 6. Sexual maturity status by body length and sex for sei whales. Numbers in the bars show the numbers of samples examined.

commercial whaling samples from the North Pacific, which was slightly higher than the value estimated in this study. However, Masaki (1976) determined the sexual maturity of males by testis weight rather than histological observation, which might have influenced the results. For females, a similar value was estimated from commercial whaling samples (13.4 m) (Masaki, 1976).

Annual ovulation rate

The corpus luteum and albicans first appeared at the age of 3 years and the number of corpora increased linearly after the age of 14 years (Figure 7). Annual ovulation rate was estimated as 0.744. The estimated value means that the majority of sei whales give birth (or ovulation occurs) three times in four years. This value was higher than 0.604 which was estimated from the 1970s commercial whaling samples (Masaki, 1976).

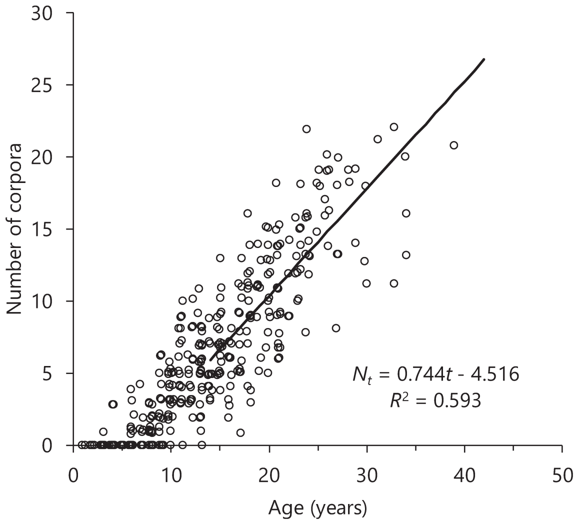

Figure 7. Jitter plot showing the relationship between age and number of corpora in sei whales. The linear regression line was fitted to ages 14 years and over.

CONCLUDING REMARKS

Substantial biological information was obtained for the North Pacific sei whale during the 17 years of JARPNII and NEWREP-NP. In particular, more age data from young individuals were obtained, resulting in improved estimates of biological parameters than those obtained in the period of commercial whaling. The analyses of biological data will contribute to the management of this whale species in the North Pacific.

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Technical Reports of the Institute of Cetacean Research (2020) pp. 54–56

Technical Report-Note (not peer reviewed)

Japan's system for monitoring of whale products sold in its domestic market through DNA registration

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As in the case of Norway and Iceland, Japan has been using a DNA-based system for monitoring (tracking origins of) large whale products sold in the retail market (Figure 1). Such a task is entrusted to the Institute of Cetacean Research (ICR) by the Government of Japan (Fisheries Agency of Japan) as an outsourced program every year.

The system is composed of two main components, i) a DNA register including genetic profiles of all large whales legally taken (including bycatches) and imported into Japan, and ii) systematic surveys of large whale products sold in the retail market. The objective of this system is to prevent illegal take of large whales and illegal import of large whale products by comparing DNA profiles of large whale products obtained in the market with those filed in the DNA register.

The Japanese DNA register (JDR) is established based on technical specifications similar to those of the Norwegian DNA register (Glover *et al.*, 2012). It contains DNA profiles of large whales sampled from different sources:

i) Scientific whaling conducted under special permit in the Antarctic (JARPA/JARPAII and NEWREP-A). This category includes Antarctic minke whales collected between 1987/88 and 2018/19, and fin whales between 2005/06 and 2011/12.

ii) Scientific whaling conducted under special permit in the western North Pacific (JARPN/JARPNII and NEWREP-NP). This category includes common minke whales collected between 1994 and 2019; Bryde's whales between 2000 and 2019; sei whales between 2002 and 2019; and sperm whales between 2000 and 2013.

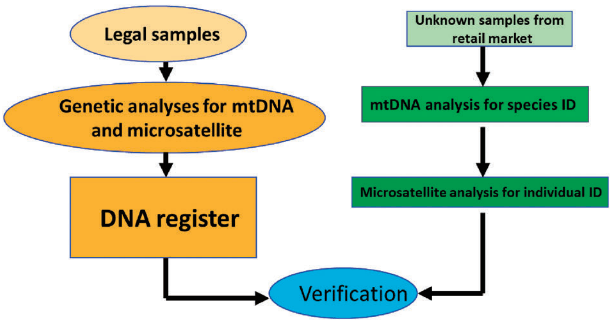

Figure 1. Flow chart of the DNA monitoring system of large whale products sold in the Japanese retail market.

iii) Commercial whaling within Japan's Exclusive Economic Zone (EEZ). This category includes common minke, Bryde's and sei whales collected since 2019.

iv) Bycatches in set nets. This category includes mainly common minke whales sold/used domestically (register system established since 1 July 2001).

v) Imports. This category includes North Atlantic common minke whales from Norway and North Atlantic fin whales from Iceland since 2008.

By the end of 2019, DNA profiles of a total of 19,000 individual whales were registered in the JDR.

In the cases of scientific whaling and current commercial whaling, samples for genetic analysis have been collected by researchers from each whale. Samples include skin samples (two or three pieces of 5×5×5 mm kept frozen or in 99% ethanol). In addition, a large amount of information is collected using established protocols from every whale sampled including species, catch date and location (longitude, latitude), body length, sex and maturity status.

The Japanese regulation on bycatches of large whales (established from 1 July 2001) requires that all animals should be registered with a DNA profile before any products derived from a bycaught animal are 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 who intends to sell the bycaught whale and sent to the laboratory at ICR (as frozen samples). Together with the genetic samples, the fishermen should provide the following information in accordance with an established protocol: species, date and location of the bycatch, type of set net, body length

Table 1

The number of whale products by sampling sites in the 2017 survey.

City/Town	Meat	Blubber	Ventral grooves	Total
Sapporo	23	2	7	32
Sendai	27	2	6	35
Niigata	3	10	3	16
Kanazawa	3	3	1	7
Noto	5	5	1	11
Anamizu	1	1	0	2
Toyama	4	0	0	4
Himi	3	5	1	9
Nagoya	13	1	5	19
Kochi	14	11	9	34
Kobe	10	8	3	21
Osaka	12	5	7	24
Taiji	5	6	2	13
Nachikatsuura	2	5	2	9
Hiroshima	11	0	4	15
Shimonoseki	18	6	10	34
Fukuoka	16	5	14	35
Nagasaki	14	6	10	30
Total	184	81	85	350

Figure 2. Sampling sites in the 2017 DNA market survey.

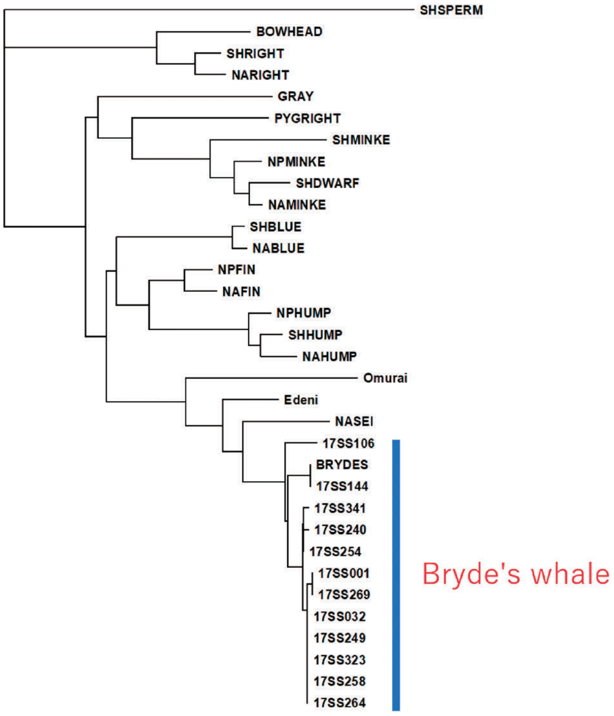

Figure 3. An example of species identification by phylogenetic analysis of 'test' and 'type' sequences of the whale products identified as Bryde's whale in the 2017 market survey. SH: Southern Hemisphere; NA: North Atlantic; and NP: North Pacific. 17SS+3 digits indicates the market samples collected in the 2017 survey.

and sex of the bycaught whale to be used (sold).

The following genetic markers are used in the JDR:

i) An approximately 500 bp fragment of the 5′-end of the mitochondrial DNA (mtDNA) control region, which is used for the purpose of identification of the origin of the whale products (species identification), through phylogenetic analyses constructed by 'test' and 'type' sequences;

ii) Microsatellite DNA (genotyping with 13–17 loci in each species), which is used for the purpose of individual identification; and

iii) Y chromosome DNA, which is used for the purpose of sex determination.

For further details, see Pastene and Goto (2006).

Regarding sampling in the market, one or two technicians, who are familiar with whale products and market operations, carry out the sampling of whale products at retail shops each year. On an annual basis, around 350 samples are collected between September and December throughout Japan, including around 18 cities or towns. Table 1 shows the cities/towns surveyed and the whale products sampled in 2017. Figure 2 shows the geographical distribution of the sampling site in the 2017 survey. The sampling attempts to cover the whole Japanese archipelago, but the method of sampling is not a random procedure. The following information is collected for each whale product sampled: kind of whale

Table 2

Results of species and individual identification of the 2017 market survey. The numbers of individual whales are shown in parenthesis. NG: Failed DNA extraction. NP: North Pacific and NA: North Atlantic.

Species identification	*N*	Individual identification	*N*
Antarctic minke whale	110	Not conducted*	—
Common minke whale: NP	87	Special permit: Offshore	1 (1)
		Special permit: Coastal	28 (13)
		Bycatch	58 (37)
Common minke whale: NA	9	Import from Norway	9 (7)
Fin whale: NA	40	Import from Iceland	40 (35)
Sei whale	84	Special permit: Offshore	84 (66)
Bryde's whale	12	Special permit: Offshore	12 (11)
Baird's beaked whale	6	Not conducted**	—
Striped dolphin	1	Not conducted**	—
NG	1	—	—
Total	350	Total	232 (170)

*Individual identification of Antarctic minke whales was not conducted because of the low probability of illegal products thereof being introduced into the Japanese market.

**Small cetaceans are not subject to the JDR.

product, sampling locality, date, weight and price. Once sampled, the products are sent to the laboratory at ICR for genetic analysis.

The laboratory work for the whale product samples were conducted with designated genetic markers for the JDR described above.

Standard phylogenetic analyses of 'test' (whale products sampled in the market) and 'type' (whale species in the JDR) mtDNA sequences are conducted to determine the species of the sampled whale products. Figure 3 shows an example of species identification by phylogenetic analysis in the case of Bryde's whales sampled in the 2017 survey.

For individual identification, matching of microsatellite genotypes of 'test' and 'type' samples was conducted using the computer program 'Cervus 3.0 software package' (Kalinowski *et al.*, 2007).

The results of the Japanese market survey in 2017 are shown in Table 2. All 350 market samples, but one, were successfully identified for the species and the origin (area) by the mtDNA analysis. One whale product (fried whale blubber or 'Koro' in Japanese) failed in the DNA extraction. The microsatellite analysis revealed that the 232 collected samples included 170 individual whales originating from the Northern Hemisphere. The genetic profiles of the 'test' and 'type' samples matched in all the cases (Table 2). Individual identification for Antarctic minke whales was not conducted because of the low probability of illegal products thereof being introduced into the Japanese market.

Results of the comparisons between 'test' and 'type' samples are summarized in the annual report to the Fisheries Agency of the Government of Japan.

Considering results of the work obtained so far, it can be concluded that the DNA-based system is an effective tool for monitoring (tracking origins of) large whale products in the Japanese retail market.

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Technical Report-Note (not peer reviewed)

Satellite-monitored tracking of Antarctic minke and fin whales under the first JASS-A survey in the 2019/2020 austral summer season

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The Japanese Abundance and Stock structure Surveys in the Antarctic (JASS-A) commenced in the 2019/20 austral summer season. The main research objectives of JASS-A are 1) the study of the abundance and abundance trends of large whale species, and 2) the study of the distribution, movement and stock structure of large whale species (GOJ, 2019; Isoda *et al.*, this issue). One of the techniques proposed for the research on main objective 2) was satellite tagging.

Implantable satellite-monitored tags (SPOT6-type; Wildlife Computers, Redmond, Washington, USA) were used for tracking Antarctic minke and fin whales during the first JASS-A survey in the 2019/20 austral summer season on board of the research vessel *Yushin Maru* No.2 (YS2) (see Isoda *et al.*, this issue). When possible, skin biopsy samples for genetic analyses were obtained from the same satellite-tagged whales. The details of the platform and equipment for satellite-monitored tagging were described in Konishi and Isoda (2019) and Konishi *et al.* (2020).

During the first JASS-A survey, the tag deployments were conducted from the end of January to early February 2020 in the western part of the International Whaling Commission (IWC) Management Area III, in the longitudinal sector between 000°E and 015°E. The tags were successfully deployed on eight Antarctic minke and ten fin whales (Table 1). Tracking information was received from five Antarctic minke (Figure 1) and nine fin (Figure 2) whales. In the case of Antarctic minke whale PTT ID 181817, which was tagged on 2 February 2020, track-

Table 1

Summary of the satellite-monitored tagging for Antarctic minke and fin whales in the 2019/20 JASS-A survey.

No.	Date of deployment	Species	School size	Latitude (°S)	Longitude (°E)	Estimated body length (m)*	PTT ID	Biopsy samples
1	2020/1/21	Fin	1	67.3	11.0	23.1	66629	Y
2	2020/1/24	Fin	2	61.6	7.6	20.1	66641	N
3	2020/1/24	Fin	1	61.2	7.3	19.7	66628	Y
4	2020/1/26	Fin	4	61.5	5.9	19.4	181812	Y
5	2020/1/26	Fin	3	61.9	5.7	20.5	181818	Y
6	2020/1/27	Fin	5	64.4	4.2	19.5	181816	N
7	2020/1/30	Ant. Minke	2	68.8	12.8	7.6	181813	Y
8	2020/2/1	Ant. Minke	1	68.7	13.7	7.5	181811	Y
9	2020/2/1	Ant. Minke	1	68.5	14.1	7.9	181821	Y
10	2020/2/1	Fin	2	68.4	14.3	19.6	181819	N
11	2020/2/1	Fin	6	68.4	14.5	20.1	181824	Y
12	2020/2/1	Fin	6	68.4	14.5	20.3	181820	Y
13	2020/2/2	Ant. Minke	2	68.5	14.6	7.6	181817	Y
14	2020/2/2	Ant. Minke	1	68.6	15.0	7.0	181815	Y
15	2020/2/2	Ant. Minke	1	68.5	14.5	6.2	181810	Y
16	2020/2/3	Ant. Minke	1	68.4	13.5	6.4	66627	Y
17	2020/2/4	Ant. Minke	1	65.8	13.4	5.7	181822	Y
18	2020/2/6	Fin	3	63.0	15.1	18.8	181814	Y

*Body lengths of whales were estimated by the researcher on board.

Tagging experiments in No. 11 and 12 were from the same school.

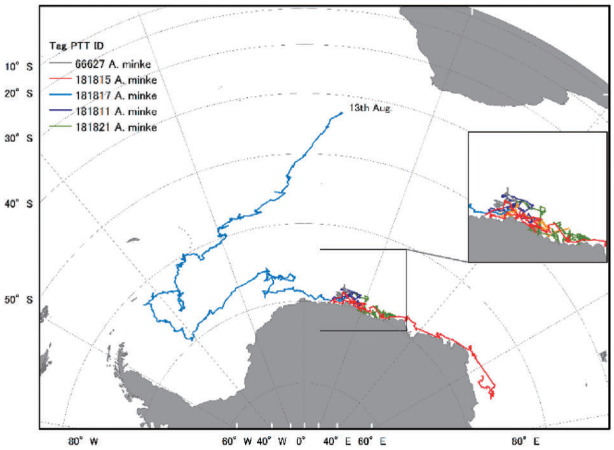

Figure 1. Tracking lines of five Antarctic minke whales tagged during the first JASS-A survey, as of 13th August 2020. The lines are plotted using all ARGOS Location Classes.

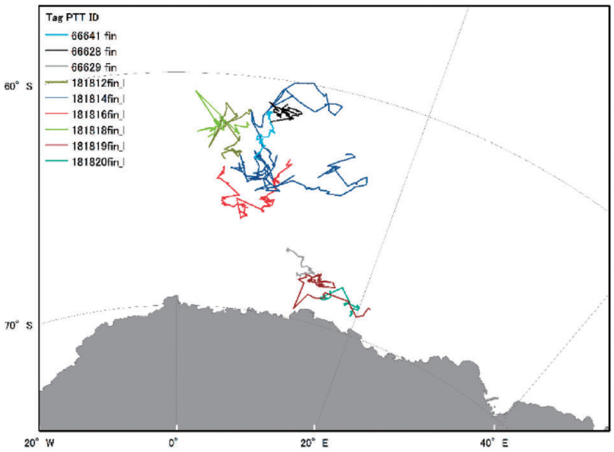

Figure 2. Tracking lines of nine fin whales tagged during the first JASS-A survey. The lines are plotted using all ARGOS Location Classes.

ing was still ongoing as of mid-August 2020. This whale showed a long longitudinal movement before starting migration to the north (Figure 1).

These tracking data will be analyzed in conjunction with previous data obtained under NEWREP-A to investigate distribution, movement and stock structures of these whales under main objective 2) of JASS-A.

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Commentary

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

Comments on the use of lethal and non-lethal techniques in the studies of large whales

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Japan designed and implemented whale research programs under special scientific permit in both the Antarctic and western North Pacific. These research programs ceased on 30 June 2019 when Japan withdrew from the International Convention for the Regulation of Whaling (ICRW). The research programs involved both lethal and non-lethal techniques. One of the issues frequently discussed among scientists was of the need to take whales for scientific purposes. I thought it would be appropriate to express my comments on this topic now that the research programs have ceased.

In my view, the following aspects are relevant when making a decision on lethal take of whales for scientific purposes: i) whether the samples and data to be collected are required for conservation and management of the whale stock (s); ii) the availability of non-lethal techniques for getting the same kind of samples and data; iii) the effect of the lethal take on the whale stock (s) involved.

Regarding i) above, it is recognized that considerable biological information is required for the appropriate conservation and management of whale resources. For example, samples and data are required to investigate relevant parameters such as growth, reproduction, recruitment, natural mortality, abundance, distribution, feeding ecology, environmental factors, interactions with other species, and the inter-annual variability of these factors (Ohsumi *et al.*, 2007). Some of this information can be obtained only through the lethal sampling of whales, for example earplugs for age estimation and sexual organs for investigation of maturity and pregnancy. These basic biological data are important for estimating several parameters such as age and length at sexual maturity, natural mortality, recruitment and so forth, which are important parameters in population dynamics models. Lethal sampling is also required to investigate stomach contents (qualitative and quantitative) which, in turn, allows us to investigate the feeding impact of the species (stock) in the ecosystem.

Regarding ii) above, scientists should make efforts to develop new non-lethal techniques to obtain the required information for conservation and management. In this sense, scientists of the Institute of Cetacean Research (ICR) have commenced several research projects to investigate the sexual maturity status of females through progesterone levels in blubber (Inoue *et al.*, 2019). They have also started researching the feasibility of the DNA methylation profiling in skin to obtain age information of whales (Tanabe *et al.*, 2020; Goto *et al.*, 2020). Skin and blubber can be obtained through biopsy sampling. The International Whaling Commission Scientific Committee (IWC SC) recommended that a field and analytical protocol should be developed to assist the evaluation of the utility of novel non-lethal techniques (IWC, 2016). In response to this recommendation, scientists from the ICR developed a protocol consisting of several questions that should be responded to in order to evaluate novel non-lethal techniques (Mogoe, 2018). The protocol consists of four questions: Question 1 is whether tissues or other kinds of samples can be obtained by a non-lethal technique. Question 2 is whether a sufficient number of samples for statistical analyses can be obtained by the non-lethal technique. Question 3 is whether the samples obtained by the non-lethal technique can produce scientific information comparable to that produced by a lethal sampling technique. Question 4 is whether the cost of obtaining the samples is reasonable for producing relevant scientific information. Question 1 and Question 2 are technical in nature. Question 3 is analytical while Question 4 is of a logistical nature. If the answers to all these questions are affirmative, then the non-lethal technique can be considered appropriate and feasible (Mogoe, 2018).

Regarding iii) above, only abundant species and stocks can be considered for lethal sampling. Furthermore, population dynamics models should be applied to evaluate the effect on the stocks of the number of whales taken lethally. For example, in the case of the former Japanese whale research under special scientific permit in the Antarctic, the effect of the take of 333 whales on the stocks of Antarctic minke whales was examined based on Hitter-Fitter model, and results suggested no negative effect on the stocks (GOJ, 2018).

In summary, the issue of lethal versus non-lethal techniques to study whales is not a 'black and-white' matter. In making a decision on the need for lethal sampling, the utility of the target data and samples for conservation and management, the availability/feasibility of non-lethal techniques to obtain the same samples, as well as the status of the stock (s) should be considered.

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Tanabe, A., Shimizu, R., Osawa, Y., Suzuki, M., Ito, S., Goto, M., Pastene, L.A., Fujise, Y. and Sahara, H. 2020. Age estimation by DNA methylation in the Antarctic minke whale. *Fish Sci* 86: 35–41.

International meetings

Participation of scientists from the Institute of Cetacean Research in International Meetings in 2020

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 subsidiary bodies of the IWC is the Scientific Committee (SC), which meets annually.

Originally, the 2020 meeting of the IWC SC was planned for Cambridge, United Kingdom, between 12 and 24 May. The in-person meeting was cancelled due to the Covid-19 pandemic. Instead, the meeting was carried out through a series of virtual sessions in the same period. A total of nine scientists from the ICR participated in the virtual meetings (Fujise, Kato, Pastene, Tamura, Matsuoka, Hakamada, Taguchi, Isoda and Katsumata). They presented a total of 11 documents: six documents at the Standing Working Group on Abundance Estimates, Stock Status and International Cruises (ASI), three as Intersessional Steering Group (ISG) reports, one at the *ad hoc* Working Group on Photo-ID (PH), and one at the Sub-Committee on Conservation Management Plans (CMP).

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

In 2020, ICR scientists also participated in the Meeting of the IWC-POWER Technical Advisory Group (TAG) and the 2020 IWC-POWER Planning Meeting, held at the Fisheries Agency of Japan (FAJ)'s Crew House, Tokyo, Japan from 18 to 21 January. On 20 April, a virtual meeting was held to discuss the effect of the Covid-19 pandemic on the logistical aspects of the 2020 IWC-POWER survey. Kato, Pastene, Matsuoka, Hakamada, Taguchi and Takahashi from the ICR participated in these meetings.

The report of these meetings can be found on the website of the IWC (https://iwc.int/home).

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

The Convention on the Conservation of Antarctic Marine Living Resources (CCAMLR) is part of the Antarctic Treaty System. The Convention was opened for signature on 1 August 1980 and entered into force on 7 April 1982, thereby establishing the Commission for the Conservation of Antarctic Marine Living Resources. Its 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 2020 CCAMLR EMM meeting was planned originally for Kochi, India, between 6 and 17 July. The in-person meeting was cancelled due to the Covid-19 pandemic. Instead, an e-mail group held virtual discussions between 6 and 13 July, on three main topics: Risk Assessment Approach, Krill Management Approach, and Research and Monitoring Plan. One scientist from the ICR participated in the meeting (Pastene).

The report of the e-mail group discussion can be found on the website of the CCAMLR (https://www.ccamlr.org/).

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

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

The 2020 meeting of the PICES was originally planned for Qingdao, China, between 22 October and 1 November. The in-person meeting was cancelled due to the Covid-19 pandemic. Instead, a business meeting of the Section on Marine Bird and Mammals (S-MBM) was carried out virtually on 16–17 October. One scientist from the ICR participated in the meeting (Tamura). He presented the document 'Estimation of prey consumption by marine mammals in the PICES regions—Update of Hunt *et al.* (2000)—'. The business meeting discussed research projects for the next five years.

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

Peer-reviewed publications

List of peer-reviewed publications based on the Institute of Cetacean Research (ICR)'s surveys up to 2020

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

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

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

JARPA/JARPAII/NEWREP-A surveys

1989 (2)

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

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

1990 (8)

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

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

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

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

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

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

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

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

1991 (9)

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

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

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

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

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

Kato, H., Zenitani, R. and Nakamura, T. 1991. Inter-reader calibration in age readings of earplugs from southern minke whale, with some notes of age readability. *Rep. int. Whal. Commn* 41: 339–343.

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

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

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

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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.

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

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

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

1994 (3)

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

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

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1995 (3)

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

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

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1996 (7)

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1997 (3)

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

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