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What do we know about whales and ecosystem in the Indo-Pacific region of the Antarctic? Part 3: population genetic structure of large baleen whales other than Antarctic minke whales

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ABSTRACT

The Institute of Cetacean Research (ICR) 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 Japan's decision to withdraw from the International Convention for the Regulation of Whaling. Japan's whale research continues in the Antarctic, using non-lethal methods only. This paper is a continuation of the series of reports on research contribution in the Antarctic by the ICR. This time, the topic is on stock structure of large baleen whale species other than Antarctic minke whales, in the Indo-Pacific sector of the Antarctic. Genetic analyses, which were based on the large biopsy sample collection of the ICR in the Antarctic, have provided important information on the stock structure and movement of baleen whales, including blue, fin, humpback and southern right whales in the Indo-Pacific sector.

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, 2015a) discussed and evaluated the large amount of data and results from these research programs.

As a consequence of Japan's change in whaling policy, the NEWREP-A ceased on 30 June 2019, the date of Japan's 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.*, 2020).

At this point, it was considered important to summarize the knowledge on whales and the Antarctic ecosystem accumulated so far by Japan's whale research in the Antarctic. This paper is a continuation of the series of reports on research contribution in the Antarctic by the ICR. This time the topic is stock structure of large baleen whale species other than Antarctic minke whales, in the Indo-Pacific sector of the Antarctic. Genetic analyses have been possible based on the large biopsy sample collection of the ICR in the Antarctic, which is one of the largest in the world. Biopsy samples were obtained during the surveys of the JARPA/JARPAII and NEWREP-A but also during the surveys of the International Whaling Commission (IWC)'s International Decade for Cetacean Research (IDCR) and Southern Ocean Whale and Ecosystem Research (SOWER) programs.

This paper is not exhaustive of the information on stock structure in Antarctic large whales. More detailed results will be presented by species in future issues of TEREP-ICR.

COLLECTION OF GENETIC SAMPLES

Biopsy sampling systems

Genetic analyses have been carried out based on biopsy samples collected by different biopsy sampling systems during systematic sighting surveys. These systems, which

Table 1

Number of biopsy samples collected by the Institute of Cetacean Research in the Indo-Pacific sector of the Antarctic between 1993/94 and 2014/15 during JARPA and JARPAII surveys.

Season	Blue				Fin				Humpback				Southern right			A. minke			Total
	III	IV	V	VI	III	IV	V	VI	III	IV	V	VI	III	IV	V	III	IV	V	
1993/94	-	-	4	-	-	-	-	-	-	20	-	-	-	5	-	-	-	-	29
1994/95	-	-	-	-	-	-	-	-	-	-	12	-	-	-	-	-	-	8	20
1995/96	1	-	-	-	-	-	-	-	2	8	-	-	-	1	-	-	-	-	12
1996/97	-	-	-	1	-	-	-	-	-	-	5	15	-	-	-	-	-	-	21
1997/98	-	1	-	-	-	-	-	-	5	19	-	-	-	4	-	1	-	-	30
1998/99	-	-	2	-	-	-	3	-	-	-	22	1	-	-	-	-	-	-	28
1999/00	1	3	-	-	-	-	-	-	10	32	-	-	-	3	-	-	10	-	59
2000/01	-	-	3	-	-	-	9	-	-	-	14	22	-	-	-	-	-	-	48
2001/02	-	1	-	-	-	4	-	-	12	14	-	-	2	14	-	-	-	-	47
2002/03	-	-	-	-	-	-	6	-	-	-	10	-	-	-	2	-	-	1	19
2003/04	2	3	-	-	4	-	-	-	27	31	-	-	-	4	-	-	-	-	71
2004/05	-	-	-	-	-	-	-	2	-	-	28	8	-	-	1	-	-	-	39
2005/06	-	5	-	-	-	9	-	-	1	6	-	-	-	15	-	-	-	-	36
2006/06	-	-	1	1	-	-	3	-	-	-	11	2	-	-	-	-	-	-	18
2007/08	4	2	-	-	-	-	-	-	1	3	-	-	-	16	2	-	-	-	28
2008/09	-	-	-	-	-	-	-	-	-	-	13	1	-	-	-	-	-	-	14
2009/10	-	-	-	-	1	-	-	-	12	26	38	-	-	1	-	-	-	-	78
2011/12	-	-	-	-	-	-	-	-	-	-	1	-	-	-	3	-	-	-	4
2012/13	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	3
2014/15	-	3	-	-	-	9	-	-	-	10	-	-	-	39	-	-	-	-	61
Total	8	18	10	2	5	22	21	2	73	169	154	49	2	102	8	1	10	9	665

Figure 1. Biopsy sampling using the Larsen system (left) and a biopsy sample from a blue whale (right).

will be described in details in future TEREP-ICR issues, include the ICR air gun system (Kasamatsu *et al.*, 1991) used between 1992/93 and 2001/02, a crossbow system used between 2002/03 and 2013/14, and a Larsen system (Larsen, 1998) (Figure 1) used from 2014/15 to the present day.

Number of biopsy samples

Surveys conducted by the ICR in the Indo-Pacific sector of the Antarctic collected a total of 665 biopsy samples between 1993/94 and 2014/15, 38 from Antarctic blue whales, 50 from fin whales, 445 from humpback whales, 112 from southern right whales, and 20 from Antarctic minke whales (Table 1). All samples collected were preserved onboard at −20°C until they were used at the ICR

genetic laboratory. An example of biopsy sample is shown in Figure 1.

OUTLINE OF THE LABORATORY WORK

The laboratory work for genetic analyses in all case studies involved the following steps: extractions of total genomic DNA, molecular sex determination, sequencing of a portion of the mitochondrial DNA (mtDNA) control region and genotyping with a set of microsatellite DNA (msDNA) loci. Technical details of the laboratory work can be found in Pastene and Goto (2016). A brief summary is presented here based on that study.

Sampled skin tissues were preserved frozen at −20°C until use. Total genomic DNA was extracted from 0.05 g of skin tissue using either the standard phenol-chloroform method or the Gentra Puregene kits (QIAGEN). Extracted DNA was stored in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

The SRY locus located on the Y chromosome was also used for sex determination following the method of Abe *et al.* (2001) with a slight modification. With the combination of loci of SRY and a microsatellite locus as a positive control, males show amplified products of both SRY and a microsatellite locus, while females show only a microsatellite locus.

An approximate 500 base pairs (bp) of the mtDNA control region were amplified by the polymerase chain reaction (PCR) using a set of primers available at the ICR. PCR products were purified using MicroSpin S-400HR columns (Pharmacia Biotech). Cycle sequencing was performed using BigDye terminator cycle sequence Kit (Applied Biosystems) and the PCR primers, following the protocols of the manufacturer. The cycle sequencing products were purified using AutoSeq G-50 spin Columns (Pharmacia Biotech). The labeled sequencing fragments were resolved using an ABI PRISM 377 or an ABI3500 Genetic Analyzers (Applied Biosystems) (Figure 2).

The samples were also genotyped at a number of msDNA loci, which varies between 14 and 17 depending on the species, in multiplex fluorescent PCRs. PCR products were electrophoresed on an ABI3500 DNA Analyzer (Applied Biosystems) (Figure 2), and allele sizes were determined using a 600 LIZ size standard (Applied Biosystems) and GeneMapper v. 4.0 (Applied Biosystems).

ANALYTICAL PROCEDURES

The analytical procedures for genetic analyses on stock structure are those used routinely in these kinds of studies, and they are mentioned briefly below, together with the progress in research.

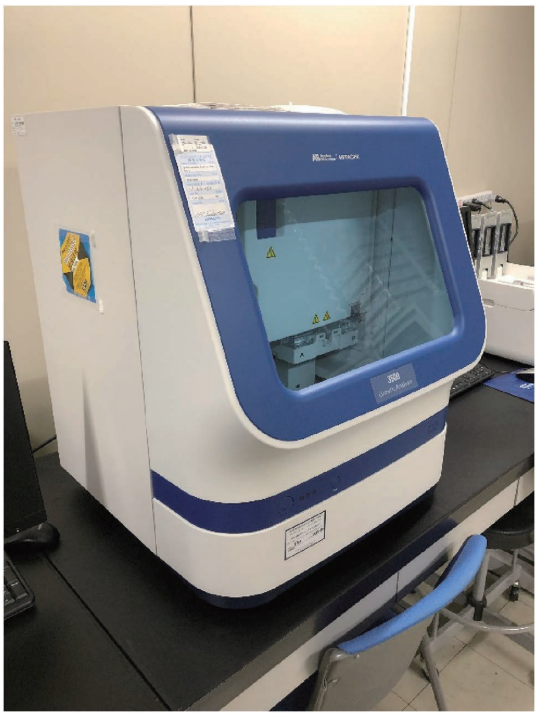

Figure 2. ABI3500 DNA Analyzer (Applied Biosystems) available at the ICR genetic laboratory.

PROGRESS IN RESEARCH

The summary of research progress on stock structure for some large whale species below are based on Pastene (in press).

Blue whales

ICR scientists have collaborated with foreign scientists in the study of population genetics of blue whales.

One example is the genetic study by LeDuc *et al.* (2007), who used samples from the Antarctic (n=30 from IDCR/SOWER cruises and n=17 from JARPA), Chile (n=16 from IDCR/ SOWER), the southern and western coasts of Australia (n=28 from IDCR/SOWER), around the Maldives (n=6 from IDCR/SOWER) and Peruvian and Ecuadorian waters (n=12 from US SWFSC research cruises). Analysis focused on investigating the pattern of genetic variation in Southern Hemisphere blue whales and the use of an assignment test to detect mixing on the feeding grounds. Genetic markers used were mtDNA control region sequences and seven microsatellite loci. Strong genetic differences were found among samples from the southeast Pacific Ocean, Indian Ocean and around the Antarctic continent. Genetic differentiation between the geographical ranges of the nominal sub-species (i.e., Antarctic vs pygmy blues in the Pacific and Indian Oceans) was not markedly greater than between populations of pygmy blue whales.

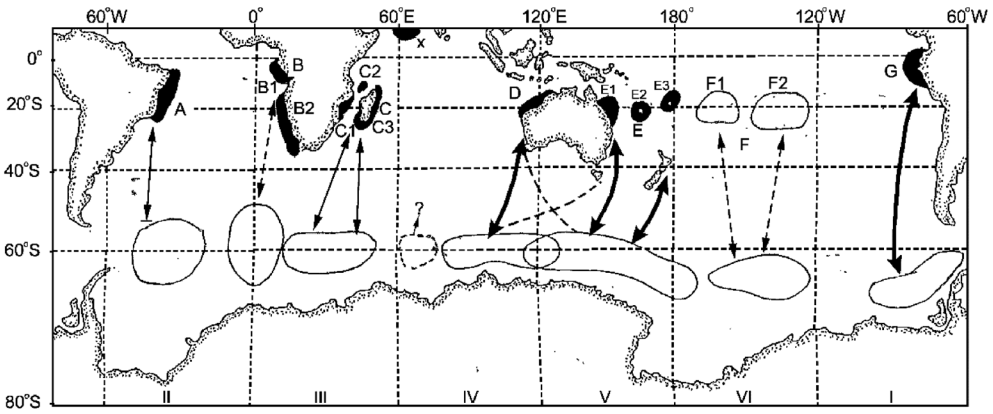

Figure 3. Hypothetical stock structure for Southern Hemisphere humpback whales. The areas and sub-areas identified reflect approximate, rather than necessarily exact, boundaries. A dotted line represents hypothetical connection, thin lines represent a small number of documented connections between areas while thick lines represent a large number of documented connections between areas (from IWC, 2005).

Fin whales

Genetic analyses on stock structure of fin whales in the Antarctic feeding grounds are very scarce. One of the few studies was conducted by Goto and Taguchi (2019).

These authors examined population genetic structure in IWC Management Areas III, IV, V and VIW based on mtDNA control region sequences (478 bp) and sixteen microsatellite loci. The analysis was based on biopsy samples collected by the JARPA and IDCR/SOWER cruises, and samples from takes during JARPAII. The analyses were conducted on the basis of three arbitrary sectors: POP1 (0°–70°E, *n*=39), POP2 (70°E–160°E, *n*=48) and POP3 (160°E–145°W, *n*=18). The study showed a high mtDNA diversity, which was comparable among groups. Genetic analyses based on heterogeneity test (mtDNA and msDNA) and STRUCTURE, PCA and test for Hardy Weinberg deviation (msDNA) failed to find evidences of stock structure in the research area. A phylogenetic analysis based on mtDNA haplotype showed no relationship between clusters and particular geographical group. The authors noted that the sample sizes were small and that the results could be due to a low power of the statistical analyses.

Humpback whales

The IWC Scientific Committee (SC) has described hypothetical stock structure and migratory corridors for Southern Hemisphere humpback whales based mainly on information such as Discovery marks, photo-identification, genetics and satellite tracks (IWC, 2005). Seven Breeding Stocks (BS) are recognised, 'A'–'G.' Some ('B,' 'C,' 'E' and 'F') were further subdivided into sub-stocks (Figure 3). The IWC SC completed the assessment of most stocks at its 2014 meeting (IWC, 2015b). For the assessment, the information on stock structure summarised below contributed to the interpretation of stock abundance and trend as well as for allocating historical catches to the various stocks.

Pastene *et al.* (2006) examined the population genetic structure in IWC Management Areas IIIE, IV, V and VI based on mtDNA control region sequences and six microsatellite loci. The analysis was based on biopsy samples collected by the JARPA and IDCR/SOWER cruises, namely: *n*=81 for Area IIIE (JARPA: 50; IDCR/SOWER: 31); *n*=172 for Area IV (JARPA: 126; IDCR/SOWER: 46); *n*=97 for Area V (JARPA: 90; IDCR/SOWER: 7); and *n*=61 for Area VI (JARPA: 44; IDCR/SOWER: 17). The analysis confirmed the high level of genetic diversity for both mtDNA and microsatellites. Both genetic markers suggested differentiation among these four Areas, with the differences being stronger for females than males. The authors did not reject the possibility of some mixing of populations on the borders of the Management Areas.

Kanda *et al.* (2014) examined the population genetic structure in IWC Management Areas IIIE, IV, V and VI based on genotypes from 14 microsatellite loci. The analysis was based on JARPA and IDCR/SOWER cruise samples: *n*=93 for Area IIIE (JARPA: 62; IDCR/SOWER: 31); *n*=218 for Area IV (JARPA: 172; IDCR/SOWER: 46); *n*=153 for Area V (JARPA: 146; IDCR/SOWER: 7); *n*=64 for Area VI (JARPA: 47; IDCR/SOWER: 17). Major genetic differences were attributed to samples from different Areas. Stronger differentiation was seen in females than in males. Despite the increase of the number of loci from six in the previous analysis to 14, the level of stock differentiation was still too low for analysis at the individual level.

Pastene *et al.* (2013) used biopsy samples obtained by JARPA/JARPAII and IDCR/SOWER surveys and mtDNA

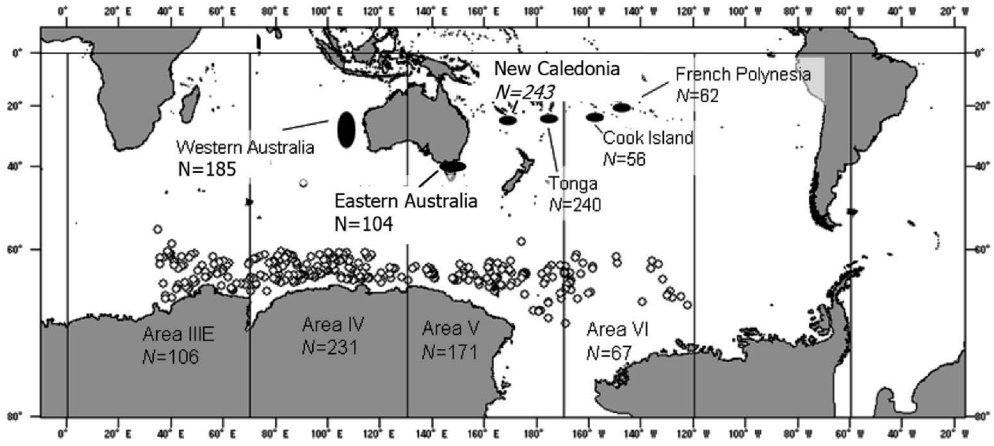

Figure 4. Geographical distribution of humpback whale samples from breeding and feeding grounds analyzed in Pastene *et al.* (2013).

control region sequences to study the distribution and mixing rates of breeding stocks BSD, BSE and BSF in Antarctic Areas IIIE–VI (Figure 4). Breeding ground samples were from Western Australia (n=185), Eastern Australia (n=104), New Caledonia (n=243), Tonga (n=240), Cook Islands (n=56) and French Polynesia (n=62). They were collected by several groups and research institutions and were provided for analysis under the IWC SC Data Availability Protocol. JARPA and IDCR/SOWER survey samples were provided as outlined above (Pastene *et al.*, 2006). According to one of the hypotheses on baseline populations, Western Australian whales were distributed mainly in Management Areas IVW (84.5% SE: 0.043; F_{ST}: 0.0015) and IVE (75.9% SE: 0.092; F_{ST}: −0.0015); whales from Eastern Australia in Area VW (64.7% SE: 0.074; F_{ST}: 0.0013); whales from New Caledonia in Area VE (83.9% SE: 0.011; F_{ST}: 0.0062), and whales from Tonga in Area VI (43.3% SE: 0.015; F_{ST}: 0.0010). Whales from Cook Islands and French Polynesia were not represented on feeding ground Areas IIIE–VI.

Southern right whales

Two genetic studies based on biopsy samples collected by the ICR have been conducted (Pastene *et al.*, 2018; *in-press*). These studies were based on 157 biopsy samples collected mainly in the Indian sector of the Antarctic. Both mtDNA control region sequencing (381 bp) and msDNA at 14 loci were used in the analyses. The mtDNA analysis suggested that whales in the Antarctic Indian sector in summer are closely related to the South West Australian breeding grounds (Pastene *et al.*, 2018). The msDNA revealed eight individual matches (four males and four females), which demonstrated that at least some males and females returned to the same feeding grounds over the years (Pastene *et al.*, in press). The matching occurred in an area where visual surveys showed aggregations of whales associated with high krill concentrations.

SUMMARY

The ICR, through its former whale research programs in the Antarctic (JARPA, JARPAII), collected a large number of biopsy samples from different baleen whale species. In fact, the set of biopsy samples from the Antarctic held by the ICR is the largest in the world. These samples were used in genetic analyses on phylogeny, stock structure and individual matching in some baleen whale species that are key components of the Antarctic ecosystem. Results of these analyses have been important for the assessment of these species conducted by the IWC SC. In the case of blue whales, the analyses contributed to understand the genetic differentiation between Antarctic and pygmy blue whales. The ICR started the genetic analysis of fin whales in the Antarctic feeding grounds. However it seems that the sample sizes are still small to allow the detection of stocks. The genetic analyses of biopsy samples of humpback whales contributed to understand the genetic diversity and degree of differentiation of the species in the feeding grounds of Areas IIIE-VI and the relationship between breeding and feeding grounds of several stocks. At least four stocks were confirmed in the Indo-Pacific sector of the Antarctic, which mix spatially in some sectors. In the case of southern right whales, genetic analysis suggested that whales in the Indo-Pacific sector of the Antarctic belong to the same stock as Western South Australia and that some whales return to the same feeding grounds over the years. More refined analyses will be conducted on these species with additional biopsy samples collected by NEWREP-A and the ongoing JASS-A program.

ACKNOWLEDGEMENTS

We thank the captain, crew members and researchers involved in the surveys of JARPA, JARPAII and IDCR/SOWER for their effort in collecting biopsy samples from different baleen whale species. The genetic analyses on stock structure summarized in this paper are based on those samples.

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