OVERVIEW OF OTHER STUDIES ON LARGE WHALES BASED ON JARPA SAMPLES

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ABSTRACT

JARPA collected systematically a comprehensive data/sample set that allowed the conduction of studies related with the four objectives of the program. Samples collected by both lethal and non-lethal components of JARPA have been also used in several studies with objectives different from the four objectives of the program. In this paper an overview of these studies are conducted. They have contributed to the whale knowledge mainly in three areas: reproductive physiology of Antarctic minke whale, phylogeny of large whales and distribution and movement of baleen whales.

INTRODUCTION

Samples and data obtained systematically during the Japanese Whale Research Program under Special Permit in the Antarctic (JARPA) have been collected mainly with the purpose to conduct studies related with the four objectives of the JARPA (SC/D06/J1). Samples and data collected by both lethal and non-lethal components of the JARPA have been also used in several studies with objectives different from the four objectives of the program.

These 'additional' studies have been reported annually to the Scientific Committee meeting through the annual report of the Institute of Cetacean Research (ICR) research activities (RAICR). Details of the studies can be found in the original papers listed in the web page http://www.icrwhale.org/JARPAReview1.htm, and in the references listed below.

The objective of this paper is to conduct an overview of the studies conducted using JARPA sample/data, which are not related to the main objectives of the program. The overview is focused mainly to three areas of research contribution: reproductive physiology of Antarctic minke whale, phylogeny of large whales and movement and distribution of baleen whales. It should be noted that in some of these studies JARPA sample/data have been a part of large data sets used.

REPRODUCTIVE PHYSIOLOGY OF ANTARCTIC MINKE WHALES

These studies have been conducted in cooperation between ICR and Obihiro University of Agriculture and Veterinary Medicine in different steps since 1994. The first step in the study of reproductive physiology in the Antarctic minke whales involved the following topics: i) studies on relationship between appearance of preantral follicles in the fetal ovary and hormone concentrations in the fetal heart, umbilical cord, and maternal blood, ii) studies on hormonal status in blood, ovary, and pituitary gland in immature and mature whales, and iii) studies on differentiation, function and morphology in testis. The second step involved the following topics: i) studies on in vitro maturation, fertilization by micro-manipulation, and embryonic development of frozen-thawed or vitrified immature oocytes, ii) studies on sperm analyses and freezing, iii) basic studies on somatic cell cloning, iv) studies on function and morphology of placenta in pregnant whales, and v) studies on milk constituents analyses.

In the third step, the following topics were addressed: i) developmental capacity of vitrified oocytes followed by in vitro maturation, and parthenogenetic activation or in vitro fertilization by intracytoplasmic sperm injection, ii) the ability of whale haploid spermatogenic cells for inducing calcium oscillations and activating oocytes, iii) contribution of spaermatozoal centrosomes to microtubule-organizing center in oocytes, iv) histological studies on the placenta and uterus in, v)

studies on composition analysis and utilization as functional materials of sphingoid base lipids from skin.

The research team successfully cryopreserved spermatozoa of Antarctic minke whales in 1994. Relationship among physiological status of whales, serum steroid hormones and morphology of follicular oocytes and spermatozoa was also studied (Fukui *et al.* 1995, 1996, 2004, Iga *et al.* 1996, Mogoe *et al.* 1998a, 1998b, 2000). After consideration on in vitro maturing (IVM) of follicular oocytes of Antarctic minke whales, they succeeded in vitro fertilization (IVF) of follicular oocytes of Antarctic minke whales in 1996. Factors affecting each step of artificial fertilization were investigated and technique on cryopreservation of spermatozoa, IVM, IVF and development of oocytes have been improved (Fukui *et al.* 1997a, 1997b, Asada *et al.* 2000, 2001a, Fujihira *et al.* 2004, 2006, Amemiya *et al.* 2004, Fukui *et al.* 2004, Iwayama *et al.* 2004). The research team recently succeeded intracytoplasmic sperm injection (ICSI) and interspecies somatic cell nuclear transfer (SCNT) of Antarctic minke whales (Asada *et al.* 2001b, Ikumi *et al.* 2004) and basic study to produce cloned whale embryos is also ongoing (Kobayashi *et al.* 2006).

The research team also proceeds with other reproductive studies in the Antarctic minke whale, such as plasma and pituitary concentration of gonadotropins (FSH and LH) (Suzuki *et al.* 2001), chemical characterization of the oligosaccharides in milk (Urashima *et al.* 2002), morphology and development of ovary (Tetsuka *et al.* 2004), relationship between preantral follicles in the fetal ovary and hormone concentrations in fetal and maternal blood (Muranishi *et al.* 2004).

PHYLOGENY OF LARGE WHALES

JARPA collected a number of biopsy samples from large whale species as follow: humpback whale: 342, right whale: 36, blue whale: 22; fin whale: 28 and sei whale: 1. At the laboratory all these samples were examined genetically to obtain mitochondrial DNA (mtDNA) control region sequences and some of them (humpback and blue whales) were examined with a set of microsatellites. Sex determination has been possible using a method developed at ICR (Abe *et al.* 2001).

Genetic data collected from the humpback whale were used in a genetic study on stock structure in the feeding ground (SC/D06/J31); mtDNA sequences collected from the fin whale were used in a genetic study focused to understand the phylogenetic relationships among fin whales from different ocean basins (Goto *et al.*, 2003), which was discussed during the 2003 Scientific Committee meeting.

DNA data obtained from biopsy samples in these baleen whales as well in minke whales sampled during JARPA were used in several genetic studies focused to understand the phylogenetic relationships among large whales as follow:

On blue whales, two collaborative genetic researches were conducted. The first was a collaboration between ICR and the Southwest Fisheries Science Center, USA. The main purpose of the study was to study the genetic differentiation between pygmy and true blue whales in the Southern Hemisphere as well among populations of the pygmy blue whales, using two genetic markers, mtDNA control region sequences and microsatellites (LeDuc *et al.*, in press). The second collaborative research is being conducted between ICR and the University of California at Davis, USA. The objective of the study is the delineation of biological stocks of blue whale worldwide using nuclear genetic markers (introns). In both projects DNA data of blue whales from the Antarctic were from JARPA.

Regarding right whales a collaborative genetic research between the American Museum of Natural History and several institutions included ICR was conducted to study taxonomy and phylogenetic relationships of this species worldwide (Rosenbaum *et al.*, 2000). MtDNA sequences of the southern right whale used in this study were from JARPA.

DNA data of several baleen whale species from JARPA were used in a genetic study focused to understand positive selection in the major histocompatibility complex (MHC) variation of the cetacean species (Hayashi *et al.*, 2003). MHC is a large multigene coding glycoprotein, which plays a key role in the initiation of immune responses in vertebrates.

DNA data of several baleen whale species from JARPA were used in a genetic study focused to determine the coding region of the SRY (sex determining region on the Y chromosome) gene and its

predicted promoter region in 20 species of cetacean. The study was also focused to construct a gene tree using the SRY sequence and its predicted promoter region to determine whether the cetacean phylogeny could be explained from the standpoint of a strictly paternal mode of inheritance (Nishida *et al*., 2003).

DNA data of several baleen whale species from JARPA were used to investigate the phylogeny and evolution of baleen whales based on the complete mitochondrial genome sequence (Sasaki *et al*., 2005) as well short interspersed repetitive elements (SINE) (Nikaido *et al*., 2006).

MOVEMENT AND DISTRIBUTION OF BALEEN WHALES

The ICR has constructed a photo-ID catalog of large baleen whale species based on the most informative pictures taken during the JARPA surveys. The ICR photo-ID catalog involves the following number of pictures: humpback whale: 502, blue whale: 153, right whale: 243. All photographs of humpback whales have been submitted to the Centralized Antarctic Humpback Whale Catalogue supported by the IWC and managed by the College of the Atlantic. Photo-ID data have been used in several studies on distribution and movement of large baleen whales as follow:

Regarding right whales, analyses on photo-ID pictures are conducted in collaboration between ICR and the Western Australian Museum, Australia. A study demonstrated movement of one animal between Adelaide, Australia and Antarctic Area IV (Bannister *et al*., 1999).

Regarding humpback whale, analyses on photo-ID pictures were conducted with the purpose to investigate movement and distribution of this species in the Antarctic feeding grounds as well between the Antarctic and low latitude areas. Rock *et al.* (in press) demonstrated movement of three individuals between Eastern Australia and Antarctic Area V. Also one animal was identified in different years in a similar longitudinal sector of Area VI.

OTHER

Sighting and distribution

Matsuoka *et al.* (1996) reported a sighting of a large school of the pygmy right whale in the southeastern Indian Ocean. The sighting was made by JARPA vessels during transit to the Antarctic research area in December 1992. Kimura *et al*. (2005) used satellite derived ocean parameters and the whale sighting location data collected under the JARPA to elucidate prevalent ocean condition where whale sighting was undertaken, and to understand its influence on the distribution of the sei whales.

Lipids composition

Several studies were focused to study the fatty acid composition of blubber oils of the Antarctic as well of the dwarf minke whales (Itoh, 1994; Itoh *et al*., 1990; 1993). A study focused specifically to understand the site and sexual differences of muscle lipids in the Antarctic minke whale (Itoh *et al*., 1998).

Contribution of biological materials to museums

JARPA has collected biological samples mainly for scientific research purposes. Some biological materials have been also collected and provided to museums, aquarium and university through Japan for exhibition purposes. JARPA biological materials (especially Antarctic minke whale skeletons) have been provided to at least to 19 museums, aquariums or universities.

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