Annex N

Report of the Working Group on DNA

Members: Pastene (Chair), Baird, Bickham, Cipriano, Gaggiotti, Hoelzel, Jackson, Lang, Øien, Palsbøll, Park, Skaug, Solvang, Tiedemann, Víkingsson, Waples, Yoshida.

1. ELECTION OF CHAIR

Pastene convened and chaired the Group.

2. APPOINTMENT OF RAPPORTEURS

Pastene acted as rapporteur.

3. ADOPTION OF AGENDA

The adopted agenda is given as Appendix 1. Items 5, 6, 7 and 8 of the Agenda are in response to requirements placed on the Scientific Committee by IWC Resolution 1999-8 (IWC, 2000), which called for annual reports on progress in the following areas.

(1) Genetic methods for species, stocks and individual identification.
(2) Collection and archiving of tissue samples from catches and bycatch.
(3) Status of and conditions for access to reference databases of DNA sequences or microsatellite profiles derived from directed catches, bycatch, frozen stockpiles and products impounded or seized because of suspected infractions.

Scientists from countries that made a statement at plenary that it was inappropriate for the SC to continue the review of the JARPA II program did not participate in the discussion of contents of papers related to JARPA II (see Item 2 of the main Scientific Committee report). These include members who have previously participated in discussions of contents of papers related to JARPA II. Therefore, it should be noted that the discussions in this report do not include the views of those members of the Scientific Committee, and therefore they may not agree with any conclusions reached.

4. REVIEW OF DOCUMENTS

Documents SC/65b/DNA01, SC/65b/BRG04, SC/65b/BRG09 and SC/65b/BRG13 were relevant for the Working Group.

5. PROGRESS ON GENETIC METHODS FOR SPECIES, STOCK AND INDIVIDUAL IDENTIFICATION

SC/65b/DNA01 was prepared in response to a recommendation from the JARPA II Review Workshop that a revised paper be submitted that explains in more detail how far the IWC guidelines for DNA data quality control were able to be followed (SC/65b/Rep2). SC/65b/DNA01 presented a full description of the protocol used by the Institute of Cetacean Research for the genetic analyses in the context of the IWC guidelines. The Group **welcomed** this document and **agreed** that it responded appropriately to the recommendation from the JARPA II Review Workshop. One member suggested that information on error rates and data consistency would be a useful addition.

SC/65b/BRG04 presented an estimate of the genome size of the bowhead whale using flow cytometry. The mean genome size (C value) was estimated to be 2.93 picograms (2.87 gigabases, Gb). There was a 3% difference in C value between the male and female bowhead which is consistent with the expected size difference resulting from the different sizes of the X and Y chromosomes. This was the first direct genome size estimate for a baleen whale, and is the lowest value reported for any cetacean. It is near the low end of values reported for cetartiodactyls and is relatively low for mammals. Most mammals possessing lower genome sizes are small mammals with high metabolic rates. The total length of the bowhead genome sequence is 2.3Gb, which is approximately 21% less than the estimate based on flow cytometry. This discrepancy is likely the result of highly repetitive DNA which cannot be assembled effectively with current methods in the genome sequence. Genome size correlates with cell size, and influences physiological processes such as metabolic rate and O2 exchange as small cells have higher surface area to volume ratios than large cells. It is also positively correlated with body size within some groups of mammals. The relatively small size of the genome of bowheads could be associated with metabolic rate, O2 exchange, or simply a plesiomorphic trait shared in common with other basal cetartiodactyls.

In discussion it was noted that genome size estimates based upon genome sequence is available for two additional baleen whales, humpback and common minke whales, and the sizes are similar to that of the bowhead whale reported in SC/65b/BRG04. It was noted that the two suggested explanations on small genome size in bowhead whale are not mutually exclusive, i.e., it can be at the same time plesiomorphic and adaptive. It was also noted that differences between directly estimated (from flow cytometry) and indirectly estimated (from sequence) genome size are considered primarily due to removal of highly repetitive sequences not included in sequence-based estimates.

SC/65b/BRG09 reported progress on the transcriptome sequence of the bowhead whale. This study compared two methods of RNA sequence to characterise the bowhead whale transcriptome including polyA RNA isolation and RiboZero which does not involve the capture of a RNA molecule by the end of its 3' tail. The latter method allowed the sequencing of somewhat degraded RNA samples as well as very long transcripts not possible with RNA derived from the polyA capture technique. The study sequenced the transcriptome from pituitary gland, adolescent testis, vibrissa follicle, mesenteric lymph node, and spleen using the RiboZero protocol and the heart, cerebellum, liver, adolescent testis, and retina from 2011 using the polyA protocol for a total of 51,637,573,518 bp of sequence. The data are being explored for the discovery of SNP loci for population genetic studies. A parallel project to sequence the bowhead genome is ongoing by collaborators and the bowhead transcriptome was used to annotate the genome sequence. A joint publication of the transcriptome and genome is planned and a web site will be created to give public access to these databases.

The Working Group **commended** the large amount of work and valuable data being produced in this study. It was noted that SNP discovery using genome and transcriptome sequence has some advantages over the use of anonymous loci, but that there are also pitfalls with this approach.

It was noted that transcriptome analysis on non-normalised RNA are biased toward highly expressed genes, while rare transcripts might go undetected. It was also noted that the approach used for SNP identification based on transcriptomes focusses exclusively on transcribed regions. Therefore, while such a strategy is well suited to identify potentially adaptive genetic variation, caution is required as to the assumption that all detected variation is selectively neutral. This assumption is typically (implicitly or explicitly) made, when genetic variation is used for population/stock structure analysis. It was further noted that it could be interesting to look for the representation of transcripts in the framework of gene ontology, in order to reveal whether certain gene functions are over-/underrepresented in the transcriptome of certain species and/or tissues.

SC/65b/BRG13 summarised results from the 2013 bowhead whale genetics project. The project had two parts, development of an mtDNA database and identification of SNP loci including the development of a SNP database. It was reported that three mtDNA loci are used: HVR1, cyt-b and ND1. This database now contains data from 650 whales, with various levels of completion of sequencing for the three loci. A total of 570 whales have been sequenced for HVR1, 480 for ND1, and 389 for cyt-b. A total of 301 of the 650 whales have been sequenced for all three loci. Appendix I of SC/65b/BRG13 contains information on all of the contigs analysed from the bowhead whale transcriptome for the presence of SNPs. It was reported that the number of SNP loci being used has increased: a total of 155 SNP loci identified for bowhead whale, including 99 new SNPs reported in SC/65b/BRG13, 14 sex-specific SNPs reported in Bickham *et al.* (2013unpublished) and 42 from the literature (Morin *et al.*, 2010). It was reported that the data in these databases will continue to be used for monitoring stock structure, population size estimates, and estimates of historical demography. Work is ongoing to fill the gaps in the mtDNA database and adding additional samples to it. Also the authors started genotyping whales for SNP the loci identified.

The Working Group again **commended** the amount of work involved in this study. In response to a question it was clarified that the transcriptome upon which the SNP loci were identified was based on multiple individuals (all from the B-C-B stock), multiple sexes, and multiple tissues. The Working Group advised that the authors should keep track of the individuals that the SNPs came from.

It was noted that using only B-C-B bowhead samples for SNP development will introduce an ascertainment bias, if these SNPs are later to be used for other populations. The authors responded that there is a transcriptome being developed from Greenland whales, and therefore they should be able to design SNPs from multiple populations to mitigate the effects of ascertainment bias.

It was also noted that SNPs located in the same contig are genetically linked, and best practice uses only a single SNP from each contig to avoid associated problems. In response the authors reported that not all SNP loci discovered would be used in the final database, and that using the transcriptome for discovery of SNP loci will allow investigation of exactly how far apart the SNP loci are in the genome, which can help to reduce the possibility of choosing linked loci.

6. REVIEW RESULTS OF THE 'AMENDMENTS' OF SEQUENCES DEPOSITED IN *GENBANK*

Last year the Committee agreed that the list of accession numbers involving inconsistencies due to a lag in the taxonomy recognised by *GenBank* or uncertainty in taxonomic distinctions currently under investigation (IWC, 2014b) should be sent to *GenBank* with a letter explaining the background and the main reasons for the inconsistencies (IWC, 2014a, p.56).

Cipriano informed the Group that he had sent the list and letter to *GenBank* during the intersessional period. A positive response was received informing that *GenBank* is willing to work with the IWC on this particular problem, and requested further explanations on the list received on accession numbers associated with problematic taxonomic designations. The Group **agreed** that Cipriano should keep in contact with *GenBank* in the next intersessional period to facilitate the work by *GenBank* staff on the correction of the inconsistencies based on the list sent.

The DNA Working Group also suggested further discussion by the IWC *ad hoc* taxonomy group on the issues regarding new species descriptions and taxonomy of baleen whales.

7. PROGRESS ON COLLECTION AND ARCHIVING OF SAMPLES FROM CATCHES AND BYCATCHES

The Committee previously endorsed a new standard format for the updates of national DNA registers to assist with the review of such updates (IWC, 2012, p.53), and the new format worked well the last two years. This year the update of the DNA registers by Japan, Norway and Iceland were based again on this new format.

Yoshida reported on the status of the Japanese register (see Appendix 2). The collection of samples is from scientific whaling in the North Pacific (JARPN-JARPN II) and the Antarctic (JARPA-JARPA II), and from bycatches. It includes coverage for 1994-2013 (JARPN-JARPN II), 1987/88-2012/13 (JARPA-JARPA II). In the case of bycatches it includes coverage for 2001-13.

Øien reported on the status of the Norwegian register (see Appendix 3). The collection of samples of North Atlantic common minke whale is from commercial catches for the period 1997 to 2013.

Víkingsson reported on the status of the Icelandic register (see Appendix 4). The collection of samples is from scientific whaling and from commercial catches. It includes coverage for 2003-07 (scientific whaling) and 2006-13 (commercial catches). Samples are presently in hand for all whales taken in 2003-13.

8. REFERENCE DATABASES AND STANDARDS FOR A DIAGNOSTIC REGISTER OF DNA PROFILES

An update of the Japanese register is shown in Appendix 2. For North Pacific minke whales sampled in 2013 mtDNA and microsatellite analyses of 100% (n=95) has been completed. For animals bycaught in 2013, mtDNA and microsatellite analyses has been completed for 100% (n=105).

For North Pacific Bryde's whales sampled in 2013, mtDNA and microsatellite analyses have been completed for 100% of the samples (n=28). No bycatch occurred in 2013. For North Pacific sei whales sampled in 2013, mtDNA and microsatellite analyses have been completed for 100% of the samples (n=100). No bycatch occurred in 2013. The single

North Pacific sperm whale sampled in 2012 was screened for mtDNA and microsatellites. No bycatch occurred in 2013.

For Antarctic minke whales sampled in 2012/13 (n=103) the mtDNA and microsatellite work has not yet been completed. No Antarctic fin whale was sampled in the 2012/13 season.

For North Pacific humpback whales 100% of the five whales bycaught in 2013 were screened for mtDNA and microsatellites. There was no bycatch of North Pacific right whales in 2013. For North Pacific fin whales 100% of the six whales bycaught in 2013 were screened for mtDNA and microsatellites. An update of the Norwegian register is shown in Appendix 3. After discounting for duplicates, missing samples and laboratory problems, 100% of the North Atlantic common minke whales caught in 2013 (n=578) were screened for mtDNA and microsatellites. An update of the Icelandic registry is shown in Appendix 4. For North Atlantic common minke whales genetic work is ongoing for the whales caught in 2013 (n=35). 100% of the fin whales caught by commercial whaling in 2013 (n=134) were screened for both mtDNA and microsatellites.

The Group **appreciated** the efforts of Japan, Norway and Iceland in compiling and providing this detailed information of their registries.

9. OTHER MATTERS

No other matters were discussed by the Group.

10. WORK PLAN

The terms of reference for the Working Group will remain the same for the next year, unless the Commission requests other information in the interim. Members of the Working Group were encouraged to submit papers relating to these terms of reference and to propose additional agenda items. Results of the 'amendment' work on sequences deposited in *GenBank* will be reported next year. Next year the Working Group will examine the technical information relevant to the TORs of the Group, contained in documents presented to other groups and subcommittees.

11. ADOPTION OF THE REPORT

The report was adopted by consensus.

REFERENCES

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Appendix 1

AGENDA

1. Election of Chair
2. Appointment of rapporteurs
3. Adoption of the Agenda
4. Review of documents
5. Progress on genetic methods for species, stock and individual identification
6. Review of results of the 'amendments' of sequences deposited in *GenBank*
7. Progress on collection and archiving of tissue samples from catches and bycatches
8. Reference databases and standards for a diagnostic DNA registries
9. Other
10. Work plan
11. Adoption of the Report

Appendix 2

AN UPDATE OF THE JAPANESE DNA REGISTER FOR LARGE WHALES

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The status of the Japanese DNA register for large whales was presented and discussed during the 2005 Scientific Committee meeting (IWC, 2006). The number of genetic samples and the number of individuals analysed and registered were reported. The status report included information of the scientific whaling in the North Pacific (JARPN II) up to 2004, of the scientific whaling in the Antarctic (JARPA) from the austral summer season 1987/88 to 2004/05, and of the bycatches and stranding up to 2005.

Update of the Japanese DNA register for large whales under the new format since the last scientific meeting is as follows.

Table 1
Update of the Japanese DNA register for large whales under the new format since the last Scientific Committee meeting.

Footnote no.	1	2	3	4	5	6	7	8	9	10	11	12
Species/year	Type	No. of whales	No. of duplicates	No. missing	No. of lab problems	No. mtDNA	% mtDNA	No. msat	%msat	Sex analysed	% sexed	Note
North Pacific minke whale												
1994-2011	SP	2,215	0	0	8	2,207	99.6	2,207	99.6	2,215	100	
2012	SP	182	0	0	0	182	100	182	100	182	100	Microsats, analysed in 2013 after SC/65a
2013	SP	95	0	0	0	95	100	95	100	95	100	
2001-11	BC	1,324	0	26	2	1,324	100	1,296	97.9	1,294	97.7	
2012	BC	114	0	0	0	114	100	114	100	114	100	
2013	BC	105	0	0	0	105	100	105	100	105	100	
North Pacific Bryde's whale												
2000-11	SP	593	0	0	3	590	99.5	593	100	593	100	
2012	SP	34	0	0	0	34	100	34	100	34	100	Microsats, analysed in 2013 after SC/65a
2013	SP	28	0	0	0	28	100	28	100	28	100	
2001-11	BC	4	0	0	0	4	100	4	100	4	100	Included two Omura's whale
2012	BC	1	0	0	0	1	100	0	0	0	0	Omura's whale
North Pacific sei whale												
2002-11	SP	884	0	0	4	880	99.5	884	100	884	100	
2012	SP	100	0	0	0	100	100	100	100	100	100	Microsats, analysed in 2013 after SC/65a
2013	SP	100	0	0	0	100	100	100	100	100	100	
North Pacific sperm whale												
2000-11	SP	52	0	0	0	49	94.2	52	100	52	100	2010 sample, no mtDNA
2012	SP	3	0	0	0	3	100	3	100	3	100	Microsats, analysed in 2013 after SC/65a
2013	SP	1	0	0	0	1	100	1	100	1	100	
2001-12	BC	2	0	0	0	2	100	2	100	2	100	
Antarctic minke whale												
1987/88-2004/05	SP	6,794	0	10	0	1,118	16.5	6,271	92.3	6,794	100	Incl. dwarf; 87/88-88/89. No microsats
2005/06-2010/11	SP	3,264	0	549	162	2,040	62.5	2,553	78.2	3,264	100	Some missing by the 3/11 tsunami in 2011
2011/12	SP	266	0	0	0	0	0	0	0	266	100	
2012/13	SP	103	0	0	0	0	0	0	0	103	100	
Antarctic fin whale												
2005/06-2010/11	SP	17	0	0	0	17	100	17	100	17	100	
2011/12	SP	1	0	0	0	1	100	1	100	1	100	Analysed in 2013 after SC/65a
North Pacific humpback whale												
2001-11	BC	37	0	0	0	37	100	37	100	37	100	
2012	BC	4	0	0	0	4	100	4	100	4	100	Microsats, analysed in 2013 after SC/65a
2013	BC	5	0	0	0	5	100	5	100	5	100	
North Pacific right whale												
2001-12	BC	2	0	1	0	2	100	1	100	1	100	Missing by the 2011 tsunami, no microsats.
North Pacific fin whale												
2001-12	BC	3	0	0	0	3	100	3	100	3	100	
2013	BC	6	0	0	0	6	100	6	100	6	100	

1. Key to sample types: SP=special permit catch, C=commercial catch, BC=bycatch, ST=stranding.
2. Number of whales that potentially entered by the previous years and enters (new year) the markets.
3. Number of occurrences (tissues) sample switching on board the vessels as detected by comparison of genetic profiles.
4. Number of individuals for which tissue samples are missing for other reasons than sample switching.
5. Genetic laboratory not able to obtain microsatellite profiles mtDNA haplotypes from tissue samples.
6. Number of samples analysed for mitochondrial control region.
7. % of total samples analysed for mitochondrial control region.
8. Number of samples analysed for microsatellites.
9. % of total samples analysed for microsatellites.
10. Number of samples analysed for sex.
11. % of total samples analysed for sex.
12. Other problems or information.

REFERENCE

International Whaling Commission. 2006. Report of the Scientific Committee. Annex N. Report of the Working Group on DNA. *J. Cetacean Res. Manage. (Suppl.)* 8:252-58.

Appendix 3

AN UPDATE OF THE NORWEGIAN DNA REGISTER FOR MINKE WHALES

Hans J. Skaug
Institute of Marine Research

Table 1

Update of the Norwegian DNA register for minke whales.

Footnote no.	1	2	3	4	5	6	7	8	9	10	11	12
Species/year	Type	No. of whales	No. of duplicates	No. missing	No. of lab problems	No. mtDNA	% mtDNA	No. msat	%msat	Sex analysed	% sexed	Note
NA minke whale												
1997-2012	C	8,278	89	54	2	8,597	100	8,597	100	8,597	100	
2013	C	588	9	1	0	578	100	578	100	578	100	

1. Key to sample types: SP=special permit catch, C=commercial catch, BC=bycatch, ST=stranding.
2. Number of whales that potentially entered by the previous years and enters (new year) the markets.
3. Number of occurrences (tissues) sample switching on board the vessels as detected by comparison of genetic profiles.
4. Number of individuals for which tissue samples are missing for other reasons than sample switching.
5. Genetic laboratory not able to obtain microsatellite profiles mtDNA haplotypes from tissue samples.
6. Number of samples analysed for mitochondrial control region.
7. % of total samples analysed for mitochondrial control region.
8. Number of samples analysed for microsatellites.
9. % of total samples analysed for microsatellites.
10. Number of samples analysed for sex.
11. % of total samples analysed for sex.
12. Other problems or information.

Appendix 4

STATUS OF THE ICELANDIC WHALE DNA REGISTER

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Practical arrangements regarding the establishment of the Icelandic DNA register were concluded in 2007. The Marine Research Institute, Reykjavik, is responsible for the establishment and maintenance of the registry that is of the same format as the Norwegian DNA registry. An ORACLE database has now been created and contains all genotyped individuals information as well as tissue collected ID of individuals collected but not genotyped. In parallel, a DNA tissue bank has been achieved and is now fully functional.

Table 1 gives the present status of the registry. Samples from all the common minke whales landed as a part of the Icelandic research program (2003-07) and all commercial NA fin whales have been genotyped and information stored in the database. Genetic analyses of NA minke whales taken for commercial purposes from 2007 to 2012 are currently ongoing. All samples from 2007-12 would be genotyped before the end of the year.

Table 1
Status of the Icelandic whale DNA register.

Footnote no.	1	2	3	4	5	6	7	8	9	10	11	12
Species/year	Type	No. of whales	No. of duplicates	No. missing	No. of lab problems	No. mtDNA	% mtDNA	No. msat	%msat	Sex analysed	% sexed	Note
North Atlantic minke whale												
2003-07	SP	189	0	0	0	189	100	189	100	189	100	
2007-10	C	186	0	0	0	166	89	169	91	169	91	
2011	C	58	0	0	0	58	100	58	100	58	100	
2012	C	49	0	0	0	48	98	49	100	49	100	
2013	C	35	0	0	0	0	0	0	0	0	0	
North Atlantic fin whale												
2006-10	C	274	0	0	0	274	100	274	100	274	100	
2013	C	134	0	0	0	134	100	134	100	134	100	

1. Key to sample types: SP=special permit catch, C=commercial catch, BC=bycatch, ST=stranding.
2. Number of whales that potentially entered by the previous years and enters (new year) the markets.
3. Number of occurrences (tissues) sample switching on board the vessels as detected by comparison of genetic profiles.
4. Number of individuals for which tissue samples are missing for other reasons than sample switching.
5. Genetic laboratory not able to obtain microsatellite profiles mtDNA haplotypes from tissue samples.
6. Number of samples analysed for mitochondrial control region.
7. % of total samples analysed for mitochondrial control region.
8. Number of samples analysed for microsatellites.
9. % of total samples analysed for microsatellites.
10. Number of samples analysed for sex.
11. % of total samples analysed for sex.
12. Other problems or information.