Annex N

Report of the Working Group on DNA

Members: Pastene (Chair), Baker, Cipriano, Danielsdóttir, Goto, Kanda, Kitakado, LeDuc, Olavarria, Palsbøll, Park, Perrin, Pomilla, Rosenbaum, Sohn, Skaug, Tiedemann, Van Waerebeek, Waples, Weinrich, Yao.

1. ELECTION OF CHAIR

Pastene convened and chaired the Group.

2. APPOINTMENT OF RAPPORTEURS

Perrin acted as rapporteur.

3. ADOPTION OF AGENDA

The Agenda is given as Appendix 1. Items 5, 6 and 7 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.

4. REVIEW OF DOCUMENTS

Relevant information was contained in SC/57/SD3, SC/57/SD4, SC/57/SD6 and SC/57/O14.

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

SC/57/SD3 presented an update on improved methods for DNA extraction and PCR amplification from small, old, and degraded tissue samples (including bone and dried soft tissue). The new methods included a modified extraction method, a set of primers to produce small, overlapping amplicons that recover about half the 5' portion of the mitochondrial control region, and use of a 'proof-reading' DNA polymerase mixture (*Expand Taq*, Roche Diagnostic Corporation), to drive the amplification. The new primers were designed using aligned sequences from a variety of cetacean species (including both mysticete and odontocete sequences) in order to locate regions of sequence conserved across cetacean species and thus maximize their utility. All but two of the newly developed primers worked well for amplifying from fresh tissue samples, so those two should be re-designed and tested again. It may be necessary to design mysticete- and odontocete-specific primers for the region where the all-cetacean primers were ineffective. Use of *Expand Taq* allowed amplification from some bone and degraded tissue extracts, so that even larger fragments (dlp1.5-dlp5, approximately 560bp) could be amplified. This method was tested by performing independent extractions from a set of bone and mummified tissue samples, and comparing the sequences obtained. Independent extractions from individuals did match each other, but differences between individuals were observed, indicating that the extraction was effective and not hampered by contamination.

The author responded to several questions. He noted that the method has not yet been tried on formalin-preserved samples but that such trials would be made. He also noted that other methods are available for this. Quantification of the DNA yield is difficult because of the very small quantities involved, but the best test of sufficiency is whether the DNA will amplify with PCR. A 5X buffer is provided with the kit. The method also has potential for use in 'bar-coding', e.g. with the cytochrome oxidase 2 gene.

SC/57/SD4 presented the results of a validation exercise for cetacean species identification using the curated reference dataset of mtDNA control region and cytochrome *b* sequences implemented in the Web-based species identification program *DNA Surveillance* (Ross *et al.*, 2003) and the non-curated sequences available in the international repository GenBank. All control region (n=1,609) and cytochrome *b* (n=1,448) sequences labelled as cetacean in GenBank, and those showing moderate similarity to cetacean sequences, were submitted to *DNA Surveillance* to re-infer their species identities. The purpose of the exercise was two-fold: (1) to evaluate the potential for taxonomic misidentification of sequences in the non-curated collection in GenBank; and (2) to evaluate the reliability of *DNA Surveillance* for identifying cetacean sequences and in recognising non-cetacean sequences. Overall agreement between GenBank and *DNA Surveillance* in the attribution of species identity was high, and there was little evidence that non-cetacean sequences in GenBank have been mislabelled as cetaceans. For the control region, 94% of GenBank were identified in *DNA Surveillance* as the same species. Control region sequences that were given different identities by the two systems formed two groups. The first group included 43 sequences (3% of cetacean) where the differences were readily attributable to nomenclatural differences arising from synonymy, from taxonomies that differ in the recognition of new species or from the resurrection of previously distinguished species. The second group of 22 sequences (2% of cetacean) involved members of the genera *Stenella*, *Delphinus*, *Tursiops* and

Lagenorhynchus, among which there was ambiguity of taxonomic identification due perhaps to apparent paraphyly of mtDNA lineages, or from potential sample misidentification from morphological evidence. The consistency of identification was similar for cytochrome *b*, although some disagreements seemed to be due to other factors. Only a small number of control region sequences identified in GenBank as sperm whale (*Physeter catodon*) are of questionable cetacean origin, but alternative identities cannot be suggested.

In the discussion that followed it was noted that some problems of misidentification from mtDNA sequences may be caused by pseudogenes (nuclear fragments), which have been encountered in *Cephalorhynchus* and *Delphinus*. It was also pointed out that inclusion of locality with submitted sequences would help in sorting out possible misidentifications.

The Group **agreed** that validation such as that described in SC/57/SD4 should be carried out routinely for cetacean sequences in GenBank and other such repositories and **recommended** that the Scientific Committee consider establishing an oversight group to coordinate this effort. The question arose of what action could be taken when an identification is found to be erroneous, in view of the fact that the original submitter owns the copyright to the entry. One possible solution suggested was that a new field could be added to the database where a challenge to the identification could be noted.

A problem in resolving identification of right whale sequences to species will be aided by the addition of more reference sequences to *DNA Surveillance.*

Kanda summarised a recent paper on the use of single nucleotide polymorphisms (SNPs) as markers in population genetics (Morin *et al.,* 2004). SNPs are genetic variation resulting from single-point mutations that produce single base-pair differences among DNA sequences. They are being used extensively in human population genetics. SNPs are found by screening DNA segments from multiple individuals for polymorphisms, which occur every 200-500bp in humans. For other species, orthologous loci that are conserved across species are targeted. One important advantage is easy standardisation of data; specific sequence changes are represented using the standard DNA code (G,A,T,C). Another potential advantage is cost effectiveness. After multiple species have been screened, the unit cost of discovery may be less than for microsatellites. A disadvantage is potential ascertainment bias (AB) through non-representative sampling of individuals or through analysing only the most variable loci (also a disadvantage for microsatellites). The bias is most problematic for estimation of population size and demographic change and least for individual identification, paternity analysis and assignment tests. AB can be avoided by sequencing across the genome in a large panel of individuals from broad geographic coverage. SNPs are bi-allelic, whereas microsatellites are multi-allelic, resulting in a need for 4-10 times more SNP loci than microsatellites for estimating genome-wide levels of variation. 50-100 SNPs = 10-20 microsatellite loci.

For the individual-identification level and related applications, reliability depends on independence of markers and their diversity (number of alleles and heterozygosity (*He*)). Maximum *He* for bi-allelic SNPs is 0.5. For individual identification, 10-20 SNPs with *He* = 0.2-0.4 and $q>0.1$ are equivalent to 4-6 microsatellites with *He* = 0.6-0.8. For paternity analysis, 40-100 SNPs = 7-14 microsatellites. For higher-order relatedness, even >100 SNPs with minor allele frequency of 0.20 were found inadequate to resolve relationships identified by ~40-50 microsatellites (*He* = 0.60-0.75*)*.

At the population level (population structure, assignment tests), SNPs are advantageous in that a large number of loci representing variation across the entire genome can be employed. Inter-locus sampling variance can be decreased. The mutation rate is low and mutations are simple. For microsatellites, the mutation rate is high and the mode of mutation is unknown, potentially leading to cryptic homoplasy and unreliable estimates of population differentiation and gene flow. However, more loci are required; one microsatellite with 11 alleles = 10 bi-allelic SNPs. In addition, AB is a potential problem.

At the species level, SNPs are attractive because they can sample the entire genome and have slow mutation rates and simple mutations. But again, AB is a disadvantage. In addition, they are subject to recombination, as opposed to mtDNA sequences.

A lengthy discussion ensued. It was noted that SNPs are difficult to find; they may be less frequent in whales than in humans, and there being no easy way to make a genome-wide scan. A directed approach used by Morin and colleagues is the use of primers for introns, which are then screened for amplifiability. Another approach, used in Berkeley by Palsbøll for ringed seals is the creation of a random library, amplification and sequencing of isolates and identification of heterozygotes. The frequency of SNPs in ringed seals found by this approach is about the same as in humans. Another difficulty noted was uncertainty about independence of loci. With a chromosome map, linkage can be avoided by use of the directed-intron method of Morin. Another question was raised about the pitfalls of including sites under selection (most methods in the past have aimed at neutral markers). The use of 'hapsters' or 'snpsters' (sequences containing both SNPs and microsatellites) can help with this problem, as they combine the benefits of both types of markers. It was also pointed out that the degree of disadvantage in using selected loci depends on the nature of the research question; in some cases (e.g. individual identification) selection is not a problem. The Chair noted that the relevant issue for DNA registers is the potentiality of this marker for individual identification. The Group **agreed** that SNPs offer considerable promise for application in the genetics of whale management, in particular in the ease of standardisation of data across laboratories.

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

Skaug reported on the status of the Norwegian register (Appendix 2). A total of 638 and 537 common minke whales were landed in 2003 and 2004, respectively. Analyses have not yet been completed for these samples.

SC/57/O14 reported that samples of skin and muscle have been collected for 36 common minke whales taken in 2003 and 25 in 2004 in the Icelandic scientific whaling operations.

Regarding collection of samples in Japan, the Group was informed that samples from scientific whaling for the Antarctic (JARPA) stored as of April 2005 were: Antarctic minke whale since 1987/88, *n*=6,778; common minke whale, *n*=16. For the western North Pacific (JARPN II) samples stored as of December 2004 were: common minke whale since 1994, *n*=1,097; Bryde's whale since 2000, *n*=243; sei whale since 2002, *n*=189; and sperm whale since 2000, *n*=31. Most of these samples have been included in

the register (see Appendix 3). In the case of scientific whaling, samples for genetic analysis are collected by cetacean researchers. These involve skin samples (two pieces of 5×5×5mm kept in 99% ethanol) and liver, heart, kidney, skin and muscle samples (one piece of 20g each kept frozen). A large amount of information on each whale sampled is collected using established protocols of JARPA and JARPN II including date and locality (longitude, latitude) of sampling, species, body length and sex.

The Japanese regulation on bycatches (established from 1 July 2001) requires that all animals should be DNA-registered before whale meat can be sold in the market. Details of the regulation and procedure can be found in the following web page: *http://www.icrwhale.org/pdf/higekujira.pdf*. The samples from North Pacific bycatch stored as of December 2004 were: common minke whale, *n*=403; Bryde's whales, *n*=3; right whale, *n*=1; and humpback whale, *n*=9. Most of these samples have been incorporated into the register (Appendix 3). Skin or muscle samples (5×5×5cm) are taken by the fisherman and sent to the laboratory at ICR (as frozen samples). The fisherman is required to provide several kinds of information (in an established format): date and locality of the bycatch, type of set net, species, body length and sex. Further details are given in Appendix 3.

A similar regulation/procedure to that for bycatches was adopted in October 2004 for stranded whales, in the case of animals utilised either for commercial or scientific purposes. Genetic samples were stored for the following stranded whales as of l December 2004: North Pacific humpback whale, *n*=1; North Pacific common minke whale, *n*=1; and North Pacific fin whale, *n*=1. These samples have been incorporated into the register (Appendix 3). Investigation and registration of frozen stockpiles of meat continues.

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

Skaug reported that genetic analyses on samples of minke whales landed in 2003 and 2004 have not yet been completed.

SC/57/SD6 provided information on procedures and standards for the Japanese register. All whales taken under special permit in the western North Pacific (through 2003) and most of those taken in the Antarctic (through 2003/04) have been incorporated into the register. All bycaught and stranded whales (through 2004) have been incorporated into the register.

As in the case of Norway, the Japanese register uses three kinds of genetic markers: mtDNA control region sequences for species identification; a set of nuclear DNA marker (microsatellites) for individual identification, and Y chromosome DNA for gender determination (Appendix 3). A total of 17 microsatellite loci are used in the case of North Pacific common minke whale, North Pacific Bryde's whale and North Pacific sei whale. In the case of the Antarctic minke whale and North Pacific humpback whale, six loci are used. Sex information for whales taken under scientific permit are provided by JARPA or JARPN II researchers (no molecular sexing is conducted for these samples). In the case of bycatches, sex is determined molecularly.

The Group thanked Japan for providing this information on its register. In response to a query about why so few microsatellites are used for Antarctic common minke whales compared to the number used for Northern Hemisphere whales (6 vs. 17), Kanda noted that the Antarctic minke whales are highly polymorphic and further that an additional five loci are used to check matches. The Group **agreed** it is important that a uniform procedure for estimating error rates be used by the several nations with DNA registers and **recommended** that this be done.

The Group expressed its gratitude to the three nations (Japan, Norway and Iceland) for supplying information on their collections and registers.

8. WORK PLAN

The terms of reference for the Working Group for the next year will remain the same as for this year, unless the Commission requests other information in the interim.

9. ADOPTION OF REPORT

The report was adopted by consensus.

REFERENCES

International Whaling Commission. 2000. Chairman's Report of the Fifty-First Annual Meeting. Appendix 9. IWC Resolution 1999-8. Resolution on DNA testing. *Ann. Rep. Int. Whaling Comm.* 1999:55.

Morin, P.A., Luikart, G., Wayne, R.K. and and the SNP Workshop Group. 2004. SNPs in ecology, evolution and conservation. *Trends Ecol. Evol.* 19(4):208-16.

Ross, H.A., Lento, G.M., Dalebout, M.L., Goode, M., Ewing, G., McLaren, P., Rodrigo, A.G., Lavery, S. and Baker, C.S. 2003. DNA surveillance: web-based molecular identification of whales, dolphins, and porpoises. *J. Hered.* 94(2):111-4.

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. Progress on collection and archiving of samples from catches and bycatches
7. Reference databases and standards for a diagnostic register of DNA profiles
8. Work plan
9. Adoption of report

Appendix 2

STATUS OF THE NORWEGIAN MINKE WHALE DNA REGISTER

Hans Julius Skaug

Year	DNA-register[1]	IWC catch statistics[2]	Not landed[3]	Landed[4]	Duplicates[5]	Missing samples[6]	Total missing[7]
1997	488	503	7	496	3	5	8
1998	609	625	11	614	1	4	5
1999	571	591	17	574	2	1	3
2000	470	487	6	481	3	8	11
2001	538	552	11	541	2	1	3
2002	625	634	9	625	0	0	0
2003	-	**647**	9	638	-	0	-
2004	-	**544**	7	537	-	1	-

[1]Number of unique individuals contained in the DNA-register (not containing duplicates). [2]Number of individuals caught by Norway, including individuals not landed. [3]Number of individuals killed, but not taken onboard the vessel. [4]Number of individuals taken onboard the vessel. [5]Number of occurrences of (tissue) sample switching on board the vessel as detected by comparison of genetic profiles. The result is that two samples have been returned from one individual, and no sample has been returned from one individual. [6]Number of individuals for which tissue samples are missing for other reasons than sample switching. [7]The difference between columns 'Landed' and 'DNA-register'.

Appendix 3

STATUS OF THE JAPANESE DNA REGISTER FOR LARGE WHALES

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Abstract

In contribution to the work of the Working Group on DNA an update of the status of the Japanese DNA register for large whales is provided. This register is based on technical specifications similar to those of the Norwegian register. On the basis of the information summarised here it is concluded that the Japanese register contains almost all the genetic profiles of whales taken legally in Japan and therefore is close to being fully diagnostic.

Introduction

The basic position of the Government of Japan (GOJ) concerning DNA identification and tracking of whale products is that matters related to market or international trade of whale products are outside the jurisdiction of the IWC. At the same time the GOJ recognises the usefulness and importance of DNA technology and it has been using this technology in its domestic management of whale products. Furthermore Japanese scientists have been contributing to IWC/SC discussions on the scientific aspects involved (e.g. genetic techniques).

Domestic management of whale products in Japan involves two main components: establishment and maintenance of a diagnostic DNA register for large whales and market monitoring through systematic DNA surveys in the retail market.

In contribution to the work of the Working Group on DNA an update of the status of the Japanese DNA register is provided. This register is based on technical specifications similar to those of the Norwegian register (Dupuy and Olaisen, 1998).

Brief description of the Japanese DNA register

Source of tissue samples

In Japan there are two main sources of tissues samples: (1) scientific whaling conducted under special permit in the Antarctic-JARPA (Antarctic minke whale since 1987/88) and western North Pacific JARPN II (common minke whale since 1994, Bryde's whale since 2000, sei whale since 2002 and sperm whales since 2000) and (2) bycatches, involving North Pacific common minke whales mainly, since 1 July 2001.

In the case of scientific whaling, samples for genetic analysis are collected by cetacean researchers. These involve skin samples (two pieces of 5×5×5mm kept in 99% ethanol); liver, heart, kidney, skin and muscle (one piece of 20g each kept frozen). A large amount of information on each whale sampled is collected using established protocols of JARPA and JARPN II including date and locality (longitude, latitude) of sampling, species, body length and sex.

The Japanese regulation on bycatches (established from 1 July 2001) requires that all animals should be DNA-registered before whale meat can be sold in the market. Details of the regulation and procedure can be found in the following web page: *http://www.icrwhale.org/pdf/higekujira.pdf.* Skin or muscle samples (5×5×5cm) are taken by the fishermen and send to the laboratory at ICR (as frozen samples). Fishermen should provide several kinds of information (on an established format) on the bycaught animal. Among these, the following relevant information is provided: date and locality of the bycatch, type of set net, species, body length and sex.

Laboratory analysis

At the laboratory, a single typing procedure is employed. The typing procedure is repeated only in the case of samples not providing satisfactory results on the first attempt. Total-cell DNA is extracted from tissues by standard phenol/chloroform extractions (Sambrook *et al*., 1989).

Specification of markers

In the Japanese register the whale DNA is composed of three parts.

SPECIES IDENTIFICATION

An approximately 500bp fragment of the 5'-end of the mitochondrial DNA control region is used. The first 490 nucleotides at the 5' end of the mitochondrial control region are amplified by the polymerase chain reaction. The oligonucleotides employed in the PCR amplification are usually MT4 (Arnason *et al*., 1993) and Dlp5R (5'-CCATCGAGATGTCTTATTTAAGGGGAAC-3'). Reactions are carried out in 50μL volumes containing 100mM KCl, 20mM Tris-HCl, 0.1mM EDTA, 1mM DTT; 0.5% Tween 20, 0.5% Nonidet P-40, 200μM dNTPs, 2.5pM of each oligonucleotide and one unit of *Taq* DNA polymerase. After an initial denaturation step at 95°C for 5 minutes, a PCR amplification cycle of 30 seconds at 94°C, followed by 30 seconds at 50°C and 30 seconds at 72°C is repeated 30 times. The amplification is completed with a final extension step of 10 minutes at 72°C. PCR products are purified by MicroSpin S-400HR columns (Pharmacia Biotech). Subsequent cycle sequencing reactions are performed with 100ng of products generated in the above PCR amplifications using the Prism™ dRhodamine Terminator Cycle Sequencing Kit (Applied Biosystems, Inc.). The oligonucleotides used to prime the cycle sequencing reaction were the same as employed in the initial PCR amplification listed above. A total of 25 cycles with 10 seconds at 96°C, 20 seconds at 56°C and four minutes at 60°C are performed. The cycle sequencing products are purified by Auto Seq G-50 Spin Columns (Pharmacia Biotech). The nucleotide sequence of each cycle sequencing reaction is determined by electrophoresis through a 5% Long Ranger™ (FMC, Inc.) denaturing polyacrylamide matrix on a DNA Prism™ 377 DNA Sequencer (Applied Biosystems, Inc.) under standard conditions. Both strand samples are sequenced in their entirety for all samples.

Standard phylogenetic analyses of 'test' and 'type' sequences are conducted to investigate the species of origin of unknown whale products.

INDIVIDUAL IDENTIFICATION

In the case of the Japanese register the microsatellite set used for population study and individual identification is composed of 17 loci, with the exception of Antarctic minke whale (10) and humpback whale (6) (see Table 1). New primers are being investigated and tested for optimising their use in the case of the humpback whale. In the case of the Antarctic minke whale the number of loci is smaller but enough for individual identification purposes because of very high genetic variability. Laboratory procedure for microsatellite analyses is as follows.

Microsatellite polymorphisms are analysed using published primers (see Table 1). Although primer sequences follow those of the original authors, an annealing temperature of each of the loci is optimised for each of the whale species in the DNA register. PCR amplifications are performed in 15μl reaction mixtures containing 10-100ng of DNA, 5 pmole of each primer, 0.625 units of *Ex Taq* DNA polymerase (Takara Shuzo), and 2mM of each dNTP, and 10x reaction buffer containing 20mM $MgCl_2$ (Takara Shuzo). Amplified products with internal size standard (GENESCAN400HD, Applied Biosystems Japan) are run on a 6% polyacrylamide denaturating gel (Long Ranger) using BaseStation100 DNA fragment analyser (MJ Research). Although alleles are visualised using Cartographer software specifically designed for the BaseStation, allelic sizes are determined manually in relation to the internal size standard and DNA of known size from a 'control' individual that is rerun on each gel.

The number and degree of variability of loci used in both registers are enough for the objective of individual identification.

SEX DETERMINATION

Data from Y chromosome DNA is used. In the case of Japan the method of Abe *et al*. (2001) is used, which involves co-amplification of SRY gene on Y chromosome and a microsatellite locus.

ERROR RATE ESTIMATIONS FOR MICROSATELLITE DATA SETS

Hoffman and Amos (2005) estimated the error rate using a large data set (*n*=2,000) of the Antarctic fur seal. The authors used several approaches (repeat-genotyping, deliberately re-sampled individuals, unintentionally re-sampled individuals, mother-offspring pairs, mismatches between pups and putative fathers) to estimate error rates. They found good concordance among the approaches used for error rate estimation with the range being 0.0013 to 0.0074 per single locus PCR. The most common errors involved the misinterpretation of allele banding patterns.

No attempts have been made to statistically estimate error rates for the microsatellite data set in the Japanese register. However side by side running to check genotypes is commonly conducted when there is some uncertainty in the pattern found. We believe that such errors are minimal in the Japanese register.

Format of individual records

In the case of the Japanese register, each registered whale has the following code.

SCIENTIFIC WHALING

'00NPM001': this specifies the year (2000), oceanic region (NP=North Pacific), species (M=minke) and individual number (001). This code allows for cross reference with the comprehensive database on JARPA and JARPN II in ICR.

BYCATCHES

'ICRY-05-028' (bycatch before DNA analysis); '30041016MI395' (bycatch after DNA analysis). In the latter code '30' is for prefecture, date (16 October 2004), species (MI=minke) and sequential number. Both codes allow for cross reference with the comprehensive database of bycaught animals in ICR.

Database structure

Two Excel files are generated, one for microsatellites and gender profiles and the other for the mtDNA sequence. In each of these, consecutive whales are numbered. In the STR/gender file, each whale is given in one row, with one column for each allele (two columns for each STR marker and for the gender locus). In the case of the Japanese register each species is given a different Excel sheet. In the mtDNA

Table 1

Microsatellite loci used for different whale species in the Japanese register.

Primer	Species designed for	SH minke whale	NP minke whale	Bryde's whale	Sei whale	Sperm whale	Humpback whale	Ref.
DlrFCB14	Beluga whale	x	x	x		x		a
DlrFCB17	Beluga whale			x	x	x		a
EV1	Sperm whale	x	x	x	x	x		b
EV5	Sperm whale					x		b
EV 14	Sperm whale		x	x	x			b
EV 21	Sperm whale		x	x	x			b
EV30	Sperm whale					x		b
EV37	Humpback whale		x			x		b
EV94	Humpback whale		x	x	x	x		b
EV104	Humpback whale	x	x	x	x	x		b
GATA28	Humpback whale	*	x	x	x	x	x	c
GATA53	Humpback whale			x	x		x	c
GATA98	Humpback whale	*	x	x	x	x	x	c
GATA417	Humpback whale	*	x	x	x	x	x	c
GGAA520	Humpback whale			x	x			c
GT001	Humpback whale			x	x	x		d
GT023	Humpback whale	x	x	x	x	x	x	e
GT195	Humpback whale	x	x					e
GT211	Humpback whale	x	x		x	x		e
GT271	Humpback whale				x	x		e
GT310	Humpback whale		x	x	x			e
GT509	Humpback whale	*	x			x		e
GT575	Humpback whale	*	x	x	x	x		e
TAA031	Humpback whale		x	x			x	c
Total number of loci used		6 (10-11)	17	17	17	17	6	

*Indicates the loci used for only individual identification. [a]Buchanan *et al.* (1996); [b]Valsecchi and Amos (1996); [c]Palsbøll *et al.* (1997); [d]Bérubé *et al.* (1998); [e]Bérubé *et al*. (2000).

file, each profile has one row. Again in the case of the Japanese register each species is given a different Excel sheet.

Status of the registry

The status of the Japanese register is shown in Table 2.

Table 2

Status of the Japanese DNA register for large whales.

Source/species	Period	Genetic samples	mtDNA	STRs	Sex
Scientific whaling					
NP minke whale	94-03	938	938	938	938
NP Bryde's whale	00-03	193	193	193	193
NP Sei whale	02-03	89	89	89	89
NP Sperm whale	00-03	28	28	28	28
Antarctic minke whale	87/88-04/05	6,778	1,103	5,808	6,778
Common/dwarf minke whale	87/88-93/94	16	16	16	16
Bycatch					
NP minke whale	01-04*	402	402	402	402
NP humpback whale	01-04*	9	9	9	9
NP right whale	01-04*	1	1	1	1
NP Bryde's whale**	01-04*	3	3	3	3
Stranding***					
NP humpback whale	04	1	1	-	-
NP minke whale	04	1	1	-	-
NP fin whale	04	1	1	-	-

*From 1 July 2001 to 31 December 2004; **including two animals identified genetically as *B. omurai (*Wada *et al*., 2003); ***November-December 2004.

All whales taken under special permit in the western North Pacific (till 2003) and most of those taken in the Antarctic (till 2003/04) have been incorporated into the register. Sex information for whales taken by scientific permit are provided by JARPA or JARPN II researchers (no molecular sexing is conducted for these samples). In the case of bycatches, sex is determined using a molecular approach.

A similar regulation/procedure to that of the bycatches was adopted in October 2004 for stranded whales, in case the animals are utilised either for commercial or scientific purposes. DNA data from some strandings which occurred after that date (whales being utilised for scientific purposes) are being incorporated into the register. There was also some investigation of frozen stockpiles of whale meat (originating from past commercial whaling), although the amount still existing is very small. Genetic analysis on these products is being conducted for incorporation into the register.

Final remarks

The IWC/SC has agreed that registers should be diagnostic, i.e. that they should contain the DNA profiles of any animals from which products might legally appear in the market (e.g. from legal catches, bycatches, ship strikes) on the understanding that products from animals not included in the register would be considered infractions.

We conclude that the Japanese register contains almost all the genetic profiles of whales taken legally in Japan and therefore is close to being fully diagnostic. Further, it should be noted that genetic data contained in the Japanese register was used by members of the IWC/SC Committee during the RMP *Implementation Assessment* of North Pacific minke whale and the RMP *pre-Implementation assessment* of North Pacific Bryde's whale.

Acknowledgements

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REFERENCES

Abe, H., Goto, M., Pastene, L.A., Dewa, K. and Naito, E. 2001. Practical use of multiplex flourescent PCR for cetacean sex identification. *Mar. Mammal Sci.* 17(3):657-64.

Arnason, U., Gullberg, A. and Widegren, B. 1993. Cetacean mitochondrial DNA control region: sequences of all extant baleen whales and two sperm whale species. *Mol. Biol. Evol.* 10:960-70.

Bérubé, M., Aguilar, A., Dendanto, D., Larsen, F., Notarbartolo di Sciara, G., Sears, R., Sigurjónsson, J., Urban-Ramirez, J. and Palsboll, P.J. 1998. Population genetic structure of North Atlantic and Mediterranean Sea fin whales, *Balaenoptera physalus* (Linnaeus 1758): analysis of mitochondrial and nuclear loci, and comparison with the Sea of Cortez fin whale population. *Mol. Ecol.* 7:585-99.

Bérubé, M., Jorgensen, H., McEwing, R. and Palsboll, P.J. 2000. Polymorphic di-nucleotide microsatellite loci isolated from the humpback whale, *Megaptera novaeangliae*. *Mol. Ecol.* 9:2,181-3.

Buchanan, F.C., Friesen, M.K., Littlejohn, R.P. and Clayton, J.W. 1996. Microsatellites from the beluga whale *Delphinapterus leucas*. *Mol. Ecol.* 5:571-5.

Dupuy, B.M. and Olaisen, B. 1998. Typing procedure for the Norwegian minke whale DNA register. Department of Forensic Medicine, University of Oslo, Norway. 32pp.

Hofmann, J.I. and Amos, W. 2005. Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. *Mol. Ecol.* 14:559-612.

Palsboll, P.J., Bérubé, M., Larsen, A.H. and Jorgensen, H. 1997. Primers for the amplification of tri- and tetramer microsatellite loci in baleen whales. *Mol. Ecol.* 6:893-5.

Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. *Molecular Cloning: A Laboratory Manual*. 2nd Edn. Cold Spring Harbor Laboratory, New York.

Valsecchi, E. and Amos, W. 1996. Microsatellite markers for the study of cetacean populations. *Mol. Ecol.* 5:151-6.

Wada, S., Oishi, M. and Yamada, T.K. 2003. A newly discovered species of living baleen whale. *Nature, Lond.* 426:278-81.