

## Annex N

# Report of the Working Group on DNA

**Members:** Pastene (Chair), An, Baker, Bravington, Cipriano, Donovan, Donoghue, Gaggiotti, Hoelzel, Kanda, Leaper, Lyrholm, Pampoulie, Perrin, Uoya, Vikingsson, Waples, Yoshida.

### 1. ELECTION OF CHAIR

Pastene convened and chaired the Group.

### 2. APPOINTMENT OF RAPPORTEURS

Cipriano and Pastene acted as rapporteurs.

### 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; and
- (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.

Agenda Item 9 is in response to requirements placed on the Scientific Committee by the Commission to review Annex {DNA} in document IWC/62/7rev.

### 4. REVIEW OF DOCUMENTS

Relevant information was contained in IWC/62/6rev, IWC/62/7rev (Annex {DNA}), SC/62/O19 and Baker *et al.* (2010).

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

No document was available for discussion under this Agenda Item. The Chair noted that at last year's meeting the Group reviewed Cipriano and Pastene (2009), which made a comprehensive review of current knowledge of techniques to extract DNA from 'difficult' samples.

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

During the first round of sequence assessment (IWC, 2009b, p.437) some inconsistencies were found but these appear to be due to a lag in the taxonomy recognised by *GenBank* or uncertainty in taxonomic distinctions currently under investigation (e.g. the number of species and appropriate names for recently described species of 'Bryde's whales').

As agreed by the Committee in previous years, any anomaly detected in the species identity assessment will be shared with members of the Committee. The original submitter would be notified of the inconsistency and a suggestion made that an amendment be made to the entry. A summary of amendments as derived from the results of the first round of sequence assessments (IWC, 2009b, p.437) is shown below:

- 23 labelled as *Balaenoptera acutorostrata* in *GenBank* were identified as *B. bonaerensis*;
- 9 labelled as *B. edeni* in *GenBank*; and
- 10 labelled as *Eubalaena glacialis* in *GenBank* were identified as *E. australis* and *E. japonica*.

The Committee noted last year that it has not yet decided on the names for the different species of Bryde's whales and that *B. edeni* is the only name accepted by the Committee to date (IWC, 2010, p.73). The Committee suggested that with regard to the nine sequences labelled as *B. edeni* no amendments should be made at this stage but that some notification should be made in *GenBank* that their taxonomic status is currently under consideration.

Following up on a task assigned by the Committee last year, the Chair informed that he had contacted *GenBank* officers to make the above indicated amendments. He was informed that only the original submitters of the sequences can make amendments to their submissions. In view of this he contacted the relevant submitter scientists encouraging them to make the relevant amendments. As a result the notification regarding Bryde's whale taxonomy was made. Amendment work by the original submitters of right and minke whale sequences is ongoing and this work will be completed during the next intersessional period.

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

An update of the status of the Norwegian register was available to the Group (see Appendix 2). The collection of samples includes commercial catches of common minke whales from 1997 to 2009. The number of samples missing from the register by year ranged from 0-11. Some of the missing samples reflect unsampled whales, while others resulted from inadvertent duplicates.

Kanda reported on the status of the Japanese register (see Appendix 3). The collection of samples is from scientific whaling in the Antarctic (JARPA and JARPA II) and North Pacific (JARPN II), bycatches and strandings. It includes complete coverage for 2009 and the 2009/10 Antarctic season.

Pampoulie reported on the status of the Icelandic register (see Appendix 4). Samples are presently in hand for all whales taken in 2003-09. Pampoulie also noted that only whales intended for export from Iceland were currently being genotyped for inclusion in that country's registry, although tissue samples from all whales were being archived, and will be genotyped as soon as possible.

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

Genetic analyses have been completed and data on mtDNA, microsatellites and sex entered in the Norwegian register for years up to 2007 (see Appendix 2). For 2008 samples, laboratory work has been completed but the results have not been analysed yet. Laboratory work is ongoing for the 2009 samples.

For the Japanese register (see Appendix 3), the genetic analyses based on mtDNA have been completed for North Pacific common minke, Bryde's, sei and sperm whales taken by scientific whaling through to 2009. Laboratory work on microsatellites for these samples is being conducted. The genetic samples of Antarctic minke whales sampled by JARPA II have not been analysed yet, except for sex and for microsatellites of 190 samples taken in 2006/07 (six loci) and 551 taken in 2007/08 (six loci). For bycatch samples, genetic analyses based on mtDNA have been completed for all samples through to 2009. Laboratory work on microsatellites for these samples is being conducted. Laboratory work is ongoing for stranded animals in 2009 for both mtDNA and STR.

For the Icelandic register (see Appendix 4) genetic analyses (mtDNA and microsatellites) were completed for common minke whales taken by scientific whaling in 2003-07. Laboratory work of samples taken under commercial whaling in 2006 and 2009 is under way. Genetic analyses were completed for fin whale commercial samples collected in 2006 and 2009.

The Group **recommended** the adoption of a standard format for the updates of national DNA registers to assist with the review of such updates in the future. The format used by the Norwegian registry update report should be used as a model for the standard format. The Chair will work interessionally with colleagues from Norway, Japan and Iceland to agree on the standard format. Also, the Group noted the addition of a 'per cent completed' column for genetic analysis of tissue samples would be useful to assist in the annual review. Víkingsson, while agreeing with these recommendations, reminded the Group that Norway, Japan and Iceland are providing update of their registries to the Group on a voluntarily basis.

The Group also noted that full technical specifications for the Japanese and Icelandic DNA registries had never been received or reviewed, and that although such information is provided voluntarily, such a review would be helpful for the Group's annual review of the status of DNA registries under its standing Agenda Items. The Chair again reminded the Group that reports of updates of registers should include a list of references including the relevant documents on protocols used.

## 9. CONSIDERATION OF REQUEST FOR ADVICE FROM THE COMMISSION

The Working Group on DNA Testing held a joint meeting with the Working Group on Bycatch to review Annex {DNA} of document IWC/62/7rev, according to the Terms of Reference provided in Annex G of IWC/62/6rev (Appendix 5). Pastene and Perrin co-chaired the sessions, which was attended by most of the members of the two Working Groups. The draft technical specifications for establishment/maintenance of diagnostic DNA registers and general approaches for design of market sampling schemes in Annex {DNA} of IWC/62/7rev were derived from the report of a workshop held from 7-9 March 2005 (IWC/M05/RMSWG5) following Terms of Reference given in Annex

B of that report. Participants at the 2005 workshop included the Specialist Group established by the RMS (Revised Management Scheme) Working Group at its meeting in Sweden in December 2004.

In the sections below, excerpts from parts of Annex {DNA} of IWC/62/7rev are indicated by:

indented type in alternate font size.

Comments and suggestions for improvements made during the discussion and additional text and footnotes **recommended** by the Working Groups (hereafter 'Group') to improve clarity and completeness of the specifications for the DNA registry and market sampling scheme are indicated below by:

*[italic text in square brackets].*

A complete and uninterrupted version of a modified Annex {DNA} is included in Appendix 6.

### 1. SPECIFICATIONS FOR THE ESTABLISHMENT/ MAINTENANCE OF A DIAGNOSTIC<sup>17</sup> DNA REGISTER/ TISSUE ARCHIVE

#### 1.1 Laboratories

##### 1.1.1 Minimum laboratory requirements

- (1) Laboratories performing DNA analysis shall be recognised by the Contracting Government under whose jurisdiction whales are harvested.
- (2) Quality control and quality assurance features shall ensure that:
  - (a) analysts have acceptable education, training and experience for the task;
  - (b) reagents and equipment are properly maintained and monitored;
  - (c) procedures used are generally accepted in the field and have been approved by the IWC Scientific Committee (see Items 1.2-1.5);
  - (d) appropriate controls are used.
- (3) Thorough laboratory records (protocols, notes, worksheets, etc.) shall be maintained and archived for possible inspection (see Item 1.7).
- (4) Changes in equipment and approved methods shall be recorded and reported annually to the IWC to allow ongoing standardisation among registers (see Item 1.7).
- (5) A suitable inventory management system shall be in place so that the whereabouts and use of each sample/aliquot over time during storage and analysis can be traced.
- (6) Portions of the tissue samples and DNA extracts should be retained and stored in an appropriate manner (see Item 1.2.3).

The Group noted that the length of time that archived samples were to be stored was not specified in item 6, but it was clear that the intent was for long-term storage. The modified text **recommended** by the Group was:

*[(6) Portions of the tissue samples and DNA extracts should be retained and stored indefinitely or until advised by the SC, using an appropriate preservation method (see Item 1.2.2).]*

- (7) The probability of errors occurring should be estimated and minimised, using standard procedures. DNA data quality/acceptability should be decided in accordance with generally accepted rules and reported annually where possible (e.g. PHRED scores for sequences, SDs of fragment length measurements for microsatellite alleles, means and SDs of peak heights for microsatellites, some evaluation of stutter for each microsatellite locus). This information should be reported annually to the IWC (see Items 1.5 and 1.7).

<sup>17</sup>A diagnostic DNA register is one that contains DNA profiles of any animals from which products *might* legally appear on the market (e.g. from legal direct catches, bycatches, ship strikes etc.). On this basis, any products found on the market that were from whales not included in the register will be from illegal whales.

The Group agreed that a variety of error-checking procedures should be followed, including *inter alia* genotyping errors, mis-labelling, identification of duplicate samples, etc.

The Group also suggested that sample quality should be checked routinely prior to genetic analysis because some samples (e.g. those derived from bycaught animals) may be degraded and thus would require increased replication to ensure accuracy. References useful for outlining such considerations and providing methods for quality control and reporting include the Guidelines agreed by the IWC Scientific Committee (IWC, 2009a) and also Morin *et al.* (2010). Modified text to clarify both requirements was **recommended** by the Group:

*[(7) The probability of genotyping errors occurring should be estimated and minimised, using standard procedures and also including provisions for detection of mis-labelling, duplicate samples, data entry errors, etc. DNA sample quality should be checked routinely prior to genetic analysis to ensure adequate accuracy in the genotyping of degraded samples (as recommended in IWC (2009a), and subsequent updates to the genetic analysis guidelines). DNA data quality/acceptability should be addressed in accordance with generally accepted rules and reported annually where possible (e.g. PHRED scores for sequences, SDs of fragment length measurements for microsatellite alleles, means and SDs of peak heights for microsatellites, some evaluation of stutter for each microsatellite locus). This information should be reported annually to the IWC (see Items 1.5 and 1.7).]*

- (8) A reference set of samples should be designated for allelic standards and an equimolar allelic ladder should be constructed by cloning and sequencing a range of alleles for each microsatellite locus.

The Group discussed whether cloning and sequencing of a range of microsatellite alleles was strictly necessary, and agreed that because many microsatellite markers had been originally derived from different cetacean species this could be an important factor in the use of data derived from such markers in other species. The Group also noted that the intent for use of allelic standards was not stated explicitly, but included inter-laboratory calibration (see section 1.1.2 below), which is one of the greatest challenges needed for ensuring accuracy in the development and maintenance of DNA registries.

- (9) The laboratory shall participate in calibration exercises with other laboratories if requested to do so by the IWC (see Item 1.1.2).

The Group noted that several different factors are important in calibration exercises and **recommended** revised wording to clarify this requirement:

*[(9) The laboratory shall participate in calibration exercises with other laboratories if requested to do so by the IWC (see Item 1.1.2), and taking into account both the analysts involved, the methods and/or software used for binning alleles, and the type of equipment used for genotyping.]*

- (10) The laboratory should be available for external evaluation and participate regularly in proficiency tests such as double-blind comparisons (e.g. see Item 1.7).

The Group noted that footnote 17 (see p.309) did not adequately describe provisions for ensuring that a DNA

register could be used to distinguish whales derived from legally sanctioned trade, and **recommended** alternate wording to satisfy this requirement:

*[<sup>17</sup>A diagnostic DNA register is one that contains DNA profiles of all animals from which products might legally appear on the market (e.g. from legal direct catches, legal imports, bycatches, ship strikes etc.). DNA profiles from legally imported whales should thus be included in the importing country's registry as one of the conditions for importation. On this basis, any products found on the market that were from whales not included in the register will be from illegally taken or illegally imported whales.]*

#### 1.1.2 Calibration of laboratories if more than one is used

Where more than one laboratory is used to generate a single register or a group of registers, or for the comparison of samples (e.g. under Item 1.8 or Item 2), appropriate calibration of microsatellite genotype scoring (e.g., absolute size or binning) must be undertaken and the results reported to the IWC. The details of the calibration exercise shall be determined by the international expert group (see Item 1.7). The calibration exercise will primarily comprise a double blind experiment with known individuals. Cloned alleles should be used to construct an allelic ladder for calibration purposes. The results of calibration exercises must be reported to the IWC. In designing calibration exercises and reviewing the results, it must be remembered that the primary function of diagnostic DNA registers is to determine whether illegal activity is taking place and that the default position is no match = illegal activity. In this regard it is important to estimate the likelihood of:

- erroneously failing to match products to an animal in the register when it is actually there – i.e. falsely implying an infraction;
- erroneously matching products to an individual in the register when it is not actually there – i.e. missing an infraction when one has occurred.

#### 1.2 Sample collection

Samples for DNA registry should be collected by trained personnel before products from them can enter the market.

The Group noted that Annex {SI} of IWC/62/7rev applies only to commercial, scientific and indigenous catches, but there was no specification for training of and information to be collected by others who may be involved in the collection of genetic samples for DNA registries including those involved in collection of samples from bycaught or stranded whales. A representative from Japan noted that written instructions and probably some initial briefing/training sessions were provided to fishermen who may be involved in collection of samples from bycaught whales. The Group **recommended** an additional footnote to specify such a requirement:

#### *[1.2 Sample collection*

*Samples for DNA registry should be collected by trained personnel<sup>18</sup> before products from them can enter the market.*

*<sup>18</sup>Contracting Governments under whose jurisdiction bycaught/stranded whales and their products may be legally marketed are responsible to develop a technical manual for collecting samples and ancillary data for inclusion in DNA registries, and for disseminating such materials and training to others who may be involved in the collection of genetic samples for such use.]*

#### 1.2.1 Size of samples

At least two samples of skin/muscle of at least 5x5x5mm must be collected from each animal for each register/archive. In addition, where possible, at least four muscle samples of 20x20x20mm should be taken and frozen as quickly as possible for each register/archive. Samples must also be obtained from any foetuses present.

### 1.2.2 Preservation

Samples should initially be preserved in 95% ethanol (in at least five times the volume of the sample, due to potential problems of dilution and evaporation) and if practical refrigerated or frozen immediately. If not able to be frozen immediately, the samples should be shipped as soon as possible (preferably within 7 days) to the analysing laboratory. This temporary storage and shipping should be in temperatures <25°C to minimise the possibility of degradation of the sample.

Long-term storage of skin/muscle samples should be in 95% ethanol at or below -20°C. The additional muscle samples should be frozen in liquid nitrogen; transport should be with dry ice. Long-term storage of frozen tissue samples should be at or below -80°C.

The Group **recommended** additional clarification of the sample preservation requirements in sections 1.2.1 and 1.2.2:

#### [1.2.1 Size of samples

*At least two samples of skin/muscle of at least 5x5x5mm must be collected from each animal for each register/archive. In addition, where possible, at least four muscle samples of 20x20x20mm should be taken. Where possible, a sample of tissue from any foetuses detected should be collected. All samples should be taken as quickly as possible and immediately placed in an appropriate preservative, and then frozen as quickly as possible at or below -20°C.*

#### 1.2.2 Preservation

*Samples should initially be preserved in 95% ethanol (in at least five times the volume of the sample, due to potential problems of dilution and evaporation) or in five times the volume of NaCl-saturated DMSO (dimethyl-sulfoxide). If not able to be frozen immediately, the samples should be shipped as soon as possible (preferably within 7 days) to the analysing laboratory. This temporary storage and shipping should be in temperatures <25°C to minimise the possibility of degradation of the sample.*

*Long-term storage of skin/muscle samples should be in 95% ethanol or NaCl-DMSO at or below -20°C. The additional muscle samples should be frozen in liquid nitrogen; transport should be with dry ice. For best preservation long-term storage of frozen tissue samples should be at or below -80°C or if that is not possible at or below -20°C.]*

#### 1.2.3 Labelling

Reliable labelling of the sample is essential. The container should be labelled on both the inside and the outside with a unique identifying code that can be related directly to the biological and other information collected for the individual (see Item 1.2.4). The label on the inside must be indelible and insoluble in alcohol to ensure that the number remains legible after storage in ethanol. The label on the outside must also be robust and remain legible if exposed to ethanol or water.

#### 1.2.4 Information to be collected

In addition to the information noted in {SI} Annex dated day/month/year to be collected for each whale (including date, locality, species, sex, and body length), the unique identifier (see Item 1.2.3) and the name (plus address if non-nominated person, e.g. in the case of bycatch) of sampling person must be recorded.

### 1.3 Tissue analysis

#### 1.3.1 Extraction of DNA

Extraction of DNA should be carried out using standard methods which have been reviewed and approved by the IWC Scientific Committee. Extracted DNA aliquots should be stored in freezers at or below -80°C.

#### 1.4 Markers and methods of analysis

Analysis of samples should be undertaken without knowledge of the biological and other information available for the whale from which the sample was taken.

Samples should be analysed for (at least):

- (1) mitochondrial DNA - primarily for identification to species and population but also contributes to profiling;
- (2) microsatellites (or Short Tandem Repeats, STRs) – for DNA profiling;
- (3) Y chromosomes - sex identification which also contributes to profiling.

#### 1.4.1 Mitochondrial DNA

Analytical methods must be approved by the international expert group (see Item 1.7). Species identification should be accomplished with an approximately 500bp fragment of the 5'-end of the control region and sequencing should occur in both directions.

#### 1.4.2 Microsatellites

Analytical methods must be approved and reviewed annually by the international expert group (see Item 1.7). Fluorescent techniques that allow electronic records to be kept should be used.

This group will ensure that the number and degree of variability of loci used in DNA registers will be sufficient to allow for an acceptable level of average probability of correctly identifying an individual.

#### 1.4.3 Sex identification

Analytical methods must be approved by the international expert group (see Item 1.7). Sex is an additional genotype that may prove useful to identify market samples and may also serve as a check on field data. Error rates (obtained by comparison with reliable field identification of sex) should be estimated and reported to the international expert group (see Item 1.7).

The Group noted that data quality standards recently adopted by the Committee were not mentioned in the text for items 1.4.1, 1.4.2, and 1.4.3 and **recommended** the following amendments:

#### [1.4.1 Mitochondrial DNA

*Analytical methods adhering to the quality standards as specified in the IWC genetic data quality guidelines (IWC, 2009a or subsequent updates) must be approved by the international expert group (see Item 1.7). Species identification should be accomplished with an approximately 500bp fragment of the 5'-end of the control region and sequencing should occur in both directions.*

#### 1.4.2 Microsatellites

*Analytical methods adhering to the quality standards as specified in the IWC genetic data quality guidelines (IWC, 2009a or subsequent updates) must be approved and reviewed annually by the international expert group (see Item 1.7). Fluorescent techniques that allow electronic records to be kept should be used. This group will ensure that the number and degree of variability of loci used in DNA registers will be sufficient to allow for an acceptable level of average probability of correctly identifying an individual.*

#### 1.4.3 Sex identification

*Analytical methods adhering to the quality standards as specified in the IWC genetic data quality guidelines (IWC, 2009a or subsequent updates) must be approved by the international expert group (see Item 1.7). Sex is an additional genotype that may prove useful to identify market samples and may also serve as a check on field data. Error rates (obtained by comparison with reliable field identification of sex) should be estimated and reported to the international expert group (see Item 1.7).]*

### 1.5 Format of individual records

Each whale is given a unique identifier that can be cross-referenced back to the biological and associated data for that animal. Records must contain:

- (a) A microsatellites and sex profile, in which each whale profile is given one row, with one column for each allele (two columns for each microsatellite marker and the sex locus).
- (b) A mtDNA sequence file, in which each profile has one row, and one column for each site where the sequence deviates from the reference sequence.

In addition, the following must be archived:

#### *General information for each sample*

- genotyping system
- software system

#### *'Raw' data*

- electropherograms
- quality scores
- raw allele sizes
- peak heights
- gel image (depending on platform used)
- number of times the genotype replicated

#### *Summary data on each locus*

- error rate and how determined
- allele frequencies in a given population
- deviations from Hardy-Weinberg equilibrium
- evidence of null-alleles, short-allele dominance (or short-allele bias due to preferential amplification) or other artefacts

### 1.6 Matching

The international expert group (see Item 1.7) will agree on software packages to be used for matching purposes.

### 1.7 External audit of DNA registers

An international expert group established pursuant to paragraph 42 shall:

- review and approve the initial technical specifications for the register(s) and any changes to those protocols;
- where necessary, decide on appropriate laboratories;
- where necessary, design calibration exercises for laboratories and review the results of those exercises;
- review annually specific information and statistics formally reported by the register(s) under Items 1.4 - 1.6;
- design and undertake periodic technical audits including the provision for trials using 'blind' control samples;
- design and arrange for periodic site visits to examine whether the agreed protocols (under Items 1.2-1.5) are being followed.

The international expert group shall submit an annual report to the IWC and its Contracting Governments for consideration two months before each Annual Meeting of the IWC.

The Group noted that whether the report of the international expert group should be submitted to the IWC Scientific Committee, the Commission, or the Secretariat was unclear and that there was also a potential change in the scheduling of IWC meetings, and **recommended** clarification in the wording of the provision for submission of the report mentioned in the last provision of section 1.7:

*[The international expert group shall submit an annual report to the Secretariat of the IWC for distribution to contracting governments and the Commission (and, if necessary subsidiary bodies of the Commission) at least two months before it must be considered.]*

### 1.8 Submission procedure for samples for comparison with registers

Submission of tissue samples to the IWC for comparison with registers:

- (1) may be made by Contracting Governments; and
- (2) shall be accompanied by officially-attested documentation of chain of custody from time of collection to submission that contains the following information:

- name and address of 'collector';
- location obtained;

- type of vendor;
- date and time of collection;
- label, if present (or verbal description of nature and origin of product offered by vendor);
- where possible, photographs; and
- comments by the Contracting Government where the market sample was collected.

Analysis of the samples shall be carried out following the procedures documented in Items 1.3–1.4 by an IWC-approved laboratory, in accordance with any necessary calibration procedures. Officially-attested documentation of chain of custody must be established for the period between submission to a Contracting Government (or appropriate intergovernmental body) and provision of analytical results.

The comparison of the resultant profile shall be made using agreed software (see Item 1.6) against the appropriate register(s).

When the matching has been completed, the IWC Secretariat shall make public the results within one week.

The Group considered all of section 1.8 in light of the stated objective of Annex {DNA}: 'to ensure a...robust, independent and transparent system'. Item 1.8 makes a crucial contribution to these objectives, by providing a mechanism for sample verification that is not reliant on national market sampling schemes, and is also not reliant on the international expert panel, whose role is to audit the system rather than to focus on individual samples. By providing an opportunity for third parties to have samples verified against an IWC-held electronic register, Item 1.8 could greatly contribute to the independence, transparency and robustness of the entire 'DNA system'. However, the current wording of Item 1.8 does not fully make clear the intent nor the mechanism.

With respect to the mechanism itself, the Group noted the following points:<sup>2</sup>

1. The physical submission of tissue samples to the IWC Secretariat (as in the current wording of the first sentence of 1.8) may be difficult because of the CITES permit issues, and is in any case normally unnecessary. Instead, it would be adequate to submit the documentation to the IWC, and the tissue itself could be sent to and analysed by a qualified laboratory\* in the country of origin. That laboratory would then genotype the sample and transmit the complete sample profile (see item 1.5 above) electronically to the Secretariat, who would then conduct the matching analysis against DNA profiles held in the central DNA database.
2. The intent of specifying how and by whom samples may be submitted (subitems 1 and 2 of section 1.8) is a safeguard against fraudulent or mischievous claims. It is, however, crucial to avoid unintended side-effects of these provisions, since item 1.8 will fail as a transparent, independent and robust safeguard unless the rules for submission can be met in practice. Since it is beyond the remit of the Scientific Committee to comment on details of chain-of-custody documentation, the Group noted that these details might warrant further consideration in a different Committee of the IWC.
3. The IWC's electronic register is to be updated annually (paragraph 42 of Annex A – draft Amendments to the Schedule, IWC/62/7rev), although this provision is not stated in Annex {DNA}. Additionally, according to the current wording of item 1.8, the results of matching

\*A qualified laboratory is one recognised by a Contracting Government that meets the standards of items 1.1.1 and 1.1.2 as specified by the international expert group.

are supposed to be made public within one week. This could lead to a sample failing to match profiles in the IWC's central register simply because the latter had not been updated at the time of sample submission. The possibility of a match cannot be excluded until after that update. This might also have implications for the timing of updates to the IWC's central register, relative to timings of IWC meetings.

In order to take account of all these difficulties with the current wording of section 1.8, the Group **recommended** the following revision of the entire section, including the requirement for submission of electronic profiles from paragraph 42 of Annex A (new item 1.9), and an additional footnote 19:

**[1.8 Mechanism for comparing samples to the IWC's central register, further to domestic market survey systems**

*A Contracting Government may request the IWC to compare any appropriately-documented tissue sample against the IWC's electronic register, regardless where the sample was collected. The tissue sample should be sent to a qualified laboratory<sup>19</sup>, not necessarily associated with the national registry. The associated documentation, which is specified below, should be sent to the Secretariat. The laboratory should send the DNA profiles (see item 1.5) to the Secretariat as soon as possible, and the sample should be kept in long-term storage (see item 1.1.1, 1.2.3).*

*The associated documentation shall describe chain of custody from time of collection to submission, including the following information:*

- name and address of 'collector';
- location obtained;
- type of vendor;
- date and time of collection;
- label, if present (or verbal description of nature and origin of product offered by vendor);
- where possible, photographs; and
- comments by the Contracting Government where the market sample was collected.

*Analysis of the samples shall be carried out following the same quality control, sample handling and calibration procedures specified above in Items 1.1 – 1.4 by a qualified laboratory<sup>19</sup>. Officially-attested documentation of chain of custody must be established for the period between submission and provision of analytical results.*

*The comparison of the DNA profile against the IWC's central register shall be made using agreed software (see Item 1.6) [Option 1: after the annual update from the relevant national register.] [Option 2: Profiles that do not match would be held in a database that would be checked against the annually-updated registry each year.] The Secretariat shall make public the results within one week.*

**1.9 Submission of DNA Profiles to the IWC's Central Registry**

*Contracting Governments under whose jurisdiction whales and whale products may be legally marketed shall maintain a diagnostic DNA register and tissue*

*bank. Before any products from a whale enter the market, samples for the DNA registry shall be collected from that whale, and submitted for inclusion in the domestic registry. DNA profiles shall be transmitted annually to a centralised archive maintained by the Secretariat.]*

**2. SPECIFICATIONS FOR THE ESTABLISHMENT/ MAINTENANCE OF MARKET SAMPLING SCHEMES**

The purpose of market sampling is twofold: to act as a deterrent to illegal activity and to detect whether such activity is occurring. Market sampling in its initial stage is not intended to determine the precise number of animals that may be involved. Rather, if illegal products are discovered, a targeted method of detecting the origin of the products and the extent of the illegal operation specific to the case should be developed.

**2.1 Design principles**

- (1) Market sampling schemes shall be case-specific. Their design shall be based on the best available information on the temporal and geographical nature of the particular market(s) and product pathways. Power to detect/deter will increase with the geographical and temporal scope of the surveys.
- (2) The design of market sampling schemes will be iterative and schemes should be reviewed periodically. Experimental testing of their potential to detect illegal products should be undertaken and reported. This should include estimation of the possibility of falsely suggesting illegal activity and missing illegal activity when it occurs.
- (3) Appropriate (e.g. not highly processed products from which it is difficult to obtain reliable microsatellite profiles) products should be chosen.
- (4) A balance between deterrence (sampling carried out openly and with publicity) and detection (undercover sampling) shall be maintained and reported.
- (5) The full range of cetacean products shall be sampled in case mislabelling occurs.
- (6) An officially-attested documentation of chain of custody from time of collection to results of matching must be collected and archived, including the information given in Item 2.3.
- (7) Analysis and matching must be carried out in an IWC-approved laboratory (with appropriate calibration if necessary) following the procedures given in Item 1 above.

**2.2 Development of appropriate market sampling schemes including audit**

The international expert group (see Item 1.7) under the auspices of the IWC shall:

- (1) co-operate in the design of and approve any market sampling scheme before it is implemented and review the associated results;
- (2) co-operate in the design of and approve experimental work and review results referring to Item 2.1 (2) above.
- (3) design and arrange for periodic site visits to ensure that the approved scheme is being implemented.

The Group noted that some 'degraded' and/or 'processed' samples from market surveys could not be analysed using exactly the same procedures as those currently used for 'fresh' and 'unprocessed' samples, but that methods could be developed to allow accurate comparison of such samples with profiles in DNA registries. The Group **recommended** one additional development goal to take into account the potential inclusion of such samples from market surveys:

*[(4) Experimental procedures should reflect the need for a standardised set of markers suited to the generation of accurate data from degraded source materials.]*

**2.3 Data to be collected**

- Product or sample of product of sufficient size to obtain DNA sample (see Item 1.2.2);
- Location obtained;
- Date and time;
- Label (or verbal description of nature and origin of product offered by vendor);

<sup>19</sup>A qualified laboratory is one recognised by a contracting government that meets the standards of items 1.1.1 and 1.1.2 as specified by the international expert group.]

- Source (e.g. wholesale market, shop, dockside etc.);
- photograph of product before sub-sampling; and
- name and contact information of person collecting.

This information should be archived in an appropriate electronic manner.

#### 2.4 Reporting

The authorities responsible for undertaking the market sampling schemes in accordance with Paragraph 42 of the Schedule shall submit an annual report of their market sampling activities to the international expert group via the IWC Secretariat at the end of February of each year. That report shall include: details of the methods used; a summary of the number and nature of the products sampled, and the geographical and temporal spread of sampling; the results of the matching exercise. The international expert group shall submit an annual report to the IWC and its Contracting Governments for consideration two months before each regular Meeting of the IWC.

The group **recommended** a slight revision of the text concerning reporting to the IWC to take into account potential changes in the meeting schedule(s) and to match the revised wording in section 1.7 above:

*[The international expert group shall submit an annual report to the Secretariat of the IWC for distribution to contracting governments and the Commission (and, if necessary subsidiary bodies of the Commission) at least two months before it must be considered.]*

### 10. OTHERS

SC/62/O19 from Baker and Brownell describes a proposal to the IWC DAG under Procedure B, requesting access to the Japanese DNA register for the purposes of evaluating the technical aspects of traceability/trackability of sei, fin and Antarctic minke whale products purchased at commercial outlets in Santa Monica, USA and Seoul, South Korea. SC/62/O19 requested that the proposal be considered for endorsement by the Group.

Kanda stated that he was not prepared to endorse the proposal in SC/62/O19 given the current policy of Japan, Norway and Iceland regarding DNA registers access and market surveys. The Group could not reach an agreement

on whether or not to endorse the proposal in SC/62/O19 although recognising that the matching exercise proposed would, in principle, be valuable for testing functionality of DNA registers for identifying and tracking whale products.

### 11. 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.

### 12. ADOPTION OF THE REPORT

The report was adopted by consensus.

#### REFERENCES

- Baker, C.S., Steel, D., Choi, Y.-C., Lee, H., Kim, S.K., Choi, S.K., Ma, Y.-U., Hambleton, C., Psihoyo, L., Brownell Jr, R.L. and Funahashi, N. 2010. Genetic evidence of illegal trade in protected whales links Japan with the US and Korea. *Biology Letters*: 5pp.
- Cipriano, F. and Pastene, L. 2009. A review of current knowledge of techniques to extract and amplify DNA from 'difficult' whale samples. Paper SC/61/SD2 presented to the IWC Scientific Committee, June 2009, Madeira, Portugal (unpublished). 5pp. [Paper available from the Office of this Journal].
- 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.
- International Whaling Commission. 2009a. Report of the Scientific Committee. Annex I. Report of the working group on stock definition. Appendix 2. Guidelines for DNA data quality control for genetic studies relevant to IWC management advice. *J. Cetacean Res. Manage. (Suppl.)* 11:252-56.
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- International Whaling Commission. 2010. Report of the Scientific Committee. *J. Cetacean Res. Manage. (Suppl.)* 11(2):1-98.
- Morin, P.A., Martien, K.K., Archer, F.I., Cipriano, F., Steel, D., Jackson, J. and Taylor, B.L. 2010. Applied conservation genetics and the need for quality control and reporting of genetic data used in fisheries and wildlife management. *J. Heredity* 101(1): 1-10.

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## Appendix 1

### AGENDA

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|--|--|
| <ol style="list-style-type: none"> <li>1. Election of Chair</li> <li>2. Appointment of rapporteurs</li> <li>3. Adoption of the Agenda</li> <li>4. Review of documents</li> <li>5. Progress on genetic methods for species, stock and individual identification</li> <li>6. Review of results of the 'amendments' of sequences deposited in <i>GenBank</i></li> </ol> | <ol style="list-style-type: none"> <li>7. Progress on collection and archiving of tissue samples from catches and bycatches</li> <li>8. Reference databases and standards for diagnostic DNA registries</li> <li>9. Consideration of request for advice from the Commission</li> <li>10. Other</li> <li>11. Work plan</li> <li>12. Adoption of the Report</li> </ol> |
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## Appendix 2

## STATUS OF THE NORWEGIAN MINKE WHALE DNA REGISTER BY MAY 2010

Hans Julius Skaug

Table 1  
Status of the Norwegian minke whale DNA register.

Year	DNA register <sup>1</sup>	IWC catch statistics <sup>2</sup>	Not landed <sup>3</sup>	Landed <sup>4</sup>	Duplicates <sup>5</sup>	Missing samples <sup>6</sup>	Lab. problem <sup>7</sup>	Total missing <sup>8</sup>
1997	488	503	7	496	3	5	0	8
1998	609	625	11	614	1	4	0	5
1999	571	591	17	574	2	1	0	3
2000	470	487	6	481	3	8	0	11
2001	538	552	11	541	2	1	0	3
2002	625	634	9	625	0	0	0	0
2003	637	647	9	638	1	0	0	1
2004	530	544	7	537	7	0	0	7
2005	626	639	6	633	3	4	0	7
2006	531	545	7	538	4	2	1	7
2007	575	597	5	592	6	11	0	17
2008 <sup>9</sup>	-	536	4	532	-	-	-	-
2009 <sup>10</sup>	-	485	1	484	-	-	-	-

<sup>1</sup>Number of unique individuals contained in the DNA-register (not containing duplicates). <sup>2</sup>Number of individuals caught by Norway, including individuals not landed. <sup>3</sup>Number of individuals killed, but not taken onboard the vessel. <sup>4</sup>Number of individuals taken onboard the vessel. <sup>5</sup>Number of occurrences of (tissue) sample switching onboard the vessel as detected by comparison of genetic profiles; i.e. two samples have been returned from an individual, and no sample has been returned for an individual. <sup>6</sup>Number of individuals for which tissue samples are missing for other reasons than sample switching. <sup>7</sup>Genetic laboratory not able to obtain microsatellite profile from tissue sample. <sup>8</sup>The difference between the columns 'Landed' and 'DNA register'. <sup>9</sup>Laboratory completed, but results not analysed. <sup>10</sup>Laboratory analyses not completed.

This table shows the number of individuals contained in the DNA-register, and the number of individuals missing. For 2008 the genetic analyses are not completed, as indicated by the '-' in the table.

## Appendix 3

## AN UPDATE OF THE JAPANESE DNA REGISTER FOR LARGE WHALES

Naohisa Kanda and Mutsuo Goto, The Institute of Cetacean Research

Table 1  
Status of the Japanese DNA register for large whales.

Source/species	Period	Genetic samples	mtDNA	STRs	Sex
<b>Scientific whaling</b>					
NP minke whale	09	162	162	*	162
NP Bryde's whale	09	50	50	*	50
NP sei whale	09	100	100	*	100
NP sperm whale	09	1	1	*	1
Antarctic minke whale	05/06	853	0	0	853
	06/07	505	0	190	505
	07/08	551	0	551	551
	08/09	679	0	0	679
	09/10	506	0	0	506
Antarctic fin whale	08/09	1	0	0	1
	09/10	1	0	0	1
<b>Bycatches</b>					
NP minke whale	09	119	119	*	*
NP humpback whale	09	3	3	*	*
<b>Strandings</b>					
NP minke whale	09	3	*	*	*
NP humpback whale	09	1	*	*	*
NP sperm whale	09	1	*	*	*

STR=microsatellites; NP=North Pacific. **Note 1:** as explained in IWC (2006), sex of the whales taken by scientific whaling was determined by scientists onboard the research vessels. **Note 2:** 0=not yet analysed at the time this table was prepared. \*Under analysis.

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 (JARPNII) 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 strandings up to 2005.

Genetic profiles of the following individuals have been added to the dataset since the last scientific meeting.

## REFERENCE

International Whaling Commission. 2006. Report of the Working Group on DNA testing. *J. Cetacean Res. Manage. (Suppl.)* 8: 252-258.



### Appendix 4

#### STATUS OF THE ICELANDIC WHALE DNA REGISTER

Christophe Pampoulie and Gisli A. Víkingsson

Table 1

Icelandic whale DNA register.

Year	Type <sup>1</sup>	No. genetic samples	Microsatellites	MtDNA	Sex
<b>Common minke whale</b>					
2003	SP	36	36	36	36
2004	SP	25	25	25	25
2005	SP	34	34	34	34
2006	SP	58	58	58	58
2006	C	1	0	0	0
2007	SP	36	36	36	36
2007	C	6	0	0	0
2008	C	38	0	0	0
2009	C	81	11	11	11
<b>Fin whale</b>					
2006	C	7	7	7	7
2009	C	125	125	125	125

<sup>1</sup>SP=Special Permit catch; C=commercial catch.

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.

Table 1 gives the present status of the registry. Samples from all the common minke whales landed as a part of the Icelandic research programme (2003-07) as well as from commercial catches of one minke whale and seven fin whales have been archived.

Genetic analyses of fin whales taken for commercial purposes in 2009 have been completed.

### Appendix 5

#### TERMS OF REFERENCE AND GUIDANCE FOR THE SCIENTIFIC COMMITTEE'S WORK WITH RESPECT TO THE 'FUTURE OF THE IWC' DISCUSSIONS (FROM ANNEX G OF IWC/62/6REV)

The Scientific Committee shall review, for clarity and completeness:

- (1) Annex {DNA} – DNA registry and market sampling scheme (this is based on the work of an earlier specialist group (IWC/55/COMMS3) and the objective is to ensure that it remains up-to-date and complete, representing a cost-effective, robust, independent and transparent system in conjunction with the other monitoring and control measures).

In particular the review of the proposed mechanism (for national schemes with international audit) will ensure that the technical specifications:

- under Section 1 (specifications for the establishment/maintenance of a diagnostic DNA register/tissue archive) remain adequate, suggesting improvements if necessary, including the clarification of details, including appropriate auditing mechanisms, such that appropriate auditing can begin during the first season of an interim arrangement; and
- under Section 2 (specifications for the establishment/maintenance of market sampling schemes) remain adequate, and in particular that a process to allow effective market sampling to occur at the start of the interim period is established, recognising, as stated under Item 2.1 that this will be an iterative process.

## Appendix 6

### REVISED ANNEX {DNA} DATED *DAY/MONTH/YEAR* (FROM DOCUMENT IWC/62/7REV)

#### Specifications and Requirements for Diagnostic<sup>17</sup> DNA Registers and Market Sampling Schemes

##### 1. SPECIFICATIONS FOR THE ESTABLISHMENT/ MAINTENANCE OF A DIAGNOSTIC DNA REGISTER/TISSUE ARCHIVE

###### 1.1 Laboratories

###### 1.1.1 Minimum laboratory requirements

- (1) Laboratories performing DNA analysis shall be recognised by the Contracting Government under whose jurisdiction whales are harvested.
- (2) Quality control and quality assurance features shall ensure that:
  - (a) analysts have acceptable education, training and experience for the task;
  - (b) reagents and equipment are properly maintained and monitored;
  - (c) procedures used are generally accepted in the field and have been approved by the IWC Scientific Committee (see Items 1.2 - 1.5); and
  - (d) appropriate controls are used.
- (3) Thorough laboratory records (protocols, notes, worksheets, etc.) shall be maintained and archived for possible inspection (see Item 1.7).
- (4) Changes in equipment and approved methods shall be recorded and reported annually to the IWC to allow ongoing standardisation among registers (see Item 1.7).
- (5) A suitable inventory management system shall be in place so that the whereabouts and use of each sample/aliquot over time during storage and analysis can be traced.
- (6) Portions of the tissue samples and DNA extracts should be retained and stored indefinitely or until advised by the SC, using an appropriate preservation method (see Item 1.2.2).
- (7) The probability of genotyping errors occurring should be estimated and minimised, using standard procedures and also including provisions for detection of mislabelling, duplicate samples, data entry errors, etc. DNA sample quality should be checked routinely prior to genetic analysis to ensure adequate accuracy in the genotyping of degraded samples (as recommended in IWC (2009), and subsequent updates to the genetic analysis guidelines). DNA data quality/acceptability should be addressed in accordance with generally accepted rules and reported annually where possible (e.g. PHRED scores for sequences, SDs of fragment length measurements for microsatellite alleles, means and SDs of peak heights for microsatellites, some evaluation of stutter for each microsatellite locus). This information should be reported annually to the IWC (see Items 1.5 and 1.7).
- (8) A reference set of samples should be designated for allelic standards and an equimolar allelic ladder should be constructed by cloning and sequencing a range of alleles for each microsatellite locus.
- (9) The laboratory shall participate in calibration exercises with other laboratories if requested to do so by the IWC (see Item 1.1.2), and taking into account both the analysts involved, the methods and/or software used for binning alleles, and the type of equipment used for genotyping.
- (10) The laboratory should be available for external evaluation and participate regularly in proficiency tests such as double-blind comparisons (e.g. see Item 1.7).

###### 1.1.2 Calibration of laboratories if more than one is used

Where more than one laboratory is used to generate a single register or a group of registers, or for the comparison of samples (e.g. under Item 1.8 or Item 2), appropriate calibration of microsatellite genotype scoring (e.g. absolute size or binning) must be undertaken and the results reported to the IWC. The details of the calibration exercise shall be determined by the international expert group (see Item 1.7). The calibration exercise will primarily comprise a double blind experiment with known individuals. Cloned alleles should be used to construct an allelic ladder for calibration purposes. The results of calibration exercises must be reported to the IWC. In designing calibration exercises and reviewing the results, it must be remembered that the primary function of diagnostic DNA registers is to determine whether illegal activity is taking place and that the default position is no match = illegal activity. In this regard it is important to estimate the likelihood of:

- erroneously failing to match products to an animal in the register when it is actually there – i.e. falsely implying an infraction;
- erroneously matching products to an individual in the register when it is not actually there – i.e. missing an infraction when one has occurred.

###### 1.2 Sample collection

Samples for DNA registry should be collected by trained personnel<sup>18</sup> before products from them can enter the market.

###### 1.2.1 Size of samples

At least two samples of skin/muscle of at least 5x5x5mm must be collected from each animal for each register/archive. In addition, where possible, at least four muscle samples of 20x20x20mm should be taken. Where possible, a sample of tissue from any foetuses detected should be collected. All samples should be taken as quickly as possible and immediately placed in an appropriate preservative, and then frozen as quickly as possible at or below -20°C.

<sup>17</sup>A diagnostic DNA register is one that contains DNA profiles of all animals from which products might legally appear on the market (e.g. from legal direct catches, legal imports, bycatches, ship strikes etc.). DNA profiles from legally imported whales should thus be included in the importing country's registry as one of the conditions for importation. On this basis, any products found on the market that were from whales not included in the register will be from illegally taken or illegally imported whales.

<sup>18</sup>Contracting Governments under whose jurisdiction bycaught/stranded whales and their products may be legally marketed are responsible to develop a technical manual for collecting samples and ancillary data for inclusion in DNA registries, and for disseminating such materials and training to others who may be involved in the collection of genetic samples for such use.

### 1.2.2 Preservation

Samples should initially be preserved in 95% ethanol (in at least five times the volume of the sample, due to potential problems of dilution and evaporation) or in five times the volume of NaCl-saturated DMSO (dimethyl-sulfoxide). If not able to be frozen immediately, the samples should be shipped as soon as possible (preferably within 7 days) to the analysing laboratory. This temporary storage and shipping should be in temperatures <math><25^{\circ}\text{C}</math> to minimise the possibility of degradation of the sample.

Long-term storage of skin/muscle samples should be in 95% ethanol or NaCl-DMSO at or below

### 1.2.3 Labelling

Reliable labelling of the sample is essential. The container should be labelled on both the inside and the outside with a unique identifying code that can be related directly to the biological and other information collected for the individual (see Item 1.2.4). The label on the inside must be indelible and insoluble in alcohol to ensure that the number remains legible after storage in ethanol. The label on the outside must also be robust and remain legible if exposed to ethanol or water.

### 1.2.4 Information to be collected

In addition to the information noted in Annex {SI} dated *day/month/year* to be collected for each whale (including date, locality, species, sex, and body length), the unique identifier (see Item 1.2.3) and the name (plus address if non-nominated person, e.g. in the case of bycatch) of sampling person must be recorded.

## 1.3 Tissue analysis

### 1.3.1 Extraction of DNA

Extraction of DNA should be carried out using standard methods which have been reviewed and approved by the IWC Scientific Committee. Extracted DNA aliquots should be stored in freezers at or below

## 1.4 Markers and methods of analysis

Analysis of samples should be undertaken without knowledge of the biological and other information available for the whale from which the sample was taken.

Samples should be analysed for (at least):

- (1) mitochondrial DNA - primarily for identification to species and population but also contributes to profiling;
- (2) microsatellites (or Short Tandem Repeats, STRs) – for DNA profiling; and
- (3) Y chromosomes - sex identification which also contributes to profiling.

### 1.4.1 Mitochondrial DNA

Analytical methods adhering to the quality standards as specified in the IWC genetic data quality guidelines (IWC, 2009, or subsequent updates) must be approved by the international expert group (see Item 1.7). Species identification should be accomplished with an approximately 500bp fragment of the 5'-end of the control region and sequencing should occur in both directions.

### 1.4.2 Microsatellites

Analytical methods adhering to the quality standards as specified in the IWC genetic data quality guidelines (IWC, 2009, or subsequent updates) must be approved and reviewed annually by the international expert group (see Item 1.7). Fluorescent techniques that allow electronic records to be kept should be used. This group will ensure that the number and degree of variability of loci used in DNA registers will be sufficient to allow for an acceptable level of average probability of correctly identifying an individual.

### 1.4.3 Sex identification

Analytical methods adhering to the quality standards as specified in the IWC genetic data quality guidelines (IWC, 2009, or subsequent updates) must be approved by the international expert group (see Item 1.7). Sex is an additional genotype that may prove useful to identify market samples and may also serve as a check on field data. Error rates (obtained by comparison with reliable field identification of sex) should be estimated and reported to the international expert group (see Item 1.7).

## 1.5 Format of individual records

Each whale is given a unique identifier that can be cross-referenced back to the biological and associated data for that animal. Records must contain:

- (a) a microsatellites and sex profile, in which each whale profile is given one row, with one column for each allele (two columns for each microsatellite marker and the sex locus); and
- (b) a mtDNA sequence file, in which each profile has one row, and one column for each site where the sequence deviates from the reference sequence.

In addition, the following must be archived:

#### *General information for each sample*

- genotyping system
- software system

#### *'Raw' data*

- electropherograms
- quality scores
- raw allele sizes
- peak heights
- gel image (depending on platform used)
- number of times the genotype replicated

#### *Summary data on each locus*

- error rate and how determined
- allele frequencies in a given population
- deviations from Hardy-Weinberg equilibrium
- evidence of null-alleles, short-allele dominance (or short-allele bias due to preferential amplification) or other artefacts

## 1.6 Matching

The international expert group (see Item 1.7) will agree on software packages to be used for matching purposes.

## 1.7 External audit of DNA registers

An international expert group established pursuant to paragraph 42 shall:

- review and approve the initial technical specifications for the register(s) and any changes to those protocols;
- where necessary, decide on appropriate laboratories;

- where necessary, design calibration exercises for laboratories and review the results of those exercises;
- review annually specific information and statistics formally reported by the register(s) under Items 1.4 - 1.6;
- design and undertake periodic technical audits including the provision for trials using 'blind' control samples; and
- design and arrange for periodic site visits to examine whether the agreed protocols (under Items 1.2-1.5) are being followed.

The international expert group shall submit an annual report to the Secretariat of the IWC for distribution to contracting governments and the Commission (and, if necessary subsidiary bodies of the Commission) at least two months before it must be considered.

### **1.8 Mechanism for comparing samples to the IWC's central register, further to domestic market survey systems**

A Contracting Government may request the IWC to compare any appropriately-documented tissue sample against the IWC's electronic register, regardless of where the sample was collected. The tissue sample should be sent to a qualified laboratory<sup>19</sup>, not necessarily associated with the national registry. The associated documentation, which is specified below, should be sent to the Secretariat. The laboratory should send the DNA profiles (see item 1.5) to the Secretariat as soon as possible, and the sample should be kept in long-term storage (see item 1.1.1, 1.2.3).

The associated documentation shall describe chain of custody from time of collection to submission, including the following information:

- name and address of 'collector';
- location obtained;
- type of vendor;
- date and time of collection;
- label, if present (or verbal description of nature and origin of product offered by vendor);
- where possible, photographs; and
- comments by the Contracting Government where the market sample was collected.

Analysis of the samples shall be carried out following the same quality control, sample handling and calibration procedures specified above in Items 1.1 – 1.4 by a qualified laboratory<sup>19</sup>. Officially-attested documentation of chain of custody must be established for the period between submission and provision of analytical results.

The comparison of the DNA profile against the IWC's central register shall be made using agreed software (see Item 1.6) [Option 1: after the annual update from the relevant national register.] [Option 2: Profiles that do not match would be held in a database that would be checked against the annually-updated registry each year.] The Secretariat shall make public the results within one week.

### **1.9 Submission of DNA Profiles to the IWC's Central Registry**

Contracting Governments under whose jurisdiction whales and whale products may be legally marketed shall maintain a diagnostic DNA register and tissue bank. Before any products from a whale enter the market, samples for the DNA

registry shall be collected from that whale, and submitted for inclusion in the domestic registry. DNA profiles shall be transmitted annually to a centralised archive maintained by the Secretariat.

## **2. SPECIFICATIONS FOR THE ESTABLISHMENT/ MAINTENANCE OF MARKET SAMPLING SCHEMES**

The purpose of market sampling is twofold: to act as a deterrent to illegal activity and to detect whether such activity is occurring. Market sampling in its initial stage is not intended to determine the precise number of animals that may be involved. Rather, if illegal products are discovered, a targeted method of detecting the origin of the products and the extent of the illegal operation specific to the case should be developed.

### **2.1 Design principles**

- (1) Market sampling schemes shall be case-specific. Their design shall be based on the best available information on the temporal and geographical nature of the particular market(s) and product pathways. Power to detect/deter will increase with the geographical and temporal scope of the surveys.
- (2) The design of market sampling schemes will be iterative and schemes should be reviewed periodically. Experimental testing of their potential to detect illegal products should be undertaken and reported. This should include estimation of the possibility of falsely suggesting illegal activity and missing illegal activity when it occurs.
- (3) Appropriate (e.g. not highly processed products from which it is difficult to obtain reliable microsatellite profiles) products should be chosen.
- (4) A balance between deterrence (sampling carried out openly and with publicity) and detection (undercover sampling) shall be maintained and reported.
- (5) The full range of cetacean products shall be sampled in case mislabelling occurs.
- (6) An officially-attested documentation of chain of custody from time of collection to results of matching must be collected and archived, including the information given in Item 2.3.
- (7) Analysis and matching must be carried out in an IWC-approved laboratory (with appropriate calibration if necessary) following the procedures given in Item 1 above.

### **2.2 Development of appropriate market sampling schemes including audit**

The international expert group (see Item 1.7) under the auspices of the IWC shall:

- (1) co-operate in the design of and approve any market sampling scheme before it is implemented and review the associated results;
- (2) co-operate in the design of and approve experimental work and review results referring to Item 2.1 (2) above;
- (3) design and arrange for periodic site visits to ensure that the approved scheme is being implemented; and
- (4) experimental procedures should reflect the need for a standardised set of markers suited to the generation of accurate data from degraded source materials.

<sup>19</sup>A qualified laboratory is one recognised by a contracting government that meets the standards of items 1.1.1 and 1.1.2 as specified by the international expert group.

### 2.3 Data to be collected

- Product or sample of product of sufficient size to obtain DNA sample (see Item 1.2.2);
- location obtained;
- date and time;
- label (or verbal description of nature and origin of product offered by vendor);
- source (e.g. wholesale market, shop, dockside etc.);
- photograph of product before sub-sampling; and
- name and contact information of person collecting.

This information should be archived in an appropriate electronic manner.

### 2.4 Reporting

The authorities responsible for undertaking the market sampling schemes in accordance with Paragraph 42 of the Schedule shall submit an annual report of their market

sampling activities to the international expert group via the IWC Secretariat at the end of February of each year. That report shall include: details of the methods used; a summary of the number and nature of the products sampled, and the geographical and temporal spread of sampling; the results of the matching exercise.

The international expert group shall submit an annual report to the Secretariat of the IWC for distribution to contracting governments and the Commission (and, if necessary subsidiary bodies of the Commission) at least two months before it must be considered.

#### REFERENCE

International Whaling Commission. 2009. Report of the Scientific Committee. Annex 1. Report of the working group on stock definition. Appendix 2. Guidelines for DNA data quality control for genetic studies relevant to IWC management advice. *J. Cetacean Res. Manage. (Suppl.)* 11:252-56.

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