Annex I

Report of the Working Group on Stock Definition and DNA Testing

Members: Lang (Convenor), Tiedemann (co-Convenor), An, Archer, Baird, Baker, Bickham, Bruniche-Olsen, Buss, Butterworth, Carroll, Cholewiak, Cipriano, Clapham, DeWoody, Donovan, Givens, Goto, Hoelzel, Inoue, Ivashchenko, Jackson, H-Y. Kim, E-M. Kim, Kitakado, Jarman, Pastene, Scordino, Širovič, Skaug, Solvang, Suydam, Taguchi, Víkingsson, Wade, Walløe, Weller, Yoshida.

1. INTRODUCTORY ITEMS

1.1 Convenor's opening remarks
Lang and Tiedemann welcomed participants.

1.2 Election of Chair and appointment of Rapporteurs
Lang and Tiedemann were appointed as co-chairs, and Cipriano acted as rapporteur.

1.3 Adoption of Agenda
The adopted agenda is given in Appendix 1. Items 2.1, 2.3, and 2.4 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.

1.4 Review of documents
The documents identified as containing information relevant to the Stock Definition and DNA Testing Working Group (hereafter, the Working Group) were: SC/67b/SDDNA01-06; SC/67b/ASI05; SC/67b/SH02-03, SC/67b/SH05-06, SC/67b/SH11, SC/67b/SH13; SC/67b/NH02; Carroll *et al.* (2018a); Carroll *et al.* (2018b); Leroy *et al.* (2017); Attard *et al.* (2018); Baker *et al.* (2018); Frasier *et al.* (2015); Tiedemann *et al.* (2018); Jost *et al.* (2018).

2. DNA TESTING

2.1 Genetic methods for species, stocks and individual identification
The Working Group discussed two papers under this agenda item: Carroll *et al.* (2018b), which reviews technological advances in genomic monitoring, including approaches useful in identifying species, stocks, and individuals; and Baker *et al.* (2018), which presents the results of a study to evaluate the feasibility of using seawater samples to collect DNA from killer whales in the Salish Sea.

Carroll *et al.* (2018b) provides a review of how genetic monitoring has been used in the field of conservation biology and looks at how high throughput sequencing (HTS) and other technological advances could aid genetic monitoring in the future. Carroll highlighted the results of the literature review to identify what genotyping methodologies are compatible with producing genotypes from minimally invasively collected samples, considering factors such as running costs and error rates. This was done in the context of genetic monitoring for conservation and management purposes, which require a reproducible set of loci that can be used with low quality and/or quantity DNA templates, but do not necessarily need the 10,000s markers that whole genome or reduced representation approaches provide. The authors acknowledge that many of these platforms do need considerable investment and resources to develop the assays at the outset, which often requires a high-quality DNA template. Among the SNP genotyping platforms that were reviewed, the Fluidigm system, MassArray, and Illumina Goldengate genotyping platforms have been used in the literature with good results. Target capture approaches included some recently published work on microsatellite genotyping via HTS (e.g. De Barba *et al.*, 2017). This could have the advantage of bridging between legacy microsatellite datasets and new HTS datasets by having a certain number of markers in common. However, it seems that the microsatellite loci need to be optimised for HTS, which could limit the number of markers used across time and platforms. The authors also covered the target capture approach known as GT-seq, which is essentially a massively multiplex PCR to amplify hundreds of markers simultaneously (Campbell *et al.*, 2015). One advantage of these target capture approaches *vs* SNP genotyping platforms is the ability to get multi-allelic or phased haplotypic data, for loci with more than one SNP, which could be more informative than single SNPs per locus.

The Working Group thanked Carroll for presenting this review.

In discussion, it was noted that much of the genetic work done to address needs of the SC has relied on generating microsatellite datasets. With advances in HTS and the generation of genome-scale data for many species, other approaches, such as SNP genotyping, are now available, often at comparable costs. SNP genotyping has several advantages over the use of microsatellites (reviewed in Morin *et al.*, 2004), and, importantly, facilitates sharing data across labs and maintaining datasets across time and changes in technology (e.g. sequencing platforms). However, in many cases, including the work done to maintain DNA registries of bycaught or special permit catches (see Appendices 2-4), 'legacy' datasets include microsatellite genotypes for hundreds to thousands of individuals. Thus, one challenge facing the SC in recent years has been determining how to take advantage of these technological advances without decreasing the utility of these large and long-term microsatellite genotype datasets. As such, microsatellite genotyping via HTS (e.g. De Barba *et al.*, 2017) could provide the means to 'bridge the gap' by maintaining the utility of these legacy datasets while also taking advantage of the newer HTS approaches. While appealing, the Working Group noted that there would be several challenges to taking such an approach, including: (1) selecting microsatellites with repeat lengths appropriate for the read lengths typically used by HTS platforms; (2) the design of primers to sequence microsatellites in the absence of genome data, which is not yet available for many species; and (3) bioinformatics difficulties associated with the alignment of sequence data

across microsatellite repeat regions that will be of differing lengths. It was noted that this switch (into sequencing of microsatellites) has been made by the human forensics scientists, and additional exploration of their process could be informative. It was also noted that pedigree data might be used to impute genotypes useful in integrating legacy microsatellite datasets into those generated via HTS sequencing. This could be mathematically challenging but might be feasible in large datasets.

In Baker *et al.* (2018), Baker and co-authors describe methods for using droplet digital (dd) PCR technology for detection and species identification of cetaceans using environmental (e)DNA collected from seawater. For this, they conducted a series of eDNA sampling experiments in the vicinity of killer whales, *Orcinus orca,* in Puget Sound (the Salish Sea). The regular habits of killer whales in these inshore waters allowed the authors to locate pods and collect seawater during 25 encounters at an initial distance of 200m and at 15-minute intervals for up to two hours after the passage of the whales. To optimise detection, they designed a set of oligonucleotide primers and probes to target short fragments of the mitochondrial (mt)DNA control region, with a focus on identification of known killer whale ecotypes. They confirmed the potential to detect eDNA in the wake of the whales for up to 2 hours, despite movement of the water mass by several kilometers due to tidal currents. Re-amplification and sequencing of the eDNA barcode confirmed that the ddPCR detection included the 'southern resident community' of killer whales, consistent with the calls from hydrophone recordings and visual observations.

The Working Group thanked Baker for presenting this paper, which presents a new approach for detecting and identifying cetacean species, including those that may be elusive to study using other methodologies. In discussion, the Working Group noted that one technical challenge associated with using this methodology is that, when sequencing such low quantities of DNA, PCR-generated sequencing errors may be more difficult to detect, and thus careful screening of the resulting data (e.g., validating haplotypes with single-base pair differences) is needed. This issue is less of a concern when using the method for species-level identifications but could be problematic when assessing questions addressing intraspecific diversity. In terms of evaluating presence/absence patterns from eDNA, additional technical considerations include the need to control for false negatives, which can occur because of the low quantity of target DNA present and/or due to the presence of PCR inhibitors in sea water, as well as for false positives that might be present due to contamination.

Although this approach has been more broadly used to detect the occurrence of species in an area (i.e., DNA barcoding), it could provide sequence data useful for stock-level identifications of cetaceans under certain circumstances (e.g., when a single animal is present). It was noted, however, that its utility in addressing questions requiring individual identification via multi-locus genotyping is, at least currently, limited for scenarios in which the water sample could contain DNA from multiple individuals. However, advances in single-cell sequencing technology may provide avenues for additional studies in the future (Lan *et al.*, 2017; Shapiro *et al.*, 2013).

Attention: SC

*The Committee **welcomes** the opportunity to review papers that take advantage of technological advances to improve the ability to detect and identify species, stocks, and individual cetaceans. It **encourages** the submission of similar papers in the future and recognizes the relevance of these techniques to the Committee's work.*

2.2 'Amendments' of sequences deposited in GenBank

GenBank is essentially an uncurated database, and inconsistencies and/or out-dated information in the metadata (e.g. taxonomic status, geographic location, locus misassignment) exist. In previous years, Cipriano has corresponded with GenBank to attempt to identify a mechanism by which these inconsistencies could be corrected. Unfortunately, Cipriano's contact at the NCBI (National Center for Biotechnology Information) passed away last year, and no further progress on this work was made.

At SC/67a, the Working Group agreed that the revised DNA quality guidelines (see Item 3.1) would contain a section discussing the precautions that should be used when including GenBank sequences in a study. Text to include in this section was drafted intersessionally and will be incorporated into the revised guidelines, which are to be completed this year.

2.3 Collection and archiving of tissue samples from catches and bycatches

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

Goto reported on the status of the Japanese register (Appendix 2). The collection of samples is from scientific whaling in the North Pacific (1994-2016 JARPN-JARPNII, NEWREP-NP 2017) and the Antarctic (1987/88-2016/17, JARPA-JARPAII and NEWREP-A), and from bycatch (2001-17).

Skaug reported on the status of the Norwegian register (Appendix 3). The collection of samples of North Atlantic common minke whales is from commercial catches for the period 1997 to 2017.

Vikingsson reported on the status of the Icelandic register (Appendix 4), which includes samples from scientific whaling (2003-07) and commercial catches (2006-17).

2.4 Reference databases and standards for diagnostic DNA registries

An update of the Japanese register is shown in Appendix 2. 100% of the samples collected from North Pacific minke whales (*n*=128) and North Pacific sei whales (*n*=134) under NEWREP-NP in 2017 have been analysed for both mtDNA and microsatellites. MtDNA and microsatellite analyses are also complete (100%) for the North Pacific minke whales (*n*=164) and North Pacific humpback whales (*n*=3) that were bycaught in 2017. No bycatch of North Pacific Bryde's, sei, right, fin, or sperm whales occurred during 2017. MtDNA and microsatellite analyses are complete (100%) for all Antarctic minke whales (*n*=333) sampled under NEWREP-A in 2017.

An update of the Norwegian register is shown in Appendix 3. With the exception of one missing sample, 100% of the North Atlantic common minke whales (*n*=431) caught in 2017 were genotyped using both microsatellites and SNPs. As noted at SC67a (IWC, 2018a, p.228-9), Norway has

discontinued mtDNA typing of samples and substituted it with SNP genotyping. The SDWG would welcome information as to the diagnostic abilities of the Norwegian SNP panel (species and/or stock identification).

An update of the Icelandic registry is shown in Appendix 4. The North Atlantic common minke whales caught by commercial whaling in 2017 (*n*=17) have not yet been screened for either mtDNA or microsatellites. No North Atlantic fin whales were caught by commercial whaling in 2017.

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

Attention: CG-A

The Committee **expresses appreciation** *to Japan, Norway and Iceland for providing updates to their DNA registries using the standard format agreed in 2011 and providing the detailed information contained in their DNA registries.*

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3. GUIDELINES AND METHODS FOR GENETIC STUDIES AND DNA DATA QUALITY

This agenda item relates to two sets of guidelines that the Scientific Committee has requested the Working Group to develop for reference in the Committee's discussions of stock structure. The DNA data quality guidelines are currently being updated (see Item 3.1 below), while the guidelines for genetic data analysis are in press with the *Journal of Cetacean Research and Management.*

In discussion, it was noted that while the DNA data quality guidelines are available on the IWC website, they are included as a link from within the Scientific Committee Handbook. The guidelines are thus difficult to find on the website. The Working Group agreed to discuss the possibility of including both sets of guidelines as a separate link under the main Scientific Committee webpage in order to ensure that they can be easily found. Lang offered to raise this issue with the Secretariat. In addition, the Working Group suggested that the guidelines would be more accessible to the broader scientific community if they were made accessible under ResearchGate, which is a web-based platform designed to facilitate collaboration and sharing of scientific information. Lang offered to explore this option as well.

Attention: SC

The Committee **emphasizes** *the importance of keeping its guidelines related to genetic data quality and analyses up to date. It therefore:*

(1) **reiterates** *the need to update these guidelines to incorporate the discussion of data quality measures used for Next Generation Sequencing data; and*

(2) **agrees** *to continue the intersessional e-mail group to review revised sections of the DNA data quality guidelines that apply to data generated from next generation sequencing platforms, including SNPs and whole genome sequencing, with the goal of posting an updated version of the guidelines on the website next year.*

3.1 Update DNA quality guidelines to include discussion of NGS data
The DNA data quality control guidelines are already available on the IWC website (*http://iwc.int/scientific-committee-handbook#ten*). In recent meetings, data derived from next generation sequencing (NGS) approaches, including SNPs, have been utilised to address stock structure questions. In light of these developments, the Working Group agreed during SC/66b (IWC, 2017b, p.264) that it would be timely to update the DNA data quality control guidelines to cover these types of data. At SC/67b, Tiedemann updated the Working Group on intersessional progress, which included revisions to the sections covering data, including SNPs, produced using NGS approaches. For SC/68b, the group will complete their review of the updated sections so that a revised version can be posted on the IWC website next year. The intersessional group formed during SC/67a will continue to work on this task intersessionally (see Workplan, Item 6.1.1).

3.2 Further applications of DNA techniques
Leroy *et al.* (2017) explores the use of quantitative metrics to detect and monitor genetic erosion. Monitoring systems should not only characterise the mechanisms and drivers of genetic erosion (inbreeding, genetic drift, demographic instability, population fragmentation, introgressive hybridisation, selection) but also its consequences (inbreeding and outbreeding depression, emergence of large-effect detrimental alleles, maladaptation and loss of adaptability). Technological advances in genomics now allow the production of data that can be measured by new metrics with improved precision, increased efficiency and the potential to discriminate between neutral diversity (shaped mainly by population size and gene flow) and functional/adaptive diversity (shaped mainly by selection), allowing the assessment of management-relevant genetic markers. The requirements of such studies in terms of sample size and marker density largely depend on the kind of population monitored, the questions to be answered and the metrics employed. The prospects for the integration of this new information and metrics into conservation monitoring programmes was discussed.

The Working Group thanked DeWoody for his presentation and noted that one of the examples given in the paper (monitoring trends in the abundance of Māui dolphins, Baker *et al.,* 2016) was reviewed by the Working Group last year.

In discussion, it was questioned whether natural selection should be included as a mechanism causing genetic erosion. Although purifying or directional selection may reduce diversity at the selected locus, that reduction is associated with increased adaptation. Selection could cause reduced diversity in loci that are linked to the locus under selection via hitchhiking ('selective sweeps'). However, this would not constitute genetic erosion acting on the locus under selection.

Jost *et al.* (2018) attempts to clarify two primary classes of population genetic structure measures: fixation metrics (F_{ST}, G_{ST}, etc) that describe how close a set of demes are to fixation, and allelic differentiation metrics (Jost's D, entropy differentiation) that describe how different the allelic frequency distribution of demes are. The paper encourages the use of D to better capture genetic diversity in populations of conservation concern.

The Working Group thanked Archer for his presentation and noted that the information builds on the discussion of 'F_{ST} and related measures' that is included in the Data Analysis Guidelines.

In discussion, the Working Group noted that since D is a measure of allelic differentiation, it is highly affected by mutation rate. This presents a challenge when calculating an

average value of D across multiple loci with different mutation rates. In such cases, weighting values of D according to the mutation rate of the locus from which it is calculated would be needed to provide an estimate of mean D that is straightforward to interpret.

The Working Group noted that D has not commonly been presented as a metric for differentiation in papers presented to the SC. This may in part be related to the fact that it is not integrated into some of the major software programs (e.g. Arlequin, Excoffier *et al.*, 2010) that are frequently used to analyse data. The Working Group noted, however, that it is possible to estimate D using the R package strataG (Archer *et al.*, 2017) as well as in Genodive (Meirmans and van Tienderen, 2004).

The Working Group also noted a recent, related paper by (Gaggiotti *et al.*, 2018) that presents a unified framework for diversity measures based on Shannon's entropy.

Finally, the Working Group noted that these two families of measures can be complementary, and that the key was understanding when it is appropriate to use each.

4. PROVIDE ADVICE ON STOCK STRUCTURE TO OTHER SUB-GROUPS

The Working Group has the task of discussing high-priority stock related papers from other sub-committees and working groups, and then providing stock structure related feedback and recommendations to those sub-committees and working groups. These discussions often refer to the genetic analysis guidelines and genetic data quality documents.

In discussion, it was noted many of the papers that the Working Group is asked to review do not include descriptions of the stock structure hypotheses that are being evaluated. The Working Group is comprised of members with varying degrees of familiarity with the work of the other sub-committees or working groups, and thus it can often be challenging to provide a thorough technical review of the paper without an understanding of the stock structure hypotheses being tested. In order to improve this process in the future, the Working Group **encouraged**: (1) presenters of stock structure-related papers provide a brief overview of the relevant stock structure hypotheses prior to discussing their paper; and (2) those who submit a ForInfo paper to consider accompanying it with a working paper that lays out the relevant stock structure hypotheses for the reader. Where possible, the Convenors can assist with the latter task when monitoring the submission of papers.

4.1 Bowhead whales
Paper SC/67b/SDDNA01 focused on stock structure of B-C-B bowheads based on population genetic data from mtDNA sequences (2,494 base pairs) and SNP genotypes (69 autosomal loci). Results from both datasets indicate that the B-C-B and Eastern Canadian Arctic (CAN) stocks are not easily distinguishable, but that the Okhotsk Sea (OK) stock is significantly different from the other two. These conclusions are based on various analyses, including F_{ST}, AMOVA, genic and genotypic differentiation, and STRUCTURE plots. Moreover, there is no evidence of sub-structuring of the B-C-B population. Sub-structure tested included spring *vs* fall harvested whales from Barrow, small *vs* large B-C-B whales (roughly corresponding to young *vs* old), and whales from St. Lawrence Island *vs* Barrow. The mtDNA and SNP results are consistent both with each other and with recent studies on B-C-B bowheads using focal microsatellites and a smaller SNP panel.

The Working Group thanked the authors for presenting this work, which is being evaluated as part of the 2018 *Implementation Review* (IR) of the Bering-Chukchi-Beaufort Seas (B-C-B) stock of bowhead whales.

In discussion, it was noted that the results presented in SDDNA01 have implications for two aspects of bowhead whale stock structure. The primary question of interest for the IR is whether substructure exists within the B-C-B stock. While a number of SNP loci showed significant deviations from Hardy Weinberg equilibrium (HWE) within the B-C-B samples, only about half of these loci exhibited heterozygote deficiencies. This pattern is inconsistent with what would be expected if the deviations from HWE were the result of a Wahlund effect (i.e. due to population substructure). An alternative explanation for the deviations from HWE is that the loci could be under selection pressure. In response, Baird noted that some of the SNPs occur within protein-coding loci, which are more likely to be under selection than non-coding regions.

The Working Group further noted that several of the comparisons previously explored using microsatellite genotypes and mtDNA sequence data (see review IWC, 2008) had been re-examined using the SNP dataset, including: temporal comparisons (whales sampled in the spring *vs* the autumn), spatial comparisons (St. Lawrence Island *vs* Barrow), and the potential for age structure, using length (large *vs* small) as a proxy for age. No significant differences were identified. Based on these results, the Working Group **agreed** that the results presented were consistent with a lack of substructure within the B-C-B stock.

The second question of interest to the SC relates to the degree of mixing between the B-C-B stock and the eastern Canadian Arctic (CAN) stock. Comparisons between these two strata revealed only small, and in some cases statistically insignificant, levels of genetic differentiation in both the mitochondrial and the SNP data. While this pattern could be related to historical connectivity between the two stocks, it could also, or additionally, be driven by some degree of contemporary gene flow. Some evidence of recent movements between these two regions exists (harpoon recovery, reviewed in Rugh *et al.*, 2003; satellite tagging, Quakenbush *et al.*, 2012). To provide increased resolution on the genetic structure within and between these two stocks, the Working Group **recommended** that the authors: (1) analyse the data using ordination methods, such as PCA and DAPC, which can potentially discriminate between groups with low levels of differentiation; and (2) analyse additional samples from the CAN stock in order to increase the power to detect genetic differentiation and to potentially allow for the detection of whales moving between regions via genetic mark-recapture.

Frasier *et al.* (2015) was reviewed as part of a joint session with the *Ad hoc* Working Group on Abundance Estimates, Status and International Cruises. A summary of the discussion can be found in Annex Q under agenda item 3.1.1.

Attention: SC, C-A

The Committee reviewed the results of new genetic analyses of bowhead whales within the Bering-Chukchi-Beaufort Sea (BCB) stock and between the BCB stock and the Eastern Canadian and Okhotsk Sea stocks. The Committee:

*(1) **agrees** that the results were consistent with a lack of substructure within the B-C-B stock;*

*(2) **agrees** that the results suggested that some level of historic or contemporary gene flow could exist between the B-C-B and the Eastern Canadian stock; and*

*(3) although not of immediate management concern, **agrees** that additional genetic analyses be conducted prior to the next Implementation Review to explore potential differentiation within and connectivity between the B-C-B and the Eastern Canadian stock, as detailed in Annex I.*

4.2 Gray whales

The population structure and status of gray whales has been the subject of a five-year long review. SC/67b/Rep07 [see Annex O, item 6.1.3] is a report of the most recent Workshop (the 'Fifth Rangewide Workshop on the Status of North Pacific Gray Whales'), which was held in Carmel, CA between 28-31 March 2018. Multiple stock structure hypotheses for gray whales in the North Pacific are being considered as part of this review, and the work presented in SC/67b/SDDNA02 and SC/67b/SDDNA03 provide information relevant to the evaluation of these hypotheses.

SC/67b/SDDNA02 uses whole-genome sequencing to investigate the demographic history of gray whales from the North Pacific and uses environmental niche modelling to make predictions about future gene flow. Sequencing efforts and habitat niche modelling indicate that: (i) western gray whale effective population sizes have declined since the last glacial maximum; (ii) contemporary gray whale genomes, both eastern and western, harbour less autosomal nucleotide diversity than most other marine mammals and megafauna; (iii) the extent of inbreeding, as measured by autozygosity, is greater in the Western Pacific than in the Eastern Pacific; and (iv) future climate change is expected to open new migratory routes for gray whales. The results indicate that gray whale genomes contain relatively low nucleotide diversity and have been subject to both historical and recent inbreeding. Population sizes over the last million years likely peaked about 25,000 years before present and have declined since then. The niche modelling suggests that novel migratory routes may develop within the next century and if so this could help retain overall genetic diversity, which is essential for adaption and successful recovery in light of global environmental change and past exploitation.

In discussion, it was noted that the trajectories of effective population size that were generated from the genome sequence data suggests that the population structure of gray whales within the North Pacific has repeatedly fluctuated in response to glaciation events, with the trajectories generated from the three genomes converging during periods of glaciation and then separating during non-glaciated periods. In more recent evolutionary time (approximately the last 10,000 to 100,000 years), the trajectories generated from the eastern sample and the trajectories generated from the two Sakhalin samples appear to be on somewhat independent trajectories, and the ENP population shows a much higher effective population size at the end of this period. Together with the results of the ABBA-BABA test (e.g. the D-statistic for different topologies), these trajectories suggest some degree of distinctiveness between the eastern North Pacific (ENP) and Sakhalin.

It was further noted that the results indicate a greater degree of inbreeding (as measured by the length and frequency of Runs of Homozygosity, ROHs) among the two whales sampled off Sakhalin when compared to the whale sampled in the ENP. This might be expected under the 'traditional' understanding of gray whale population structure, in which the Sakhalin whales were presumed to represent a small and largely isolated remnant population that remained in the WNP year-round. However, the contemporary view of gray whale stock structure is much more complex, and at least some of the Sakhalin whales (referred to in the stock structure hypotheses as the Western Feeding Group whales, or WFG) are known to migrate to and overwinter in the ENP. The time frame over which the signal of increased inbreeding in the two Sakhalin samples could be generated was discussed. It was noted that recombination could decrease the length of ROHs in a period of several generations, suggesting that the increased length of ROHs in the Sakhalin whales could have developed over ecological time scales. While it seems unlikely that the increased signal of inbreeding in the genomes of the Sakhalin whales would be present if these whales are representative of WFG whales that interbreed at random with the large ENP population (e.g. a large gene pool), such a signal might be generated if WFG whales largely interbreed with each other while on migration and are thus representative of a smaller gene pool. It was noted that one of the Sakhalin whales (WGW1) is known to have migrated to the ENP. In terms of the frequency of ROHs and the total length of ROHs, the inbreeding values for WGW1 fell in between those of the other Sakhalin whale and the ENP sample. Thus it was also questioned if the inbreeding measured in WGW1 could be representative of admixture between WFG whales and whales that are part of the larger ENP population that feeds north of the Aleutians.

The authors noted that it is difficult to draw broad conclusions on the basis of the three genomes that have currently been analysed and that they plan on extending this study by analysing the genomes of a larger number of individuals.

SC/67b/SDDNA03 used a panel of SNPs to investigate the genetic diversity and population structure within the species. Results indicate the gray whale gene pool is differentiated into two lineages (i.e. 'sub-stocks'), that each lineage contains similar levels of genetic diversity, and that both the Eastern and Western geographic samples were derived from mixed-stock aggregations composed of two distinct lineages. Overall, the data are inconsistent with the idea that the gray whale gene pool consists of a single population at equilibrium.

Several of the population structure inferences drawn in SDDNA03 were made using the R package LEA (Frichot and Francois, 2015), and much of the discussion focused on the comparability of this approach with that employed by the program STRUCTURE (Pritchard *et al.,* 2000), which has been broadly used in the context of the SC, particularly with regard to the genetic patterns assessed to form clusters. Both programs can be used to estimate admixture coefficients and to infer the number of genetic clusters present in genetic data. LEA has been designed to run more quickly and efficiently with large numbers of loci. However, the underlying algorithms used by these two programs differ. While STRUCTURE forms clusters such that adherence to Hardy-Weinberg-Equilibrium and linkage equilibrium across loci is maximised within clusters, LEA calculates ancestry coefficients using a least-squares method and uses an 'entropy criterion' and a cross-validation approach to estimate the number of ancestral populations that best explain the genotypic data (Alexander and Lange, 2011; Frichot *et al.*, 2014). It is unclear whether the differences in the underlying algorithms used by these two approaches affects how the results should be interpreted. However, in the case of SC/67b/SDDNA03, both STRUCTURE and LEA were run, and the two programs produced similar results.

The Working Group noted that when making inferences that test against equilibrium assumptions, loci are assumed to be neutral, as balancing/disruptive selection can bias inference, be it at the analysed locus itself or a closely linked locus. The SNPs used in this analysis were derived from genes with known functions in cetaceans. While selection cannot be entirely ruled out, the authors reported that no evidence of selection had been detected for the assessed SNPs at any of the loci (e.g. they have similar measures of expected and observed heterozygosity).

Additional discussion focused on the interpretation of the admixture coefficients calculated for the individuals sampled off Sakhalin. Under the assumption that two genetic clusters were present in the dataset, three patterns were evident: (1) some individuals assigned strongly the Sakhalin cluster (i.e., the cluster comprised primarily of individuals sampled off Sakhalin); (2) some individuals assigned strongly to the Mexico cluster; and (3) some individuals showed intermediate assignment values suggestive of mixed ancestry. The Working Group noted that while the individuals falling in the latter category could represent admixed individuals, it is also possible that the apparent admixture reflects individuals being incorrectly assigned due to a lack of resolution in the dataset, which included genotypes of 95 SNP loci. In addition, the majority of parent-offspring pairs (POPs) identified within this dataset included one individual that assigned strongly (Q>=0.95) to the Mexico cluster and another that assigned strongly to the Sakhalin cluster. If the individual representing the putative offspring in this POP was the result of mating between a Sakhalin and a Mexican whale (and assuming these groups represent separate populations), the admixture coefficient would be expected to reflect a more even distribution of ancestry to the two groups (e.g. a Q-value near 0.5). An explanation for this pattern has not been identified, although it was noted that such results could also reflect mis-assignment in either the parentage analysis or the LEA analysis. There were, however, a few putative parent offspring pairs which more closely fit the expected pattern, in which the Q-value for one member of the pair was more intermediate (0.65-0.72).

The authors noted that in the future, the results of the analyses presented in SC/67b/SDDNA03 will be used to inform the selection of samples for additional whole genome resequencing. Including samples representing individuals falling into each of the three categories identified above could address some of the questions raised above. In addition, integrating the genetic dataset with the existing photographic evidence could provide further insight into these questions.

The Working Group thanked the authors for presenting the work in SC/67b/SDDNA02 and SC/67b/SDDNA03 and noted the value of having genome data for this species. The Working Group **encouraged** the continuation of work to produce additional genomic data from these samples, which may aid in better evaluating the stock structure hypotheses currently under consideration for North Pacific gray whales.

Attention: SC

*In reviewing the results of new genetic analyses of gray whales in the North Pacific, the Committee **advises** that the genetic and photographic data for this species be combined to better assess stock structure-related questions. Given the potential for genomic data to aid in better evaluating the stock structure hypotheses currently under consideration for*

*North Pacific gray whales, the Committee **encourages** the continuation of work to produce additional genomic data from sampled gray whales.*

4.3 North Pacific right whales

SC/67b/NH02 presented the results of a genetic study on stock structure in North Pacific right whale, based on new and published mitochondrial DNA (mtDNA) control region sequences (399bp) of individuals from the western (*n*=29) and eastern (*n*=23) North Pacific. A sub-sample from the western side (*n*=18) was examined with 13 microsatellite loci to investigate the level of nuclear DNA diversity. Striking mtDNA differences were found between western and eastern North Pacific right whales. The F_{ST} between western and eastern North Pacific right whales was high (0.0929) and statistically significant (*P*=0.0002). This result is consistent with the hypothesis that separate populations inhabit the eastern and western North Pacific. Levels of nucleotide and haplotype diversities were high, 0.0174/0.8916 and 0.0165/0.8538 in the western and eastern populations, respectively. For the microsatellite data, the average expected heterozygosity in the western population was estimated at 0.595. The observed multimodal mtDNA mismatch distribution rejected a model of historical sudden expansion in both populations. Furthermore, Bayesian skyline plots (BSP) generated from the mtDNA data suggested a similar historical trend of female effective population size (Ne_f) for the two populations, with a stable Ne_f over time followed by a recent sharp decline. The timing of the decline ranged between 25,000 and 60,000 years ago (considering different populations and two assumptions of mutation rates), which coincide with the last glaciation period in the Pleistocene. Rapid climate changes during this period could have affected the habitat and prey resources of the North Pacific right whales, resulting in the sharp decline in their abundance. No signal of recent recovery was observed in the BSP analysis; however, this could be due to a lack of resolution for contemporary population size as shown in other studies.

The Working Group thanked Pastene for presenting this work, which was recommended by the NH sub-committee at SC/67a (IWC, 2018a, p.27). It was noted that, at least in the United States, right whales in the eastern and western North Pacific have been managed as separate stocks based on a gap in the distribution of sightings, and it is valuable to see this assumption confirmed by genetic comparisons. The Working Group looks forward to hearing more about this work and **encouraged** the authors to consider its publication in the future.

Attention: SC

*The results of new genetic analyses support the recognition of separate stocks of right whales in the eastern and western North Pacific. Given the importance of this work, the Committee **encourages** the publication of this information in the near future.*

4.4 Southern Hemisphere blue, fin, right and sei whales
4.4.1 Non-Antarctic Southern Hemisphere blue whales
A pre-assessment of Southern Hemisphere blue whales was conducted by the SH sub-committee this year. Multiple papers on the stock structure of non-Antarctic Southern Hemisphere blue whales were discussed, including SC/67b/SH03, SC/67b/SH05, SC/67b/SH11, and Attard *et al.* (2018). Review of these papers was conducted as part of a joint

SH/SDDNA session, and details of the discussion can be found in Annex H under agenda item 3.3.

4.4.2 Antarctic blue whales
Paper SC/67b/SH02 presents a comparison of contemporary and historical mitogenome diversity in Antarctic blue whales using 20 blue whale bones collected from the islands at 54°26'00"S/36°33'00"W and the Antarctic Peninsula. This paper was discussed as part of a joint session of the SDDNA Working Group and the SH sub-committee, and details of the discussion can be found under agenda item 3.2 of Annex H.

During the discussion, it was noted that the depletion of tissue samples collected from Antarctic blue whales during the SOWER surveys remains a concern. This issue has been raised in the past, when an intersessional email group was formed to discuss approaches towards mitigating depletion of blue whale biopsy samples from the SOWER cruises (IWC, 2012b p.220). However, given the advances in high throughput sequencing that have occurred over the last several years, the Working Group agreed that revisiting this topic would be beneficial. An intersessional Working Group was formed, with the task of providing recommendations on genomic approaches that would maximise the utility of these and other samples (see Item 6.1.3).

4.4.3 Southern Hemisphere fin whales
Paper SC/67b/SH13 compares mtDNA control region sequence data generated from fin whales sampled off the north-central coast of Chile with published data from the North Pacific, North Atlantic, and the Southern Hemisphere (Archer *et al.,* 2013). Statistically significant levels of genetic differentiation were identified between the two Hemispheres as well as between the North Atlantic and North Pacific, as had been previously reported. However, no significant differentiation was identified between geographic areas in the Southern Hemisphere. This paper was discussed as part of a joint session between the SD&DNA Working Group and the SH sub-committee, and details of the discussion can be found under Annex H, agenda item 4.1.

4.4.4 Southern right whales
Paper SC/67b/SH06 presents the results of a genetic mark-recapture study of southern right whales in Antarctic Area IV. Eight whales were recaptured, suggesting that individual whales tended to return to the same location in the Antarctic in subsequent years.

Carroll *et al.* (2018a) used a dataset of mitochondrial DNA sequences and microsatellite genotypes from 17 loci to examine circumpolar population structure, historical demography, and effective population size in Southern Hemisphere right whales. While significant differentiation was observed amongst wintering grounds for both mtDNA and microsatellites, analyses of nuclear variation identified two clusters corresponding to the South Atlantic and Indo-Pacific Ocean basins.

Both of these papers were discussed in a joint session with SH, and the details of that discussion are provided in Annex H, agenda item 5.1.

4.4.5 Southern Hemisphere sei whales
SC/67b/SDDNA05 reports on progress with genetic analysis of samples collected from sei whales that were part of the largest mass mortality event that occurred in southern Chile in March 2015 (Haussermann *et al.*, 2017). MtDNA control region sequences have been produced from 50 of the 160

skin and bone samples that were collected during this event, revealing relatively high levels of mtDNA diversity among the stranded whales. This study is part of a recently funded project to examine the historical and contemporary population structure of sei whales, and future work will include the collection of additional samples from this and other regions of the Southern Hemisphere.

The Working Group **welcomed** the information provided in this report, noting that there are very few biopsies available from sei whales and thus little is known about population structure in this species.

Attention: SC

*In reviewing the results of stock structure analyses of Southern Hemisphere whale stocks, the Committee **expresses concern** regarding the depletion of tissue samples in existing collections (including those collected during the IWC SOWER surveys). Given recent advances in high throughput sequencing technology, the Committee **agrees** that an intersessional working group should be formed to provide recommendations on genomic approaches to maximize the utility of these samples for future studies.*

4.5 North Pacific common minke whales
A preparatory workshop for the upcoming *Implementation Review* on North Pacific common minke whale (NPM) was held in Tokyo, Japan, on 12-13 February 2018 (SC/67b/Rep05). This new approach of a preparatory workshop was chosen because NPM stock structure has been a matter of controversial debate without agreement, such that all current stock structure hypotheses were ranked 'medium plausibility'. This unresolved issue makes the implementation potentially complicated. During the workshop, the stock-structure related work since the last *Implementation Review* was reported. This work included about 2000 new samples and new types of stock structure inference, i.e. DAPC and kinship analysis. Based on 16 microsatellite loci, STRUCTURE consistently inferred two genetic clusters. Specimens with at least 90% assignment to one of the clusters were assigned to J and O stock, respectively, rendering about 10% of the samples unassigned. Investigating the stock affinity of the unassigned specimens was considered crucial to inform stock structure hypotheses for implementation. Therefore, prior to the first *Implementation Review* workshop, two types of analyses were recommended.

SC/67b/SDDNA06 addressed one of two recommendations on stock structure analysis from the 'Workshop on Western North Pacific common minke whale stock structure in preparation for the start of the *Implementation Review* in April 2018' which was 'Analysis 1'. This study was conducted to review the stock assignment threshold which was currently set at 90% for the program STRUCTURE analysis, using two types of datasets with 26 microsatellite loci: (1) sub-samples with sample sizes that are balanced but not necessarily equivalent among sub-areas (*n*=336); and (2) sub-samples including all available data (*n*=538). Each dataset was randomly split into training and test data, and the assignment probability in each sample was estimated for 16 and 26 loci using respective training and test data in the program STRUCTURE. All samples were assigned to stock based on respective threshold of 65%, 70%, 80% and 90% probabilities. The comparison of the results from 16 loci with 26 loci using the training data suggested that the stock assignment with 16 loci was confirmed with 26

loci in 96% of the total cases under the current threshold, and more than about 70% of the unassigned samples with 16 loci went to either of J or O stocks with 26 loci. The likelihood of reversed assignment between J and O stocks was very low regardless of the levels of thresholds, which was at most 2.2%. On the other hand, the mismatch assignment rate between the unassigned and the assigned was higher under the 90% (4.0-4.5%) than the lower (0.8-2.7%) thresholds in both datasets. The performance test using test data for the thresholds of 65% and 70% which were tentatively selected for 16 loci showed a predictive accuracy of around 0.9 in all combinations of datasets and thresholds.

The Working Group thanked the authors for presenting their results and confirmed that the workshop's recommendation for Analysis 1 has been properly completed by this work. It was further clarified that STRUCTURE had been run with the same default parameters for all analysis with 'locprior' disabled.

In discussion, the Working Group noted that a reconsideration of the most appropriate threshold for STRUCTURE assignment is motivated by the idea to leave out J-type specimens in order to enhance resolution for a joint analysis of O-type and unassigned specimens, as kinship analyses indicated a closer affinity of unassigned to O-type specimens (Pampoulie and Daníelsdóttir, 2013). The division of the 26 loci dataset into a training data set and a test data set had reduced sample size for analysis 1 by 1/3. While this allowed for testing of a new threshold/classification rule in a separate data set (i.e. the test data set), the reduction in sample size (from 538 to 336) increased uncertainty in the consistency measures.

In comparing the results of STRUCTURE based on the 16-locus dataset with those generated using the 26-locus dataset, the assignment of some individuals (based on the given threshold) was 'reversed', such that an animal assigned to the O stock using the 26-locus dataset was assigned to the J stock using the 16-locus dataset and vice versa. It was noted that these differences cannot be directly interpreted as error rates (as the true assignment is unknown), but rather comprise measures of concordance/consistency. It was further noted that these reversed assignments underscore the possibility of misassignments (e.g. assigning a 'true' J stock whale to the 'O' stock or the reverse), albeit the frequency of such incidences was low (0 for the 90% threshold and <1% for the 80% threshold). In this context, it was further noted that lack of assignment in STRUCTURE may be due to different reasons, notably noise due to low resolution, admixture, or additional stocks that are less differentiated from either J or O.

After considerable discussion on the effects of changing thresholds for assignment, the Working Group **agreed** to run two types of assignments on the full 16-locus data set, i.e., one with the established threshold of 90% and a second with a threshold of 80%. The latter was chosen based on the finding that this threshold reduced the percentage of unassigned almost by half, while retaining consistency with the 26-locus assignment in >94% of specimens.

In earlier days before genetics, J and O were distinguished by differences in breeding seasons and it was asked whether there is any way to include that type of information for informing the analysis. While this would introduce a new classification rule, a previous investigation showed that classification by breeding season matched with genetic assignment. There has been reported concordance between genetic assignment and fetus lengths for certain time periods. In summary, the Working Group **encouraged** the inclusion

of non-genetic biological data to inform stock structure where possible.

Subsequently, the stock structure-related further genetic data analyses (Analysis 2 in SC/67b/Rep05) were discussed. An agreement was reached that South Korea will provide mtDNA data from specimens from subareas 5 and 6W for inclusion in the analyses. Most of these samples have been also typed for microsatellites. Although there has not been any cross-validation in microsatellite typing across Japanese and South Korean laboratories, the Working Group **encouraged** the inclusion of these data in the upcoming analyses.

It was noted in discussion that emphasis shall be on analyses with higher resolution power than STRUCTURE. It was further noted that – in the *Implementation Review* – stock structure inference has the prime function to inform trial structure. The previously used mixing matrix has been invalidated, such that a new mixing matrix has to be compiled. In this context, stock structure should be discussed in light of its relevance for running trials.

Based on the workshop recommendations, the Working Group **agreed** that the following analyses should be performed prior to the implementation workshop (notwithstanding that further analyses are welcome where feasible and appropriate):

(1) F_{ST}, F_{IS}, heterozygosities, haplotype diversity, and related measures;
(2) PCA (or FCA) analyses, including partitioning based on multiple components, and DAPC;
(3) spatially explicit analyses (especially Geneland, but also BAPS, TESS; spatial pattern of diversity measures);
(4) updated kinship analyses including most recent samples; and
(5) (if possible) Wahlund analyses as undertaken by Waples in 2011 (Tiedemann *et al.*, 2014).

A workplan, including details on available data and sample stratification, is provided in Appendix 5 (also see Workplan Item 6.1.3). As specified in SC/67b/Rep05, the primary analyses will be organised and performed by ICR (Pastene and coworkers), under the advice and assistance of the advisory group, where appropriate.

Data will be available under the Data Availability Agreement, Procedure A.

Attention: SC, C-A

The Committee reviewed new results of genetic analyses that were recommended at the intersessional Workshop (SC/67b/Rep05) to better evaluate the use of genetic data to assign stock affinity in North Pacific common minke whales.

The Committee:

(1) **agrees** *that future analyses should incorporate a range of assignment thresholds to encompass uncertainty;*
(2) **supports** *the additional genetic analyses described in Annex I Appendix 5 relating to the second recommendation of the intersessional workshop and* **agrees** *that they should be performed prior to the next intersessional workshop; and*
(3) **encourages** *the inclusion of non-genetic biological data to inform stock structure where possible.*

4.6 North Atlantic common minke whales
An intersessional workshop focused on the development of *SLA*s (*Strike Limit Algorithms*) for the Greenlandic hunt was

held between 20-24 March 2018 in Copenhagen, Denmark (SC/67b/Rep06). During the workshop, results of genetic analyses focused on further evaluation of the four stock structure hypotheses under consideration for NA minke whales were presented (see Fig. 1 in Tiedemann *et al.,* 2018). After reviewing this new information, the Workshop **agreed** that one of the hypotheses (Hypothesis IV) was not supported and that another (Hypothesis III) was less plausible than the remaining two hypotheses. At SC/67b, the Working Group reviewed the results of the genetic analyses presented at the Workshop.

Tiedemann *et al.* (2018) utilised currently available genetic data (mtDNA typing of 1,563 specimens, 15 typed microsatellite loci for 1,732 specimens) for NA common minke whales to evaluate the current stock structure hypotheses. Results of Parent-Offspring analysis were not fully compatible with the hypothesis III of complete panmixia among NA minke whales. Further, there was no pervasive occurrence of positive inbreeding coefficients (F_{IS}) within subareas, as would be expected under hypothesis IV, in which all feeding grounds contain a mixture of two separate stocks in any subarea. Hypothesis IV is hence not supported by the genetic data.

Subsequent analyses concentrated on the western (W) and central (C) areas (i.e. not using data from area E) in order to assess the plausibility of hypotheses I and II. The subarea-specific inbreeding coefficient (F_{IS}) was significantly positive for subareas WG and CIC, indicating that minke whales in these areas originate from more than one breeding stock. There is some indication in the present data for 2 W stocks (hypothesis I): The genetic data exhibit a high genetic diversity in WC, which appears separated from the other subareas in both spatial Principal Component Analyses (sPCAs). WG is separated from CIC according to the sPCAs of both marker systems. CG appears intermediate between WG and CIC.

There are significant temporal fluctuations in the genotype composition in WG and CIC, suggesting an influx of deviant genotypes in certain years. The observed genotype patterns are best reconciled in a scenario where WG and CIC are predominantly used by two different (albeit genetically similar) stocks. In some years the more western stock moves also into CIC, in some years the more eastern stock moves into WG. In the light of these inferences, hypotheses I and II were modified to allow for migration of W2/W into CIC (see SC/67b/Rep06). There is no indication that mixing among W/W2 and C stock affects one sex preferentially.

With this mixing scenario in mind, one may aim to identify particular years in which the mixing would be low, in order to use them as a reference year for genetic characteristics of the respective stock. Such a year should be expected to show a low mtDNA diversity and low F_{IS} values in both stocks and across sexes. Across all analyses performed, these criteria are well met for year 2007.

Using 2007 as a reference year, PC values provided by the spatial Principal Component Analysis of genotype data were utilised to assign single specimens to putative stocks, based on 6-dimensional vector distance (3PCs for each microsatellites and mtDNA). This approach yielded estimates for year specific mixing rates for 2003 to 2016, with average proportions of W:C stock as follows: WG 66:34; CG 61:39; CIC 33:67. The underlying assumptions (identification of a reference year; stock affinity reflected in proximity of individual PC values to stock mean PC values) remain so far untested. As true mixing proportions are unknown, validation of the estimated proportions is currently not feasible. The estimated mixing proportions may nonetheless prove useful in compiling mixing matrices, as they may constitute the only quantitative information available.

In discussion, it was clarified that these analyses relied on the same genetic markers as previous stock structure inferences on this species and that microsatellite scores originating from different laboratories had been made comparable by re-typing a representative subset of samples for inter-lab calibration. As a general feature of microsatellite fragment length typing, it was further noted that homoplasy, i.e. identical size of different alleles, cannot be excluded.

The subsequent discussion centered around the utilisation of an ordination approach (here, sPCA) to provide estimates of stock mixing proportions. It was clarified that this analysis stratifies the genetic data (here, microsatellite and mtDNA data) along principal components (PCs), taking into account also sampling location. It was further clarified in discussion that this method provides a classification rule for which the probability of correct individual assignment is not known. This contributes to uncertainty in the assignment. There is however no reason to expect a bias in the assignment to one or the other stock, as long as the standard deviations for any utilised PC are similar across stocks for the reference year (as was the case for year 2007 used as reference here). It was also clarified that a hypothetical random classification rule should – on average – result in mixing ratios of 50:50 in all areas, while the application presented here yielded average mixing rates significantly different from 50:50, i.e., around 65:35 and 35:65 for WG and CIC, respectively.

The Working Group **agreed** that inferred mixing rates – despite of associated uncertainties – comprise a step forward for AWMP/RMP simulation trials, as previously used mixing rates were not based on any specific empirical data. It was further noted that the approach used here could be used to infer stock structure below the resolution level of the STRUCTURE approach and that the mixing scenario suggested here was compatible with an earlier assessment, applying DAPC to NA common minkes (Hoelzel *et al.*, 2014). The precision [albeit not the accuracy] of the mixing rate estimation could be assessed with a resampling approach (e.g. jackknife).

Further discussions compared the approach used here (using only the first three PCs for any marker set) to DAPC. The latter may utilise a substantially higher number of PCs and may hence tend to overclustering, i.e. identify more clusters than biologically relevant.

The Working Group **encouraged** the attempt to utilise genetic data to estimate mixing rates and encouraged its utilisation in other IWC-related contexts and for further genetic loci (i.e. SNPs). As this study mostly focused on the Central and Western North Atlantic, an extension of the study to the Eastern North Atlantic was also encouraged.

Attention: SC, C-A

The Committee reviewed the use of a new approach that utilized ordination analyses of genetic data to assign stock mixing proportions. While this new approach requires making certain assumptions about the data, the Committee:

(1) **agrees** that the inference of mixing rates was informative for AWMP/RMP simulation trials in the absence of empirical data; and
(2) **encourages** the attempt to use genetic data to estimate mixing rates in the context of other IWC-related tasks.

4.7 Further stock structure advice
SC/67b/ASI05 was reviewed during a joint session of the SDDNA Working Group and the Ad hoc Working Group on Abundance Estimates, Status and International Cruises. A summary of the discussion can be found in Annex Q under item 3.1.2.

5. NEW STATISTICAL AND GENETIC ISSUES RELATING TO STOCK DEFINITION

5.1 Simulation tools for spatial structuring (e.g. TOSSM)
TOSSM was developed with the intent of testing the performance of genetic analytical methods in a management context using simulated genetic datasets (Martien *et al.*, 2009), and more recently the TOSSM dataset generation model has been used to create simulated datasets to allow the plausibility of different stock structure hypotheses to be tested (Archer *et al.*, 2010; Lang and Martien, 2012). The Working Group noted that while TOSSM has been particularly valuable in informing the interpretation of results of stock structure related analyses, it has not been broadly utilised within the IWC Scientific Committee for this purpose.

A wide-range of software packages are now available for producing simulated datasets that can be used for statistical inference and/or validating statistical methods (reviewed in Hoban, 2014; Hoban *et al.*, 2012). At SC67a, the Working Group agreed that reviewing the available packages and evaluating their utility to address issues of interest to the IWC Scientific Committee would be useful, and an email correspondence group was formed to conduct this review intersessionally (IWC, 2018b). The group was unable to report on their findings this year and thus agreed to continue work on this item intersessionally (see Work Plan Item 6.1.4), with the goal of providing a summary at SC/68a. In addition, the possibility of bringing in an Invited Participant with specialised expertise in this topic to present an overview of the applicability of this approach to the SC was discussed. The Working Group **agreed** that this strategy would facilitate making progress on this item, and Lang and Tiedemann offered to look into this possibility.

Attention: SC

*The Committee noted that while simulation-based approaches have been particularly valuable in informing the interpretation of results of stock structure-related analyses, they have not been broadly utilised within the Committee for this purpose. The Committee **agrees**:*

(1) to continue an intersessional review via an e-mail correspondence group (Annex I Table 2, ICG-3) of the available simulation tools and their potential utility to the Committee; and

(2) to consider bringing in invited expertise to present an overview of the applicability of such approaches in order to expedite progress on this agenda item.

5.2 PCA, DAPC, and related methods
Tiedemann *et al.* (2018) employed a novel approach to utilise the results of ordination-based analyses (sPCA) to estimate mixing rates in North Atlantic common minke whales. Details of this discussion are provided above under Item 4.6 above. In addition, SC67b/SDDNA03 (see Item 4.2) and Carroll *et al.* (2018a) (discussed in Annex H, item 5.1) used a Discriminant Analysis of Principal Components (DAPC) to evaluate population structure within gray and southern right whales, respectively.

5.3 Terminology
The status of the glossary on key terms in stock definition was revisited. It was suggested to restrict this discussion first to only those terms of most relevance to discussions of baleen whales, and see if there could be agreement on those within SD.

It was noted that, although the SC has not formally agreed on the terminology suggested earlier in the Working Group, the consistent use of key terms by members Working Group (e.g. using 'stock' only for breeding stocks') has overall increased consistency in terminology in SD related issues.

In its current version, the glossary uses the term 'biological stock'. While the current definition is well grounded in population genetics where it is similar to a 'population', it was noted that this term is hardly ever used outside the Working Group. It was further noted that, while stock definition typically incorporates information on genetic population structure, a defined 'stock' may additionally reflect management rationales.

One could maintain this approach to define 'stock' as close to some biological reality or, alternatively, use 'stock' as a management term (as in fisheries), and then explain how 'population' or 'deme' relate to 'stock'. In the IWC context, a stock is a unit that is managed separately and simulated as a separate unit in IWC *Implementations*. To define stocks constitutes hence a core concept for how IWC implementations are performed.

The Working Group **agreed** that the term 'stock' refers to a breeding assemblage ('biological stock'), while feeding grounds may be used by different stocks (mixed-stock (adj.) feeding aggregation). The potentially complex scenarios of differential migration among breeding grounds and feeding areas can be classified in 'archetypes', as has been forwarded during the development of TOSSM.

The Working Group further **agreed** to establish an intersessional e-mail group (see Work Plan Item 6.1.5) to revisit in more detail the current terminology and suggest revisions where appropriate for consideration at SC/68a.

Attention: SC

*The Committee **agrees** to establish an intersessional email group to revisit terminology with specific reference to the implications of inferred stock structure in other sub-committees, particularly those that deal with large whale assessments, and suggest revisions where appropriate for consideration at SC/68a.*

5.4 Close-kin mark-recapture
During last year's meeting (SC/67a), an overview of the close-kin mark-recapture (CKMR) approach (Bravington *et al.,* 2016) was presented to the SC. CKMR uses multi-locus genotyping to find close relatives among tissue samples from dead and/or live animals; the number of kin-pairs found, and their pattern in time and space, can be embedded in a statistical mark-recapture framework to infer absolute abundance, parameters like survival rate, and even stock structure.

No papers applying the CKMR approach were reviewed by the Working Group this year, although the value of integrating data from epigenetic aging (see discussion below, Item 5.5) into CKMR was noted. Given that CKMR has multiple applications that fall within the Scientific Committee's scope of work, the Working Group **encouraged** the submission of papers utilising this approach in the future.

*Given that close-kin mark-recapture has multiple applications that fall within the Committee's scope of work, the Committee **encourages** the submission of papers utilising this approach in the future.*

5.5 Epigenetic aging

Epigenetic (DNA-methylation) aging has been successfully used to estimate age in humpback whales (Polanowski *et al.*, 2014). As noted above, epigenetic aging is particularly valuable in the context of estimating abundance with the close-kin mark-recapture (CKMR) approach, as it can increase precision in such estimates by allowing the parent to be distinguished from the offspring. It may further be informative in the context of RMP implementation. Given the utility of these methods for the work of the Scientific Committee, at SC/66b the SH sub-committee endorsed a proposal to organise an open presentation on new epigenetic developments for measuring whale age, with the goal of introducing the SC to the concept and methodological developments in the technique (IWC, 2017). At SC/67b, the SH sub-committee, in coordination with the Working Group, invited Jarman, who was the lead scientist on the humpback whale work, to give this presentation, which was organised as a special night session in order to enable participation across sub-committees and Working Groups. Following this open presentation, the Working Group also heard the results of SC/67b/SDDNA04, which focussed on evaluating the feasibility of using this technique to estimate the age of Antarctic minke whales.

Jarman's presentation focussed on the prospects for age estimation in cetaceans by DNA methylation analysis. Cetacean population biology is commonly studied through a variety of analyses based on skin biopsy samples and generating age information from these samples will be valuable for addressing many questions. Age estimation of human tissues by DNA methylation analysis is now established as the method of choice for biological age estimation in medical research; and as a proxy from chronological age estimation in forensic studies. Similar work on mice and non-human primates have demonstrated the effectiveness of this class of methods in other mammals. One published study on age estimation in humpback whales (Polanowski *et al.*, 2014) demonstrates the potential of these sorts of methods for cetacean age estimation. 'Epigenetic clock' like change in DNA methylation at specific CpG sites in different mammal genomes is a mechanism for regulation of expression specific genes in an age-related manner. The scale of change in DNA methylation at age-regulated CpG sites appears to scale to lifespan and display some conservation among mammal species. Jarman then spoke about issues specific to age estimation in cetaceans, including why DNA methylation-based age estimation are likely to work well in cetaceans and what current and near-future prospects there are for this class of methods.

SC/67b/SDDNA04 presented the results of the feasibility study of the DNA-methylation (DNA-M) technique to determine age in the Antarctic minke whale. A total of 100 Antarctic minke whale samples, for which earplug readings were considered excellent or good, were selected for the DNA-M feasibility study. Seven CpG sites in three genes (TET2, CDKN2A and GRIA2) were selected for this study because they showed significant correspondence between CpG methylation levels and age in a previous study on humpback whales. In addition, SDDNA04 investigated changes in the DNA-M rate among different positions of the whale's body, some involving dorsal side (expose to sunlight) and others on the ventral side. DNA-M rate of the seven CpG sites were scored successfully, and regressions of each CpG methylation against age determined by earplug were conducted. Coefficients of determination (R^2) of all CpG sites were lower than that of the previous humpback whale study. The assay predicted age from skin samples with a standard deviation of 8.865 years. For some loci DNA-M rate fluctuated among 8-10 positions of the whale body. The authors concluded that age determination of Antarctic minke whale based on the seven DNA-M sites (from three genes) used in this study is not feasible particularly for use in population dynamics models such as SCAA.

The Working Group thanked Goto for presenting these results. While this study was initiated in response to a recommendation made during the Expert Panel review of the proposal by Japan for NEWREP-A (IWC, 2016, p.17), it was noted that identifying methods to estimate age in cetaceans is valuable not only in the context of the RMP implementation, as in the NEWREP-A exercise, but has multiple uses in the context of the SC, including the ability to discriminate between the parent and offspring among genetically identified parent-offspring pairs, which can inform both assessment of stock structure as well as genetic mark-recapture estimates of abundance (e.g. CKMR).

In discussion of the technical aspects of the paper, the Working Group noted that while it was reasonable to first evaluate the utility of DNA methylation studies for estimating age in Antarctic minke whales using the seven sites that proved useful in humpbacks, screening additional loci would be beneficial. This could be done by identifying loci correlated with age in humans or mice, and then making use of the now available minke whale genome to localise the homologous loci in this species. For the Antarctic minke whale analysis presented here, one site (TET-3) showed a relatively high age-related effect, while two others demonstrated a more minor effect (TET-2 and Cdkn2a3). Other loci (e.g. Cdkn2a1 and Cdkn2a2 and F) did not show any correlation. All seven loci were integrated into the model used to estimate age, and thus using only those loci that appear to have an age-related effect might reveal a stronger relationship.

The Working Group further noted that the information provided in SDDNA04 on positional sampling was useful, and that some of the differences identified between tissues collected from different regions of the body could have been driven by sampling a mix of cell types from different tissues rather than from environmental influences.

Importantly, during the discussion it was noted that a humpback whale age assay had a precision of 3.7 years, measured as the mean absolute difference (MAD) between estimated and known ages (Polanowski *et al.*, 2014). That was a preliminary study demonstrating the fundamental feasibility of this approach, and is not as accurate or precise as tests developed for humans and mice based on analysis of many more CpG sites. While precision is expected to be improved with the inclusion of more CpG sites, the maximum precision possible for any DNA methylation-based age estimator is likely limited by the imperfect relationship between chronological age and biological age. To date, that precision measured as MAD/lifespan has ranged from 3.9% in humpback whales (Polanowski *et al.,* 2014, assuming a 95-year lifespan), to 3.2% of lifespan in

humans (e.g Horvath, 2013) and 1.7% of lifespan in mice (Stubbs *et al.*, 2017). These observations indicate that the SD and 95% CI for age estimation described in Polanowski *et al.* (2014) and in SC/67b/SDDNA04 could be substantially improved before an inherent limit is reached. It was further noted that these precision estimates adhere to age determination in individual specimens. Hence, averaged age estimates over cohort will improve over larger sample sizes and may be more precise.

The Working Group noted that the implications of this upper limit on precision in estimating age for individuals would need to be evaluated in the context of the specific application for which the age data were being used. For example, although additional precision is helpful, CKMR studies may be informed by relatively crude estimates of age allowing the parent to be discriminated from the offspring (i.e. ordinal age).

In conclusion, the Working Group **agreed** that: (1) the results presented in SC/67b/SDDNA04 were not sufficient to provide individual age estimates that would be appropriately precise to use in the population dynamics modelling exercise recommended for NEWREP-A; and (2) that screening of additional loci would likely allow more precise age estimates to be provided in the future. Given that there is an upper limit on the degree of precision that can be achieved, however, the SC needs to evaluate whether, if optimal precision is achieved, epigenetic-based age estimates will be useful in the specific context of the NEWREP-A recommendation.

Attention: SC

*The Committee welcomed the results of the study to evaluate the feasibility of using epigenetic techniques to estimate age in Antarctic minke whales and **agrees**:*

(1) that the current set of loci did not provide sufficient precision for use in the population dynamics modelling exercise recommended for NEWREP-A;

(2) that identification of additional sites with an age-related DNA-methylation pattern is encouraged, as it would likely allow more precise estimates of age to be made in the future; and

(3) given that there is an upper limit to the degree of precision that can be achieved using this technique, evaluating the utility of epigenetic age estimation to the Committee should be further evaluated by the sub-committees concerned with regard to the degree of precision needed for the specific application of interest.

6. WORK PLAN

6.1 Work Plan
6.1.1 DNA quality guidelines
The e-mail group formed to discuss updating the DNA quality guidelines will continue intersessionally. The draft guidelines have been revised to incorporate sections covering data, including SNPs, produced using next generation sequencing (NGS) approaches. For SC/68a, the group will complete their review of the updated sections, such that a revised version can be posted on the IWC website next year. The group was convened under Tiedemann and included Archer, Baird, Baker, Bickham, Carroll, DeWoody, Hoelzel, Goto, Jackson, Lang, Palsbøll, Pampoulie, Solvang, Taguchi, and Waples.

6.1.2 Recommendations to maximise utility of tissue samples
An intersessional e-mail group was convened to provide recommendations on genomic approaches that would maximise the utility of tissue samples, including those collected as part of IWC surveys, that are in danger of becoming depleted in the future. The group was convened under Lang and included Baker, Bickham, Carroll, Goto, Taguchi, and Tiedemann.

6.1.3 North Pacific minke whale genetic analyses
The Working Group agreed that additional genetic analyses should be performed prior to the *Implementation Review* for North Pacific minke whales. A work plan with details of the analyses is included in Appendix 5. As specified in SC/67b/Rep05, the primary analyses will be organised and performed by ICR (Pastene and coworkers), under the advice and assistance of the advisory group, where appropriate.

6.1.4 Simulation tools
The intersessional e-mail group that was convened at SC67a to discuss the utility of simulation tools for evaluating spatial structure will be continued. The focus of this intersessional email group will be to: (1) review available software packages for conducting genetic and/or genomic simulations; and (2) evaluate the utility of these packages to address issues of interest to the Working Group. A summary of these intersessional discussions will be provided during SC/68a. The group was convened under Lang and included Archer, Bickham, Carroll, DeWoody, Hoelzel, Kitakado, and Tiedemann.

6.1.5 Terminology
An intersessional e-mail group will be re-convened to discuss the use of stock structure-related terms within the Scientific Committee reports and in papers submitted to the Scientific

Table 1
Summary of the Workplan.

Topic	Intersessional 2018/19	2019 Annual Meeting (SC/68a)	Intersessional 2019/20	2020 Annual Meeting
3.1 DNA quality guidelines	Intersessional email group to review recent revisions to the DNA quality guidelines that pertain to data produced using NGS approaches.	Report and finalise updated guidelines		
4.4.2 Recommendations to avoid sample depletion	Intersessional email group to provide recommendations on genomic approaches to maximise the utility of tissue samples that are in danger of becoming depleted in the future.	Report and provide advice		
4.5 North Pacific minke whale stock structure	Perform genetic analyses detailed in Appendix 5; report results at intersessional workshop on the North Pacific minke whale *Implementation Review*.	Review results and provide advice		
5.1 Simulations	Intersessional email group to review software packages and evaluate utility to the SD&DNA.	Report	Continue as needed	Report (if needed)
5.3 Terminology	Intersessional email group to continue discussions of the use of stock structure-related terms within the SC.	Report	Continue as needed	Report (if needed)

Table 2
Intersessional e-mail Groups.

SC Agenda Item/ Sub-Committee	Type	Group (short name)	Terms of Reference	Members
Item 3.1/SD&DNA	ICG-1	DNA quality	Review recent revisions in sections of the DNA quality guidelines that pertain to data produced using NGS approaches.	Tiedemann (Convenor), Archer, Baird, Baker, Bickham, Carroll, DeWoody, Hoelzel, Goto, Jackson, Lang, Palsbøll, Pampoulie, Solvang, Taguchi, and Waples
Item 4.4/SD&DNA	ICG-2	Sample depletion	Discuss and provide recommendation on genomic approaches to maximise the utility of tissue samples, particularly those in danger of depletion.	Lang (Convenor), Baker, Bickham, Carroll, Goto, Taguchi, Tiedemann
Item 5.1 Simulation tools	ICG-3	Simulations	(1) review available software packages for conducting genetic and/or genomic simulations, and (2) evaluate the utility of these packages to address issues of interest to the Working Group.	Lang (Convenor), Archer, Bickham, Carroll, DeWoody, Hoelzel, Kitakado, Tiedemann
Item 5.3 Stock structure-related terminology	ICG-4	Terminology	Revisit the definitions that were previously put forward for stock-related terms at IWC 2014, particularly those related to large whale assessments, and revise them where necessary.	Tiedemann (Convenor), Baird, Bickham, Carroll, Cipriano, Lang, Scordino

Table 3
Summary of the 2-year budget request for SDDNA.

RP no.	Title	2019 (£)	2020 (£)
Other			
	Collaborative analysis of WNP minke whale stock structure using Japanese microsatellite DNA database and spatially explicit population structure analyses	£6,247	
	Total request	**£6,247**	

Committee. The focus of this group will be to revisit the terminology definitions that were previously put forward (IWC, 2014), particularly those related to large whale assessments, and revise them where necessary. This group will be convened under Tiedemann and will include Baird, Baker, Bickham, Carroll, Cipriano, Lang, and Scordino.

6.2 Budget requests for 2019-20
The Working Group received one budget request for 2019-20 (see Table 3). This request was put forward by Hoelzel and addresses the need to complete recommended analyses on the stock structure of North Pacific minke whales prior to the 2019 intersessional Workshop on the North Pacific minke whale *Implementation Review*. Specifically, the funding requested is to help complete the work included in the 'Analysis 2' recommendation made in SC/67b/Rep05. This project would represent a collaborative effort with Pastene and his colleagues, who would provide access to the Japanese microsatellite data. The Working Group **agreed** that completing this work prior to the intersessional Workshop is important and **recommended** that this work be funded.

7. ADOPTION OF REPORT
The report was adopted at 17:00 on 2 May 2018.

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Appendix 1

AGENDA

1. Introductory items
   1.1 Convenor's opening remarks
   1.2 Election of Chair and appointment of Rapporteurs
   1.3 Adoption of Agenda
   1.4 Review of documents
2. DNA testing
   2.1 Genetic methods for species, stocks and individual identification
   2.2 'Amendments' of sequences deposited in GenBank
   2.3 Collection and archiving of tissue samples from catches and bycatches
   2.4 Reference databases and standards for diagnostic DNA registries
3. Guidelines and methods for genetic studies and DNA data quality
   3.1 Update DNA quality guidelines to include discussion of NGS data
   3.2 Further applications of DNA techniques
4. Provide advice on stock structure to other sub-groups
   4.1 Bowhead whales
   4.2 Gray whales
   4.3 North Pacific right whales
   4.4 Southern Hemisphere blue, fin, right and sei whales
       4.4.1 Non-Antarctic Southern Hemisphere blue whales
       4.4.2 Antarctic blue whales

Appendix 2

AN UPDATE OF THE JAPANESE DNA REGISTER FOR LARGE WHALES

Mutsuo Goto, Hiroyuki Oikawa and Mioko Taguchi

The Institute of Cetacean Research, 4-5 Toyomi-cho, Chuo-ku, Tokyo, 104-0055, Japan

Notes:	1 Type	2 No. of whales	3 No. of duplicate	4 No. missing	5 Lab problem	6 No. mtDNA	7 % mtDNA	8 No. msat	9 % msat	10 Sex analysed	11 % sexed	12
NP minke whale												
1994-2016	SP	2,680	0	0	8	2,672	100	2,672	100	2,680	100	
2017	SP	128	0	0	0	128	100	128	100	128	100	
2001-16	BC	2,008	0	26	2	2,008	100	1,980	99	1,978	99	
2017	BC	164	0	0	0	164	100	164	100	164	100	
NP sei whale												
2002-16	SP	1,354	0	0	4	1,350	100	1,354	100	1,354	100	
2017	SP	134	0	0	0	134	100	134	100	134	100	
NP Bryde's whale												
2000-17	SP	730	0	0	3	727	100	730	100	730	100	
2001-16	BC	5	0	0	0	5	100	4	80	4	80	Include three Omura's whale and one from the East China Sea stock
2017	BC	0	0	0	0	0	0	0	0	0	0	No BC
NP humpback whale												
2001-16	BC	63	0	0	0	63	100	63	100	63	100	
2017	BC	3	0	0	0	3	100	3	100	3	100	
NP right whale												
2001-16	BC	3	0	1	0	3	100	2	67	2	67	Missing by the 2011 tsunami, no microsats
2017	BC	0	0	0	0	0	0	0	0	0	0	No BC
NP fin whale												
2001-16	BC	11	0	0	0	11	100	11	100	11	100	
2017	BC	0	0	0	0	0	0	0	0	0	0	No BC
NP sperm whale												
2000-17	SP	56	0	0	0	56	100	56	100	56	100	
2001-16	BC	2	0	0	0	2	100	2	100	2	100	
2017	BC	0	0	0	0	0	0	0	0	0	0	No BC
Antarctic minke whale												
1987/88-2004/05	SP	6,794	0	10	0	1,118	17	6,271	92	6,794	100	Including dwarf; 87/88-88/89. no microsats.
2005/06-2015/16	SP	4,217	0	549	162	2,978	71	3,506	83	4,217	100	Some missing by the 3/11 tsunami in 2011.
2016/17	SP	333	0	0	0	333	100	333	100	333	100	
Antarctic fin whale												
2005/06-2011/12	SP	18	0	0	0	18	100	18	100	18	100	

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

The status of the Japanese DNA register for large whales was presented and discussed during the 2005 IWC SC meeting (IWC, 2006). Since then, the number of genetic samples and the number of individuals analysed and registered have been reported to the IWC SC annual meetings. The annual reports include information of whales taken by the scientific whaling in the North Pacific (JARPN/JARPNII and NEWREP-NP) and the Antarctic (JARPA/JARPAII and NEWREP-A), and from bycatches and stranding. The most recent full description of the protocol used by the Institute of Cetacean Research for the genetic analyses in the context of the IWC guidelines was presented by Kanda *et al.* (2014).

The update of the Japanese DNA register for large whales till 2017 is as follows.

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Appendix 3

AN UPDATE OF THE NORWEGIAN MINKE WHALE DNA REGISTER

Hans J. Skaug

University of Bergen and Institute of Marine Research

Notes:	1	2	3	4	5	6	7	8	9	10	11	14		12
	Type	No. of whales	No. of duplicates	No. missing	Lab problem	No. tDNA	% mtDNA	No. msat	% msat	Sex analysed	% sexed	SNP	% SNP	
NA minke whale														
1997-2016	C	11,307	109	75	2	11,121	100	11,121	100	11,121	100	578	5	-
2017	C	431	3	1	0	0	0	430	100	430	100	430	100	-

Notes:
1. Key to sample types: SP = special permit catch, C=commercial catch, BC=bycatch, ST=stranding.
2. Number of whales that potentially entered by the previous years and enters (new year) the markets.
3. Number of occurrences (tissues) sample switching on board the vessels as detected by comparison of genetic profiles.
4. Number of individuals for which tissue samples are missing for other reasons than sample switching.
5. Genetic laboratory not able to obtain microsatellite profiles or mtDNA haplotypes from tissue samples.
6. Number of samples analysed for mitochondrial control region.
7. % of total samples analysed for mitochondrial control region.
8. number of samples analysed for microsatellites.
9. % of total samples analysed for microsatellites.
10. Number of samples analysed for sex.
11. % of total samples analysed for sex.
12. Other problems or information.
13. Discontinued starting from 2016.
14. Started in 2016.

Appendix 4

STATUS OF THE ICELANDIC WHALE DNA REGISTER

Christophe Pampoulie and Gisli A. Víkingsson

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

The table gives the present status of the registry. Samples from all the common minke whales landed as a part of the Icelandic research program (2003-07) and recent commercial catches (2008-17), as well as from commercial NA fin whale catches have been genotyped and information stored in the database.

Notes:	1	2	3	4	5	6	7	8	9	10	11	12
Species/year	Type	No. whales	No. duplicate	No. missing	No. lab problem	No. mtDNA	% mtDNA	No. msat	%msat	Sex analysed	% sexed	
NA minke whale												
2003-07	SP	189	0	0	0	189	100	189	100	189	100	-
2008-16	C	414	0	0	0	362	87	365	88	367	89	-
2017	C	17	0	0	0	0	0	0	0	0	0	-
NA fin whale												
2006-16	C	688	0	0	0	688	100	688	100	688	100	-

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

Appendix 5

WORKPLAN FOR GENETIC DATA ANALYSIS RECOMMENDED PRIOR TO THE FIRST *IMPLEMENTATION* WORKSHOP ON NORTH PACIFIC COMMON MINKE WHALE (BASED ON DISCUSSIONS IN SDNA-NPM SMALL GROUP)

This work plan is based on the recommendations from the Workshop on Western North Pacific common minke whale stock structure in preparation for the start of the *Implementation Review* (SC/67b/Rep05) and takes into account that the recommended *Analysis 1* has already been completed (SC/67b/SDDNA06).

For *Analysis 2*, the Workshop **agreed** on the importance of trying to better understand the nature of unassigned individuals and suggested several analyses to resolve this issue. This work plan specifies available genetic data, sample partitions to be compiled, sample stratification for specific analyses, and analytical methods to be applied.

Available data
The following table lists available genotyped samples from South Korea (subareas 5 and 6W; data hold by Hyun Woo Kim and coworkers) and Japan (other subareas; Pastene, Goto, Taguchi). The South Korean scientists have kindly agreed to provide their mitochondrial DNA sequence data to Pastene and co-workers for joint analyses.

Sample partitions
The recommended analyses are to be performed for the entire data set available. In this context, both the 16 loci and the 26 loci data set should be utilised.

Further, two types of partitions are to be analysed:

The first will include O-stock together with the unassigned individuals, using both the 80% and 90% thresholds for assignment (based on 16 microsatellite loci).

The second will not be based on the STRUCTURE results but rather will include only the relevant geographic areas that are not dominated by J-stock (i.e. subareas 7, 8 and 9).

Clustering in the PCA/DAPC analyses may identify putative J-stock individuals as a strongly supported cluster that could be excluded in further analyses if this facilitated the resolution of more weakly differentiated clusters. The objective is to diminish or eliminate the strong signal identifying the distinction between O and J stocks to increase the potential to identify a weakly differentiated stock. However, the priority should be to resolve local patterns by the selection of geographic samples without post-hoc purging if possible.

	Sub-area													
Marker set	1E	2C	5	6W	6E	7CN	7CS	7E	7WR	8	9	10	11	Total
mtDNA**	69	338	114	922	916	1,178	925	49	89	251	541	15	129	5,536
16 microsat loci**	69	338	-*	-*	916	1,178	925	49	89	252	541	15	129	4,501
26 microsat loci	26	28	-	-	126	42	148	27	27	35	39	15	25	538

*Microsatellites were also typed in South Korea, but have not yet been cross-validated with Japanese typings.
**Japanese samples from 2016 not yet included.

Stratification

All available samples will be stratified as follows: 1. By year and subarea; 2. By month and subarea.

In this stratification, by-catches shall be flagged to facilitate analyses as to the effect of inclusion/exclusion of by-caught specimens. Depending on the number of available samples per year/month and subarea, adjacent years/months may be combined to increase sample size per stratum (e.g., looking at two years or two months periods).

Analysis

It was agreed that the following analyses should be performed prior to the implementation workshop (notwithstanding that further analyses are welcome where feasible and appropriate):

(1) F_{ST}, F_{IS}, heterozygosities, haplotype diversity, and related measures;

(2) PCA (or FCA) analyses, including partitioning based on multiple components, and DAPC;

(3) spatially explicit analyses (especially Geneland, but also BAPS, TESS; spatial pattern of diversity measures);

(4) updated kinship analyses including most recent samples; and

(5) (if possible) Wahlund analyses as undertaken by Waples in 2011 (Tiedemann *et al.*, 2014).

As specified in SC/67b/Rep05, the analyses will be organised and performed by ICR (Pastene and co-workers), under the advice and assistance of the advisory group, where appropriate.

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