## Annex I

## Report of the Working Group on Stock Definition and DNA Testing

## 2.1 Northern and Southern Hemisphere blue whales

**Members:** Lang, Tiedemann (co-Convenors), Arguedas, Baird, Baker, Bickham, Bravington, Burkhardt, Butterworth, Cipriano, Cooke, Cunn, de Moor, DeWoody, Elwen, Filatova, Fruet, Funahashi Goodman, Goto, Gunnlaugsson, Herr, Hjort, Hoelzel, Hall, Isoda, Jackson, Kumakiri, Leslie, Litovka, Mallette, Mate, Maeda, Miller, Mizroch, Morishita, H. Morita, Y. Morita, Nakamura, Pampoulie, Park, Pastene, Paudel, Reeves, Rosenbaum, Scordino, Širović, Skaug, Solvang, Suydam, Taguchi, Tamura, Torres-Florez, Tsuno, Walters, Wade, Walløe, Weller, Yoshida, Zerbini.

### **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 elected as co-Chairs, and Cipriano acted as rapporteur.

## 1.3 Adoption of Agenda

The adopted agenda is given in Appendix 1.

Items 4.1, 4.3, and 4.4 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/67a/SDDNA01-05; SC/67a/NP01; SC/67a/SH11; SC/67a/Rep07; Malde *et al.* (2017); Baker *et al.* (2017); Bravington *et al.* (2016b), Leduc *et al.* (2017); Lah *et al.* (2016), Pastene *et al.* (2012), Hamner *et al.* (In press) and Taguchi *et al.* (2017).

## 2. SCIENTIFIC ADVICE ON STOCK STRUCTURE PROVIDED TO OTHER GROUPS

The Stock Definition and DNA Testing Working Group (hereafter, the Working Group) has the task of discussing high-priority stock related papers from other subcommittees 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. Leduc et al. (2017) builds on previous studies of population structure in Southern Hemisphere blue whales (LeDuc et al., 2007; Torres-Florez et al., 2014) by incorporating additional samples collected in the eastern North Pacific (ENP) and eastern Tropical Pacific (ETP). Using mtDNA control region sequences and genotype data derived from seven microsatellite loci, significant nuclear and mitochondrial differentiation was identified between blue whales sampled in the Indian Ocean (IO), eastern South Pacific (ESP), Antarctic (ANT), ENP, and ETP. Within the Southern Hemisphere, these results are consistent with those of previous studies, which have shown that the pygmy-type blue whales in the IO are as different from the pygmy-type blue whales in the ESP as they are from the ANT whales. The magnitude of mtDNA differentiation identified between the ENP and ESP strata, however, was markedly lower than that found among the SH strata. When the ETP stratum was subdivided into northern (nETP) and southern (sETP) regions, no significant differences were identified in the nuclear comparisons of the nETP with the ENP or of the sETP with the ESP. Similar results were observed in the genetic assignment test, where samples from the nETP were generally assigned to the ENP while samples from the sETP were assigned to the ESP. These results suggest that, at least during the months in which the ETP was sampled (September to November), the sETP was being primarily visited by whales moving up from Chilean waters or other parts of the ESP, with the nETP being primarily used by whales from the ENP. However, temporal and spatial segregation of blue whales in the ETP is likely to be more complex than shown by the general trend, as the pattern of assignment for some individuals was equivocal (i.e. close to parity). As with previous studies, the pattern of genetic variation identified in the Southern Hemisphere is compatible with the recently proposed subspecies status of Chilean blue whales. However, the low degree of differentiation between ESP and ENP whales indicates additional study is needed to better elucidate the range of the Chilean subspecies and its relationship to the ENP.

In discussion, it was noted that including the Indian Ocean blue whales as a single stratum, as was done in Leduc *et al.* (2017), is problematic, given that blue whales in the southwestern IO off Madagascar, those in the Northern Indian Ocean, and the Indonesia-Australia blue whales are acoustically differentiated and likely to comprise separate genetic stocks. While the limited number of samples available from the southwestern and northern portions of the Indian Ocean preclude making genetic comparisons with those areas, future comparisons should consider the Indonesia-Australia stratum separately from samples collected in other regions of the IO.

In discussion of the genetic assignment test results, Lang clarified that while the results supported a general tendency for whales sampled in the sETP to assign to the ESP and whales sampled in the nETP to assign to the ENP, the assignment probabilities of many individuals were equivocal (near 0.5), which could be interpreted as evidence that some

of the whales using this area are admixed individuals. The Working Group suggested that examining the mtDNA haplotype identities of potentially admixed individuals could provide insight into how the assignment probabilities should be interpreted, although the utility of this approach could be limited in this case given the high proportion of haplotypes shared between the ESP and the ENP. The Working Group further questioned if the ambiguity in the results of the assignment tests could reflect sampling of ETP whales that utilise unknown and/or unsampled feeding areas in the ENP. Photo-identification effort conducted off Costa Rica Dome (Chandler et al., 1999; Douglas et al., 2015) found that only a small proportion of the photographed whales could be matched to photo-identification catalogues in the ENP. These unmatched whales could be whales from the ESP or whales that utilise less well-studied regions of the ENP during summer and fall. Lang confirmed that most of the ENP biopsy samples were collected within the region of high photo-id effort but noted that the potential effect of an unsampled feeding ground on the results of the ETP assignment test has not been explored.

It was further asked if any temporal patterns in the proportion of individuals assigning more strongly to the ENP or ESP had been observed. Such a pattern might be expected given that the sampling period roughly corresponded to the start of the ENP wintering season and the end of the ESP wintering season. Lang noted that, while not shown in the paper, some efforts had been made to explore this possibility, but a clear pattern in the probability of assignments to each area over time was not detected. Given that the level of differentiation between the ENP and ESP is lower than that seen between the other strata in the study, the use of a relatively small number of microsatellite loci used in the study may have limited the power of the analyses to detect such patterns if they exist. Lang reported that most of the samples utilised in Leduc et al. (2017) have been incorporated in an ongoing project focused on using full mitogenome sequences and SNP genotypes at ~300 loci to better understand the stock structure and subspecies taxonomy of blue whales. This work will be presented at SC/67b.

It was noted that the initial proposal that ESP whales represent a separate subspecies of blue whale was based on analysis of total lengths from whaling catch data, which showed that the whales caught off Chile were intermediate in length between the IO pygmy-type blue whales and Antarctic blue whales (Branch et al., 2007). Length data derived from aerial photogrammetric studies, however, show a somewhat different pattern, with the lengths of blue whales in the ENP, ESP, ETP, and IO being similar (Durban et al., 2016; Gilpatrick and Perryman, 2008). This discrepancy could be associated with differences in the biases associated with each dataset. In addition, temporal biases may also be present within the catch data, either due to differences in incentives (e.g. whalers being compensated based on the length of the whale) and/or differences in how whale lengths were estimated. With respect to the ESP, ETP, and ENP, the genetic results presented in Leduc et al. (2017) are consistent with the morphological data from photogrammetry, although differences between the IO pygmy blue whales and the ESP blue whales continue to be supported with the additional data. Branch is currently re-analysing the catch data to further assess the reported size distributions, which may provide insight into the source of some biases inherent in this dataset.

Pastene noted that historical blue whale catch information collected over two years by technicians aboard Japanese

whaling ships off the coast of Chile has recently been uncovered. The data associated with a subset of these catches includes measurements that are relevant to comparisons of body proportions, which have been shown to differ between pygmy and Antarctic blue whales (Ichihara, 1966). This data will be analysed in collaboration with Branch.

#### 2.2 Western North Pacific common minke whales

Genetic analyses on the stock structure of North Pacific common minke whales have been conducted by Japanese scientists following specific recommendations made at the Expert Workshop to Review the ongoing JARPNII Programme held 26-30 January 2009 in Yokohama, Japan (IWC, 2010). Results of these analyses were reviewed at the Expert Panel of the Final Review of the Western North Pacific Japanese Special Permit Programme (JARPNII) held 22-26 February 2016 in Tokyo, Japan (IWC, 2017a) and at the subsequent IWC Scientific Committee meeting in 2016 (IWC, 2017b). At SC/67a, the Working Group reviewed new results addressing these recommendations (SC/67a/SDDNA01) as well as a summary of previously conducted work (SC/67a/SDDNA05).

SC/67a/SDDNA01 presents the results of using a dataset of complete genotypes at 16 microsatellite loci, accompanied with mtDNA and biological information, in 4,554 North Pacific common minke whales to infer Parent-Offspring (P-O) relationships, using a Maximum-Likelihood approach. The relationship between False Discovery Rate (FDR) and Power (P) was evaluated by simulation. Of 145 inferred P-O pairs at an estimated FDR of 0.1, 141 were further evaluated by typing 10 additional microsatellite loci. 75 were confirmed (among them 26 mother-foetus pairs), 66 pairs were ranked 'False Positives', yielding an overall observed FDR of 0.468. FDR<sub>o</sub> was substantially reduced when J and O stock were analysed separately. While observed and estimated values for Power were in the same range of magnitude, observed FDR was always substantially higher than estimated FDR. This was attributed to the fact that FDR<sub>1</sub> was estimated via simulation, implicitly assuming a single panmictic population, an assumption clearly not met in the present data set. This interpretation is corroborated by the reduced FDR<sub>o</sub> when stocks were analysed separately. The dataset with  $2\overline{6}$ microsatellites clearly outperformed the 16 microsatellite data sets. At  $FDR_{E}$ =0.001, Power was at or close to 100%  $(P_E = 0.989 \text{ and } P_O = 1.000)$  and the observed False Discovery Rate was FDR<sub>0</sub>=0.128. Among the validated P-O pairs, O stock pairs were significantly overrepresented, while pairs between J and O stock individuals were absent. Specimens neither assigned to J nor O stock ('unassigned') exhibited a stronger affinity to the O stock. The J stock seems to appear on both sides of Japan closer to the coast, while the O stock occurs mostly east of Japan, both close to the coast and far offshore. This analysis provides no evidence for further stock structure in the area covered by this data set.

This study demonstrates that a modest increase in the number of loci investigated (here, from 16 to 26 microsatellite loci) may already substantially improve kinship inference under Maximum Likelihood. It further addresses recommendations made at both the JARPNII final review and the 2016 IWC Scientific Committee meeting regarding kinship analysis in North Pacific common minke whales.

In discussion, concern was expressed about the lack of independence that is incurred when the same dataset (the 16-locus genotype data) is used to assign individuals to stocks (Pastene *et al.*, 2016), estimate the likelihood of possible POP relationships within those stocks, and then make inferences about the plausibility of stock structure hypotheses based on these findings. Alternative stratification schemes, such as using geography or a second set of independent microsatellite loci to stratify the samples into genetic clusters, would circumvent this concern. It was noted that the lack of independence does not invalidate the inferred POPs, but could bias the estimates of FDR. This bias is expected to result in additional FPs, as individuals assigned to stocks in this way would be genetically more similar to each other than to the broader sample set. This pattern can be seen in the separate analysis of the J stock minke whales, in which no FPs were identified. The two known J-Stock POPs (i.e. mother-foetus pairs) were not detected, neither in the complete dataset nor when the J stock minke whales were analysed separately.

As part of the analysis, two LOD scores were reported; one based on the genotypes of all samples at 16 microsatellite loci and a second that included genotypes at 26 microsatellite loci, but was calculated based on only those samples identified in putative POPs using the original 16-loci dataset. It was noted that for some pairs, the LOD scores changed markedly between the two calculations, while for other pairs the LOD scores remained similar. It was explained that this pattern suggests that when only the 16 loci genotypes were used, it is possible for some pairs to be assigned a high LOD score by chance. However, when the additional 10 loci are added, that possibility is greatly reduced, and identifying mismatching genotypes at even a single locus for a pair previously suggested to represent a POP can potentially decrease the LOD score markedly.

It was noted that, rather than calculating LOD scores based on a simulated randomly mating population, a permutation test performed on the individuals in the data set itself would better address the influence of stock structure. One issue with this approach is that if actual individuals are used for permutation, then some circularity is introduced, given that these individuals are treated as unrelated, despite sharing a PO relationship. Given the relatively small number of POPs, the effect of this bias may be small. It was further noted that while this suggestion should be evaluated, it is not known what the impact of using this approach would be in terms of decreasing deviations between estimated and observed FDR.

Among inferred O-stock POPs, many included one individual sampled near the coast and one sampled in offshore waters. It was asked whether the biological data associated with these individuals suggested a pattern of offspring being found close to shore and the parent being found offshore. It was confirmed that this general pattern was present, and it included not only mother-offspring pairs, but also fatheroffspring pairs. It was further referred to SC/67a/SDDNA05 for information on this issue.

It was queried if the sex ratio was close to parity within sampled whales assigned to the J and O stocks and used in the kinship inference. Tiedemann noted that in the assigned O-stock whales, the number of sampled males is markedly larger than the number of sampled females. This data is provided in Appendix 2.

In concluding the discussion of the technical aspects of this paper, the Working Group commented that this work provides a good example of the value of increasing the number of loci in analysis of kinship, as was also highlighted in the discussion of Bravington *et al.* (2016b). Furthermore, the Working Group noted the value of having biological data associated with the individuals used in kinship-based analyses and encouraged the inclusion of such data when available. The plausibility of the POPs identified in the 16-locus analysis was verified by examining the biological data associated with each pair; pairs that were not biologically compatible with sharing a PO relationship were then flagged and not used in subsequent analysis. Only three of the pairs identified in the 16-locus analysis and also verified by biological data were not supported when the additional ten loci were genotyped.

The Working Group thanked Tiedemann and his coauthors for this presentation and for the work done to address the recommendations of the JARPN II panel review and final report. Discussion of how these results fit in with the stock structure hypotheses under consideration was delayed until after the presentation of SC/67a/SDDNA05.

SC/67a/SDDNA05 presented a brief summary of the updated analyses on the stock structure of western North Pacific common minke whales conducted following recommendations from the Scientific Committee. The refined analyses on hypothesis testing (including evaluation of the statistical power), morphometric, STRUCTURE, DAPC, catch-at-age and kinship, provided strong support to stock structure Hypothesis A (proposing only J and O stocks), with a single O stock exhibiting a pattern of sexual and age segregation during migration. The authors consider that Hypothesis C (proposing two J stocks and two O stocks) is contradicted by the data, and consequently such hypothesis should now be rejected.

The Working Group thanked Pastene and Taguchi for presenting this summary. The technical discussion of SC/67a/SDDNA05 focused on how samples were selected for inclusion in the exercise evaluating how genotyping additional microsatellite loci affected the proportion of individuals that could not, using the 16-loci dataset, be assigned to either the J or O stock with confidence (Tamura et al., 2017). Individuals selected for this exercise were chosen at random from the subset of samples collected in subareas 6 and 7, with the intent of generating a dataset that would include a relatively equal proportion of J and O stock whales. The Working Group noted that this dataset was representative of only a portion of the region being considered, while other areas, such as the Sea of Japan and the Yellow Sea, were not included. This could result in a bias in the assignment probabilities generated by STRUCTURE. The Working Group suggested that an analysis in which the additional loci were genotyped in samples collected from a broader region would be a more appropriate test. However, the Working Group, while also recognising the logistical difficulties inherent in genotyping additional samples, welcomed the typing of additional loci.

The Working Group then discussed the implications of the results presented in SC/67a/SDDNA01, as well as those summarised from past discussions in SC/67a/SDDNA05, in evaluating the plausibility of the stock structure hypotheses included in the *IST*s for Western North Pacific minke whales.

In general, the Working Group noted that several gaps in understanding persist for western North Pacific common minkes; in particular, the breeding areas for these animals remain unknown, and current hypotheses only partially consider the potential for mixing of whales on migratory routes or wintering grounds. It was further noted that the results presented in SD5 do not contribute to an understanding of the heterogeneity that has been identified in some previous studies within the O-type whales (Wade and Baker, 2012).

The Working Group further noted that, while the table illustrating the location and number of inferred POPs within and between regions suggests connectivity between areas, it does not provide information on how those numbers compare to the numbers of sampled animals in each region for which no POP relationships were inferred. Including such information would provide insight into the relative magnitude of connectivity between areas.

Although questions about the stock structure of minke whales in the western North Pacific may not be fully resolved, particularly in the absence of knowledge about the location of breeding grounds, the Working Group noted the importance of evaluating the evidence at hand with respect to the stock structure hypotheses under consideration. As such, the Working Group agreed that the results of the kinship analysis are inconsistent with the mixing matrices associated with Hypothesis C as currently implemented in the RMP trials among sub-areas 7CS, 7CN, 8 and 9.

#### 2.3 North Pacific Bryde's whales

Taguchi et al. (2017) presented the results of a Discriminant Analysis of Principal Components (DAPC) to examine the stock structure of the Bryde's whales in the North Pacific. A total of 1,019 whales collected in sub-areas 1W, 1E and 2 till 2014 was examined using seventeen microsatellite DNA loci. Bryde's whales from the eastern South Pacific off Peru, western South Pacific off Fiji and eastern Indian Ocean off Java were used for comparative purposes. The DAPC analyses revealed no structure within the North Pacific however, it showed that Bryde's whales from the North Pacific, eastern and western South Pacific and eastern Indian Ocean belong to four distinct stocks. The negative results of DAPC analysis for the North Pacific were explained by the low  $F_{\rm ST}$  estimates among the three sub-areas (1W, 1E and 2), and these results were consistent with the previous STRUCTURE results. A previous heterogeneity test showed no differences within sub-area 1, but significant differences between sub-areas 1 and 2, for both mitochondrial and microsatellite DNA. Therefore the combined results suggest the occurrence of two stocks in the sub-areas, which are weakly differentiated.

SC/67a/Rep07 utilises the genetic information presented by Taguchi *et al.* (2017) for a further analysis of spatial genetic structure. Specifically, the area was divided longitudinally into slices of 5° longitude each. Using a moving average approach over 10° longitude (i.e. two slices), mean values were calculated for microsatellite heterozygosity ( $H_E$  and  $H_o$ ) and mitochondrial haplotype diversity. Further, mean values of the first two principal components (PCs) of the DAPC value were analysed according to the same scheme. Patterns of spatial heterogeneity were revealed in the mitochondrial haplotype diversity and both PCs of the DAPC, but not in the microsatellite heterozygosity.

It was noted that the initial DAPC analyses were not informative about stock structure. The additional spatially explicit analyses, however, provided information relevant to stock-structure which was used in conjunction with biological information for stock structure inference [summarised in table 4 of SC/67a/Rep07]. It was further noted that spatially explicit analysis of information captured in single principal components (PCs) in a DAPC or other Principal Component Analyses (PCAs) may unravel stock-structure patterns not as easily detected in representations combining several PCs and/or geographic regions in a single visualisation. A further example of this approach can be found in Lah *et al.* (2016).

#### 2.4 Other

The Working Group also provided stock structure related feedback and recommendations on South American Bryde's

whales (Pastene *et al.*, 2012), North Pacific gray whales (SC/67a/NH11), Māui dolphins (Baker *et al.*, 2017), and Hector's dolphins (Hamner *et al.*, In press). The latter two papers were focused on the use of genotype-based estimates of abundance and effective population size, and were thus discussed as part of a joint session with the ASI and SM subcommittees. A summary of the discussion of those papers is included in Annex Q.

Pastene et al. (2012) presented the results of a genetic analysis based on mitochondrial DNA control region sequences to investigate both species identity and populations genetic structure of South American Bryde's whales. The genetic analysis was based on historical, biopsy and stranding samples from Chile (n=10) and Brazil (n=8). For comparative purposes published sequences of the Bryde's whales from different localities of the Indian and Pacific Oceans (including Peru, n=24) were incorporated into the analysis. Results of the phylogenetic analysis identified the Bryde's whales of South America as Balaenoptera brydei1. No statistically significant genetic differentiation was found between Chilean and Peruvian Bryde's whales. However, striking differences were found between western South Atlantic (Brazil) and eastern South Pacific (Peru and Chile) animals. In addition, striking genetic differences were found between all South American localities and those from the western North Pacific, Fiji and Java. These results suggest movement of B. brydei in the eastern South Pacific in the latitudinal range corresponding to Chile and Peru. These results also suggest no or very limited movement of whales between the South Pacific and the South Atlantic Oceans. This is consistent with the notion that *B. brvdei* is not distributed further south of approximately 40°S on both sides of South America.

The Working Group thanked Pastene for presenting this work. Discussion focused on how to interpret these results in the context of studies of regional variation in Bryde's whales in other areas. While in other areas, such as New Zealand and Brazil, Bryde's whales exhibit some degree of residency within coastal areas (Lodi *et al.*, 2015; Wiseman *et al.*, 2011), the whales off Chile appear to be make southnorth movements from southern Chile (~38 degrees) to the waters off Peru (Pastene *et al.*, 2012).

The Working Group was tasked with reviewing the aspects of SC/67a/NH11 that relate to stock structure. This paper, which is summarised in Annex O, describes the results of a population assessment of the gray whales feeding off Sakhalin Island (SI) and the southern coast of Kamchatka, Russia. This assessment is an update of that presented in Cooke et al. (2016) and contains new data from multiple sources, including the photo-identification data collected from gray whales off Kamchatka (Yakovlev et al., 2013; 2014). In addition, the output of the population model underlying the assessment was, for the first time, compared to the results of a genetic paternity test (Lang, 2010) aimed at identifying putative fathers for calves brought to the SI feeding ground by their mothers. This comparison indicated that the Sakhalin feeding aggregation is probably not genetically closed but that the SI and Kamchatka feeding aggregations, taken together, may be genetically closed. Of note, however, genetic data from Kamchatka would be required to confirm this.

The Working Group thanked Cooke for presenting this paper. In discussion, Cooke clarified that the hypothesis testing scheme utilised in SC/67a/NH11 assumed random

mating between all whales in a specified group and then compared the number of paternities detected in the model output for that region with the observed number of paternities derived from the empirical data. For this exercise, the defined group was initially restricted to only those whales that utilise the Sakhalin feeding ground and was then extended to include whales using either or both the Sakhalin and southern Kamchatka (hereafter referred to as SKNK for consistency with the current stock structure hypotheses. SC/67a/Rep04) feeding grounds. When the model assumed that all fathers were part of the Sakhalin group, the predicted number of detected paternities was significantly higher, albeit by a small number, than that observed in the empirical study. However, when the model assumed that all fathers were present within the combined SI and SKNK regions, the predicted number of detected paternities was less than the observed number. It was concluded that Sakhalin whales mate preferentially, but not exclusively, with each other, but that it is possible that Sakhalin and Kamchatka whales, taken together, mate only within the combined group. However, it was noted that the population model does not specify where such mating is occurring, i.e. it cannot distinguish between a scenario in which Sakhalin and southern Kamchatka whales breed with each other on the wintering grounds or a scenario in which those animals breed with each other while migrating or on the feeding ground.

With respect to the stock structure hypotheses under consideration, the results of SC/67a/NH11 may have implications on two fronts. First, an estimate of the number of whales utilising the combined SI and SKNK regions is provided. This estimate provides data that could be used to assess the plausibility of hypotheses, such as hypotheses 3b and 4b, which assume connectivity between the SKNK and SI feeding grounds but demographic independence of this combined area from the larger feeding ground in the Northern Bering-Southern Chukchi region. Secondly, the results of SC/67a/NH11 are consistent with a scenario in which whales feeding off SI and SKNK are mating with each other preferentially. Such a scenario is represented in hypothesis 4b, although in terms of the modelling framework hypotheses 4b and 3b are represented in the same way.

The Working Group noted that the results of SC/67a/ NH11 highlight the need for additional data to be collected off southern Kamchatka. Although a small number of biopsy samples have been collected from this area (see Table 1, SC/67a/Rep04), no biopsy efforts are known to have been made in more recent years (after 2011). While the model in SC/67a/NH11 is useful in evaluating whether hypotheses, such as preferential mating between SI and SKNK whales, are consistent with the model output, paternity analysis based on samples from both areas are necessary to determine if such mating occurs.

Finally, the Working Group noted that genetic analysis of historical specimens collected from western North Pacific migratory routes and/or wintering grounds are needed to evaluate the relationship between the historic western breeding stock (i.e. the stock that was subjected to past commercial whaling in the western North Pacific) and the whales that currently utilise SI and/or those represented in contemporary records of gray whales in Japanese and Chinese waters. Lang noted that mtDNA control region haplotype data had been obtained from the baleen of one such specimen (AMNH M-34260). This baleen was collected in Ulsan, South Korea, by R.C. Andrews in 1912. However, the mtDNA haplotype identified from this baleen is common among contemporary samples collected from gray whales in both the eastern and western North Pacific, which, in the absence of additional sequence data from this or other historic specimens, is not informative with respect to evaluating such relationships.

## **3. DNA TESTING**

## **3.1** Genetic methods for species, stock and individual identification

The Working Group first discussed four papers (SC/67a/SDDNA02-03, (Lah *et al.*, 2016; Malde *et al.*, 2017) that utilise Single Nucleotide Polymorphisms (SNPs) to look at population or species-level questions.

SC/67a/SDDNA02 presents an update on a paper (DeWoody *et al.*, 2016) presented in 2016 that reported the results of the genome sequences of two western gray whales from Sakhalin Island and one eastern gray whale from northern Alaska, and the development and validation of a SNP panel for gray whales. A modified version of that paper has been accepted for publication in the journal *Biological Bulletin*. The genome sequences are now available through NCBI and the SNP data will be archived by the journal.

The gray whale genome sequences and SNP panel and the ongoing collection of a larger dataset of SNPs from western and eastern gray whales will help to resolve issues regarding gray whale stock structure currently being considered under the Rangewide Review of the Population Structure and Status of North Pacific Gray Whales. Other useful applications include genetic fingerprinting for the identification of individual whales from their biopsies, estimates of relatedness and other population genetics parameters that inform of structure, genetic diversity, and aspects of behavior and reproduction. The SNP panel will provide a useful platform for future studies of gray whales because the results are directly comparable from lab to lab and study to study.

The Working Group thanked Bickham for the presentation and expressed their appreciation for this work, noting that having a publicly available gray whale genome sequence will be a valuable resource for future studies.

In discussion, it was noted that biopsies were collected from the gray whales off Sakhalin Island, Russia, by the Russian Gray Whale Project (formerly the Russia-US research program), between 1995 and 2007 (Lang et al., 2010), while the samples analysed in SC/67a/SDDNA02, as well as those which will be sampled in the future, were collected in 2011 and later. Given that this time span covers over two decades, the Working Group noted that it would be useful to compare the genetic composition of whales sampled early in the study with those sampled in more recent years to determine if any shifts in the genetic composition of the whales feeding off Sakhalin had occurred during this time period. The value of using a SNP panel, such as the one designed in SC/67a/ SDDNA02, to conduct such analyses was also highlighted, as SNP data can be compared among studies and over time without the need for the cross-study calibration that is necessary with microsatellites (Morin et al., 2004).

Bickham noted that future plans included using the SNP panel described in SC/67a/SDDNA02 to genotype samples that will be collected from gray whales in the three primary wintering lagoons in Baja California, Mexico. It was noted that, should analysis of additional samples be warranted, genetic samples are available from US and Canadian waters that encompass much of the migratory range of gray whales in the eastern North Pacific as well as the feeding grounds in the Bering and Chukchi Seas and the Pacific Northwest (see Table 1, SC/67a/Rep04).

SC/67a/SDDNA03 summarises progress made on the bowhead genetics program with respect to building a mtDNA database and developing a new panel of 96 SNPs. For mtDNA, 3 parts were sequenced (HVR1, ND1, and cytb). The mtDNA database now has 435 samples sequenced for all 3 parts. From these samples, 72 unique haplotypes were identified. The B-C-B stock shares haplotypes with both the Okhotsk and Eastern Canadian Arctic stocks. whereas the Okhotsk and Canadian stocks do not have any shared haplotypes. All 3 stocks contain private haplotypes. Regarding the SNP data, SC/67a/SDDNA03 updates a panel of SNPs presented in Baird et al. (2016). 53 SNP loci were carried over from the earlier panel, and newly developed SNPs were derived from protein-coding sequences from Greenland bowhead genome sequences to increase the SNP panel to 96 loci. 475 bowhead samples were genotyped using the Fluidigm method, including 411 from B-C-B, 34 from Canada, and 30 from Okhotsk stocks. Quality control methods included genotyping duplicate samples, using mother/foetus pairs, and samples from earlier studies. There was low genotyping error rate for this method, calculated to be 0.7%. The authors note that the benefit of using nonanonymous loci is that the data are replicable across labs and methods. Additionally, the error rate of the Fluidigm method described here is low. These data will be used in future studies to examine  $F_{\rm \scriptscriptstyle ST}$  and migration among stocks, relatedness, and historical demography.

In discussion, it was asked whether this panel of SNPs has been used for population genetic inference. Baird noted that such analyses were in progress and the results would be presented at SC/67b.

The Working Group thanked Baird for her presentation and looks forward to hearing more about this work during the bowhead whale *Implementation Review* that begins next year.

Malde *et al.* (2017) presented an array of SNP markers displaying fixed or nearly fixed allele frequency differences among the minke whale species. Five panels of putatively diagnostic markers were established on a genotyping platform for validation of allele frequencies; two panels (26 and 24 SNPs) separating the two species of minke whale, and three panels (22, 23, and 24 SNPs) differentiating the three subspecies of common minke whale. Two statistical methods for inferring the degree of back-crossing in hybrid individuals had been developed. The SNP panels were validated against a set of reference samples, demonstrating the ability to accurately identify back-crossed individuals up to three generations.

The Working Group thanked Skaug for presenting this work. In discussion, it was noted that the panel of SNPs used in Malde *et al.* (2017) was designed specifically for the detection of hybrid and back-crossed individuals across species and would not be appropriate, given the number of markers and the panel design, for examining population structure or kinship-based questions within species. However, a similar approach could be used to design a SNP panel appropriate for addressing population-level questions.

Lah *et al.* (2016) presents information on the population structure of a highly mobile marine mammal, the harbor porpoise (*Phocoena phocoena*). In the Atlantic shelf waters, the population structure of this species follows a pattern of significant isolation-by-distance. The population structure of harbor porpoises from the Baltic Sea, which is connected with the North Sea through a series of basins separated by shallow underwater ridges, however, is more complex. Here, the population differentiation of harbor porpoises in European Seas was investigated with a special focus on the Baltic Sea and adjacent waters, using a population genomics approach. 2,872 single nucleotide polymorphisms (SNPs) were used, derived from double digest restrictionsite associated DNA sequencing (ddRAD-seq), as well as 13 microsatellite loci and mitochondrial haplotypes for the same set of individuals. Spatial principal components analysis (sPCA), and Bayesian clustering on a subset of SNPs suggest three main groupings at the level of all studied regions: the Black Sea, the North Atlantic, and the Baltic Sea. Furthermore, a distinct separation was observed between the North Sea harbor porpoises and the Baltic Sea populations, as well as a split between porpoise populations within the Baltic Sea. A notable distinction was observed between the Belt Sea and the Inner Baltic Sea sub-regions. Improved delineation of harbor porpoise population assignments for the Baltic based on genomic evidence is important for conservation management of this endangered cetacean in threatened habitats, particularly in the Baltic Sea proper. In addition, SNPs outperformed microsatellite markers in particular in the assignment of individual specimens to genetic clusters. The paper demonstrates the utility of RADtags from a relatively small, opportunistically sampled cetacean sample set for population diversity and divergence analysis. It can further serve as basis for the development of a panel of informative SNP loci used in population genetic and kinship analyses of Harbour porpoises in European waters

The Working Group thanked Tiedemann for presenting this work. In discussion, it was noted that this study demonstrated the utility of opportunistically sampled specimens (e.g. strandings) in genomic analyses, which typically rely on obtaining high quality DNA which is not always present in degraded samples.

The paper also identified similar divergence patterns when the large SNP panel and the smaller number of microsatellites were used. However, individual-level distinctions were better revealed using SNPs.

The Working Group noted that including multiple SNPs within loci and inferring haplotypes has been shown to have increased power when compared to unlinked SNPs (Morin *et al.*, 2009). This increased power would also be expected to result when using SNPs linked to microsatellite loci, e.g. 'SNPSTRs' (Mountain *et al.*, 2002). Tiedemann noted that only unlinked loci were used in the study, but that exploring the use of linked loci could be beneficial.

In reviewing these papers, it is important to evaluate whether the approach used is suitable for the question being addressed. The first three studies (SC/67a/SDDNA02, SC/67a/SDDNA03, and Malde *et al.* (2017) utilised SNP panels designed from whole genome sequences. The number of SNPs used to identify interspecies hybridisation was low, but the SNPs chosen have high diagnostic power. Both SC/67a/SDDNA03-04 utilised a moderate number of SNPs, many or all of which were chosen from genes known to be under selection. The utility of this approach (choosing SNPs potentially under selection) could be limited in population genetic analyses that assume neutrality. When genome data is available, it is however straightforward to design additional panels for use in specific analyses.

The fourth study (Lah *et al.*, 2016) utilised a ddRAD approach, in which SNP discovery and genotyping is simultaneously conducted. While this approach can identify thousands of loci, the number of loci shared among samples decreases when additional sample libraries are sequenced as the SNPs produced are essentially randomly selected from

across the genome. However, the ddRAD sequence data produced could be used to design a SNP panel for use with an amplicon-based approach, which would provide higher consistency in genotyping success across specimens.

Discussion then focused on the importance of understanding if loci used are under selection. Expectations for such loci vary with the type of selection; while positive selection may result in divergence between groups, little variation would be expected in loci under purifying selection. In addition, some analysis (e.g. unbiased population inference) may assume that loci are neutral and thus may not be appropriate to use with data from loci under selection. Finally, it should be noted that SNPs derived from coding regions are not necessarily under selection themselves; in many studies, little evidence of strong selection has been detected even when SNPs are derived from coding regions.

The final paper (SC/67a/SH11) discussed under this agenda item focused on species identification from bone fragments. The author's summary for this paper is included in Annex H.

In discussion, it was questioned whether there had been any attempt to collect a specific type of bone (e.g. the left jaw) to maximise the number of individuals and minimise duplicates. Baker noted that while it would be ideal to focus on collecting a specific bone, permit and availability issues constrained such efforts. However, over 70,000 whales were taken on South Georgia, and thus the chance of collecting bone fragments from the same individual were low. Samples sharing the same mitogenome sequence can also be flagged as potential duplicates.

The mitogenome sequences were produced using a shotgun sequencing approach. The Working Group suggested that using a hybrid capture approach could be useful with historic and particularly with ancient samples. Such an approach could integrate nuclear SNPs as well.

Elwen noted that approximately 100 skulls are available from the northern coast of Namibia, although given the hot and wet environment, degradation may be an issue. The Working Group noted that degradation from weathering should mainly effect the surface area, and new extraction approaches (Damgaard *et al.*, 2015; Korlević *et al.*, 2015) are available that have been successful with, for example, the South Georgia whale bones.

A summary of the discussion of this paper in the Southern Hemisphere sub-committee is included in Annex H.

### 3.2 'Amendments' of sequences deposited in GenBank

In previous years, Cipriano has corresponded with *GenBank* to attempt to identify a mechanism by which inconsistencies identified in the metadata (e.g. taxonomic status, geographic location, locus misassignment) of some entries could be corrected. Unfortunately, Cipriano's contact person at the NCBI (National Center for Biotechnology Information) passed away this year, and no further progress on this work was made on this front.

It was noted by the Working Group that *GenBank* is essentially an uncurated database, and that there is value in retaining the 'raw data' that it represents. Although experienced users may be aware that additional sequence validation may be needed when using *GenBank* sequences, the concern is that less experienced users will be unaware of the associated caveats and may inadvertently worsen the problem by utilising sequences that have been erroneously assigned to a locus or a taxon.

Cipriano agreed to continue efforts to work with GenBank staff to find a mechanism for dealing with

identified inconsistencies. The Working Group also agreed that the revised DNA quality guidelines (see Item 4.1) would contain a section discussing the precautions that should be used when including *GenBank* sequences in a study.

## **3.3** 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, 2012a, 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 their register (see Appendix 3). The collection of samples is from scientific whaling in the North Pacific (1994-2016 JARPN-JARPNII) and the Antarctic (1987/88-2015/16, JARPA-JARPAII and NEWREP-A), and from bycatch (2001-16).

Skaug reported on the status of the Norwegian register (see Appendix 4). The collection of samples of North Atlantic common minke whale is from commercial catches for the period 1997 to 2016.

Pampoulie reported on the status of the Icelandic register (Appendix 5), which includes samples from scientific whaling (2003-07) and commercial catches (2006-16).

## 3.4 Reference databases and standards for diagnostic DNA registries

An update of the Japanese register is shown in Appendix 3. For North Pacific minke whales bycaught or sampled under JARPN II in 2016, mtDNA and microsatellite analyses of 100% (n=169, bycatch; n=37, JARPNII) has been completed. For North Pacific Bryde's whales and North Pacific sei whales sampled under JARPNII in 2016, mtDNA and microsatellite analyses have been completed for 100% of the samples (n=25, Bryde's whales; n=90, sei whales). No bycatch of North Pacific Bryde's whales or North Pacific sei whales occurred in 2016. No sampling or bycatch of sperm whales occurred in 2016. Bycatches of North Pacific humpback whales (n=2), North Pacific right whales (n=1), and North Pacific fin whales (n=1) occurred; mtDNA and microsatellite analyses is complete for 100% of these samples.

For Antarctic minke whales sampled under NEWREP-A in 2016, mtDNA and microsatellite analyses have been completed for 100% of the samples (n=333).

With regard to the Japanese register, it was noted that no gray whales were listed in the register, despite reports of some bycaught whales being mentioned in the Japanese progress reports. Japan responded that these specimens have been genotyped but are not included in the register, because the register only concerns market products and sales of gray whale are prohibited by domestic law.

An update of the Norwegian register is shown in Appendix 4. After discounting for missing samples, 100% of the North Atlantic common minke whales (n=578) caught in 2016 were screened microsatellites.

An update of the Icelandic registry is shown in Appendix 5. 100% of the fin whales caught by commercial whaling between 2006 and 2016 (n=688) were screened for both mtDNA and microsatellites. The North Atlantic common minke whales caught by commercial whaling in 2016 (n=36) have not yet been screened for either mtDNA or microsatellites.

During presentation and discussion of the Norwegian register, the Working Group was informed about the

discontinuation of mtDNA analysis on Norwegian samples, as well as an eventual replacement of microsatellite typing by SNP analysis. Regarding this issue, the following was noted: Last year the Committee welcomed Norway's plan to add SNPs in its register and noted that SNP genotyping should be seen as a complement, not as a replacement of the current microsatellite genotyping. No technical details of the plan were available last year, and therefore, the Committee recommended that those details are provided at future meetings so that the Committee can provide technical advice (IWC, 2017b, p.71). Following the new information from Norway as to the discontinuation of mitochondrial DNA and eventually microsatellite analyses, there were concerns among the SDWG as to the comparability of the DNA registers in the future. The Working Group reiterates the recommendation from the Committee's last year. The SDWG acknowledges that DNA registries are maintained on a voluntary basis: it encourages coordination of all DNA registers so that they are based on comparable genetic markers.

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

## 4. GUIDELINES AND METHODS FOR GENETIC STUDIES AND DNA 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. Both sets are subject to ongoing update as appropriate.

#### 4.1 DNA quality

The DNA data quality control guidelines are already available as a 'living document' on the IWC website (http:// *iwc.int/scientific-committee-handbook#ten*). In recent meetings, data derived from next generation sequencing approaches, including SNPs, have been utilised to address stock structure questions. In light of these developments, the Working Group agreed that it would be timely to update the DNA data quality control guidelines to cover these types of data. During SC/67a, Tiedemann presented a draft of the updated guidelines, which included added text addressing issues associated with SNP genotyping, next generation sequencing, and sequencing of nuclear genes. During discussion, several suggestions on topics to add to the draft were mentioned. An intersessional email group was formed to implement these suggestions and discuss any additional revisions [see Item 8.2]. A revised version of the guidelines will be presented at SC/67b.

#### 4.2 Genetic analysis guidelines

This document provides guidelines for some of the more common types of statistical analysis of genetic data that are employed in IWC management contexts. The main section is intended as guidance for managers and also contains examples of management problems that are regularly faced by the Committee. There is also an extensive appendix of genetic analysis techniques for specialist readers. This guidelines document was completed intersessionally and has been accepted for publication in the *Journal of Cetacean Research and Management*. In discussion, the Working Group suggested that it would be valuable to make these guidelines, as well as those discussed in Item 4.1 when completed, available electronically as well as through the journal. Lang offered to follow up with the journal on this suggestion.

Given that this intended to act as a 'living document', it may be subject to updates in the future as the Working Group sees fit.

## 4.3 Other issues

No other issues were discussed.

## **5. TERMINOLOGY**

Following a recommendation arising in 2012 (IWC, 2012b, p.219), the Working Group began compiling a 'go-to' glossary of stock related terms, with the aim of encouraging consistent use of stock structure related terms within Scientific Committee reports and in papers submitted to the Scientific Committee and within SC reports and discussions. Initial work on this glossary focused on defining terms most commonly used in assessments of baleen whales. At SC/65b and SC/66a, joint sessions of the SDWG and the Small Cetaceans sub-committee were held to evaluate how the terms in this glossary aligned with terminology used in the SM sub-committee discussions (IWC, 2015, p.231; 2016, p.290). During these discussions, concerns were raised regarding the application of these terms to small cetaceans, in part due to differences in the behaviour and life history of small cetaceans relative to baleen whales. There is also some reluctance as to changing terminology which may be well established within a particular sub-committee and the related scientific community. Limited progress was made in addressing the concerns of the SM sub-committee, and the Working Group noted that even within sub-committees that focus on assessments of baleen whales, stock-structure related terms continue to be used inconsistently.

The Working Group decided to revisit this issue at SC/67b, with a focus on coming to an agreement within the group with respect to how terms are defined. At SC/67b the Working Group plans to invite Punt and Butterworth to provide a short tutorial on how the advice of the Working Group is utilised by the RMP and other sub-committees. While this exercise is intended to increase understanding of the role filled by the Working Group in the context of other sub-committees' work, it will also provide an opportunity to get feedback from the presenters as to how stock-structure related terms are utilised within other sub-committees.

#### 6. NEW STATISTICAL AND GENETIC ISSUES RELATING TO STOCK DEFINITION

## 6.1 Simulation tools for spatial structuring (e.g. TOSSM, Testing of Spatial Structure Models)

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). The Working Group agreed that reviewing the available packages and evaluating their utility to address issues of interest to the Scientific Committee would be useful. An email correspondence group was formed to conduct this review intersessionally and to provide a summary of their findings at SC/67b (see Item 8.3). In addition, the Working Group looks forward to reviewing papers demonstrating the utility of simulation-based approaches to inform stock structure questions in future sessions.

#### 6.2 Other

## 6.2.1 Close-kin mark-recapture

At SC/67a, Bravington was invited to provide a presentation to the Scientific Committee on the close-kin mark-recapture (CKMR) approach (Bravington et al., 2016b) and the utility of linking it to epigenetic aging from DNA samples. These are new techniques (5-10 years), based on tissue samples, which could be very useful to the IWC Scientific Committee for reliable and relatively inexpensive population assessment - e.g. in evaluating the conservation significance of bycatch and/or directed takes. 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 markrecapture framework to infer absolute abundance, parameters like survival rate, and even stock structure. The spatial distribution of kin-pairs has been used qualitatively for stock structure investigation several times in the SC (e.g. SC/67a/ SDDNA01 and SC/67a/SDDNA05). CKMR for abundance estimation is much more recent (and requires greater surety in the genotyping); it has been successfully applied to southern bluefin tuna (Bravington et al., 2016a), and is being used in several current international projects on endangered sharks and commercial fish stocks. CKMR is not to be undertaken lightly, since the genotyping and the statistical modelling are demanding and sample-size requirements must be thought through carefully, but it is cheap and powerful provided enough samples can be collected. Although CKMR should be useful without additional information in many cetacean stock delimitation applications, it will yield precise results much faster if age can be estimated, even roughly. While age can already be obtained in some situations (e.g. bycatch of odontocetes where teeth can be obtained and sectioned), the utility of CKMR for cetaceans will be now increased given the new capability to use the same tissue-samples for epigenetic ageing which (after many unsuccessful attempts) has in the last few years been successfully demonstrated in humpback whales and other mammal species (Jarman et al., 2015; Polanowski et al., 2014). Although species-by-species or even population-specific calibration of epigenetic age is of course challenging for species with few or no reference, Bravington suggested that it may be possible to infer the calibration indirectly in the course of a CKMR study, with the two approaches giving mutual support.

Whether hidden population structure is problematic when using this method was discussed. Bravington noted that, in the absence of differential sampling, having multiple, unrecognised stocks would not bias an estimate of overall abundance. While estimates of relatedness, which are calculated using allele frequency information, can be artificially inflated if unrecognised population structure is present, CKMR requires that identified parent-offspring pairs (POPs) match at least one allele at every locus (i.e. no mismatching loci are allowed) and thus is not as sensitive to undetected structure if a sufficiently large number of informative loci is typed. However, CKMR requires that genotyping error rates be stringently controlled to prevent the identification of true POPs from being obscured by a large number of false positives. The value of integrating data from epigenetic aging into CKMR was noted (see discussion below, Item 6.2.2). It was noted that, as research into epigenetic aging in model species, such as mice, has progressed, the techniques used have become more reliable as well as more affordable. However, methylation rates may be specific to species or even populations, and thus epigenetic age estimates need to be verified. This may be easier with odontocetes, where epigenetic age estimates could be calibrated by comparison to ages estimated by counting growth layer groups in teeth (Perrin and Myrick, 1980). It was noted that while estimates of the actual age of animals is needed for some applications, inference of relative age is sufficient in other cases. Such inferences can be used in calibration of epigenetic methods when long-term close kin sampling is pursued.

In conclusion, the Working Group thanked Bravington for presenting an overview of this approach, which has multiple applications within the Scientific Committee's scope of work. The Working Group looks forward to reviewing more papers using CKMR in the future.

#### 6.2.2 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 Scientific Committee to the concept and methodological developments in the technique (IWC, 2017c). Although it was not possible to coordinate such a presentation for SC/67a, the Working Group agreed that learning more about the applicability of epigenetic aging to the work of the Scientific Committee is important and encouraged that submission of papers relevant to this topic be presented next year.

# 6.2.3 Inference of demographic history using whole genome sequences

SC/67a/SDDNA04 explored the use of genome sequence data from two western gray whales (WGW) sampled near Sakhalin Island and one putative eastern gray whale (EGW) from Barrow, Alaska to reveal the demographic history and structure of populations. Notwithstanding that this analysis is based on a small sample size, a genome possesses an extensive record of the ancestry of an individual and individuals belonging to the same population are expected to exhibit the signatures of shared historical events not present in genomes from individuals of different populations. Our ability to reconstruct these histories has increased recently due to the reduced cost of genome sequencing and advances in bioinformatics and analytical methods largely originating from human population genomics.

Results indicated that gray whale genomes contain substantial nucleotide diversity even though effective population sizes have declined substantially since the last glacial maximum. Contemporary gray whale genomes, both eastern and western, contain levels of autosomal nucleotide diversity that exceed levels found in many endangered species. The extent of recent historical inbreeding is shown here to be greater in WGW genomes, as measured

Summary of the work plan.										
Item	Intersessional 2017/18	2018 Annual Meeting (SC/67b)								
Item 4.1 DNA quality guidelines	Intersessional email group to discuss updating guidelines to include data produced using next generation sequencing approaches	Review intersessional progress								
Item 6.1 Simulation-based tools	Intersessional email group to review software packages and evaluate utility to the SDDNA WG	Review intersessional progress								

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by autozygosity, compared to the single EGW genome. It appears that individuals from the western Pacific have been subject to recent inbreeding that likely stemmed from bottlenecks induced by commercial whaling in the 20th century. However, the status of the Sakhalin whales as belonging to either, or both, of the EGW or WGW population is not resolved as some of the analyses employed in this study fail to differentiate them. In discussion, the authors recognised that the current study was based on a very small sample size of whales and they indicated that follow on work will involve re-sequencing the genomes with shallow coverage of 20 to 30 individuals each of WGW and EGW.

The Working Group thanked the authors for their presentation, which focuses on an analysis that has not previously been presented to the group. In discussion, the Working Group noted that the inferred trajectories of effective size over time derived from the eastern and two western genomes seemed to be generally similar until the late Pleistocene. While these results are interesting, the authors noted that sequencing of additional samples was needed to have confidence in the inferred trajectories. This work was largely intended as a 'proof of concept' exercise to demonstrate the feasibility of using this approach with the gray whale genome data, and sequencing of the genomes of additional samples is planned.

The Working Group noted that some limitations are inherent in this approach. First, the analysis is not informative with respect to recent population history. Secondly, both the inferred dates and the estimates of effective size  $(N_{i})$ over time depend on parameter values used for generation time and mutation rate; particularly in the latter case, there is uncertainty about the best estimate to use. Thus while the estimates of  $N_{\rm o}$  and divergence times may not be that accurate, higher confidence can be placed in the trends in  $N_{,}$ which are independent of the generation time and mutation rate used.

#### 7. OTHER ISSUES

No other matters were discussed by the Working Group.

### 8. WORK PLAN

#### 8.1 DNA testing

The terms of reference for the DNA Testing agenda item will remain the same for the next year, unless the Commission requests other information in the interim. Members of the Working Group were encouraged to submit papers relating to these terms of reference and to propose additional agenda items. Comparison of methods for SNP development and assessment will be continued next year. Any progress on efforts to identify a mechanism to amend misclassified sequences in GenBank will be reported.

### 8.2 DNA quality guidelines

The email group formed to discuss updating the DNA quality guidelines will continue intersessionally. Using the draft updated guidelines produced during SC/67a, the group

will continue to review and update sections covering data, including SNPs, produced using next generation sequencing (NGS) approaches. Topics to be addressed include analytical procedures to process the raw NGS data (trimming, filter settings, etc.) as well as issues arising from biological phenomena related to the markers of choice (e.g. linkage, selection vs neutrality, locus orthology). The group was convened under Tiedemann and included Baird, Baker, Bickham, DeWoody, Goto, Hoelzel, Jackson, Lang, Leslie, M., Natoli, Palsbøll, Pampoulie, Rosel, Skaug, Taguchi, and Waples.

## 8.3 Simulation tools for spatial structuring

An intersessional email group will be convened to discuss the utility of simulation tools for evaluating spatial structure. 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/67b. The group was convened under Lang and included Bickham, DeWoody, Hoelzel, Kitakado, and Tiedemann.

#### 9. ADOPTION OF REPORT

This report was adopted at 12:00 on 17 May 2017.

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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. Provide scientific advice on stock structure to other sub-groups
  - 2.1 Southern and Northern Hemisphere blue whales
  - 2.2 Western North Pacific common minke whales
  - 2.3 North Pacific sei and Bryde's whales
  - 2.4 Other
- 3. DNA testing
  - 3.1 Genetic methods for species, stocks and individual identification
  - 3.2 'Amendments' of sequences deposited in GenBank

- 3.3 Collection and archiving of tissue samples from catches and bycatches
- 3.4 Reference databases and standards for diagnostic DNA registries
- 4. Guidelines and methods for genetic studies and DNA data quality
  - 4.1 DNA quality guidelines
  - 4.2 Genetic analysis guidelines
  - 4.3 Other developments
- 5. Terminology
- 6. New statistical and genetic issues relating to stock definition
  - 6.1 Simulation tools for spatial structuring (e.g. TOSSM, Testing of Spatial Structure Models)
- 6.2 Other
- 7. Other issues
- 8. Work plan

### Appendix 2

## SEX RATIOS IN PARENT-OFFSPRING PAIR INFERENCE AMONG WESTERN NORTH PACIFIC COMMON MINKE WHALES

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This appendix provides information on sex ratios in datasets used for kinship inference in western North Pacific common minke whales (SC/67a/SDDNA01).

		C C			
Sex	J*	0*	Unassigned	Foetuses	Total
Male	844	1,660	278	0	2,782
Female	921	657	141	0	1,719
Unidentified	0	0	0	53	53
Total	1,765	2,317	419	53	4,554

\*Without foetuses.

#### **Appendix 3**

## 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

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 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 2016 is as follows.

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						Т	Table 1						
Japan DNA register.													
Footnote no.	1	2	3	4	5	6	7	8	9	10	11	12	
Species/Year	Type	No. whales	No. duplicates	No. missing	No. lab problems	No. mtDNA	% mtDNA	No. msat	% msat	Sex analysed	% sexed	Note	
North Pacific minke whale 1994-2015 2016 2001-2015 2016	SP SP BC BC	2,643 37 1,839 169	0 0 0 0	0 0 26 0	8 0 2 0	2,635 37 1,839 169	100 100 100 100	2,635 37 1,811 169	100 100 98 100	2,643 37 1,809 169	100 100 98 100		
North Pacific Bryde's what 2000-2015 2016 2001-2015	le SP SP BC	705 25 5	0 0 0	0 0 0	3 0 0	702 25 5	100 100 100	705 25 4	100 100 80	705 25 4	100 100 80	Include 3 Omura's whale and 1 from the East China Sea stock	
2016	BC	0	0	0	0	0	0	0	0	0	0	No BC	
North Pacific sei whale 2002-2015 2016	SP SP	1,264 90	0 0	0 0	4 0	1,260 90	100 100	1,264 90	100 100	1,264 90	100 100		
2002-2015 2001-2015 2016	SP BC BC	56 2 0	0 0 0	0 0 0	0 0 0	56 2 0	$\begin{array}{c} 100\\ 100\\ 0 \end{array}$	56 2 0	$\begin{array}{c}100\\100\\0\end{array}$	56 2 0	100 100 0	No BC	
Antarctic minke whale 1987/88-2004/05 2005/06-2013/14	SP SP	6,794 3,884	0 0	10 549	0 162	1,118 2,645	17 68	6,271 3,173	92 82	6,794 3,884	100 100	Incl. dwarf; 87/88-88/89 no. microsats Some missing in the 03/11 tsunami in 2011	
2015/16	SP	333	0	0	0	333	100	333	100	333	100		
<b>Antarctic fin whale</b> 2005/06-2013/14	SP	18	0	0	0	18	100	18	100	18	100		
North Pacific humpback w 2001-2015 2016	hale BC BC	61 2	0 0	0 0	0 0	61 2	100 100	61 2	100 100	61 2	100 100		
North Pacific right whale 2001-2015	BC	2	0	1	0	2	100	1	50	1	50	Missing by the 2011 tsunami, no	
2016	BC	1	0	0	0	1	100	1	100	1	100	micrsats	
North Pacific fin whale 2001-2015 2016	BC BC	10 1	0 0	0 0	0 0	10 1	100 100	10 1	100 100	10 1	100 100		

<sup>1</sup>Key to sample types: SP=special permit catch, C=commercial catch, BC=bycatch, ST=stranding.

<sup>2</sup>Number of whales that potentially entered by the previous years and enters (new year) the markets.

<sup>3</sup>Number of occurrences (tissues) sample switching on board the vessels as detected by comparison of genetic profiles.

<sup>4</sup>Number of individuals for which tissue samples are missing for reasons other than sample switching.

<sup>5</sup>Genetic laboratory not able to obtain microsatellite profiles mtDNA haplotypes from tissue samples.

<sup>6</sup>Number of samples analysed for mitochondrial control region.

<sup>7</sup>% of total samples analysed for mitochondrial control region.

<sup>8</sup>Number of samples analysed for microsatellites.

<sup>9</sup>% of total samples analysed for microsatellites.

<sup>10</sup>Number of samples analysed for sex.

<sup>11</sup>% of samples analysed for sex.

<sup>12</sup>Other problems or information.

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### **Appendix 4**

## AN UPDATE OF THE NORWEGIAN MINKE WHALE DNA REGISTER

Hans J. Skaug

University of Bergen and Institute of Marine Research

Table 1

			Ν	Vorwegian	minke wha	ale DNA regi	ster.					
Footnote no.	1	2	3	4	5	6	7	8	9	10	11	12
Species/Year	Type	No. whales	No. duplicates	No. missing	No. lab problems	No. mtDNA	% mtDNA	No. msat	% msat	Sex analysed	% sexed	Note
NA minke whale												
1997-2015	С	10,721	109	67	2	10,552	100	10,552	100	10,552	100	-
2016	С	586	0	8	0	0	0	578	100	578	100	-

<sup>1</sup>Key to sample types: SP=special permit catch, C=commercial catch, BC=bycatch, ST=stranding.

<sup>2</sup>Number of whales that potentially entered by the previous years and enters (new year) the markets.

<sup>3</sup>Number of occurrences (tissues) sample switching on board the vessels as detected by comparison of genetic profiles.

<sup>4</sup>Number of individuals for which tissue samples are missing for reasons other than sample switching. <sup>5</sup>Genetic laboratory not able to obtain microsatellite profiles mtDNA haplotypes from tissue samples.

<sup>6</sup>Number of samples analysed for mitochondrial control region.

<sup>7</sup>% of total samples analysed for mitochondrial control region.

<sup>8</sup>Number of samples analysed for microsatellites.

<sup>9</sup>% of total samples analysed for microsatellites. <sup>10</sup>Number of samples analysed for sex.

<sup>11</sup>% of samples analysed for sex.

<sup>12</sup>Other problems or information.

## Appendix 5

## 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. Table 1 gives the present status of the registry. Samples from all the common minke whales landed as a part of the Icelandic research program (2003-07) and recent commercial catches (2008-16), as well as from commercial North Atlantic fin whale catches have been genotyped and information stored in the database.

Icelandic whale DNA register.												
Footnote no.	1	2	3	4	5	6	7	8	9	10	11	12
Species/Year	Type	No. whales	No. duplicates	No. missing	No. lab problems	No. mtDNA	% mtDNA	No. msat	% msat	Sex analysed	% sexed	Note
NA minke whale												
2003-07	SP	189	0	0	0	189	100	189	100	189	100	-
2008-15	С	378	0	0	0	362	97	365	97	367	98	-
2016	С	36	0	0	0	0	0	0	0	0	0	-
<b>NA fin whale</b> 2006-16	С	688	0	0	0	688	100	688	100	688	100	-

<sup>1</sup>Key to sample types: SP=special permit catch, C=commercial catch, BC=bycatch, ST=stranding.

<sup>2</sup>Number of whales that potentially entered by the previous years and enters (new year) the markets.

<sup>3</sup>Number of occurrences (tissues) sample switching on board the vessels as detected by comparison of genetic profiles.

<sup>4</sup>Number of individuals for which tissue samples are missing for reasons other than sample switching.

<sup>5</sup>Genetic laboratory not able to obtain microsatellite profiles mtDNA haplotypes from tissue samples.

6Number of samples analysed for mitochondrial control region.

<sup>7</sup>% of total samples analysed for mitochondrial control region.

<sup>8</sup>Number of samples analysed for microsatellites.

% of total samples analysed for microsatellites.

<sup>10</sup>Number of samples analysed for sex.

<sup>11</sup>% of samples analysed for sex.

<sup>12</sup>Other problems or information.