Annex I

Report of the Working Group on Stock Definition

Members: Jackson (Convenor), Baker, Bickham, Bravington, Broker, Collins, Double, Cipriano, Elvarsson, Gaggiotti, Hoelzel, Kanda, Kasuya, Lang, Palsbøll, Pampoulie, Park, Pastene, Perkins, Perrin, Rosenbaum, Scordino, Skaug, Solvang, Tiedemann, Urbán, Víkingsson, Wade, Waples, Weller.

1. INTRODUCTORY ITEMS

1.1 Opening remarks
Jackson welcomed participants.

1.2 Election of Chair and appointment of rapporteur
Jackson was elected as Chair and Lang acted as rapporteur.

1.3 Adoption of Agenda
The adopted agenda is given in Appendix 1.

1.4 Review of documents
The documents identified as containing information relevant to the Working Group were: SC/65a/SD02, SC/65a/RMP01, SC/65a/RMP03, SC/65a/IA06, SC/65a/SH13, SC/65a/BRG16, Anderwald *et al.* (2011), SC/65a/Rep03 (Item 5.2) and Weller *et al.* (2013).

2. GUIDELINES 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 (hereafter SDWG) to develop for reference in the Committee's discussions of stock structure. The DNA Data Quality guidelines are already available as a 'living document' on the IWC website, and the Genetic Data Analysis guidelines will be available in this form by SC/65b. Both are subject to ongoing update as appropriate.

2.1 Genetic data analysis guidelines document
The 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. During SC/65a some additional Appendix sections were completed and SDWG members reviewed the guidance section and made progress on the management problems sections. This work is anticipated to complete intersessionally (see the work plan in Item 7.1).

2.2 Genetic data quality review
During SC/65a, additional sections were added to the guidelines on marker validation and systematic quality control. These sections provide guidance on: quality control and development of single nucleotide polymorphism data, quality control and development of other marker types not discussed in the original document (e.g. major histocompatibility complex genes), the quality of next generation sequencing data and guidelines regarding acceptable levels and types of errors occurring in DNA data.

3. STATISTICAL AND GENETIC ISSUES RELATING TO STOCK DEFINITION
The Stock Definition 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 (IWC, 2013b). These discussions often refer to the genetic analysis guidelines and genetic data quality documents, the latter of which can be found at *http://iwc.int/scientific-committee-handbook#ten.*

Some general comments were made which are relevant to many papers submitted to the Scientific Committee.

The SDWG discussed the fact that with new next generation sequencing tools it is now relatively inexpensive to increase the number of loci analysed (e.g. by developing single nucleotide polymorphisms, SNPs and using Double Digest Restriction Associated DNA sequencing, ddRADseq, approaches) so that more information could be gained from each sample in a population study. More genetic markers are often called for in circumstances where the existing marker set cannot detect differentiation due to lack of discriminatory power or lack of subdivision. Increasing the number of loci increases the power to detect subtle population structure using both traditional and clustering analyses and can facilitate future studies of relatedness patterns among sampled animals. Whilst the addition of more markers to a study is generally valuable, it was also cautioned that the value of this is truly in the context of the questions being addressed - for example, do the existing markers already have sufficient power to exclude the possibility of demographically independent sub-stocks within the sample? Simulation analysis of the power of data to measure departures from panmixia and to reject demographically significant (i.e. sufficiently high) migration rates between putative differentiated clusters are useful in this regard. Increased numbers of loci can increase power to detect subtle population structure and also allow for improved inference of the population history underlying the substructure. However, they can increase resolution to the point where even individuals can be discriminated and can also amplify spurious signals from genotype errors and small departures from random sampling. Therefore it is important to consider the level at which structure needs to be detected in order for it to be of management concern.

A general caution was issued regarding removal of relatives from genetic datasets when analysing population differentiation. A major characteristic of small populations is that they are inbred and therefore contain close relatives. Removal of relatives can therefore bias the population sample. However, this can be justified if there is reason to believe that the population sample is not random. For example when mother-calf pairs are sampled together, sampling is not independent, so calves can be removed from such datasets.

3.1 Population structuring and migration rates
3.1.1 Revised Management Procedure
SC/65a/RMP03 summarises the genetic stock structure studies performed on North Atlantic fin whale. It presented a summary table including allozyme, microsatellite loci and mitochondrial DNA (mtDNA) and gave information on levels of structure observed. SC/65a/RMP03 emphasised the generally low levels of differentiation observed except at some allozyme loci. The allozyme results are then discussed in relation to a new manuscript (Olsen *et al.*, In press) suggesting that allozyme patterns at the two most informative loci (*MPI* and *MDH-1*) were not detected by DNA results. These new results suggested that the observed pattern at these loci may therefore not reflect genetic drift, migration or even selection at those loci. The results of the allozyme studies should consequently be interpreted with caution. SC/65a/RMP03 also presents additional work which has been done on estimates of numbers of migrants (gene flow) and relatedness analysis (logarithm of odds, or LOD score) and emphasised the need to develop these methods further in the absence of strong genetic differentiation. SC/65a/RMP03 noted that the development of new genetic markers, such as SNPs, might provide greater power to detect structure than do the existing markers. SC/65a/RMP03 also emphasises the need for more cooperative work and more effort to combine all data/samples available to get a better picture of the stock structure of North Atlantic fin whale.

In discussion, it was questioned whether there was any evidence in the results to suggest that animals were travelling in stable groups as they moved from area to area. The author noted that a pair of animals identified as a mother/son pair were sampled in close temporal and spatial proximity; however, the female of this pair was also identified as being related to another animal that was sampled further away (SC/65a/RMP01). Concordant with this, Daníelsdóttir (2006b) found allozyme differences between years; one plausible explanation for this finding was that animals were traveling together in groups. However it was also noted that Olsen *et al.* (In press) recently sequenced two of these allozyme loci and found no non-synonymous DNA substitutions (see IWC, 2013a, pp.238-39), suggesting that these allozymes are not informative about population structure in the way previously interpreted in Daníelsdóttir (2006b). If allozymes are not considered, the mitochondrial DNA and microsatellites are consistent in showing low genetic differentiation between management areas. In this regard it was observed that the use of recently developed methods such as ddRADseq (Peterson *et al.*, 2012) would allow sequencing of thousands of genetic loci, including both neutral loci and those under selection. Additional loci increase the potential for identifying diagnostic markers and would enable a broader range of questions to be addressed, particularly enabling better estimation of migration rates.

SC/65a/RMP03 emphasised the value of integrating available data and samples from North Atlantic fin whales, particularly in light of future efforts to develop new markers. In discussion, it was noted that data from different sources had been integrated for some previous papers (Daníelsdóttir, 2006a; Pampoulie *et al.*, 2008), and that efforts to collaborate were ongoing.

3.1.2 In-depth assessment
SC/65a/IA05 presented the results of microsatellite DNA analysis conducted on the North Pacific sei whale samples obtained from 2010-12 IWC-POWER. The samples came from the IWC-POWER cruises that surveyed 173°E-172°W area of the central North Pacific in 2010

(*n*=13), 170°W-150°W area of the central North Pacific in 2011 (*n*=29), and 150°W-135°W area of the eastern North Pacific in 2012 (*n*=35). All of the areas were north of 40°N. The POWER genetic data from 14 microsatellite loci was then analysed with previously reported genetic data from the JARPN II samples (*n*=489) collected from the western North Pacific between 143°E and 170°E in 2002-07 and the commercial whaling samples collected from the central North Pacific between 180° and 150°W in 1972-73 (*n*=57) and from the eastern North Pacific between 150°W and 139°W in 1973 (*n*=64). Analyses of these samples allowed the authors to detect temporal (40 years apart) and spatial (143°E to 135°W area divided into western, central, and eastern) genetic differences among the North Pacific sei whales. The results showed:

(1) very similar levels of genetic diversity among the POWER, JARPNII and commercial whaling samples;
(2) no evidence of genetic differences among the three POWER samples;
(3) no evidence of the temporal genetic differences between the recent POWER and past commercial whaling samples collected from the same area; and
(4) no evidence of spatial genetic differences among the western, central, and eastern samples.

This study supports authors' previous view that the open waters of the North Pacific were occupied by the individuals from a single stock of sei whales.

The SDWG thanked the authors for presenting this work, noting that the number of samples analysed was large and covered not only a large fraction of the North Pacific but also a large temporal scale (~40 years). This time span represents at least 2-3 generations and the fact that no differentiation over time or space was identified is important. On the other hand, a couple of potential limitations of the datasets and analyses were identified. First, it was pointed out that the microsatellite markers used in this study were developed for species other than sei whales, and that in some cases this can result in reduced data quality. Others, however, pointed out that use of non-species specific markers is widespread within the IWC, and that the Japanese laboratory has a strong record of producing high quality data. The second limitation is one that arises often in interpreting genetic data for cetaceans - the absence of information about breeding grounds, which means that samples are taken from potentially mixed aggregations of individuals from different stocks. This latter scenario complicates interpretation of results of statistical tests comparing samples from different spatial and temporal strata.

In response to a question, it was explained that this study did not include analysis of mtDNA data, although previous analyses (which did not include POWER samples) did not detect evidence of differentiation among North Pacific sei whales using mtDNA (Kanda *et al.*, 2009). The Working Group also asked whether differentiation between the JARPNII and 2012 POWER samples had been measured, as such a comparison was not reported in this paper. It was noted that while it is unlikely that differences would be identified given the results in SC/65a/IA05, these samples represent the most spatially segregated sample sets available from recent surveys and as such it might be of interest to conduct this comparison.

Discussion of these issues led to a more general discussion of the potential advantages of considerably boosting the number of genetic markers (e.g. by identification of large numbers of SNPs) to provide greater resolution in situations

when levels of genetic differentiation, if any, are likely to be low and samples from putative breeding populations are not available (see Item 3).

The authors noted that no power analysis of the current dataset has been conducted. However, simulations have been used to test the power of a subset of this dataset to detect stock differentiation (Pastene *et al.*, 2009). These simulations indicated that if the magnitude of differentiation was similar to or even smaller than that observed between the J and O stocks of North Pacific minke whales, population structure could be detected using the current set of the microsatellite markers and sample sizes. It was noted however that since North Pacific sei whales inhabit the open ocean, the level of differentiation between any existing breeding stocks might still be lower than their simulations. The Working Group **recommended** that the power of the current dataset to detect subtle population structure be analysed. However they observed that without knowing how the analysed samples correspond to breeding populations, the results of such a power analysis must be interpreted with caution. For example, it is possible to have a situation in which two breeding stocks appear in the same proportions in all areas and all time periods over which the feeding ground has been sampled. In this case, no differentiation would be detected in any of the comparisons of samples collected on feeding grounds. Although this scenario is unlikely given the spatial and temporal coverage of the samples analysed in SC/65a/IA05, it illustrates the difficulty of assessing power given the sampling design.

The Working Group discussed the value of clustering analyses (e.g. STRUCTURE, which does not require *a priori* stratification of samples) to detect population structure within North Pacific sei whales. STRUCTURE was used in a previous study utilising the JARPNII samples; no structure was detected (Kanda *et al.*, 2009). The magnitude of the F_{ST} value reported in Kanda *et al.* (2009) was similar to that identified in SC/65a/IA05. It was noted that STRUCTURE has little power to detect clusters when F_{ST} values are low and structure is weak, but can at least be informative as to whether or not strong population structure exists. It was **recommended** that clustering analysis, using STRUCTURE or a similar approach, should be conducted with the current data set.

Relatedness analyses can provide insight into population structure in scenarios where low but meaningful levels of differentiation exist (reviewed in Palsbøll *et al.*, 2010). These analyses can be informative even when only a small number of relatives are detected, and the number of available samples is expected to increase if the POWER surveys continue. The Working Group **recommended** that relatedness analysis be conducted in the future to provide insight into whether subtle structure exists among North Pacific sei whales.

3.1.3 Bowhead, right and gray whales
SC/65a/BRG16 and Appendix 2 both discuss genetic evidence for stock structuring of gray whales, so were presented together. Appendix 3 summarises the hypotheses laid out in both documents.

SC/65a/BRG16 reviews the issue of stock structure of North Pacific gray whales and the status of the western gray whale population. The authors present five stock structure hypotheses, which are not intended to be exhaustive of possible hypotheses but sufficient to frame a discussion of the issue. Because telemetry played such a key role in changing the previous view about seasonal migration habits and possible stock structure, additional tagging

would have value in further evaluating these hypotheses. It was recommended that historic or ancient samples of the pre-depletion western gray whale be investigated to help determine if all or any of the animals that summer in the Sea of Okhotsk are descendants of the pre-depletion stock. It was also recommended that a more exhaustive survey of genetic variation to cover the extensive range of the eastern gray whales is necessary for an adequate understanding of the status of the Sea of Okhotsk population. Recommendations were also made to increase the number of mitochondrial genes studied and to change from microsatellites to SNPs for nuclear DNA studies. In this way, inter-laboratory comparisons are possible for nuclear loci and the methods could be applied to both historical and current samples. Finally it was recommended that a gray whale genome project be undertaken to provide the development of better analytical methods and a deeper understanding of gray whale biology.

Appendix 2 presented the results of nuclear microsatellite genetic comparisons between whales sampled off Sakhalin Island and Eastern North Pacific (ENP) whales sampled north of the Aleutians. Three stock structure hypotheses were put forward, and the results were evaluated in light of recently discovered movements of gray whales between Sakhalin Island and the ENP.

In discussion, it was observed that at least two major factors are at play when considering the population genetics and dynamics of the western gray whale. From a historical perspective, it is important to understand the evolutionary history and biogeography of the western gray whale, for the purpose of understanding population identity, and through this whether the feeding ground members are a historical 'relict' population (i.e. ancestors of the current feeding group used this feeding ground prior to exploitation), or are a result of recent immigration from the Eastern North Pacific. Ancient DNA analyses of western gray whale material could potentially resolve this question. It was noted that ancient DNA analyses of gray and bowhead whales from the Eastern North Pacific and North Atlantic suggest large scale changes in distribution possibly related to environmental changes in the past, indicating that feeding and breeding ground locations can be quite fluid (Alter *et al.*, 2012; Foote *et al.*, 2013). From a management perspective, genetics is applied to understand the current level of genetic and therefore demographic distinctiveness of the western gray whale from the Eastern North Pacific population in order to determine management decisions, regardless of the age or historical distinctiveness of the feeding aggregation in question. This is the principal objective of work carried out by the SDWG.

In discussion of Appendix 2, it was observed that even with the various stratifications of the dataset, significant differentiation continued to be observed when Sakhalin Island whales were compared with gray whales sampled north of the Aleutian Islands. It was further observed that the estimates of nuclear DNA diversity in each subsample were difficult to compare because they were not corrected for sample size. It would therefore be useful to see allelic richness values as well as confidence intervals for the point estimates of differentiation presented.

It was commented that nuclear DNA diversity in the Sakhalin Island whales is high, which would not necessarily be expected if the Sakhalin island whales were a true 'relict' population. However if the Sakhalin sample contains some animals that are migrating from the ENP, then those animals could increase the diversity identified in the Sakhalin stratum. It was further observed that many sub-structured

baleen whale populations have high genetic diversity despite clear evidence of recent population bottlenecks (e.g. humpback whales, bowhead whales) and that current levels of bowhead diversity are similar to the levels reported here (Givens *et al.*, 2010; Morin *et al.*, 2012). In this regard, the high diversity may be because the Sakhalin animals are a mixture of whales from eastern and western wintering grounds. There is no direct evidence from tagging that Sakhalin animals travel south towards China (a putative wintering ground). However indirect evidence is available, as one of the animals observed off Japan was first identified as a calf off Sakhalin (Weller *et al.*, 2008).

Some suggestions were made for intersessional analyses of the data presented in Appendix 2. It was **recommended** that these analyses be repeated for each sex to see whether there were any sex-specific differences in F_{ST} values. It would be useful as previous analyses by sex (Lang, 2010) for a subset of these data suggested mainly male mediated gene flow between Sakhalin Island and the Eastern North Pacific. It was also **recommended** to measure F_{IS} for each locus. It was also noted that if a good demographic model can be obtained for this species, it would be possible to use the number of pairs of related individuals to infer migration rates (Peery *et al.*, 2008). A further analysis of interest would be to derive the genotypes of the 'missing fathers' from the genotypes of the mother-calf pairs. This would allow calculation of allele frequencies of the missing fathers, which could then be compared to the other gray whale strata (Sakhalin and Eastern North Pacific) to determine paternal similarity. It was also observed that kinship reconstruction would be possible if more markers were generated.

Given that there is weak evidence for departures from Hardy-Weinberg and linkage equilibrium in Appendix 2, Table 4, the approach outlined in Waples (2011) was also **recommended** for measuring the degree of population sub-structuring at Sakhalin Island. Waples (2011) observed that the magnitude of the Wahlund effect in population mixtures is expected to be highest at loci which differ the most between the two (or more) contributing populations. Specifically, F_{IS} at individual gene loci in the mixture should be proportional to F_{ST} between the contributing populations, and r^2 (an index of linkage disequilibrium) for a pair of loci in the mixture should be proportional to the product of F_{ST} at the two loci. This implies an expected linear correlation between F_{IS} and F_{ST} at single loci and between r^2 and $F_{ST}(1)$ * $F_{ST}(2)$ at pairs of loci. It was noted that among the tests for Hardy-Weinberg equilibrium, the significant *p*-values were only marginally significant, so the current evidence for a Wahlund effect appears to be weak. It was also observed that for any analysis that requires the *a priori* determination of allele frequencies per population, this would be difficult or impossible in the case of the 'western Pacific population' since the relevant individuals cannot be independently identified, i.e. the identity of the population is unknown. However, in order to better understand the structuring of the Sakhalin feeding ground relative to the Eastern North Pacific gray whales the SDWG **agreed** that it would be useful to conduct this test using the stratifications described in Appendix 2. The authors agreed to conduct an update of this analysis considering the suggestions above and to present this new information to IWC SC/65b.

It was noted that if the whales utilising the Sakhalin feeding ground include a mixture of whales that breed in the WNP and in the ENP, then evidence for the Wahlund effect should be detected. However, the Wahlund effect disappears in a single generation of random mating, and so no signature would be detected if only offspring of ENP and WNP whales were sampled. Single locus tests of Hardy Weinberg equilibrium would however be expected to detect admixture if the sample set included parent ENP and WNP whales and their offspring. Given this scenario, the approach outlined in Waples (2011) would be expected to detect residual admixture disequilibrium for two to four generations. In addition, if new ENP animals are immigrating to the Sakhalin feeding ground over multiple years and generations, the Wahlund effect could be observed in each generation. By comparing the one-locus test and two-locus test it might be possible to discriminate between a scenario involving mixing of ENP and WNP whales on the feeding ground but no interbreeding and a scenario in which interbreeding of ENP and WNP whales occurs by comparing the results of these two methods.

It was also suggested that it would be useful to consider whether a type of Allendorf-Phelps effect (Waples, 1998) could be contributing to the observed levels of differentiation between ENP and WNP samples. The Allendorf-Phelps effect is related to the better-recognised founder effect, but it does not require any permanent population subdivision; it arises when progeny of a local breeding event involving a small number of parents are sampled before they become mixed with the larger population. Examples of how a combination of AP and founder effects could generate levels of differentiation consistent with those seen among whales in the ENP and WNP are provided in Appendix 4. While the examples discussed utilised nuclear DNA, it was noted that the same process could be measured in mitochondrial DNA with some modifications to the formulas used. While the examples shown in Appendix 4 suggest that it is plausible that this effect could be generating the levels of divergence seen between whales in the ENP and WNP, this is not the only scenario in which the observed differentiation could arise. Therefore a positive result may not necessarily mean this process is the underlying mechanism, but consideration of the potential role of AP/founder events could provide a useful context for interpreting empirical data. It was noted that these examples do not incorporate overlapping generations or age structure in the population, and more detailed hypothesis testing could be complicated. The main take-home message from the simple examples in the Appendix is that it is relatively easy to generate the levels of divergence observed between WNP and ENP samples, using various combinations of small numbers of individuals and one or a few generations of recent isolation. It was noted that it might be easier to prove that this effect was not the primary process creating the observed differentiation using information on movements of animals or parent-offspring relationships between individuals.

It was noted that this method is not informative with respect to evaluating the plausibility of hypotheses that assume that some of the whales sampled off Sakhalin are a remnant of the pre-exploitation population of western North Pacific gray whales. However, it could be informative with respect to evaluating the demographic processes currently influencing stock structure in gray whales.

A novel approach was also proposed for distinguishing between gray whales that feed near Sakhalin Island or the ENP, using biopsy samples to identify the micro flora living on whale skin, using meta-genomic sequencing of the *16S* locus (Caporasoa *et al.*, 2011).

It was noted that the synthetic likelihood approach described in Wood (2010) could be useful in discriminating between stock structure hypotheses. This approach proposes

a method to estimate parameters in scenarios where it is difficult or impossible to calculate the true likelihood. This approach uses simulations from which a large number of statistics of interest can be calculated. Observed values of these statistics can then be compared to the distribution generated from the simulations to evaluate the likelihood, given the specific parameters and hypothesis used. It was noted that this type of approach might be valuable when complicated overlapping hypotheses exist and where the effects on any one statistic are difficult to tease out. As such, this approach might be of general interest to the Scientific Committee, where there are often many hypotheses to discriminate between. Lang and Gaggiotti offered to investigate this approach further and report back to the SDWG at SC/65b.

Weller *et al.* (2013) reports on a workshop held by the US National Marine Fisheries Service (NMFS) to assess gray whale stock structure. Currently a single stock of gray whales, the eastern North Pacific stock, is recognised in US waters. More recently, however, new information has suggested the possibility of recognising two additional stocks in US waters:

(1) the Pacific Coast Feeding Group (PCFG); and
(2) the western North Pacific (WNP) stock.

To assess this possibility, NMFS established a scientific Task Force comprised of eight NMFS scientists with expertise in fields relevant to stock structure assessment. The objective of the Task Force was to provide an objective scientific evaluation of gray whale stock structure as defined under the US Marine Mammal Protection Act (MMPA) and implemented through the NMFS Guidelines for Assessing Marine Mammal Stocks (NMFS, 2005). The Task Force reviewed new information relevant to gray whale stock structure, including the results of genetic, photo-identification, tagging, and other studies. The Task Force agreed on a series of questions relevant to evaluating if the PCFG and/ or the WNP gray whales qualify as stocks under the MMPA and the GAMMS guidelines. A structure decision-making process was used whereby Task Force members allocated likelihood points to categories reflecting their certainty as to how well each question could be answered given the currently available scientific evidence. The Task Force concluded that there was substantial uncertainty regarding whether the PCFG represents a separate stock under the MMPA and GAMMS guidelines and was unable to provide definitive advice as to whether the PCFG is a population stock under the MMPA and the GAMMS guidelines. The Task Force did, however, provide unambiguous advice that the WNP stock should be recognised as a population stock under the MMPA and the GAMMS guidelines. The Task Force provided recommendations for future work, including the continuation of field studies as well as additional analysis of the existing photo-identification and genetic data.

In discussion, it was noted that the use of likelihood point allocation to measure support for stock structure hypotheses could be biased by strong certainty on the part of a minority of Task Force members, and that there could be future value in including scientists with no prior involvement in the research findings that were being assessed. It would also be valuable to explore other methods of compiling the scores of expert panels, such as that used for Olympic diving, where the highest and lowest score are removed before averaging the score of the rest of the panel. This would help alleviate concerns that the scores of potentially biased or invested individuals could dictate the interpretation of a collective group opinion.

3.1.4 Icelandic Special Permit Research Program
The following two papers were presented to the SDWG following discussions at the Expert Workshop to Review the Icelandic Special Permit Research Program (Marine Research Institute, Reykjavik, 18-22 February 2013, see SC/65a/Rep03).

SC/65a/SD02 is a paper requested by the Expert Workshop, and is an integrated paper incorporating information from genetics, morphometry, telemetry, biological parameters, stable isotopes, fatty acids and pollutants (recommendation 12.1.2 (1) of the report of the Expert Workshop: Produce a fully integrated paper incorporating the information from genetics, morphometrics, telemetry, biological parameters, stable isotopes, fatty acids and pollutants). This multidisciplinary approach is based on Annex D of the Report of the Expert Workshop to Review the Icelandic Special Permit Research Programme, presenting the summary of potential indicators of structure developed by proponents during the workshop. While the genetic studies performed during the Icelandic Scientific Permit did not reveal any genetic pattern, which should be of concern for the IWC (evident lack of genetic differentiation), other biological information suggested that stock structure might exist among the IWC stock boundaries, and that, in addition, some subtle structure might exist on breeding grounds.

The SDWG thanked the authors for presenting this commendable compilation of data, which addresses a specific recommendation of the Expert Workshop. In particular, the value of Annex 1 (a summary of potential indicators of structure, with priority rankings) was highlighted as it is a useful summary of the various datasets available and represents an important first step. The next step is to use these data to decide what information should be given more importance or is most relevant to stock structure questions. It was noted that the compilation suggests that there is biological heterogeneity within the feeding ground, but the underlying processes creating these differences are currently unknown. Interpretation of indicators is also complicated by limited knowledge of the timescale over which most are informative, and therefore how they relate to migration and unidentified seasonal wintering grounds. Caution is therefore advised in interpreting these data in the context of those processes.

Some specific comments were raised during discussion of the various indicators. The SDWG noted that it is hard to interpret information from diet composition, isotopes and fatty acids in a stock structure context. The turnover time for both isotopes and fatty acids is variable and can be of short duration (e.g. Caut *et al.*, 2010). While the available evidence suggests that minke whales eat little if anything during winter, it is not known for sure if they fast. There are indications, however, that minke whales may accumulate less fat reserves during summer than do other baleen whales (SC/65a/SP01, SC/65a/Rep03), raising the possibility that they may not be as extreme in fasting as some baleen whales are. If this is the case, then some of the indicators which pertain to diet (i.e. diet composition, isotopes, fatty acids) may be useful for discerning wintering ground differences if collected just after arrival on feeding grounds.

The SDWG noted that it is hard to interpret information from isotopes and fatty acids in a stock structure context, since the turnover time for both of these indicators is unknown. While the available evidence suggests that minke whales eat little if anything during winter, it is not known for sure if they fast. There are indications, however, that minke whales may accumulate less fat reserves during summer than do

other baleen whales (SC/65a/SP01, SC/65a/Rep03), raising the possibility that they may not be as extreme in fasting as some baleen whales are. If this is the case, then some of the indicators which pertain to diet (i.e. diet composition, isotopes, fatty acids) may be useful for discerning wintering ground differences if collected just after arrival on feeding grounds.

Telemetry data seems to suggest autumn movements south towards a wintering ground, with signals received from one whale off the west coast of Africa in early December. Efforts were made to collect biopsy samples from tagged animals but they were unsuccessful. It was noted that the analysis of organic contaminants (e.g. Auðunsson and Víkingsson, 2013-rev) used several different markers and different tissue types to compare different areas, resulting in a large number of pairwise comparisons. In such circumstances the likelihood of a significant result occurring by chance is moderate, so it was suggested that a correction for multiple tests be applied. The authors noted that this had been discussed during the Expert Workshop, and that a revised analysis was planned. It was also questioned whether any sex differences in the organic pollutant levels and trace element signatures were observed in the study. It was noted that differences between the sexes are known to exist for other baleen whales, for example some females have lower contaminant levels due to the offloading of pollutants in their milk during nursing (e.g. Aguilar et al., 1999). No further differences were identified, although it was noted that samples sizes were small after subdividing by sex.

A question was raised as to whether it would be possible to look at the genetic patterns among parasites to provide more information on stock structure. Given that the life cycle of parasites is so much shorter than of whales, patterns could be amplified among the parasites. This approach has been successfully used in baleen whales before (e.g. right whales, Kaliszewska et al., 2005). It was noted that this approach usually requires dead whales, but that this was a possibility worth looking into.

Anderwald et al. (2011) is a published paper that was made available for the Icelandic Special Permit Expert Review Workshop as SC/F13/SP16 because it discusses stock structuring of minke whales in the North Atlantic.

Anderwald et al. (2011) investigated minke whale stock structuring using nuclear microsatellites and 300 samples from 8 locations in the North Atlantic (NA) and 1 from the Sea of Japan. No clear signal was found in the NA using the clustering program STRUCTURE (K=1), however analysis of the NA dataset together with Japan identified K=3, indicating two clusters in the NA. Although the authors acknowledge that K=1 in the NA is the best supported result from STRUCTURE, the features of these two clusters ('putative breeding stocks'; PBS1 and PBS2) were nevertheless thoroughly assessed to test the null hypothesis that K=1 (not to confirm the apparent differentiation indicated in STRUCTURE when K=3). These tests concerned independent data on the shape of ordination clusters, the possibility that previous (published) indications of structure instead reflected differential mixing of two stocks in different locations, and consistency with mtDNA (for structure, diversity and population history). For example, both microsatellite and mtDNA data showed lower diversity in PBS1 and population splitting and expansion just after the last glacial maximum (LGM). None of the tests were fully consistent with K=1. The authors concluded that although STRUCTURE finds no clear signal for multiple stocks in the NA, their further analyses suggest that more

research is needed to investigate possible cryptic structure, and at present cannot support a hypothesis of panmixia. Anderwald et al. (2011) suggested the need for a study with greater power (using e.g. 4,000-5,000 SNP loci), preferably in combination with satellite telemetry.

Anderwald et al. (2011) is a published paper but was discussed in SC/65a/Rep03 because it was included in the material available to the Review on Stock Structure. Comments on this paper are given in SC/65a/Rep03. The Panel noted that the analyses of nuclear genetic data that followed the partitioning of the samples based on results from STRUCTURE suffered from problems of circularity because the same data were used to both partition the samples and then test the resulting groups for differences. It was noted that there needs to be evidence that analyses based on data from a panmictic population do not produce comparable results when analysed similarly.

The corresponding author does not agree that the same data were used to both partition the samples and then test the resulting groups for differences, and offered the following comments:

The report raises three issues: (1) the way STRUCTURE was used; (2) the need for simulation tests; and (3) problems associated with circularity. The report is of course accurate about the use of STRUCTURE, but this was fully acknowledged in the paper and the strategy clearly described. As reported in the paper, the best estimate from STRUCTURE for the North Atlantic (NA) is for K=1. The authors explored the putative partitioning in the NA from STRUCTURE when K=3 to further test the hypothesis that K=1. With respect to simulation studies, Palsbøll had undertaken preliminary simulations that showed that STRUCTURE could generate subsamples from a single panmictic population that were differentiated when compared by F_{ST}. It would in any case be circular to simply use the same genetic data for differentiation from STRUCTURE to test for differentiation by some other test, and this was never the intention. The authors therefore devised tests to test predictions associated with K=1. For example, if K=1 they expected a single factorial component analysis (FCA) cluster, with the two putative populations representing different overlapping portions of that cluster ('2 halves of a ball'). If K=1 there should be no useful inference from comparing the relative proportions of the two putative stocks in different geographic regions, in the context of earlier indications of stock structure. Since the mtDNA genome is not expected to hitchhike on the nuclear genome in this species, if K=1 there should be no correlation between apparent differentiation based on microsatellite loci and differentiation at mtDNA loci. The process by which STRUCTURE separated these groups, where F_{ST} was significant, could in theory affect the analysis in IMa. While this should be small compared to what was observed (since any subsamples from a single panmictic population will share the same coalescent history), the key inference from this analysis was again related to the comparison with mtDNA results. From the microsatellite data PBS1 was less diverse and the apparent splitting time between PBS1 and PBS2 was around the time of the LGM. From mtDNA the inference was the same, with PBS1 less diverse and an expansion signal for just after the LGM. Since none of these tests were consistent with the null hypothesis of K=1, the authors suggest the possibility of cryptic structure. The degree to which these tests are robust varies, and none were individually very strong (the strength of inference related more to the congruence among them), but the author disagreed that they represent a circular

analysis of the genetic data. Each depends on independent data (mtDNA, previous publications on stock structure, an assessment of the shape of ordination clusters, etc.), not the original genetic data to test predictions about what would be expected if the STRUCTURE result was an artefact. Since all showed some indication that the structure may be real, the authors felt the implication was strong enough to require further investigation, and this was their specific recommendation.

In discussion, the SDWG **recommended** some further analyses of the North Atlantic minke whale data to help resolve the uncertainty:

- conduct clustering analysis with a set of new, independently segregating markers to see whether the same groups of individuals are identified with the new dataset;
- complete simulations (already in progress) of panmictic populations using STRUCTURE, setting $K=2$; and
- try discriminant analysis of principal components as an alternative way of identifying structure within the dataset (Jombart *et al.*, 2010).

The authors **agreed** to try the above intersessionally and further noted that they were planning a SNP analysis of these samples, which would increase the number of available markers and thereby increase the resolution for identifying populations within a mixed assemblage. It was also noted that more regional samples are now available to add further geographical resolution to this dataset.

3.2 Population assignment and mixing
3.2.1 Other Southern Hemisphere whale stocks
SC/65a/SH13 presented the results of a mtDNA analysis of 575 humpback whales obtained in the Antarctic during surveys of the JARPA/JARPA II and IDCR/SOWER, and 1,057 whales from low latitude localities of the South Pacific and eastern Indian Ocean. The analysis was carried out in response to a recommendation from the IWC Scientific Committee in 2012 to calculate mixing proportion of breeding stocks D, E and F in the Antarctic feeding grounds of Areas IIIE, IV, V and VI. Genetic samples from breeding grounds were obtained mainly by biopsy sampling but also from sloughed skin and beachcast whales: Western Australia (WA, $n=167$, 1990-2002; $n=185$, 2007), Eastern Australia (Eden, Tasmania) (EA, $n=104$), New Caledonia (NC, $n=243$), Tonga (TG, $n=240$), Cook Islands (CI, $n=56$) and French Polynesia (FP, $n=62$). In the Antarctic feeding grounds, samples were obtained by biopsy sampling: Areas IIIE ($n=106$), IV ($n=231$), V ($n=171$) and VI ($n=67$). Genetic samples of both data sets were examined for approximately the first half of the mtDNA control region. Duplicated samples were excluded from the analysis. In the case of mother/calf pairs only one sequence was used. Sequences from both data sets were aligned to produce a single data set comprising 137 haplotypes. Two kinds of analyses were conducted: mixing proportion and F_{ST} under two stock structure hypotheses (six stocks and four stocks as baseline samples for the stocks proportion analysis). In general results were consistent with the geography. Under the six-stock hypothesis, the largest proportion in Area IIIE was of the WA stock. The largest proportion in Areas IVW and IVE was of the WA stock. The largest proportion in Area VW was of the EA stock. The largest proportion in Area VE was of the NC stock. The stock with the largest proportion in Area VI was the TG stock. None of the Antarctic Areas investigated was represented by whales of the FP and CI

stocks, or just with a limited representation in Area VI (case of the CI stock). In general results of the mixing proportion analysis were consistent with the results of the F_{ST}, with a few exceptions.

In SC/65a/SH13, breeding ground samples are grouped into strata for analysis based on two stock structure hypotheses chosen from fig. 6 of IWC (2011): one medium plausibility and one high plausibility. This work is an update of Pastene *et al.* (2011), last discussed in IWC (2013a, p.236).

In discussion, the Working Group suggested that additional stock structure hypotheses would be worth exploring. These stock structure hypotheses will be discussed further in the sub-committee on other Southern Hemisphere whale stocks (Annex H of this volume). It was also observed that in the mixture proportion analysis, the French Polynesia breeding ground was not estimated to contribute substantially to any of the Antarctic feeding areas analysed. The SDWG **agreed** that additional biopsy sampling in Area I and eastern Area VI would provide more insight into where the whales that breed off French Polynesia are feeding.

3.2.2 Revised Management Procedure
3.2.2.1 NORTH ATLANTIC FIN WHALES
SC/65a/RMP01 presents a new method for genetic relatedness analysis based on a three-step procedure. First LOD scores were computed for three kinds of relationships (Half-siblings, Parent-offspring, and First cousin), then p-values were estimated and finally a False Discovery Rate (FDR) procedure was applied. Using this relatedness analysis based on the likelihood odds score (LOD) and false discovery rate (FDR) methods, SC/65a/RMP01 found relationships among 15 individuals caught in 2009 and 2010 in Icelandic waters (out of the 34,959 pairs comparisons), exhibiting different types of relationship, from grandparent to grandchild, to parent and offspring and half-sibling. One female was found to be related to two other animals. This female was the mother of a male and half-sibling with another female. SC/65a/RMP01 also suggested that this new three-step procedure supported by p-values should be applicable to stock structure issues raised by the IWC, in terms of different levels of relationships observed among IWC 'stock boundaries'.

In response to a query about possible genotyping errors, the authors noted that they had amplified all genotypes three times. All loci that showed mismatches were excluded, and only individuals with complete genotypes were used in the analysis. Because of this, the analysis was assumed to contain no genotyping errors. It was noted that the resolving power to detect relationships is limited by the number of loci used in the study (15 microsatellites), giving a false discovery rate of 10%. Therefore age was used as an additional consistency check, to evaluate whether the relationships most strongly supported by LOD scores are biologically feasible. Increasing the number of loci utilised in the study would allow errors to be more easily detected and would provide greater resolution to discriminate between relationships. It was commented that it is possible to distinguish between half-siblings and other relations using genetic evidence (Epstein *et al.*, 2000), although this would require many additional loci.

SC/65a/RMP01 utilised simulations to estimate p-values associated with each LOD score. This process involved simulating individuals by drawing alleles independently with replacement from a gene pool with the same allele frequencies as the empirical dataset. It was noted that this process is equivalent to simulating a population with an

infinite effective population size, while in real populations the finite effective size creates linkage disequilibrium and random departures from Hardy Weinberg equilibrium. It seems possible that this might lead to high LOD scores as a consequence of finite effective size rather than relatedness. The potential effects of this assumption on the estimation of *p*-values for each LOD score should be explored. In the context of this study, the authors noted that another implementation based on the work of Skaug *et al.* (2010) has been performed on these data and supported the same sets of pairs as those identified in this study, suggesting that assumptions about effective size have not influenced the current results.

4. TOSSM (TESTING OF SPATIAL STRUCTURE MODELS)

No new items were presented on this topic during SC/65a. The SDWG noted that last year some long-term TOSSM work was suggested for the Pacific Coast Feeding Group (PCFG) of gray whales (IWC, 2013a, p.239). Some of this work is in progress. Weller *et al.* (2013) also made a recommendation for additional TOSSM simulations to be conducted to further explore plausible levels of immigration into the PCFG. The SDWG looks forward to seeing further progress on these TOSSM recommendations at SC/65b.

5. TERMINOLOGY AND THE UNIT-TO-CONSERVE

The SDWG discussed a series of tentative definitions of stock related terms, which are intended to be a useful reference point for the Scientific Committee (see Appendix 5). This document has been developed with the aim of encouraging consistent use of stock related terms within Scientific Committee reports and in papers submitted to the Scientific Committee. Once these definitions have been agreed by the Scientific Committee, the SDWG would like to make this set of definitions available by web-link both on the IWC website and to have them referred to in future calls for papers made by the IWC Scientific Committee.

It was noted that in reality, biological structure often exists along a continuum and it is challenging to identify distinct breakpoints along that continuum to define what units are important to conservation and management, both in terms of temporal and spatial breakpoints. Some members suggested that one way of better representing this problem spatially may be to use the term 'deme', a commonly used identifier in population biology. A definition of this, and how it relates to other stock related terms, is provided in Appendix 5. This concept is unlikely to rapidly spread outside the SDWG to the rest of the Scientific Committee, but in our discussions it may provide a useful language bridge between IWC work (as discussed by the SDWG) and the field of population biology. Additional work was identified to better develop the scope of the definitions laid out in Appendix 5 (see work plan Item 7.3).

6. OTHER ISSUES

The SDWG noted that with the rapid recent developments in NGS technology and analysis, there are a number of emerging issues of relevance to the Scientific Committee, in terms of: (1) assessment of NGS data quality, and how best to curate such data; and (2) new methods for measuring stock structuring and measurement of other statistical quantities of interest to the SDWG (such as inbreeding) using NGS data. New and published papers were therefore solicited

on these topics for discussion at SC/65b, where they will be discussed and considered in the context of the existing guideline documents on DNA analysis and quality.

7. WORK PLAN

7.1 Genetic analysis guidelines
The genetic analysis guidelines are anticipated to be completed intersessionally (convened under Waples) and will be ready to circulate within the Scientific Committee by the end of 2013.

7.2 Gray whale stock structure
An intersessional email group was formed with the sub-committee on bowhead, right and gray whales to develop hypotheses of western gray whale stock structure, convened under Lang. Members are Bickham, Scordino, Hoelzel, Rosenbaum, Mate, Jackson, Baker, Broker, Urbán, Dupont, Brownell, Litovka, Reeves, Tyurneva and Waples.

The terms of reference are:

(1) to agree a series of hypotheses of gray whale stock structure, with a focus on evaluating the stock identity of the whales feeding off Sakhalin;
(2) to decide on the plausibility of hypotheses based on available data; and
(3) to discuss tests and methods to discriminate between the agreed hypotheses.

Results from this exercise will be reported at an intersessional Workshop (see Annex F) to assess the population structure and status of North Pacific gray whales.

Additionally, Lang and Gaggiotti have agreed to investigate the utility of synthetic likelihood methods as a means of better discriminating competing stock structure hypotheses and will report back to the SDWG in 2014.

7.3 Stock definition terminology
An intersessional email group was formed to decide appropriate stock definitions (using the terms laid out in Appendix 5), with reference to available data, for an example set of cetacean populations that have been the focus of Scientific Committee discussions over the last five years. Results from this exercise will be presented in SC/65b. The group was convened under Jackson and included Lang, Scordino, Pampoulie, Kanda, Double, Hoelzel, Cipriano, Waples, Palsbøll, Tiedemann, Bickham and Baker.

8. ADOPTION OF REPORT
This report was adopted at 19:30 on 12 June 2013.

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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. Guidelines for genetic studies and DNA data quality
   2.1   Genetic data analysis guidelines document
   2.2   Genetic data quality review
3. Statistical and genetic issues relating to stock definition
   3.1   Population structuring and migration rates
       3.1.1   Revised Management Procedure

      3.1.2   In-depth assessment
      3.1.3   Bowhead, right and gray whales
      3.1.4   Icelandic Special Permit research programme
   3.2   Population assignment and mixing
       3.2.1   Other Southern Hemisphere whale stocks
       3.2.2   Revised Management Procedure
4. TOSSM
5. Terminology and unit-to-conserve
6. Other issues
7. Work plan

Appendix 2

ADDITIONAL ANALYSES ON THE POPULATION STRUCTURE OF GRAY WHALES, WITH A FOCUS ON THE GRAY WHALES MOVING BETWEEN SAKHALIN ISLAND, RUSSIA AND THE EASTERN NORTH PACIFIC

A.R. Lang

Genetic comparisons have identified significant differences in mtDNA haplotype and microsatellite allele frequencies between the Sakhalin whales and eastern North Pacific (ENP) whales that feed north of the Aleutians (Lang *et al.*, 2011). In light of recent information demonstrating the movements of some Sakhalin whales to the ENP, these genetic differences need further examination. Differences in mtDNA haplotype frequencies, in combination with reduced haplotype diversity identified among whales feeding off Sakhalin, indicate matrilineal fidelity to this feeding area. This is consistent with observations of the return of whales first identified as calves to the Sakhalin feeding ground. These mtDNA differences could develop whether the animals feeding off Sakhalin include only whales which overwinter in the ENP or if whales feeding off Sakhalin consist of a mix of whales which overwinter in the ENP and whales that overwinter in the western North Pacific (WNP). Irrespective of the wintering origin of these whales, mtDNA differences support the demographic distinctness of the Sakhalin feeding ground whales. These results are consistent with results of a recent population assessment indicating little or no immigration into Sakhalin in recent years SC/65a/BRG27.

However, the mechanism driving the observed nuclear DNA (nDNA) differences is less clear. Although other explanations exist, here three potential hypotheses that could create nDNA differences among the animals feeding off Sakhalin and those feeding in the ENP are discussed.

(1) The Sakhalin feeding ground is utilised by a mix of whales that overwinter in the ENP and whales that overwinter in the WNP. Whales that overwinter in the ENP are randomly mating with ENP whales feeding in other areas. The nDNA signal is generated by interbreeding among whales remaining in the WNP year-round.
(2) The Sakhalin feeding ground is utilised largely or exclusively by whales that overwinter in the ENP. Much

of the breeding for this group of animals occurs early in the migration when the whales are still west of the bulk of the ENP population; this interbreeding is generating the nDNA signal.

(3) The Sakhalin feeding ground is utilised largely by whales that overwinter in the ENP and mate randomly with ENP whales feeding in other areas. The nDNA signal is created by the high levels of maternal relatedness among the animals feeding off Sakhalin.

Currently, genetic data (sex, mtDNA control region sequences, and genotypes for 12 microsatellite loci) are available for 142 gray whales sampled while feeding off Sakhalin between 1995 and 2007. These samples include 83% of all gray whales photographically identified off Sakhalin during this time period. Twenty-two of the Sakhalin gray whales that have been recorded in the ENP are included in this genetic dataset. Here we stratify the Sakhalin dataset with respect to the animals that have been recorded in the ENP and re-analyse the genetic data. Results are evaluated in light of the previously stated hypotheses.

Methods

All data was generated as part of earlier comparisons; sample collection methods, laboratory processing, and analytical methods are described in Lang *et al.* (2011). The strata utilised in the comparisons below include:

(1) Sakhalin (*n*=142): This stratum includes all whales sampled on the Sakhalin feeding ground (1995-2007).
(2) Sakhalin – no ENP migrants (*n*=108): This stratum is identical to the previously described stratum (#1) except that all of the Sakhalin whales that have been recorded in the ENP have been removed. In addition, whales that have been photographically identified (and genetically confirmed) as the mothers, calves, or maternal half-siblings of those whales recorded in the ENP have also been removed.

(3) Sakhalin-ENP migrants (n=22): This stratum includes Sakhalin whales that have been recorded in the ENP.

(4) Sakhalin – no relatives (n=84): This stratum is identical to the first stratum (no. 1) except that in cases where both individuals of a photographically identified (and genetically confirmed) cow-calf pair were sampled, the calf has been removed from the dataset.

(5) North (n=110): This stratum includes all whales sampled north of the Aleutians; the majority of these samples were collected as part of the aboriginal harvest off Chukotka, Russia.

Results and discussion

Limitations exist with respect to the analyses presented here. First, sample sizes representing the Sakhalin-ENP migrant group are small and likely do not represent all of the animals feeding off Sakhalin that travel to the ENP during winter. Similarly, although maternal relatives identified photographically (e.g. based on identified mother-calf pairs) were removed from either stratum prior to analyses, it is likely that additional maternal relatives of the Sakhalin-ENP migrants exist among the animals remaining in the Sakhalin dataset. These limitations need to be kept in mind when interpreting the results presented here.

Examination of the haplotypes carried by the Sakhalin-ENP migrants did not reveal a clear pattern. While some Sakhalin-ENP migrants carried haplotypes that are rare among Sakhalin whales, others carried the two very common haplotypes found off Sakhalin (Table 4). However, one of those two haplotypes is also common among ENP whales, and the other is found among a moderate number of ENP whales. Haplotypes carried by the Sakhalin-ENP migrants were dispersed throughout the median joining tree, and no pattern was evident (Fig. 1).

Mixing of whales that overwinter in the ENP and the WNP on the Sakhalin feeding ground would be supported by a finding of Hardy-Weinberg (HW) disequilibrium among whales sampled off Sakhalin. Although one locus was out of HW equilibrium in the Sakhalin stratum, this was similar to the results in the North stratum (Table 3). However, the power of HW tests to detect admixture is relatively low and thus these results are not necessarily informative with respect to discriminating between the first and second hypotheses.

Under hypothesis 1, the Sakhalin-ENP migrants would be expected to be more similar to ENP whales than to Sakhalin whales that remain in the WNP year-round. In contrast, the Sakhalin-ENP migrants were generally more similar to the remaining animals sampled off Sakhalin than they were to the animals in the North stratum (Tables 3 and 7). As aforementioned, however, it is plausible that some of the whales remaining in the Sakhalin stratum could be maternally related to the Sakhalin-ENP migrants, which would reduce differentiation between these groups. As such, the results of this comparison are difficult to interpret.

Table 1

MtDNA diversity summary statistics.

Strata	n	No. of haplotypes	Haplotype diversity	Nucleotide diversity (%)
Sakhalin	142	22	0.77	1.57
Sakhalin - no ENP migrants	108	18	0.77	1.62
Sakhalin - ENP migrants	22	8	0.81	1.11
Sakhalin - relatives removed	84	22	0.82	1.45
North	107	33	0.95	0.84

Table 2

Results of mtDNA comparisons.

Comparison	F_{ST}	p-value	χ^2 p-value
Sakhalin (n=142) vs North (n=107)	0.085	<0.001	<0.001
Sakhalin - no ENP migrants (n=108) vs North (n=107)	0.084	<0.001	<0.001
Sakhalin - ENP migrants (n=22) vs North (n=107)	0.065	<0.001	0.009
Sakhalin - no ENP migrants (n=108) vs Sakhalin - ENP migrants (n=22)	0.031	0.062	0.008
Sakhalin - no known relatives (n=84) vs North (n=107)	0.058	<0.001	<0.001

Table 3

Results of Hardy-Weinberg comparisons for heterozygote deficits.

Locus	HWE p-value (prob test)	
	Sakhalin	North
EV14t	0.1107	0.2395
EV37	0.9697	0.1608
EV94t	0.7404	0.0988
Gata028t	0.8035	0.6708
Gata098	0.4934	0.3994
GATA417t	0.823	0.5242
GT023t	0.3174	0.1864
RW31t	0.858	0.0186
RW48t	0.021	0.371
SW10t	0.7549	0.4233
SW13t	0.7294	0.6783
SW19t	0.9144	0.0877

Table 4

MtDNA haplotype frequencies.

HapID	North	Sakhalin	Sakhalin - no ENP migrants	Sakhalin - ENP migrants
1	**10**	**51**	**36**	**9**
2	**4**	**44**	**37**	**3**
3	15	9	9	
4	**5**	**5**	**2**	**3**
5	1	3	3	
6		1	1	
7	7	2	2	
8	**1**	**2**		**2**
9	**1**	**1**		**1**
10		**1**		**1**
11	3			
12	5	1	1	
13	6	2	2	
14	1	1	1	
15	2			
16	1			
17	1	1	1	
18	3			
20	6	1	1	
21	2			
22	1	1	1	
23	5			
24	2			
25	6	1	1	
26	**2**	**1**		**1**
28	2	3	3	
29	3			
31	1			
33	5	1	1	
35	**1**	**7**	**3**	**2**
36	1			
38	1	3	3	
40	1			
42	1			
43	1			
Total	107	142	108	22

Table 5

Number of significant (*p*<0.05) comparisons in the linkage disequilibrium test (total comparisons *n*=66).

Strata	Number of significant comparisons
North	3
Sakhalin	9
Sakhalin – no ENP migrants	5
Sakhalin – no known relatives	5

The number of pairs of loci in linkage disequilibrium (LD) was markedly higher among whales sampled off Sakhalin than it was among the North stratum (Table 5). When known relatives were removed from the comparisons, the number of pairs of loci in LD was reduced. The significant nDNA differences between Sakhalin and the North stratum remained after removal of known relatives, although the magnitude of differentiation was less (Table 7). While the lower magnitude of differentiation might suggest that the inclusion of related animals has inflated measures of genetic differentiation, the fact that the comparisons remain significant could also be interpreted as evidence that the nDNA signal is not entirely driven by maternal relatedness among the Sakhalin animals.

An additional consideration in the evaluation of the hypotheses laid out above is the results of paternity analyses of whales first identified as calves on the Sakhalin feeding grounds (Lang *et al.*, 2010). Analysis of 57 mother-calf pairs and 42 males sampled off Sakhalin identified putative fathers for 46-53% of the calves. These results supported interbreeding among the whales feeding off Sakhalin. Given that 83% of the animals photographically identified during the same time period had been genetically sampled, however, they also raised questions regarding the identity of the 'missing' fathers.

The paternity results are not consistent with hypothesis 3, in that they support interbreeding among whales sampled off Sakhalin. The paternity results could be consistent with hypothesis 1, in which case the calves that were not assigned fathers would be the calves of mothers that travel to the ENP and interbreed with ENP males. The results could also be consistent with hypothesis 2. Under this hypothesis, all or most of the Sakhalin whales are migrating to the ENP but interbreeding among them is occurring while relatively far west on the migratory route. However, it is likely that not all females would mate early in the migration, and thus the unassigned paternities would represent cases where some females interbred later in the migration when they were intermixed with ENP animals migrating from other feeding areas. It is unclear how much of this interbreeding (between Sakhalin and ENP whales) would have to occur before the nDNA signal would be erased. Of note, most mothers had at least one calf assigned to a putative Sakhalin father in the analysis, and some of the Sakhalin-ENP migrants
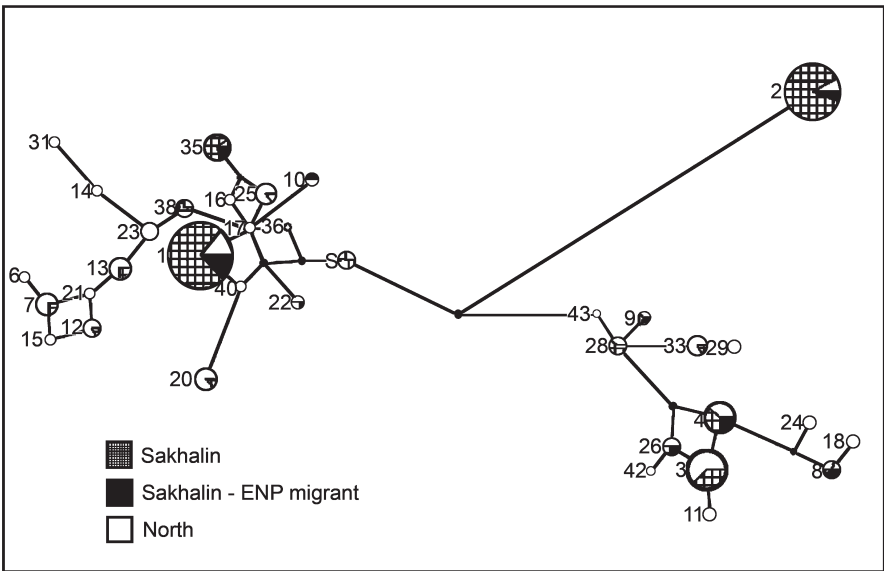

Fig. 1. Median-joining tree.

Table 6

Microsatellite diversity summary statistics.

Strata	n	Nb alleles	He	Ho
North	110	8.75	0.729	0.714
Sakhalin	142	8.33	0.690	0.702
Sakhalin - no ENP migrants	108	8.08	0.686	0.697
Sakhalin - ENP migrants	22	6.75	0.716	0.720
Sakhalin - known relatives removed	84	8.17	0.699	0.721

Table 7

Results of microsatellite comparisons.

Comparison	F_{ST}	F_{ST} p-value	G``$_{ST}$	G``$_{ST}$ p-value	χ^2 p-value
North (n=110) vs Sakhalin (n=142)	0.02	0.001	0.04	0.001	0.001
Sakhalin - no ENP migrants (n=108) vs Sakhalin - ENP migrants (n=22)	0.00	0.374	-0.04	0.441	0.258
North (n=110) vs Sakhalin - ENP migrants (n=22)	0.00	0.065	-0.03	0.057	0.015
North (n=110) vs Sakhalin - no known relatives removed (n=84)	0.01	0.001	0.02	0.001	0.001

were assigned as putative fathers. These results provide some support for hypothesis 2 over hypothesis 1; further evaluation of the paternity results is ongoing.

The results presented here suggest that analysis of the existing genetic data may not be able to discriminate between hypotheses 1 and 2. In the future, use of a simulations-based approach may be helpful in further evaluating the plausibility of hypothesis 3. In addition, future work will involve increasing the number of microsatellite loci genotyped on the Sakhalin whales to facilitate a study of relatedness patterns among these whales. The increased number of loci will also strengthen the paternity analysis and allow better evaluation of those results.

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Appendix 3

**POSSIBLE STOCK STRUCTURE HYPOTHESES FOR NORTH PACIFIC GRAY WHALES**

Aimee Lang, John Bickham, Jon Scordino, Jennifer Jackson

Here we present possible stock structure hypotheses for North Pacific (NP) gray whales, with the intent of facilitating discussion of methods to discriminate between hypotheses. Of note, discussion of these hypotheses is focused on evaluating the stock identity of the whales feeding off Sakhalin; no attempt is made to evaluate the Pacific Coast Feeding Group of whales.

Each hypothesis is accompanied by a description and a figure representing the scenario.
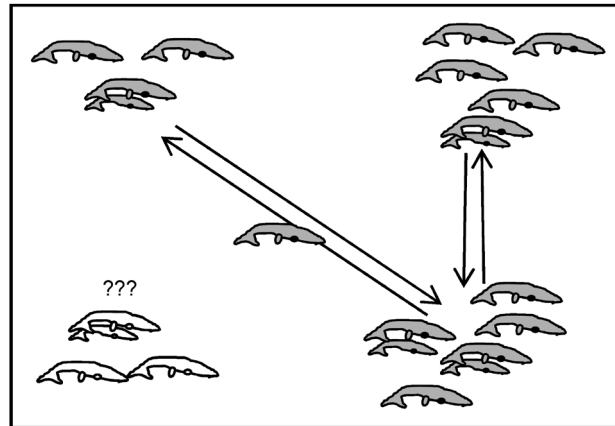
1. Panmixia – persistent

No population structure (e.g. panmixia) is present among feeding grounds used by NP gray whales; individuals move between feeding areas and exhibit random mating. Panmixia has been present over long time scales (prior to exploitation). Gray whales in the North Pacific use multiple migratory routes and wintering grounds with high levels of gene flow [animals randomly choose feeding grounds and randomly choose migratory routes and wintering grounds].
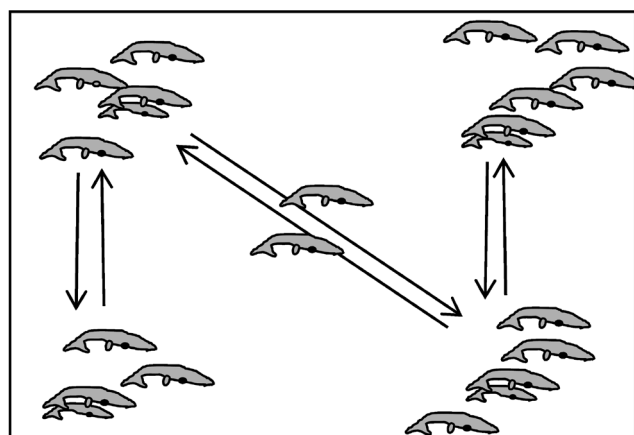

Key for Figs 1-7

Pre-exploitation western gray whale
Eastern gray whale
Gray whales with fidelity to Sakhalin Island
Whales of uncertain provenance
Mother-calf pair
Inferred migratory movements

2. Panmixia – post-exploitation - [SC/65a/BRG16, Hypothesis 1]

No population structure (e.g. panmixia) is present among feeding grounds used by NP gray whales; individuals move between feeding areas and exhibit random mating. Panmixia developed post-exploitation, and the pre-exploitation population of western gray whales (e.g. 'true' western gray whales) is extinct or utilises unidentified feeding areas in the western North Pacific (WNP). Whales off Sakhalin represent a random (e.g. different each year) subset of Eastern North Pacific (ENP) whales. All whales feeding off Sakhalin migrate to the ENP during winter months and breed randomly with other ENP whales.

3. Maternal feeding ground fidelity, one wintering ground, random mating - [SC/65a/BRG16, Hypothesis 2 and Appendix 2, Hypothesis 3]
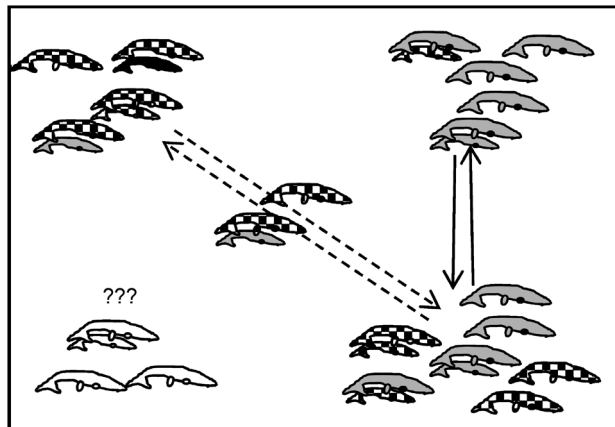

Utilisation of feeding areas is influenced by internal recruitment, with calves following their mothers to feeding grounds and returning in subsequent years. Mating is random with respect to feeding ground affiliation. The Sakhalin feeding ground is utilised by a subset of whales that show matrilineal fidelity to the feeding ground; these whales overwinter in the ENP and mate randomly with whales from other feeding grounds. The pre-exploitation population of western gray whales (e.g. 'true' western gray whales) is extinct or utilises unidentified feeding areas in the WNP.

4. Maternal feeding ground fidelity, one wintering ground, assortative mating with respect to feeding ground - [Appendix 2, Hypothesis 2]
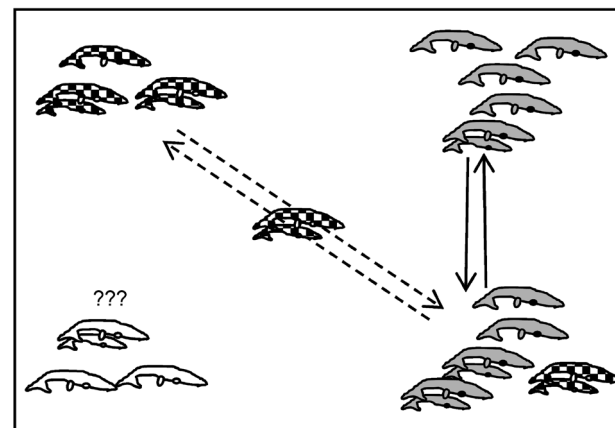

Utilisation of feeding areas is influenced by internal recruitment, with calves following their mothers to feeding grounds and returning in subsequent years. Mating is not random with respect to feeding ground affiliation. Whales using Sakhalin migrate to and overwinter in the ENP; however, some interbreeding occurs early in the migration when Sakhalin animals would be more likely to interbreed with each other than with animals feeding in other areas. The 'true'/pre-exploitation western gray whales are extinct or utilise unidentified feeding areas in the WNP.

5. Maternal feeding ground fidelity, two wintering grounds, random mating with respect to wintering grounds - [SC/65a/BRG16, Hypothesis 3 and Appendix 2, Hypothesis 1]
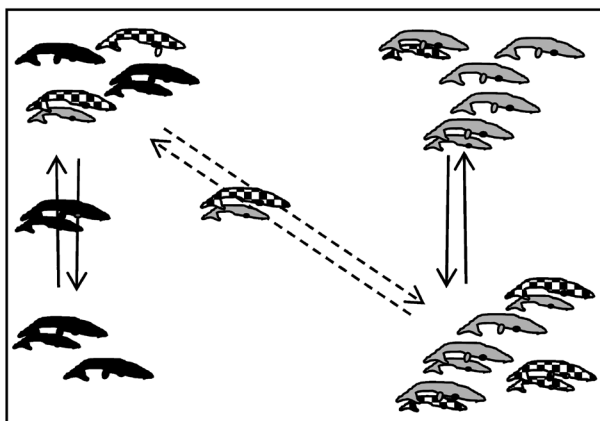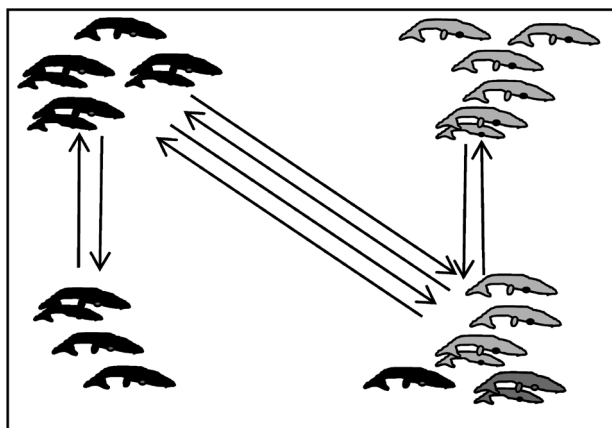

The Sakhalin feeding ground is utilised by whales that show matrilineal fidelity to this feeding ground. Some proportion of these whales migrate to the ENP and interbreed with other ENP whales, while the remainder represent 'true'/pre-exploitation western gray whales that migrate in the WNP and interbreed with each other.

6. Maternal feeding ground fidelity, two wintering grounds, random mating with respect to wintering grounds - [SC/65a/BRG16, Hypothesis 4]
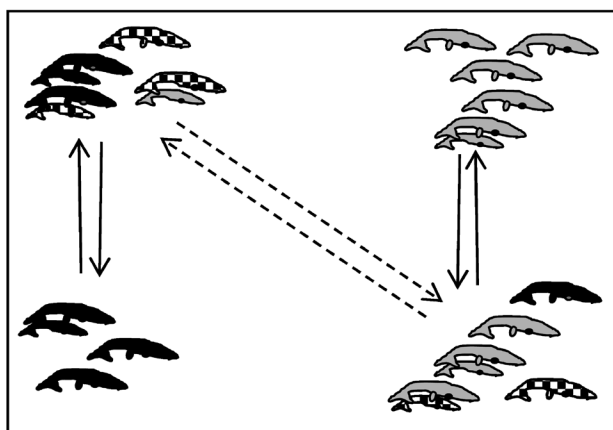
The Sakhalin feeding ground is utilised by whales that show matrilineal fidelity to this feeding ground. These whales include 'true'/pre-exploitation western gray whales that migrate to and overwinter in both the ENP and WNP with some interbreeding with eastern gray whales (EGW) as well as EGW that have colonised this summer feeding ground (i.e. it is a mixture of eastern and western gray whales, and the latter migrate in either direction).

7. Maternal fidelity to feeding grounds, two wintering grounds, assortative mating with respect to feeding ground - [SC/65a/BRG16, Hypothesis 5]

The Sakhalin feeding ground is utilised solely by whales that are the descendants of the 'true'/pre-exploitation western gray whales. They overwinter in both the ENP and the WNP with no interbreeding with the eastern gray whales.

Appendix 4

POTENTIAL RELEVANCE OF THE ALLENDORF-PHELPS EFFECT TO UNDERSTANDING GENETIC DIFFERENCES BETWEEN EASTERN AND WESTERN NORTH PACIFIC GRAY WHALES

R.S. Waples

The purpose of this Appendix is to suggest that a type of 'Allendorf-Phelps' (AP) effect might be contributing to observed levels of differentiation between ENP and WNP samples of gray whales. The AP effect is related to the more widely known founder effect, but it does not require any permanent population subdivision; the AP effect arises when progeny of a local breeding event involving a small number of parents are sampled before they become mixed with the larger population. Here is how the authors (Allendorf and Phelps, 1981) originally described the scenario: A lake has a single population of fish, but each year they spawn at random in different source streams. Let's say in one year a small number of adults spawn in stream A and a different, also small, number of adults spawn in stream B. If the adults are sampled, their genetic profiles would be expected to differ significantly no more often than the nominal Type I error rate (say 5% of the time). But if juvenile progeny are sampled before they mix in the lake, their allele frequencies will differ based on the chance differences in the two sets of parents, plus an episode of genetic drift. Also, the sample size of juveniles could be fairly large, further increasing the likelihood that the differences would appear to be statistically significant. But there is no permanent population subdivision in the system, because when they mature the juveniles randomly pick a stream to spawn in. Therefore, the apparent genetic differences between samples of offspring from different streams is an artifact caused by sampling after an episode of local genetic drift but before the offspring become mixed back into the larger population.

Waples (1998) showed that the expected magnitude of inflation in F_{ST} due to this type of sampling is equal to $1/(2\tilde{N}_e)$, where $\tilde{N}_e$ is the harmonic mean effective number of breeders in the two (or more) locations. It seems to me that a type of Allendorf-Phelps effect might be contributing to the genetic signal in WNP gray whales, and it could involve a variation of Hypothesis 1 in SD/WP1. A possible scenario might be something like this: each generation, some small, random subset of ENP gray whales breed in the WNP, perhaps behaviourally enticed to follow others across the Pacific. Their progeny will differ in allele frequencies from the larger ENP population by a type of Allendorf-Phelps effect, with magnitude determined primarily by the effective number of breeders that move to the WNP each generation. Some of those progeny remain and breed in the WNP, while others return to the ENP populations. Such a system might be quasi-stable for many generations, or it might be quite ephemeral. regular rcolonisation by new ENP 'founders' would keep the overall divergence modest.

A few simple examples illustrate how consideration of the AP effect might be useful, at least in providing context for interpreting the empirical data. According to Appendix 2, F_{ST} for the ENP (termed North in that paper) and WNP is about 0.02. I assume this is an unbiased F_{ST} that accounts for sample size effects. Using the AP effect, we can ask questions such as: What type(s) of simple, contemporary demographic processes could produce an F_{ST} of that magnitude? The examples below should be regarded as only rough approximations to that question, because the standard models used assume a single population and discrete generations, whereas gray whales have overlapping generations and (at least) two interacting groups of individuals.

Scenario 1: simple AP effect over one generation. In this scenario, a small number (N_1) of whales leave the ENP population, migrate to the WNP, and reproduce there. Their offspring are sampled and compared with samples from the much larger (N_2) ENP population. What is the expected F_{ST}? As noted above, $E(F_{ST})$ for this scenario is $1/(2\tilde{N}_e)$. If we assume that the census size to effective size ratio in both populations is 0.5, the $E(F_{ST})$ is $1/\tilde{N}$, where $\tilde{N}$ is the harmonic mean N. Since N_2 is much larger than N_1, the harmonic mean of N_1 and N_2 will be very close to $2N_1$. So, for this scenario we conclude that $E(F_{ST}) \approx 1/(2N_1)$. This implies that the empirical F_{ST} of 0.02 could be produced by a simple AP effect over one generation involving about 25 individuals $[1/2 \times 25) = 0.02]$.

Scenario 2: After the one-generation founding event described above, the WNP population breeds in isolation for t generations before samples are taken to compare with ENP. Each generation of isolation would increase F_{ST} by approximately $1/(2\tilde{N}_e)$, which again can be well approximated by $1/(2N_1)$. So, after t generations following the founding event, $E(F_{ST})$ would be approximately $1/(2N_1)+t/(2N_1)=(t+1)/(2N_1)$. If population size in the WNP changes during this time period, one would use the harmonic mean N_1 in the denominator. For example, if descendants of the original founders bred for more than 4 generations in isolation before sampling, then F_{ST} of 0.02 could be produced by a founding (and subsequently constant) population size of about 125 individuals $[(1+4)/(2 \times 125)=0.02]$.

The ratio N_e/N could be lower than 0.5, especially if N is taken to be all individuals in the population (not just adults). If actual N_e/N is lower than assumed above, the empirical F_{ST} of 0.02 could be produced with more individuals in the WNP population. More complicated scenarios could be developed that involve multiple generations of one- or two-way migration, but these scenarios are not so simple to evaluate. In general, reverse migration from WNP to ENP should not have much effect on F_{ST} (unless it appreciably changes N_1), but recurrent migration from ENP to WNP would tend to retard divergence.

None of the above is meant to imply that these simple scenarios are likely to accurately describe contemporary processes involving WNP gray whales. However, scenarios involving some combination of AP and founder events seem quite plausible, and the examples above show that levels of divergence equal to those currently found can be generated fairly rapidly when small numbers of individuals are involved.

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Appendix 5

KEY STOCK DEFINITION TERMS FOR THE IWC SCIENTIFIC COMMITTEE

Recognising there is considerable potential value in standardising use of terminology related to 'stocks' and 'populations' within the IWC, the SDWG has assembled the following list of definitions of commonly used terms. Initially, these are intended to guide usage of documents prepared within the SDWG; eventually, we hope they might lead to more consistent usage in the broader IWC community.

We separate key definitions into two sets: (1) aggregations (a group of whales that occur predictably in a particular location and are persistent for at least part of a year); and (2) defined aggregations (i.e. biological information is available to identify stock composition/demes within the aggregation). We propose that the Scientific Committee use whenever possible the most exclusive (i.e. stock delineating) term among the definitions presented here. So for example if further information is not known regarding stock composition, a regularly occurring group of whales will be called an aggregation, but if additional relevant information is available this may for example be described as a mixed-stock aggregation, or a breeding stock. We also try to relate these definitions to population biology, using the deme concept defined in Section (3).

(1) Aggregations
Aggregation - a group of whales delineated by an area where many individuals of a species are aggregated part of the year, or by a location used for some important function in their life history, or alternatively where some structural property or ecological process occurs with high density (Derous *et al.*, 2007; DFO, 2004). Aggregation relates to events that repeatedly occur at a specific time and location, and can be inferred for both 'mating/breeding' and 'feeding aggregation' if additional information regarding behaviour or ecology is known. These terms are currently used in management of other marine organisms exhibiting migration over large distances from one area to another, mainly from breeding to feeding locations. Other relevant aggregation terms where data on stock composition are not available are 'wintering ground', 'breeding ground' and 'feeding ground'.

(2) Defined aggregations
Biological Stock – all the individuals in an area that are part of the same reproductive process, forming a self-contained unit, with emigration/immigration rates far lower than the intrinsic rate of population growth. This is similar to the ecological definition of a biological population, where immigration (or emigration) rates are insufficient to influence population demographics[1]. In this definition, a 'reproductive process' is a group of individuals sharing a common breeding ground during the breeding season.

Relationship to population biology: a collection of one or more demes among which interbreeding takes place such that there is demographic dependency within a population. Examples: West Australia breeding ground (humpback whales), New Zealand calving ground (right whales).

[1]The transition between demographic dependence and independence occurs at a point where migration (*m*) between populations is still high; see Waples and Gaggiotti (2006) for consideration of how small *m* must be before two populations are demographically independent. Hastings (1993) suggests from simulation that $m \approx 10\%$.

Sub-stock - a consistently identifiable subunit of a stock, distinguished on ecological, behavioural or genetic grounds. While gene flow among sub-stocks can be high, i.e. they may share a breeding ground with individuals from other sub-stocks; removal of individuals from one sub-stock may result in depletion of individuals within their particular niche (i.e. feeding group) over management relevant time scales. Sub-stock 'boundaries' can be difficult to define.

Relationship to population biology: a sub-stock can be one or more entire demes, part of a single deme, or include parts of more than one deme. Tentative examples: Pacific Coast Feeding Group (gray whales), West Greenland Feeding Aggregation (humpback whales), Mauritius and La Réunion breeding ground (humpback whales).

Mixed-stock (adj.) The SDWG discourage the use of 'mixed stock' as a noun and recommend instead the more precise and less ambiguous 'mixture of stocks'. We recommend use of mixed-stock as: *a compound adjective used to indicate that the modified noun involves a mixture of individuals from different stocks (e.g. a mixed-stock fishery or a mixed-stock aggregation).* Therefore 'mixed-stock', can be used appropriately as a compound adjective, as in 'the mixed-stock harvest took individuals from several different stocks`. The preferred term to describe situations where individuals from different stocks or populations coexist is therefore a 'mixture of stocks'.

Relationship to population biology: a mixed-stock aggregation contains multiple partial or complete demes. Examples of mixed-stock aggregations: mixed-stock wintering grounds in Dominican Republic (humpback whales), Mexican lagoons (gray whales), mixed-stock feeding Areas in the Southern Ocean (humpback whales).

(3) Relationship with population biology
Deme - the largest group of conspecific individuals within which matings can be considered to occur largely at random. Given this definition, a *population* can be described as a collection of one or more demes, among which interbreeding takes place such that there is demographic dependency within a population. Populations within the species are isolated enough from one another that they are demographically independent and merit separate conservation status. Note that determining the threshold or tipping point level of population isolation for demographic independence still requires reference to management objectives. Note also that although a population can include more than one deme, a single deme or parts of a deme cannot occur in more than one population.

(4) Relationship with IWC Revised Management Procedure
The biological stock is generally used as fundamental unit in RMP/AWMP trials, and is often the first focus of trials to meet conservation requirements.

Management stock - (RMP working definition, see Donovan, 1991) is a deliberately vague term and is defined as an area of ocean to which a catch limit is applied. This is ideally equivalent to a biological stock (above) but may be a mixed-stock aggregation, a sub-stock, a group of sub-stocks, or partial and temporal components of any of these types of stocks. The combination is specific to each

management stock. Historically, this term arose from the drawing of 'stock boundaries' when information defining those boundaries was scant or based on catch distributions or recovery of implanted tags. It must therefore be borne in mind that many 'management stocks' as defined by the IWC may contain only a part of a breeding stock, or mixed-stock feeding aggregation.

(5) Relationship to government management objectives and the 'Unit to Conserve'

The 'unit to conserve' depends partly on biology and partly on the level of political and economic interest in the species. The unit to conserve is often an amalgam of the unit that best matches societal ideals (a policy driven decision) and units that exist in nature, with a lot of iteration between both elements (Taylor, 2005). Taking the precautionary approach, the unit to conserve might be the smallest division of the population that can be determined as a semi-autonomous unit (here the 'sub-stock', for example). At the other end of the spectrum, the unit to conserve might be the species itself, regardless of range or population structure. In the IWC context the unit to conserve may be considered equivalent to a management stock. With respect to government

management objectives, the unit to conserve tends to be the priority focus and the term 'stock' is often applied to this unit (which may be a sub-stock or deme, or part of a biological stock). Since the IWC receives documents from many different governments, some of which discuss stock structure, it is useful to acknowledge that in these documents the term stock is often therefore defined differently, but that this term can easily be reinterpreted using the core SDWG stock related concepts defined above.

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