Annex I

Report of the Working Group on Stock Definition

Members: Jackson (Convenor), Baird, Baker, Bickham, Bravington, Cipriano, Double, Funahashi, Gaggiotti, George, Hoelzel, Kitakado, Lang, Palsbøll, Park, Pastene, Prewitt, Rosenbaum, Scordino, Skaug, Solvang, Tiedemann, Urbán, Víkingsson, Vladimirov, Waples, Weller, Yoshida.

1. INTRODUCTORY ITEMS

1.1 Opening remarks
Jackson welcomed participants.

1.2 Election of Chair and appointment of rapporteurs
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/65b/SD01-04, SC/65b/SH07, SC/65b/SH17, SC/65b/BRG02, SC/65b/IA08, SC/65b/IA13, Alexander *et al.* (2013), Cunha *et al.* (2014), Dick *et al.* (In review), Polanowski *et al.* (2014), Torres-Florez *et al.* (2014), SC/65b/RMP05, SC/65b/RMP09, SC/65b/Rep02 (Item 5) and SC/65b/Rep04 (Item 3.1).

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 first set is already available as a 'living document' on the IWC website, and the second will be available in this form before SC/66a. Both sets 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/65b some additional appendix sections were completed. This work is anticipated to be completed intersessionally (see the work plan, Item 6.1).

A number of papers were discussed that present new methodologies of relevance for the genetic data analysis guidelines.

Polanowski *et al.* (2014) used information on age-associated DNA methylation in human and mouse genes to identify homologous genes for humpbacks, using skin samples of individuals of known age. Of the 37 cytosines assayed, seven had significant age related profiles and these were used to calibrate relationships between cytosine methylation and age. The three most informative markers were used to develop a humpback epigenetic age assay, with an r^2 of 0.787. This predicts age from skin samples with a standard deviation of 2.991 years. In trials this method predicted the 'parent' among the parent-offspring pairs in >93% of a set of 12 samples.

The SDWG thanked Double for presenting Polanowski *et al.* (2014) and noted that this technique could potentially have broad applications. For example this method could be particularly useful in complementing genetic studies of parentage, as it can help to determine which member of a putative parent-offspring pair is the parent and which is the offspring. In close-kin mark recapture studies, this can increase the information content available for abundance estimation and measurement of population structure.

In discussion, it was questioned whether using this method requires a new calibration every time it is applied to a new population, or if a calibration derived from a population/species in one area can be applied to a population/species in another area. The authors calibrated the methylation levels in a reference population of known age from the Gulf of Maine and applied this calibration to a sample set of unknown age from east Australia, and therefore consider that this method should be broadly applicable within a species. However, *de novo* calibration of methylation patterns would be required to develop this for different species (as shown for sperm whales in this paper), as inter-species methylation patterns and accumulation rates are not consistent.

It was queried whether precision of the approach might increase with the number of loci analysed. The authors were not present to address this but it was suggested that this is not likely to improve much.

Alexander *et al.* (2013) reports on quality control related to next-generation sequencing (NGS) of mitogenomes from sperm whales – a species reported to have low levels of mitochondrial (mtDNA) control region diversity on a global scale (Lyrholm *et al.*, 1999). The authors sequenced 20 mitogenomes from 17 sperm whales representative of worldwide diversity using two NGS platforms: Illumina GAIIx, and Roche 454 GS Junior. Over 93 Mbp of NGS sequence data was generated for the 20 sperm whale mitogenomes representing 17 individuals. For the 13 mitogenomes sequenced with Illumina, this provided an average sequencing depth of 359X. For the seven mitogenomes sequenced with 454, this provided an average sequencing depth of 174X. Average mapping quality exceeded 36 (BWA: PHRED quality) for sperm whale mitogenomes sequenced with Illumina, and exceeded 63.5 (GS Reference Mapper 454 quality) for samples sequenced with 454. An additional 43kbp of Sanger sequence was used to validate variable sites in the multiple sperm whale alignment and to estimate sequencing error of the NGS platforms. Resequencing of three individuals with both NGS platforms and partial Sanger sequencing showed low discrepancy rates: 454-Illumina: 0.0071%; Sanger-Illumina: 0.0034%; and Sanger-454: 0.0023%. These error rates were an order of magnitude less than the overall nucleotide diversity of the 17 mitogenomes, calculated to be 0.096%, confirming suitability of both NGS platforms for investigating low mitogenomic diversity.

The SDWG thanked Baker for his overview of this paper, which was submitted in response to a request by the SDWG to identify papers that discuss NGS technology and quality control methods for this year's meeting.

In discussion, it was noted that much of the NGS work that has been completed so far focuses on phylogenetic analyses, where small error rates are not a big concern. Given that sperm whales have very low variation, it was important to ensure that sequencing errors did not impact diversity estimates. However, comparison of sequences generated by both NGS platforms as well as by Sanger sequencing technology found relatively low rates of discrepancy, indicating that with careful quality control these NGS approaches are appropriate for use with low diversity species.

It was noted that the accuracy of NGS methods increases with read depth. Read depths were highly variable in this study, and only regions with a minimum of 15x coverage were utilised. For some regions with few reads, Sanger sequencing was used to fill in the gaps.

It was noted that use of 454 sequencing is declining as alternate technologies become available. For example new approaches, such as Nanopore sequencing, may see increased use in the future. Given that similar technology underlies both of these sequencing approaches, the SDWG noted that the comparison presented in Alexander *et al.* (2013) was very instructive.

2.2 Genetic data quality review

A paragraph concerning SNP data will be developed intersessionally by Tiedemann, Hoelzel and Palsbøll for addition to the data quality document.

2.3 Other developments

Dick *et al.* (In review) presented *geneGIS*, a suite of computational tools to facilitate visual exploration and spatial analyses of individual-based records from DNA profiles and photo-identification records. *geneGIS* uses open-source programming language Python 2.7 and ArcGIS 10.1 software to create a user-friendly, menu-driven toolbar linked to a Python Toolbox containing customised geoprocessing scripts. For ease of sharing and installation, the *geneGIS* toolbox is compiled into an ArcGIS Python Add-In, available for download from the website *http://www.genegis.org*. An increasing number of studies of long-lived, mobile or migratory species, include multiple sources of individual identity, such as photo-identification and DNA profiling. These studies often include numerous encounters with individuals over time, in some cases over many years and in different migratory habitats (e.g. feeding and breeding grounds for baleen whales). *geneGIS* is intended to help users visually explore these linked, spatio-temporal records and to provide a computational environment for spatial analyses in the context of molecular and spatial ecology. At present, *geneGIS* consists of 12 tools grouped into four categories (Import, Export, Genetic Analysis, and Geographic Analysis), plus a Help category that links to the *geneGIS* website. A key goal of *geneGIS* is to allow novel ways of data exploration through visualisation, spatial selection, data extraction and basic analyses of genetic data in relation to the marine environment. This information is critical during hypothesis development for spatially explicit analyses, such as landscape or seascape genetics. This software is not intended to duplicate the efforts of other specialised software packages for molecular ecology such as GenAlEx (Peakall and Smouse, 2006; 2012), Genepop (Raymond and Rousset, 1995; Rousset, 2008), Alleles in Space (Miller, 2005), and SPAGeDi (Hardy and Vekemans, 2002), but instead enable exploratory analyses and data export in an appropriate format to those programmes for further analyses. *geneGIS* also offers data export as a Keyhole Markup Language (KML) file for use with Google Earth and a SRGD file format compatible for data upload into the *Wildbook* relational database management framework[1].

In addition, *geneGIS* provides two tools (Summarise Encounters, Compare Encounters) invoked with buttons from the toolbar that allow the user to interactively spatially select two different groups of points and provide some basic statistics about that selection including the number of samples, the number of unique individuals, and the number of unique individuals common to both selections. Examples provided in Dick *et al.* (In review) include application of the tools to an integrated database of photo-identification records and DNA profiles (e.g. mtDNA, microsatellite genotypes and sex) from more than 18,000 encounters with humpback whales as part of the SPLASH programme (Baker *et al.*, 2013; Calambokidis *et al.*, 2008). These examples are broken down into the five current key functions of *geneGIS*: (1) data visualisation; (2) spatially explore, display and select data; (3) export data; (4) data extraction from environmental layers; and (5) conduct basic spatial analyses.

The SDWG thanked the author for summarising this work, and noted that they looked forward to hearing more about *geneGIS* in the future.

In discussion, it was questioned how this application was different from what is used to study terrestrial vertebrates. It was noted that the approach is similar to those utilised with terrestrial vertebrates, in that it represents a way to integrate the use of spatial and temporal tools with data from genetics and photo-identification.

It was suggested that incorporating habitat data into the *geneGIS* platform (e.g. using MGET[2]) could be valuable for exploring patterns between environmental variables and whale movements and structure. This integration is planned for the future.

3. STATISTICAL AND GENETIC ISSUES RELATING TO STOCK DEFINITION

The SDWG 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, 2012). These discussions often refer to the genetic analysis guidelines and genetic data quality documents[3].

3.1 Population structuring and migration rates

During SC/65b, the SDWG discussed close-kin mark recapture methods, which are currently being developed for a number of species including blue whales (SC/65b/SH17) and North Atlantic minke whales (Annex D, item 3.3.1).

SC/65b/RMP05 reported the use of a probabilistic likelihood-based approach to look for related individuals across a dataset of Icelandic minke whales (n=244), using 16 microsatellite loci. With no microsatellite error, detection power of duplicates is 100% and false discovery rates are low for parent-offspring pairs. Detection rates were measured for identification of parent-offspring pairs (POPs), full siblings

[1] *http://www.splashcatalog.org/mmuwildbook/.*
[2] *http://www.mgel.env.duke.edu/mget.*
[3] See *http://iwc.int/scientific-committee-handbook#ten.*

and half siblings using a simulation approach with the numbers of microsatellite loci and sample sizes used in the dataset. Different false discovery rates were then applied to obtain the relationship between false discovery rate and detection power. A dataset of 15 mother-foetus pairs was used to compare the simulation predictions with detection probabilities estimated from a set of known parent offspring pairs. The comparison correlated well, supporting this approach.

In a similar analysis applied to a broader dataset of North Atlantic minke whales (Annex D, adjunct 3), seven parent-offspring pair relationships were identified between Iceland (CIC) and Norway (EW), suggesting a degree of movement between these two regions. Given that the total number of comparisons involved in each pairwise search varies with the sample size, it was suggested that calculation of the fraction of comparisons that identified putative parent-offspring pairs relative to the total number of comparisons would allow standardisation across areas. The suggestion in the RMP pre-meeting to develop a null ('panmictic') expectation of the number of POPs identified given the number of pairwise comparisons performed was followed. Indeed, the number of observed hits did not differ significantly from the null expectation under a single panmictic population.

Palsbøll (2014) used kinship analysis to measure the proportions of related animals of different categories (parent-offspring, full and half sibling-sibling) seen both between and within individual North Atlantic sampling areas. Proportions were similar, potentially suggesting a lack of structuring between regions. It was noted that when the false discovery rate is high, as is likely for more distant relationships (e.g. sibling-sibling), then a high proportion of pairs identified as sharing a given relationship could be false positives, and it is not possible to distinguish the truly related component from noise.

The SDWG also discussed the implications of the high number of female-female POPs identified relative to expectation in the Gulf of St Lawrence sample (and compared to West Greenland). This, combined with earlier sampling of one female and three of her first order kin within the Gulf of St Lawrence, suggests that there may be some maternally driven site fidelity to this region. The SDWG agreed that more samples would be needed to evaluate this further.

SC/65b/SH17 (see summary in Annex H, item 5.1.1.4) was briefly discussed under this topic and presents a proposal to incorporate relatedness analyses into a planned, mark-recapture based circumpolar abundance estimate of Antarctic blue whales. This builds on recent work by Bravington *et al.* (2014) to measure spawning biomass of bluefin tuna through close-kin identification. The importance of using a large number of loci was stressed as a better way to keep the false discovery rate low than attempting to account for uncertainty in a mark-recapture framework. The use of a large number of loci provides sufficient detection power, even if keeping the absolute number of false positives below one. It also makes false negatives very easy to detect. Given the large number of loci utilised, error was evaluated using an exclusion criterion, such that every pair identified as related was accepted as long they had been genotyped at enough loci.

The SDWG welcomed the work presented on kinship methods. This approach has broad utility for the work of the Scientific Committee as it can increase the stock structure-related information content available from existing sample collections. The SDWG encouraged the continuation of this developing methodology and reporting to the SDWG on this topic.

3.1.1 Revised Management Procedure

The following work was presented to the SDWG following discussions at the AWMP/RMP joint Workshop on the stock structure of common minke whales (Copenhagen, 14-17 April 2014; see SC/65b/Rep04) and during the IWC Scientific Committee pre-meeting on North Atlantic common minke whales.

SC/65b/RMP09 reported analyses of a dataset of around 1,200 North Atlantic minke whale samples using 16 microsatellite loci and 369bp mtDNA control region sequences. These represented – according to IWC stock assignment – the Western (West Greenland), the Central (east Greenland, Iceland), and the Eastern stock (Norway, Spitsbergen, Barents Sea, North Sea). Most of the genetic variation (over 99%) is assigned to the lowest level of geographic stratification in both microsatellites (i.e. the individual level) and mtDNA (i.e. the locality level). Nonetheless, there is a consistent tendency towards a subtle differentiation among the stocks. In all analysis, West Greenland and Eastern stock are slightly more differentiated. The Central stock is intermediate, with a closer affinity towards West Greenland. Locus-specific analysis reveals that: (1) significance in the microsatellite data is due to divergence at a single locus; (2) levels of differentiation at mitochondrial DNA are similar to those revealed in a previous study; and (3) microsatellite F_{ST} values – even if corrected for within population variability – are considerably lower than values derived from an earlier allozyme study. Possible reasons for these differences are discussed. This study is generally compatible with the IWC-three stock hypothesis (W, C, E), but would not contradict a two stock hypothesis (W+C, E) either, as none of the analyses revealed any difference between W and C stock.

In discussion, it was noted that the results of SC/65b/RMP09 highlighted the importance of examining locus-specific estimates of divergence. When the full dataset (n=16 loci) was analysed, the overall signal was significant. However inspection of locus-specific estimates showed that only a single locus, SAM25, demonstrated significant differences between strata, with an F_{ST} value much higher than that of the other loci. Reanalysis of the overall dataset without this locus yielded a non-significant result. It was noted that SAM25, like all of the loci utilised in this study, is a non-focal locus. However, the notable characteristic of this locus is its unusual allele size pattern, as two high frequency alleles are separated by only one base pair and thus may be prone to mis-scoring. Two steps were taken to further evaluate whether scoring errors were present in the genotypes of this locus: (1) the genotypes of 12 samples that were shared between two of the labs were compared; and (2) the genotypes for 15 mother-foetus pairs were examined to ensure that they shared at least one allele. No significant source of error was identified using either of these two methods.

Discussion of the utility of looking at locus-specific estimates of F_{ST}` continued during the SDWG review of SC/65b/Rep04. In the intersessional Workshop, it was noted that studies evaluating whether stock structure exists in minke whales in the North Atlantic have drawn different conclusions, with some studies suggesting substantial levels of differentiation exist between areas and others identifying little to no differentiation. Evaluating why these differences exist is complicated, as differences in sample size, areas sampled, years sampled, marker types, and potentially laboratory protocols exist between studies.

In a first step towards understanding factors contributing to differences in the results of these studies, Adjunct 4 of Annex D compares locus-specific F_{ST} values across five

datasets (Andersen *et al.*, 2003; Anderwald *et al.*, 2011; Daníelsdóttir *et al.*, 1992; Palsbøll, 2014; SC/65b/RMP09) to examine the effect that locus variation and marker type may have on stock structure inference. This comparison revealed that markers varied substantially in F_{ST}' between studies. Some differences in the geographic distribution and intensity of sampling exist; however, the overlap between studies was large enough that this also seemed unlikely to fully explain the F_{ST}' differences. Therefore, although no formal tests were conducted, it seems unlikely that the F_{ST}' differences between studies can be attributed entirely to this aspect.

It was noted that, in addition to those listed in SC/65b/Rep04, another factor that could cause contrasting F_{ST}' between studies is scoring errors. For example, in SC/65b/RMP09 (see above) all loci but one generated low locus-specific F_{ST} scores. Although retyping this locus did not reveal any genotyping errors, because of the allele distribution at this locus, it is likely to be associated with technical issues. Given that there is overlap between the samples and loci used in the studies, a first step toward evaluating possible error would be to compare the genotypes from samples that were used in multiple studies to identify any inconsistencies. This might provide some insight into whether examining differences between laboratories should be a priority. It was noted that the loci in Adjunct 4 of Annex D were non-focal, which may contribute to the inconsistencies seen; though see Appendix 2. An additional possibility (also discussed during the Workshop) is that if there is some cryptic structure in the North Atlantic with temporally shifting mixing patterns in some areas (e.g. due to migration), the different studies might yield different patterns because they were sampling over different time periods.

The SDWG noted that during the intersessional workshop five lines of investigation were proposed to try and understand the differences in levels of genetic differentiation between allozymes and microsatellites for North Atlantic minkes (SC/65b/Rep04, p.6). Since this time it has been possible to exclude two of these as causative factors (Q1 and Q4) for the microsatellite-allozyme comparison (Annex D, Adjunct 4). For the current comparison between microsatellite studies Q1 can also be excluded (Q4 is not applicable). The remaining possibilities (Q2, Q3 and Q5) concern temporal and geographic sampling heterogeneity and other non-random sampling factors which could potentially influence measurement of stock structure. One approach that might be helpful in better understanding the effects of these factors is to use simulations to evaluate the effects of limited sample size, cryptic structure, and other possibilities.

It was noted that ddRAD sequencing was underway to identify a large number of SNPs in North Atlantic minkes in order to investigate population structure. This will result in another dataset and marker type to use in comparisons. While the ddRAD approach will generate many loci, the number of individuals genotyped using this approach will be relatively small and therefore extension of the ddRAD sequencing analyses to include methods such as close-kin (as has been done with microsatellite data, see Item 3) will have limited scope because of the cost required. In this regard the continued development and analysis of the large existing microsatellite dataset for North Atlantic minkes remains very valuable. With continued use of these markers, the work presented in Annex D (Adjunct 4) will be critical both for helping to develop a consensus view on stock structure in the North Atlantic and for highlighting any problems with particular microsatellite markers.

The SDWG expressed strong appreciation for these efforts to combine the allozyme and microsatellite datasets together for a locus-specific reanalysis. They noted that it is of central importance to the ongoing assessment to resolve what factors may be contributing to the lack of concordance among studies of North Atlantic minke stock structure. In addition, determining the factors underscoring the different signals in this dataset may have wider implications for other studies of interest to the IWC Scientific Committee. In most cases, multiple datasets are not available for comparisons of results, thus discordant signals such as those presented in RMP Annex D (Adjunct 4) could be present but unrecognised in other studies.

It was suggested that it might be beneficial to rank which factors were thought to be most likely to contribute to the lack of consistency between the results of these studies. The difficulty of this task was recognised, and a small working group was convened under Waples, (Hoelzel, Palsboll, and Tiedemann) to take on this task. Items for further intersessional work are listed in Annex D, item 3.3.3.

3.1.2 Bowhead, right and gray whales

SC/65b/BRG02 reports a meta-analysis of population genetics studies of sharks, whales, dolphins and porpoises that included estimates of genetic diversity and population differentiation based on F_{ST} estimates. The presentation focused on issues related to microsatellite diversity and standardised F_{ST} (Rousset, 1997) and the influence of locus selection on these measures. Specifically, the influence of focal loci (markers derived from the species being studied) and non-focal loci (application of a marker to a different species) was examined. Based on an analysis of 711 microsatellite loci from 84 studies of 74 large vagile marine species, the paper reports significant differences in microsatellite experimental design among groups of researchers. Whale and dolphin studies were based on a significantly (p=0.0001) lower proportion of focal loci (0.24±0.07; 0.22±0.06 respectively) than in the porpoise and shark literature (0.93±0.1; 0.64±0.07 respectively). The effective number of alleles (1/(1-He)) was coincident with this; being significantly (p<0.05) lower in whale and dolphin studies (3.66±0.16 and 2.86±0.09 respectively), than in studies of sharks and porpoises (5.94±0.33; 3.8±0.27). The full and minimal models for the complete data set suggest that the effective number of alleles for the average non-focal locus is 7% in the full and 5% in the minimal models below that estimated for the average focal locus. It is shown in SC/65b/BRG02 that this reduction in allelic diversity is associated with higher estimates of standardised F_{ST} (locus type effect p<0.001). This consistent ascertainment bias wherein non-focal microsatellite loci yield lower estimates of genetic diversity and higher estimates of F_{ST} is consistent with both an empirical study of bowhead whales and theoretical considerations of F_{ST}. Specifically, the calculation of F_{ST} is heavily influenced by the frequency of the most common allele and thus methods that affect allelic diversity can also affect F_{ST}. An empirical test of this was the study of bowhead population genetics that focused on stock structure as measured by microsatellites. In that study non-focal microsatellites not only differentiated recognised stocks but suggested additional differences among sample locations or temporal samples of a single migratory population. Focal microsatellites, and focal plus non-focal combined, showed these differences to be incorrect while still differentiating among the recognised stocks(Givens *et al.*, 2007; Givens *et al.*, 2010). The authors of SC/65b/BRG02 strongly recommend the use of non-focal microsatellites to estimate

F_{ST} should be avoided in future studies since this might lead to inflated estimates of F_{ST} that are potentially statistically significant. In some cases this could lead to conclusions of population differentiation where none exist.

The potential implications of the main conclusion made in SC/65b/BRG02 are far reaching, and resulted in extensive discussions, both in the SDWG and in a smaller group convened to consider the conclusion further. The outcome of those discussions is presented in detail in Appendix 2. Briefly, the authors of SC/65b/BRG02 noted that the level of variation at 'non-focal' (heterologous) microsatellite DNA loci is about 5% lower compared to 'focal' (homologous) loci. The authors argued that the consequently higher measures of F_{ST} (due to well-established population genetic principles) for non-focal loci could lead to the over-diagnosing of stock structure. This inference has ramifications for the many studies based on non-focal loci in cetaceans and more widely in the field of molecular ecology. If the authors' assertion were correct, this would call into question the conclusions of those studies and the consequent recommendations agreed by IWC sub-committees. In discussion many in the SDWG argued that the conclusion from SC/65b/BRG02 about the over-diagnosing of structure was based on a methodological misconception. The essential reasoning is as follows (see Appendix 2 for further details). F_{ST} has two components: locus-specific effects, and population-specific effects. While highly diverse loci may not reflect a sufficient proportion of the population-specific effects to detect real population structure, it is not conversely true that markers of low variation will detect non-existent structure. Indeed, if this was the case, commonly used low diversity markers such as allozymes and SNPs would routinely, artificially detect population structure, and this is demonstrably not the case. Regardless of the specific magnitude of F_{ST}, a statistical assessment determines significance with a controlled magnitude of type I error.

3.1.3 Small cetaceans

Cunha *et al.* (2014) reported on a new study on the population structure of the franciscana dolphin. This species is possibly the most endangered small cetacean in South America, primarily because of high and likely unsustainable bycatch levels throughout its range, which includes coastal waters extending from the central coast of Brazil to the central coast of Argentina. Currently four management areas (or FMAs) have been established for the franciscana labeled FMA I to IV (Secchi *et al.*, 20031-3). In 2004, the Scientific Committee reviewed the status of the franciscana and made a number of recommendations to improve knowledge of the species stock structure (IWC, 2005).

Cunha *et al.* (2014) analysed mitochondrial DNA sequences generated from samples (n=162) collected throughout range of the species, including sites on the northern range that were previously unsampled. Results of AMOVA analyses suggested the existence of two evolutionary significant units (ESU): the North ESU (comprising ES and RJN sites) and the South ESU (from RJS south to ARG). The existence of two ESUs is supported by reciprocal monophyly of DNA lineages, as well as strong quantitative differentiation in AMOVA analysis. In addition, authors suggested there is evidence to split the northern ESU into two management areas (termed FMAIa and FMAIb) and to split FMAII into two areas: FMAIIa and FMAIIb.

Although the mtDNA sequence-based analyses of Cunha *et al.* (2014 failed to detect a significant difference between FMAIII and FMAIV, the authors believe that separation between these areas should be maintained because microsatellite data from other studies reported on small-scale genetic differentiation within these areas and because three different countries must manage franciscanas in that region. In addition, due to this micro-geographic differentiation, authors adopted the subdivisions of FMAIII and FMAIV as proposed by Mendez *et al.* (2010a) and Costa-Urrutia *et al.* (2012). The authors conclude that there is a need to further investigate population structure and demographic parameters in the most poorly known and possibly smaller populations in the northern range of the species.

The SDWG reviewed Cunha *et al.* (2014) in light of past recommendations made by the Scientific Committee. Cunha *et al.* (2014) utilises mtDNA control region sequences generated from samples (n=162) collected throughout the range of the franciscana dolphin in the southwest Atlantic in order to measure population structuring. The range of the franciscana has previously been subdivided into four main management areas (FMAs). This paper addresses a recommendation made in 2004 (IWC, 2005) to evaluate whether or not there is a barrier to gene flow across a distributional hiatus within Area I, and also addressed a recommendation made in 2010 (IWC, 2011) to investigate additional sub-structure within the other management areas.

The SDWG noted that these results are consistent with restricted maternal gene flow within FMAI across the distributional hiatus, as well as within regions included in FMAII. However, analysis of nuclear markers is needed to determine whether male-mediated gene flow between these regions exists. It was noted that additional lines of evidence for restricted movements, including the limited movements of franciscana dolphins satellite-tagged within FMAIV and the presence of significant environmental breaks that likely limit dolphin movements between areas (Mendez *et al.*, 2010a), are concordant with the results of these mtDNA analyses. While recognising the difficulty of adding samples to the analyses, the SDWG also noted that sample sizes used to represent some strata were very small and the inclusion of additional samples would be beneficial.

Although the addition of nuclear markers would be beneficial in further assessing management unit boundaries, the mtDNA results presented in Cunha *et al.* (2014) provide evidence that additional substructure within FMAs may exist. If the goal of management is to be risk-averse, then it is important to consider this possibility in developing finer level management boundaries, given that high levels of by-catch are occurring. Within at least FMA IV, there is evidence to suggest the joint entanglement of mother-offspring and reproductive pairs (Mendez *et al.*, 2010b). The SDWG noted that previous analyses of population structure within FMAIV using microsatellites (Mendez *et al.*, 2010a) upheld the sub-structuring conclusions originally drawn based on mtDNA analysis (Mendez *et al.*, 2008), suggesting that inter-regional migrations are limited for both males and females of this species.

In summary, the SDWG **recommended** that:

(1) additional analyses using nuclear markers be conducted to evaluate management unit boundaries for both males and females;
(2) additional samples be included in future analyses if available in order to improve resolution of FMAs; and
(3) attempt to resolve the biologically critical dispersal rates in terms of management goals, and determine what levels of genetic differentiation such dispersal rates are expected to generate.

3.1.4 Other Southern Hemisphere whale stocks

Torres-Florez *et al.* (2014) examined genetic relationships between the blue whales from southeastern Pacific (SEP) areas of southern Chile (SCh), northern Chile (NCh) and the Eastern Tropical Pacific (ETP) and Antarctic blue whale on feeding grounds using seven microsatellite loci and mtDNA Control region sequences. Significant differences between Antarctica and the other three areas of the SEP were found, while no significant differences were found in comparisons between the two areas in Chile, or between the ETP and both Chilean areas. The Bayesian clustering analysis using STRUCTURE revealed two clusters: one composed of whales from SCh, NCh and ETP, while the other one was composed of whales sampled in the Antarctic. Although two genetic groups were clearly identified with classical and Bayesian analyses, some animals sampled in the Antarctic as well as some animals sampled in the Pacific oceans seems to be vagrants. The effective number of migrant analyses (using the programme MIGRATE) suggested stronger population structure when maternally inherited markers were studied. This finding suggests a low number of migrants between the ANT and the SEP clusters based on mitochondrial markers, while a larger number of migrants with nuclear markers. Based on the lack of differentiation between blue whales in the ETP and those off Chile, the ETP could potentially be a breeding site for blues from SCh and NCh. However, there is an absence of samples immediately north of the equator and other lines of evidence (satellite tagging, photo-ID, acoustics, sighting survey data) should be considered. While ANT blue whales show significant genetic differentiation from the SEP cluster, some gene flow may occur and it is possible that some males from other ANT areas could also use the ETP region as a breeding ground, which may explain the observed number of migrants between ANT and SEP clusters. While no evidence of the Antarctic blue whale morphotype has been found in the ETP, acoustic records of Antarctic blue whales in the ETP do exist. While data and current analyses support the hypothesis that blue whales sampled in the SEP belong to a unique population, additional and more systematic sampling efforts are needed across this expansive range, particularly in the South Pacific Gyre, the ETP and the west coast of the Antarctic Peninsula. Analyses now underway will be build upon this dataset by including eastern North Pacific blue whale samples with the aim of a better understanding blue whale population structure in the North and South Pacific Oceans.

In discussion it was queried whether recaptures of the seven microsatellite loci used in this study were evaluated by eye, or using a likelihood based method. It was confirmed that the probability of identity was calculated and was reasonably low using the seven loci.

3.1.5 JARPA II Special Permit Research Programme

The following four papers were written following discussions at the Expert Workshop to review the Japanese JARPA II Special Permit Research Programme (Tokyo, 24-28 February 2014; see SC/65b/Rep02).

Scientists from countries that made a statement at Plenary that it was inappropriate for the Scientific Committee to continue the review of the JARPA II programme did not participate in the discussion of contents of papers related to JARPA II (see Item 2 of the main Scientific Committee report). These included members who have previously participated in discussions of contents of papers related to JARPA II. Therefore, it should be noted that the discussions in this Item do not include the views of those members of the Scientific Committee.

The SDWG was asked to consider whether recommendations given by the Expert Workshop (see SC/65b/Rep02, item 5) had been addressed in these papers, as well as to identify any other methodological or analytical issues if evident. Summaries of these papers can be found in item 5.1 of SC/65b/Rep02. SC/65b/IA13 was also discussed in In-Depth Assessments (see Annex G, item 2.1) following discussion in the Working Group.

SC/65b/SD01 is a revised version of Pastene *et al.* (2014) presented to the JARPA II Review Workshop, which took into consideration some short-term recommendations from the Review Panel. Previous genetic and non-genetic results were consistent with the occurrence of at least two stocks and an area of mixing in the central sectors (involving mainly sector VW). In this study genetic samples obtained during surveys of the JARPA II (2005/06-2010/11) were examined using mtDNA control region sequencing and microsatellite DNA to test the previous hypothesis on stock structure derived from JARPA research. A total of 2,278 samples were considered in the mtDNA analysis and 2,551 in the microsatellite analysis. The hypothesised mixing Area VW was not considered in the analysis. For mitochondrial and nuclear markers, significant statistical genetic differences were found between whales from the western and eastern sectors, for females, males and total samples in the case of the mtDNA, and for females and total samples in the case of microsatellites. Furthermore yearly variation was found for females and total samples, mainly in the western sector, in the case of the microsatellites. Therefore genetic results based on JARPA II samples were consistent with the previous hypothesis of at least two stocks in the research area, one in the most western part and the other in the most eastern part (I and P stocks). Furthermore microsatellite analyses suggested substantial yearly variation mainly within the I stock and especially for females. These yearly differences can be explained by the dynamics of the I and P stocks, which mix with each other in different proportions in different years in part of the western sector (SC/65b/IA13), or by the sporadic intrusion of an unknown third stock occurring in the western part of Area III. In this paper additional analyses were conducted in response to the Review Panel recommendations that locus-specific F_{ST} values should be provided to identify which loci are responsible for the differences in the heterogeneity tests and that the F_{IS} values should be provided for the test on HW equilibrium (SC/65b/Rep02).

The SDWG **agreed** that the short-term recommendations in SC/65b/Rep02 have all been addressed. Discussion then focused on the longer-term recommendation to identify whether the patterns found in this paper are consistent with an isolation-by-distance (IBD) scenario for Antarctic minke whales, since some additional patterns shown in SC/65b/IA13 appeared to be consistent with this hypothesis. It was one of the tasks of the JARPA II review workshop to identify the range of scenarios consistent with the available data. In this regard it was observed that the results in SC/65b/SD01 might be consistent with a one-stock IBD hypothesis, since one would expect to find genetic differences when comparing samples collected at the two ends of the survey area. However, in response it was noted that there is additional non-genetic data which supports the two-stock hypothesis (Pastene, 2006), and that structuring within this population is very subtle, so it is hard to evaluate the IBD hypothesis with these data as the effect size is small. Furthermore areas of high sighting density have been identified in low latitude areas of both eastern Indian Ocean and western South Pacific (SC/65b/SD01, fig. 2), north of the research area, which

could correspond to breeding stocks migrating in summer into the western and eastern sectors of the research area in the Antarctic, respectively (Pastene, 2006). Regardless, this alternative hypothesis will be investigated intersessionally. Further discussion of this scenario followed the presentation of SC/65b/IA13 below.

SC/65b/IA13 is an updated version of Kitakado *et al.* (2014), which was submitted at the JARPA II review meeting (see SC/65b/Rep02), to show information on what was has been added since the review meeting and what analysis will be conducted in the future to reflect the recommendations by the review panel. This study presented an integrated approach, by using genetic and morphometric data, for estimating longitudinal segregation of two populations for Antarctic minke whales taken by the JARPA and JARPA II surveys during the austral summers from 1989/90 to 2010/2011 in Antarctic areas III-E, IV, V and VI-W. The method allows a soft boundary to vary by year and sex although it assumed baseline populations. A joint conditional likelihood function was defined for the estimation of mixing proportions, which is expressed as linear logistic models with population-specific parameters. It was observed that the morphometric data had statistically dominated information compared to the genetic data and it helped convergence in the optimisation. The result indicates that the spatial distribution of the two populations has a soft boundary in Area IV-E and V-W, which depends on the year. It also suggested possible sex differences along the boundary. The authors will incorporate random effects to the yearly mixing parameters toward better precision.

In discussion, the SDWG agreed that the short-term recommendations made in the JARPA II review had been addressed. The SDWG noted that the use of integrated data from two different sources (morphometric and genetic) is an interesting and valuable approach and appreciated the work of the authors to address the recommendations made at the JARPA II workshop.

The SDWG agreed that it would be valuable to look at the consistency between the signal derived from the morphometric data and the signal derived from the genetic data. As the authors of SC/65b/IA13 mentioned in their paper, a random effects model would help to clarify this by providing the appropriate framework to address signal consistency and better estimate year-to-year variability. In particular, if the data are stratified by year under the two-stock with mixing model, are the boundaries between stocks that are identified by the genetic and morphometric data generally in the same area? It was noted that, in some years, the boundaries identified using the genetic data alone were dramatically different from the boundaries identified using the combined morphometric and genetic dataset. The authors noted that looking at possible environmental correlates, and how they might shift between years, would be valuable.

One interesting finding in SC/65b/IA13 was that the morphometric data showed a stronger signal than the genetic data. The SDWG noted that this result could potentially be explained if the morphometric data are affected by selection or phenotypic plasticity (different phenotypes arising when the same genotypes are exposed to different environments). Although there is no apparent reason to expect strong phenotypic plasticity in Antarctic minke whales (for example, all feed primarily on the same major food – krill), each species' niche has many dimensions that we don't fully understand, so it is possible that some unstudied aspects of the species' environment causes different plastic responses in different geographic areas.

The SDWG noted that the approach taken in this paper was based on the assumption that samples collected at both extremes of the survey area represent pure stocks. Some of the data are consistent with expectations under this hypothesis (Pastene, 2006). For example, the plots of mixing proportions versus longitude based on the genetic data show a sigmoid pattern (SC/65b/IA13, fig. 6). As would be expected under a hypothesis that separate stocks inhabit the extremes of the survey area while mixing occurs in the middle regions, the mixing proportions are stable at the western and eastern ends of the survey area (where samples representing the pure stocks were collected), and decline in a stepwise manner across the central (putative mixing) region.

The SDWG then discussed the possibility that a single stock with IBD could explain the data as an alternative to the two-stock hypothesis (as noted above for SC/65b/SD01). It was noted that plots of the morphometric data versus longitude (SC/65b/IA13, fig. 3) do not show plateaus at either end of the survey area, as would be expected under the two-stock mixing hypothesis, but instead appear to show a continuous cline. However, it was also noted that these plots integrate data over 22 years, and temporal variability might obscure the pattern within any given year. The author stated that, unfortunately, results from individual years are too sparse to be usefully plotted in this way.

In the general discussion of implications of SC/65b/SD01 and SC/65b/IA13, it was acknowledged that the data utilised in these two papers were collected only from feeding areas, making the biological mechanism for an IBD effect difficult to understand. Nevertheless, the Workgroup agreed that it would be useful to determine whether the genetic/morphometric data are consistent with a single-stock IBD hypothesis and recommended that appropriate evaluations be conducted intersessionally.

As noted above, two areas of high density of whales in low latitudes north of the survey area (SC/65b/SD01, fig. 2) during winter months has been identified and may represent an area used for breeding. The difficulty of collecting samples from this and other northern areas during winter months was recognised. However such sampling efforts, along with related approaches such as satellite tagging, are considered very valuable to further improve our general understanding of minke whale stock structure.

The SDWG also recognised that the data analysed in this paper represented only a partial survey (~42%) of Antarctic waters. Under a two-stock model that assumes whales are distributed throughout the Southern Ocean, it is possible that these stocks would have another area of mixing where they meet on the other side of Antarctica. It is also possible that this second area of mixing does not exist, but rather there is a distributional hiatus at some point across this unsampled part of the range. Such a scenario could represent the distribution of a ring species. To assess these possibilities, it would be useful to analyse samples from the other side of Antarctica. While this goal is of less relevance for regional RMP analyses, analysis of samples in this area would increase overall understanding of the biology of the species in the Southern Hemisphere. Some historical commercial samples from this area exist (Yoshida *et al.*, 1998), but given their pack-ice location and timing of collection, such samples may not be able to address the questions of interest to the group (Goto *et al.*, 1998).

Among the longer-term recommendations made during the JARPA II Review Workshop, only two were discussed by the SDWG: the model formulation in a random effects framework, and the possibility of an-isolation-by distance mechanism explaining the observed data.

In summary, **recommendations** made by the SDWG include the following.

(1) The consistency between the results derived from the morphometric and genetic datasets should be further examined, particularly when the data are stratified by year. The possibility of using one dataset to identify boundaries between stocks and then testing whether differences in the other dataset were observed if the data were stratified according to that boundary should be explored.
(2) Alternate models such as the hypothesis of a single stock model with isolation-by-distance should be explored (this reiterates the longer-term recommendation from SC/65b/Rep02).
(3) Genetic samples from lower latitude areas, which may represent breeding stocks for these Antarctic minke whales, should be collected and analysed. This echoes previous SC recommendations and many other situations considered by the Scientific Committee where only samples from feeding grounds exist.

SC/65b/SD02 is a revised version of Kanda *et al.* (2014) presented to the JARPA II Review Workshop, which takes into consideration some short-term recommendations from the Review Panel. In this study a total of 581 humpback whale biopsy samples obtained from Areas III to VI during surveys of the JARPA/JARPA II and IDCR/SOWER up to 2010/11 season were analysed using 14 microsatellite DNA loci in order to describe their stock structure in the Antarctic feeding ground. After exclusion of duplicates, 528 samples were used for further analyses at stock level. Although a few cases of small temporal differences were detected within the Areas, major genetic differences were observed among Areas IIIE, IV, V and VIW. Stronger differentiation was seen in females than in males. Despite the increase of the number of loci, the level of the stock differentiation (F_{ST} = 0.003) was still too to conduct a clustering analysis at the individual level. In this paper additional analyses were conducted in response to the Review Panel recommendation that the F_{ST} values should be included in the tables showing results of the heterogeneity tests (SC/65b/Rep02).

In discussion it was noted that the short-term recommendations in SC/65b/Rep02 have all been addressed and that this work might be of interest also to the Southern Hemisphere sub-committee, as it is concluding the Southern Hemisphere humpback assessments.

SC/65b/SD03 is a revised version of Goto *et al.* (2014), which responds to some recommendations from the Review Panel. In this study genetic samples (catches and biopsies) of fin whales obtained by JARPA/JARPA II were analysed with two genetic markers, mtDNA control region sequencing (479bp-segment) and microsatellite DNA (16 loci), to investigate stock structure of this species in the Antarctic feeding grounds. Genetic samples were available from Areas IIIE (n=6), IV (n=23), V (n=24) and VIW (n=2). No statistical significant difference in mtDNA haplotype frequencies was found between Areas IIIE+IV and Areas V+VIW. Large number of singletons and small sample sizes could have decreased the power of the mtDNA statistical analysis. The microsatellite analysis showed a statistically significant deviation from the Hardy-Weinberg equilibrium in Area V, and the heterogeneity test showed significant differences between Areas IV and V. Results of the genetic analyses therefore suggested the possibility of genetic structuring of fin whales in the JARPA II research area, which should be further explored with the analyses of a large number of samples in the future. In this paper additional analyses were conducted in response to the Review Panel recommendations, among them that the F_{IS} values should be provided for the tests of Hardy-Weinberg equilibrium (SC/65b/Rep02).

The authors have addressed all the short-term recommendations in SC/65b/Rep02. No additional comments on this paper were received.

This concluded the review of papers that were revised to address recommendations made at the JARPA II Expert Review Workshop. Kitakado and Pastene thanked the SDWG for their review of these papers.

3.2 Population assignment and mixing

3.2.1 Small cetaceans

SC/65b/SD04 presented the results of a pilot study that uses the double digest restriction-site associated DNA sequencing (ddRAD-seq) genotyping-by-sequencing method on harbour porpoise (*Phocoena phocoena*) specimens from the Baltic Sea, eastern North Sea, Spain and the Black Sea. From a single Illumina lane and a set of 49 individuals, around 6,000 SNPs were obtained. These markers were used to estimate population structure and differentiation. Splits were identified between porpoises from the North Sea and the Baltic, and within regions in the Baltic Sea (between the Belt Sea and the Inner Baltic Sea). The SNP analysis confirms population structure elucidated by previous mtDNA/microsatellite studies. This paper demonstrates the feasibility of SNP analysis on opportunistically sampled cetacean samples, with varying DNA quality, for population diversity and divergence analysis.

In discussion the SDWG welcomed the presentation of these results, which demonstrate the great potential of ddRAD approaches for generating large single nucleotide polymorphism (SNP) datasets over a relatively short timeframe and within a reasonable budget. It was noted that in addition to the ddRAD approach utilised in SC/65b/SD04, other genomic sequencing techniques, such as the use of existing genomic resources to develop SNP panels, can be used to identify a large number of SNPs. Unlike ddRAD approaches, which result in the discovery of a large number of unmapped variable loci, SNPs detected using these techniques can be mapped to the genome and thus associations with known genes identified. An advantage of using the ddRAD approach is that SNP genotyping is completed in a single step, while the techniques based on SNP discovery from reference sequences require additional work to design assays and genotype samples. Although using these techniques is more time consuming and expensive, once assays are designed they can be used to generate additional SNP datasets for any number of additional samples with relatively high reproducibility. The one-step approach used in ddRAD is rapid and is particularly useful for studies with small sample sizes, but it is more difficult to add samples to a project at a later stage

One issue with using the large numbers of SNPs generated using ddRAD approaches (>6,000 in SC/65b/SD04) is that many loci will not be independent. This lack of independence should be considered when generating p-values using permutation tests, which assume that the loci being analysed are independent. Although at least one programme, PHASE (Stephens and Donnelly, 2003), is available to statistically infer which loci are linked, the SDWG agreed that this issue is a generic problem and will need to be addressed in the future.

The SDWG queried whether the porpoise population clusters identified using SNP loci were generally consistent

with those previously generated using microsatellite data. The author observed that the breaks were roughly congruent but cautioned that the number of individuals used in this study is small, so inference is somewhat limited by this aspect. However the ddRAD approach can generate much higher resolution information per individual than microsatellite data within a reasonable budget, so represents a promising approach for future studies.

3.2.2 Other Southern Hemisphere whale stocks

SC/65b/SH07 investigates the level of connectivity between humpbacks migrating through New Zealand and breeding/calving grounds in the South Pacific. Historically humpback whales (*Megaptera novaeangliae*) migrating past New Zealand have been linked to the east Australia migratory corridor, western South Pacific breeding grounds and IWC Antarctic Area V feeding grounds. Due to the largely opportunistic nature of sightings, to date most studies have analysed small datasets. Here 211 samples were genetically analysed (193 biopsy samples as part of a dedicated Cook Strait survey of whales on their northern migration, and 18 from dead, beach cast whales) with samples largely collected between 2003 and 2010 (n=210). The 190 DNA profiles that passed quality control represented 167 unique whales. Comparison of the 167 whales to the Oceania (n=1,052 individuals) and east Australia (n=865 individuals) DNA registers revealed six matches to New Caledonia and five matches to east Australia; there were no matches to any other Oceania region. This study shows that humpback whales passing New Zealand on their northern migration show the least genetic difference to New Caledonia. However, they don't appear to show the same fidelity to the migratory corridor as they do to the breeding grounds. The low rate of between-year resightings and matches to east Australia suggests more variability in the use of migratory corridors. Possible connections to an east Australian breeding ground in the Great Barrier Reef could not be explored fully due to a lack of data from this area but given the level of matches to the east Australian migratory corridor this would be of interest in the future.

In discussion it was observed that the low number of re-sights in the dataset could be explained if humpbacks have low fidelity to the New Zealand migratory stream or alternatively if the migratory stream was large. In this regard it was questioned whether independent data are available to determine the size of the migratory stream and begin to distinguish these hypotheses. The authors were not present to respond, but it was suggested that some data may be available from surveys of the humpback migration in Cook Strait.

It was also noted that if you consider the numbers of genetic samples available from both east Australia and New Caledonia in proportion to their population sizes (east Australia is much larger than New Caledonia), the similar numbers of recaptures found between New Zealand-east Australia (5) and New Zealand-New Caledonia (6) may in fact suggest good connectivity with east Australia, since the probability of recapture of east Australian whales is likely to be much lower. In future this interchange might be usefully investigated in a quantitative multi-strata framework, which could take into account the different capture probabilities in the two breeding populations.

3.2.3 In-Depth Assessment

SC/65b/IA08 reports on the uncertain stock origins of sei whales represented by 71 products purchased in Japanese market from 1997 to 2009. Based on reported catches of sei whales, the authors of SC/65b/IA08 expected that sei whale products could have originated from two sources: (1) the importation and long-term storage (up to 10 years) of scientific whaling in the North Atlantic by Iceland prior to 1989; and (2) the Japanese scientific whaling in the North Pacific (JARPN II), where sei whales are reported to form a single stock (Kanda *et al.*, 2006; 2009; 2013). Instead, phylogenetic reconstruction and matching of mtDNA control region sequences with 26 available reference sequences from the North Atlantic, the North Pacific and the Southern Hemisphere provided evidence for market products originating from three sources or stocks of sei whales. For the 11 products purchased prior to the inclusion of sei whales in JARPN II in 2002, three showed a phylogenetic affinity with reference sequences from the North Atlantic and eight showed an affinity with reference sequence from the Southern Hemisphere. Although phylogenetic support (i.e. bootstrap) was weak for identification of North Pacific or Southern Hemisphere, three of the 11 were an exact match to reference sequences from the Southern Hemisphere. After 2002, the majority of products (n=47) showed a phylogenetic affinity with reference sequences from the North Pacific, consistent with an origin from the JARPN II hunt, but a substantial proportion (n=13) showed an affinity to reference sequences from the Southern Hemisphere, similar to that of the products purchased before the JARPN II hunt. The authors consider two alternate explanations for the 21 products showing an affinity with the Southern Hemisphere reference sequences: (1) there are at least two stocks of sei whales in the North Pacific, one of which shows a phylogenetic relationship with the Southern Hemisphere; or, (2) there is an Illegal, Unreported or Unregulated (IUU) source of sei whale products originating from the Southern Hemisphere. The authors noted the importance of hypothesis 1 for the current In-Depth Assessment of sei whales in the North Pacific.

In response, Yoshida and Pastene commented that it is not possible to infer stock structure based on market samples because the origin of such samples is unknown. They observed that there is not a clear diagnostic genetic signal for North Pacific and Southern Hemisphere sei whale samples and presented preliminary phylogenetic analyses of mtDNA haplotypes (480bp) from samples from JARPN II (488), POWER (31), past commercial surveys in the North Pacific (304), North Atlantic (1) and Southern Hemisphere (4), which showed no clear separation between North Pacific and Southern Hemisphere whales. Considering this phylogeny, they stated that assignment of samples of unknown origin is not possible because there is no clear separation between Southern Hemisphere and North Pacific sei whales, and observed that a comprehensive study on worldwide genetic structure of sei whale based on samples of known origin is required.

Discussion in SDWG focused on the high diversity and weak phylogeographic structure in the phylogeny presented in SC/65b/IA08 (fig. 1). Assigning sei whales of unknown origin to either the North Pacific or Southern Hemisphere using mtDNA is hampered by uncertainty regarding underlying stock structure. The phylogeny suggests there may be weak bootstrap support for differences between mitochondrial lineages occurring in the North Pacific *vs*. Southern Hemisphere. Neither North Pacific nor Southern Hemisphere mtDNA haplotypes formed monophyletic clades. It was noted that the phylogeny might be better resolved with additional sequencing of mtDNA.

The authors of SC/65b/IA08 suggested that exact haplotype matches between samples provide evidence that both samples come from a similar geographic origin.

In this regard the SDWG observed that it would be useful to visualise these data in a haplotype network depicting both haplotype frequency and location, for example to understand whether high frequency haplotypes are restricted or widespread in distribution. It was noted that no haplotypes shared by both the Southern Hemisphere and North Pacific have yet been identified, though the group observed that this may be a function of the pattern and intensity of sampling to date. It would be valuable to formally test whether there is a non-random distribution of mitochondrial haplotypes with regard to region of origin (North Pacific *vs*. Southern Hemisphere).

It was observed that if the market samples included in this dataset have a North Pacific origin, this suggests higher mtDNA diversity in the North Pacific than has been estimated by previous studies of the sei whale (Kanda *et al.*, 2009). These differences may be a function of the geography and intensity of current sampling in the North Pacific.

The SDWG **agreed** that the global stock structure for this species requires additional investigation, with careful geographic sampling design and sequencing of markers additional to the mtDNA control region to understand the origins of market products given the reported catch records. They noted that this paper was associated with a request for the mtDNA sequences reported in Kanda *et al.* (2009 representative of the North Pacific sei whale to be made available through the Data Availability Group Procedure B, as well as representative Icelandic sequences from the North Atlantic, in order to enable a more comprehensive reference comparison with the sei whale samples collected from the market, and that this request will be discussed further in IA (Annex G, item 4.1).

4. TOSSM (TESTING OF SPATIAL STRUCTURE MODELS)

No new items were presented on this topic during SC/65b. The SDWG noted that some long-term TOSSM work on the Pacific Coast Feeding Group (PCFG) of gray whales is still underway (IWC, 2013). Weller *et al.* (2013) also made recommendations for additional TOSSM simulations to be conducted to further explore plausible levels of immigration into the PCFG. The SDWG looks forward to seeing further results from these TOSSM recommendations at SC/66a.

5. TERMINOLOGY AND THE UNIT-TO-CONSERVE

Following a recommendation arising in 2012 (IWC, 2013), to compile a 'go-to' glossary of stock related terms, the SDWG made some progress on this document both intersessionally and during SC/65b. 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 (IWC, 2014). During SC/65b the SDWG decided further work was needed to agree the definition for 'mixtures of stocks', as well as substantial additions to the developing glossary in order to align the terms used in SDWG with terminology already in use by the small cetaceans sub-committee. It was agreed that this work would be conducted intersessionally (see work plan).

In a joint meeting with the sub-committee on small cetaceans, several concerns were raised, including *inter alia* the following.

(1) Within the SDWG, use of the term 'aggregation' was proposed to make a distinction between situations where no information on the stock composition of a group of animals is available and situations in which information about the stock composition of the group is available. However, when used to describe small cetaceans, use of the term 'aggregation' is often interpreted as having a temporal component, which could be problematic when describing a group of small cetaceans that occur in an area year-round but for which no information on stock composition is known.
(2) Within small cetaceans, groups of individuals may be considered a discrete unit based on behavioural observations, including evidence for long-term associations between individuals. For example, residency is one of the behavioural characteristics that is often used to describe what are considered populations of small cetaceans. Alternatively (at least historically), stocks of whales considered in IWC discussions were often based solely on distribution relative to sometimes arbitrary geographic criteria.
(3) The relationship between the terminology proposed for use by the SDWG and the terminology utilised by the IUCN needs to be clarified.

It was noted at the end of the joint SD/SM session that while it would be ideal to align the terms used for small cetaceans with those proposed by the SDWG, if an agreed-upon set of terms cannot be reached, then it will be important to make sure that the relationships between the terminology used by the SM sub-committee and that proposed by the SDWG is clear. The primary purpose of the terminology and unit-to-conserve discussions within the SDWG are to facilitate clarity in descriptions of 'stocks' and other units in general and the distinctions on which such units are based, and this objective does not require use of exactly the same terminology.

6. WORK PLAN

6.1 Genetic analysis guidelines

The genetic analysis guidelines are anticipated to be completed intersessionally (group convened under Waples) and should be ready to circulate within the Scientific Committee at SC/66a.

6.2 Stock definition terminology

An intersessional email group was formed to agree to a revised set of stock definitions (IWC, 2014) with special reference to small cetaceans, following discussions at SC/65b. Results from this exercise will be presented at SC/66a. The group was convened under Cipriano, and included Bjørge, Currey, Fortuna, Hoelzel, Jackson, Lang, Natoli, Reeves, Rosel, Rosenbaum and Thomas.

7. ADOPTION OF REPORT

The report was adopted at 14:01 on 20 May 2014.

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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
   - 2.3 Other developments
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 Bowhead, right and gray whales
     - 3.1.3 Small cetaceans
     - 3.1.4 Other Southern Hemisphere whale stocks
     - 3.1.5 JARPA II Special Permit research programmeme
   - 3.2 Population assignment and mixing
     - 3.2.1 Small cetaceans
     - 3.2.2 Other Southern Hemisphere whale stocks
     - 3.2.3 In-depth assessment
4. TOSSM
5. Terminology and unit-to-conserve
6. Work plan

Appendix 2

TECHNICAL COMMENTS ON SC/65B/BRG02

The following section contains technical comments from the SDWG regarding the genetic analyses and microsatellite choices made in the studies analysed by SC/65b/BRG02.

SC/65b/BRG02 reports approximately 5% lower variation at 'non-focal' (heterologous) microsatellite DNA loci for cetacean studies, which is consistent with earlier studies for other taxa. However, it was noted that this reduction in heterozygosity is small compared to the range of heterozygosity values among different microsatellite markers within a species. As expected based on well-established theory, F_{ST} was higher for loci with lower variation (including the non-focal loci). The authors propose that this effect in turn will lead to over-diagnosing the level of stock structure when non-focal loci are employed. The SDWG argue that this interpretation of the correlations detected in SC/65b/BRG02 is erroneous.

The observed effect of locus-specific variation is the natural result of differences in mutation rates across loci. In fact, it is possible to partition F_{ST} into two components; a locus-specific effect that, in the case of neutral loci, is determined by the locus-specific mutation rate; and a population-specific effect that depends on both population size and migration rate. It is this latter component that we want to capture by measuring genetic differentiation using neutral markers, but this is not a simple matter because of differences in mutation rates among loci. Loci with high mutation rates are more variable and lead to downwardly biased estimates of F_{ST}. On the other hand loci with low mutation rates will lead to upwardly biased estimates of F_{ST}. Consequently, there is no such thing as a single genome-wide 'true' F_{ST}. Instead, F_{ST} is a property of each locus and obtaining a genome-wide estimate requires either a standardisation of locus-specific F_{ST} to take into account differences in locus variation (Hedrick, 2005) or explicitly modelling F_{ST} in terms of locus-specific and population-specific effects (Gaggiotti and Foll, 2010).

Regardless of the magnitude of differentiation, the estimation of F_{ST} is subjected to a statistical assessment in order to control the Type I error rate. Therefore, while highly diverse loci may not reflect a sufficient proportion of the population-specific effects to detect real population structure, it is not conversely true that markers of low variation will detect structure that isn't actually there. Indeed, if this was the case, low variability loci, such as allozyme loci and SNPs, should yield 'significant' population genetic structure at a higher rate than highly variable loci, which is not the case.

It is a long established fact that the manner in which genetic markers are selected can introduce a bias in subsequent population genetic estimates, such as diversity and divergence (Ellegren *et al.*, 1995; Morin *et al.*, 2004). Indeed, some sort of bias is involved in the selection of markers to use in almost all genetic studies. Whether this bias creates a problem for inference depends on the type of analysis. In terms of microsatellite loci, most laboratories isolating and characterising novel loci tend to aim for the most variable loci (in terms of number of alleles and/or heterozygosity). The result is a bias towards loci with high levels of variation. The same biased selection procedure is often applied when selecting among published non-focal (and focal) microsatellite loci. However, the overall number of high quality loci (i.e., that conform to best laboratory practices) will likely be lower and hence microsatellite loci (as is the case for SNPs as well) tend to be less variable in non-focal species. This well-studied phenomenon, especially in studies involving humans, is known as 'ascertainment bias'. Numerous procedures have been developed to correct for ascertainment bias, which is especially prominent in bi-allelic markers, such as SNPs (Nielsen, 2004).

Assuming that microsatellite loci are genotyped following best laboratory practices, the ascertainment bias introduced during the locus selection in the focal species does not apply

to sub-sequent non-focal studies. Ascertainment bias in a population genetic assessment only becomes a potential issue if the microsatellite loci were selected based upon microsatellite data generated from a non-random sample of individuals (e.g., one specific population segment in the targeted species).

The work on Bering-Chuckhi-Beaufort (B-C-B) bowhead whales was put forward in SC/65b/BRG02 as an example of 'erroneous' population genetic structure due to the use of non-focal microsatellite loci. During an assessment of the stock structure in B-C-B bowhead whales, focal microsatellite loci failed to detect structure identified with an independent panel of focal and non-focal loci (Givens *et al.*, 2007; Givens *et al.*, 2010; Jorde *et al.*, 2007). SC/65b/BRG02 attributed this 'discrepancy' to F_{ST} 'inflation' in the non-focal loci. However, if the initial study had a higher genotyping error rate, this could also explain the differences (Morin *et al.*, 2009). Other possible explanations for marker discrepancy also exist, as outlined in Item 3.1.1 and Annex D, adjunct 4. While additional work is necessary to elucidate the underlying mechanism giving rise to the correlations reported in SC/65b/BRG02, the general inference made in SC/65b/BRG02 concerning 'inflated' levels of population structure is due to the use of non-focal loci is incorrect. Overall, the SDWG agreed that conservation decisions based on genetic studies can have multi-level impacts and reiterated the importance of using the best possible methodology in order to do this, as described above.

Discussion then focused on interpretation of the correlations described in SC/65b/BRG02.

It was observed that the statistical analyses in this paper may have overestimated the level of significance of the observed relationships because they have not accounted for pseudo-replication arising from treating different species with shared phylogenetic history as independent data points (Garland, 1992).

The temporal spread of studies used in the meta-analysis was also noted as a potentially confounding factor. Early studies, many of which were focused on species of high conservation concern, had limited choices in terms of available microsatellite loci to develop and limited ability to detect and minimise error. These could potentially explain some of the results presented (which show an increase in non-focal, low diversity microsatellites for IUCN threatened compared to non-threatened species). Along these lines, it was cautioned that whilst the results in SC/65b/BRG02 demonstrate non-random associations between the variables being tested, the paper makes the error of interpreting correlation in terms of cause and effect. Causal relationships can only be established after careful consideration of a wide variety of other covariates (such as temporal trends in microsatellite development) that might influence the observed relationships.

In regard to the relationship found between IUCN categories, F_{ST} values and non-focal microsatellites (SC/65b/BRG02, Fig. 5), it was noted that in most cases IUCN status is designated for a species throughout its global range. It is therefore unclear how to interpret the associations identified between IUCN status and F_{ST}, since these values were measured for subunits within a species. Perhaps the best use of results from this paper would be to help formulate hypotheses that can be evaluated in more detail in subsequent studies.

In summary, the SDWG **recommended** that the data used in the meta-analysis be made available so that the SDWG can better understand the associations identified in SC/65b/BRG02. This will allow examination of alternative explanations for the results, which is needed before drawing conclusions for SDWG.

Bickham commented as follows. Discussion of SC/65b/BRG02 in the meeting and in a small group meeting and via email has focused on alternative interpretations of the data presented in the paper as well as generic caveats about correlation analyses and cause and effect. The latter points apply to all meta-analyses, since they are of fundamental nature correlative. It is always possible that some unmeasured correlate is at the root of an observed significant association, such as the ones discussed in Waples (1991). The issue of phylogenetic multiple sampling has also been raised but the authors do not agree that these are likely the cause of the effects reported in their study. First of all, there is a clear difference in practice between whale and shark studies, the former having a lot more non-focal loci. Secondly, the relationship between non-focal loci and diversity and F_{ST} hold up within each phylogenetic group. Perhaps the various groups of cetaceans could apply here as phylogenetic replicates, but not so the sharks. So, this seems to be a general attribute of non-focal microsatellites not restricted to a particular phylogenetic lineage. The authors of SC/65b/BRG02 understand that correlation analyses are not the strongest way to arrive at inferences, but meta-analyses provide a broad scale perspective on issues that cannot be obtained by more experimental approaches.

Bickham noted that what is compelling in this paper is that statistical analyses across a large number of studies, theoretical considerations of F_{ST}, and their own personal experiences with the application of focal and non-focal microsatellites are consistent with their conclusions. He felt that seldom does a single study have such strong substantiation. He noted that it is possible that the author's interpretation that the significantly different results obtained by these two classes of markers can lead to incorrect conclusions about management units when non-focal microsatellites are misapplied will not be borne out by future tests. However, Bickham observed that at this point the only study to critically analyse this is the work presented. The bowhead studies (Givens *et al.*, 2007; Givens *et al.*, 2010; Morin *et al.*, 2012) showed that the application of non-focal microsatellites produced significant F_{ST} values that subsequently disappeared upon application of focal microsatellites, as well as SNPs, and which had no sensible biological support. He noted that since his work in 2007 the structure of the B-C-B bowhead population has been further confirmed by the development of an extensive database of whale movements using satellite tagging which is an entirely independent test of their hypothesis. The sub-stock structure indicated by the significant F_{ST} values produced by the non-focal microsatellites failed when tested by a set of more appropriate microsatellite markers, SNPs, and by the movement studies. Therefore we think our conclusions reached in the bowhead studies are sound and the data in SC/65b/BRG02 convince us that this is not an isolated, unique incident but just an example of a broader phenomenon.

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