

Annex I

Report of the Working Group on Stock Definition

Members: Lang (Convenor), Baird, Baker, Bell, Bickham, Brownell, Burkhardt, Butterworth, Cipriano, DeWoody, Double, Scordino, Hielsches, Hoelzel, Findlay, Friedlaender, Jackson, Litovka, Mallette, Mizroch, Natoli, Olson, Palka, Pampoulie, Park, Pastene, Rosel, Rosenbaum, Širović, Skaug, Solvang, Tiedemann, Torres Florez, Wade, Waples, Yoshida, Zharikov

1. INTRODUCTORY ITEMS

1.1 Convenor's opening remarks

Lang welcomed participants.

1.2 Election of Chair and appointment of rapporteurs

Lang was elected as Chair and Cipriano 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/66b/SD01, SC/66b/BRG07, SC/66b/SH17-SH18, SC/66b/Rep06, Attard *et al.* (2016), Alexander *et al.* (2016), Amaral *et al.* (2016), and Meschersky *et al.* (2015).

2. GUIDELINES AND METHODS FOR GENETIC STUDIES AND DNA QUALITY

This agenda item relates to two sets of guidelines that the Scientific Committee has requested the Working Group (hereafter SDWG) to develop for reference in the Committee's discussions of stock structure. Both sets are subject to ongoing update as appropriate.

2.1 Guidelines for genetic data analysis

This document provides guidelines for some of the more common types of statistical analysis of genetic data that are employed in IWC management contexts. The main section is intended as guidance for managers and also contains examples of management problems that are regularly faced by the Committee. There is also an extensive appendix of genetic analysis techniques for specialist readers. This guidelines document is near completion and is expected to be available by SC/67a (see Item 6.1).

2.2 DNA quality

The DNA data quality control guidelines are already available as a 'living document' on the IWC website¹. In recent meetings, data derived from next generation sequencing approaches, including SNPs, have been utilised to address stock structure questions. In light of these developments, the SDWG agreed that it would be timely to update the DNA data quality control guidelines to cover these types of data. An intersessional e-mail group was formed to begin addressing this issue (see Item 6.2 and Annex V).

2.3 Other issues

SC/66b/SH17 and SC/66b/SH18 report on the construction of validated registers of mtDNA control region haplotypes for Southern Hemisphere blue and humpback whales, respectively. These papers were discussed in a joint session with the Sub-committee on Other Southern Hemisphere Whale Stocks (SH), and the discussion is included in Annex H item 8).

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. These discussions often refer to the genetic analysis guidelines and genetic data quality documents.

3.1 Population structuring and migration rates

3.1.1 Bowhead, right, and gray whales

SC/66b/BRG07 reports on ongoing effort to build SNP and mtDNA databases for bowhead whales. The SNP panel was designed from transcriptome data plus previously designed SNPs from Morin *et al.* (2010). We started with a panel of 96 SNPs and analysed 285 samples using the Fluidigm SNPtype platform. These samples included 252 B-C-B and 33 Okhotsk individuals. Several quality control mechanisms were described including duplicate samples, mother/fetus pairs, and duplicating samples/SNPs from the Morin *et al.* (2010) study. We analysed autosomal SNPs for genetic distances, F_{ST} , and expected/observed heterozygosities for each population. Results showed that of the 96 loci, 55 loci passed quality control standards (including 48 autosomal, 1 Y chromosome and 6 X chromosome loci). Complete replication of data from the Morin *et al.* (2010) study was attained, and all fetuses showed at least one allele shared with their mother for each locus. Only one pair of samples had identical genotypes (probably due to mis-labelling), therefore indicating that DNA fingerprinting is possible. B-C-B population showed 12 loci deviating from Hardy-Weinberg Equilibrium, while the Okhotsk population showed two loci deviating. The F_{ST} value between B-C-B and Okhotsk was 0.05 (and was significant), a similar value to previous studies using mtDNA and microsatellites. The advantage of the above method for SNP genotyping will be to have a panel that can be used to build a database comparable among labs, as the loci are not anonymous. In the future additional SNPs, including sex chromosome SNPs, will be combined with mtDNA haplotype data to explore historical demography.

The SDWG thanked the authors of SC/66b/BRG07 for this update. It was noted that the number of loci demonstrating heterozygote excess compared to HW expectations in the B-C-B stock was higher than would be expected by chance, while only two loci deviated from Hardy Weinberg equilibrium in the Okhotsk Sea stock. This pattern is opposite of what would be predicted, as in largely isolated populations heterozygote excess is expected to be found in more loci in small versus large populations. Possible

¹<http://iwc.int/scientific-committee-handbook#ten>.

explanations for this pattern include scoring error, selection for heterozygotes, and assortative mating, although it is unlikely these explanations would result in a large proportion of loci being affected. The authors noted that all loci that produced ambiguous genotype calls were discarded, and that the quality control measures implemented (replication of genotypes, comparison of mother-foetus pairs) did not indicate issues with scoring.

The authors further noted that, compared to what was seen in a similar project identifying SNP loci in gray whales (SC/66b/DNA04), a much higher proportion of the initial loci screened were discarded due to amplification failure or difficulty in reliably scoring genotypes. The gray whale SNP panel, which was developed using genome sequence data from three gray whales, includes 92 autosomal nuclear markers from protein-coding genes specifically targeted because of evidence for selection. The bowhead loci were derived from two different sources: (1) transcriptome sequences, which included some intronic regions; and (2) sequences from microsatellite flanking regions (Morin *et al.*, 2010). Exploring whether the pattern of locus failure and/or heterozygote excess was associated with SNPs derived from a particular source could be informative.

The authors of SC/66b/BRG07 mentioned that further analysis using mtDNA was planned, and it was suggested that, if possible, sequencing of the mitogenome, which would encompass the segments currently being sequenced (HVRI portion of the mtDNA control region, cytochrome-*b*, and ND1), would provide greater resolution in studies of historical demography.

Two additional papers discussing the development of SNP panels (SC/66b/DNA02 and SC/66b/DNA04) in minke whales and gray whales, respectively, were discussed in the DNA Working Group (Annex O, Item 5).

Meschersky *et al.* (2015) presents data on the frequencies of mitotypes (combined mtDNA control region, cytochrome-*b*, and ND2 gene sequences, representing ~1,800 bps total) in gray whale samples collected from four regions in the North Pacific (the Chukotka Peninsula, the Koryak coast, eastern Kamchatka, and Sakhalin Island, Russia). While the number of mitotypes per region decreased markedly when moving from the northernmost (Chukotka Peninsula) to the southernmost (Sakhalin Island) sampling site, the mitotypes that were predominant among the Sakhalin Island samples are also found in moderate to high frequencies in samples from the northernmost site (Chukotka). One mitotype (mitotype 2) is highly divergent from all other mitotypes identified and is also one of the mitotypes found in high frequency among the Sakhalin Island sampled whales. Although the full mitotype sequences presented in Meschersky *et al.* (2015) are not available for other regions, comparison of the control region sequences generated in this study with previously published sequence data indicates that this divergent lineage is also present among whales utilising wintering and calving areas in the eastern North Pacific, suggesting that the prevalence of mitotype 2 among sampled Sakhalin whales does not necessarily reflect the origin of that lineage in the western North Pacific. However, given that sequencing of the cytochrome B and ND2 regions revealed multiple variants associated with the same mtDNA control region haplotype, caution should be used before drawing conclusions based on the mtDNA control region sequences alone.

The SDWG thanked the authors for contributing this work, which followed up on a suggestion made during previous gray whale stock structure discussions to increase

the length of the mtDNA region sequenced (IWC, 2011). The SDWG questioned if it was possible that the highly divergent lineage (mitotype 2) presented here could be an artifact generated by sequencing nuclear insertions of mitochondrial DNA sequence (NuMts). It was noted that while this possibility had not been considered explicitly, multiple labs have produced this same control region sequence and have not identified any sequencing issues, and thus this sequence is possibly a remnant of ancient divergence. However, when the gray whale genome becomes available, the SDWG advised that the nuclear genome be checked for the presence of NuMts.

3.1.2 Other Southern Hemisphere whale stocks

3.1.2.1 RIGHT WHALES

Carroll *et al.* (2015) investigated the role of maternally directed learning of migratory habitats, or migratory culture, on the population structure of the endangered Australian and New Zealand southern right whale. The work builds on the previous genetic analyses of population structure for right whales in the region (Carroll *et al.*, 2011) and includes new results from stable isotopes to investigate maternal fidelity to feeding grounds (Valenzuela *et al.*, 2009). Using DNA profiles, comprising mitochondrial DNA (mtDNA) haplotypes (500 bp), microsatellite genotypes (17 loci) and sex from 128 individually identified whales, the authors find significant differentiation among winter calving grounds based on both mtDNA haplotype ($F_{ST}=0.048$, $\Phi_{ST}=0.109$, $p<0.01$) and microsatellite allele frequencies ($F_{ST}=0.008$, $p<0.01$), consistent with long-term fidelity to calving areas. However, most genetic comparisons of calving grounds and migratory corridors were not significant, supporting the idea that whales from different calving grounds mix in migratory corridors. The authors also report a significant relationship between $\delta^{13}C$ stable isotope profiles of 66 Australian southern right whales with both mtDNA haplotypes and kinship inferred from microsatellite-based estimators of relatedness. The authors suggest that this indicates migratory culture influences genetic structure on feeding grounds. This fidelity to migratory destinations is likely to influence population recovery, as long-term estimates of historical abundance derived from estimates of genetic diversity indicate the South Pacific calving grounds remain at <10% of pre-whaling abundance (Carroll *et al.*, 2013; Jackson *et al.*, 2016).

No comments on this paper were received. The SDWG thanked Baker for his presentation of this work.

3.1.3 In-depth assessment

3.1.3.1 SPERM WHALES

Alexander *et al.* (2016) discussed the genetic structure of sperm whales on a global scale. This paper was reviewed by the SDWG, and the authors' summary is provided in Annex G (item 5.1).

The SDWG thanked Baker for presenting this information on sperm whale stock structure, noting the difficulty of studying a species with complex social structure and strong sex-specific dispersal patterns.

In discussion, it was noted that several haplotypes were found in high frequencies in multiple regions, and the question was raised as to whether this pattern was driven by lineage sorting or infrequent gene flow. Baker noted that the results indicate that sperm whales in different ocean basins share a recent common ancestor, and thus shared haplotypes are likely due to incomplete lineage sorting as a result of recent divergence. However, this pattern may be complicated by male-mediated gene flow between regions.

In comparison to the other regions sampled, the Pacific showed relatively little regional differentiation in comparison with social structure. It was noted that while differences in regional strategies for sampling groups could be a potential confounding influence, several other factors, including the disruption of social bonds due to recent whaling, have been proposed as possible explanations for differences between regions (Whitehead *et al.*, 2012).

The SDWG further noted that some areas where sperm whales are known to occur, such as New Zealand and Alaska, were not represented in the sample set analysed in Alexander *et al.* (2016), and questioned whether these sampling gaps could influence the interpretation of the observed patterns. Baker noted that these two areas are used primarily by adult males, and samples of males from these areas were not utilised given that the paper focused on understanding factors influencing structure among nursery groups in tropical and temperate regions. However, the South Atlantic represents a sampling gap and efforts are underway to identify samples available from that area. Baker suggested that the sampling of equatorial/tropical nursery groups could be used as a basis for stock structure hypotheses, if males from more polar waters could be allocated based on mtDNA or direct kinship measures.

3.2 Population assignment and mixing

3.2.1 Other Southern Hemisphere whale stocks

3.2.1.1 BLUE WHALES

Attard *et al.* (2016) presented analyses of population structure within Antarctic blue whales using both mtDNA control region sequences and microsatellite genotypes ($n=20$ loci). This paper was discussed as part of a joint SH/SD session, and a summary of that discussion is included in Annex H (item 5.2.1).

3.2.1.2 HUMPBACK WHALES

Amaral *et al.* (2016) presented an assessment of the stock structure of humpback whales on feeding grounds in the Antarctic. In the Southern Hemisphere, seven primary humpback whale breeding stocks have been identified based on tropical distribution between which there is limited gene flow. In the summer, these stocks are distributed among six Management Areas (Areas I-VI) defined by the IWC in the Southern Ocean feeding grounds. The extent to which different breeding stocks mix on these feeding grounds, and the genetic structure and relationships between them has remained unclear. This study is the first to analyse the circumpolar genetic structure of humpback whales in their feeding aggregations. Sequences of the mitochondrial control region and 10 microsatellite DNA genotypes were obtained for 399 humpback whale samples, obtained within the six Antarctic Management Areas. The results obtained suggest a marked pattern of population differentiation between feeding aggregations across the feeding range. Management Area I surrounding the Antarctic Peninsula, associated with Breeding Stock G in the southeastern Pacific Ocean, was highly differentiated from all other feeding aggregations. Management Area III, which has been associated with Breeding Stock C in the western Indian Ocean, also showed differentiation from adjacent Areas I, II and IV. When comparing remaining Areas, little significant differentiation was seen, suggesting interchange of individuals and overlap of breeding stocks on those Southern Ocean feeding grounds. Genetic diversity was higher in Areas II, III and IV and lower in Areas I and V. The higher genetic diversity seen in Areas II and III may indicate mixing of the genetically distinct Breeding Stocks B and C

on the feeding grounds or the existence of a single feeding ground. The results obtained provide some of the first steps towards a full investigation of interchange between breeding stocks occurring on feeding grounds in the Southern Ocean.

The SDWG thanked the authors for presenting this work, which pulls together results of several analyses that have been presented to the Scientific Committee at different times in the past.

In discussion of Amaral *et al.* (2016), the SDWG questioned the use of the IWC whaling Management Areas to stratify the samples into putative stocks. The boundaries of these IWC Areas do not appear consistent with the distribution of sampled whales and do not reflect the stock delineations used in the most recent assessment work. Rosenbaum noted that this was a 'legacy' paper for publication, and that the current accepted IWC boundaries were used for the Comprehensive Assessment and are also in a paper that is being prepared for submission.

The SDWG noted that the pattern of genetic differentiation identified between Areas was somewhat inconsistent with the individual assignment values resulting from the STRUCTURE analysis assuming two 'clusters', or stocks, are present in the sample set. Area I was significantly differentiated from most other areas for both the mtDNA and the microsatellite analysis, and whales sampled within Area I had relatively high STRUCTURE assignment probabilities to one cluster. However, whales in Areas IV-VI, and to a lesser extent Area II, also assigned with relatively high probabilities to that cluster, while whales sampled in Area III were largely assigned to the second cluster.

In addition, the SDWG expressed concern over the impact of using prior location information in the STRUCTURE analysis, as was done when the results noted above were generated. When the analysis was rerun without incorporating prior location information, the assignment probabilities of individuals in all Areas were equivocal, suggesting that the assignment of individuals in the analysis mentioned above was driven by location information rather than derived from the genetic data. In addition, recent work suggests that the ability of STRUCTURE to recover the correct population structure decreases when strata of markedly different sample sizes are present (Puechmaille, 2016). As such, the high sample size of Area III, which contains over half of the analysed samples, may in part be driving the apparent difference between this area and the others in the STRUCTURE plot results. Rerunning the analysis after subsampling the Area III data to make the sample sizes per Area more compatible across regions could provide insight into whether the sample size disparity is influencing the STRUCTURE results. Rosenbaum noted that in the updated analyses, the revised STRUCTURE analyses were consistent with points made by the SDWG.

A final question was whether the data presented here could be combined with data generated from whales sampled on the breeding grounds to better evaluate mixing of breeding stocks on the feeding ground. Rosenbaum reported that a follow-up paper is nearing submission for publication that: (1) includes the results of analysis of both feeding and breeding ground samples; and (2) stratifies samples according to the boundaries laid out in the most recent assessment. The SDWG looks forward to reviewing this follow up paper in the future.

3.2.2 Western North Pacific Japanese Scientific Permit Programme (JARPEN II)

An Expert Panel was convened 22-26 February 2016 to conduct a final review of the western North Pacific Japanese

Scientific Permit Programme (JARPN II, Tokyo, Japan; see SC/66b/Rep06). As part of this review, new information on the stock structure of North Pacific minke whales, Bryde's whales, and sei whales was presented and reviewed by the Expert Panel, resulting in a series of recommendations by the Panel (SC/66b/Rep06) and a subsequent response addressing the short-term recommendations by the proponents (SC/66b/SP01). The RMP sub-committee requested that the SDWG evaluate the new information on stock structure for North Pacific minke whales and Bryde's whales (see Appendix 2). As part of this evaluation, the SDWG also considered the new information on sei whale stock structure that was presented to the Expert Panel.

3.2.2.1 NORTH PACIFIC MINKE WHALES

One of the recommendations made by the 2016 Expert Panel was to provide an estimate of genotyping error rates. Paper SC/66b/DNA01 responded to this recommendation and is reviewed in Annex O (item 5). For discussion among the SDWG, Pastene provided a review of four papers related to North Pacific minke whale stock structure that were presented to the 2016 Expert Panel: Pastene *et al.* (2016b), Pastene *et al.* (2016c), Bando and Hakamada (2016), and Kitakado and Maeda (2016). New information included in these papers is summarised in Appendix 3. Two additional Appendices discussing the evaluation of stock structure hypotheses in North Pacific minke whales are included as Appendix 4 and 5.

At the 2016 Expert Panel review, preliminary results of an ongoing analysis to identify parent-offspring pairs among sampled North Pacific minke whales were presented (SC/66b/Rep06). This analysis addresses a recommendation by the 2009 Expert Panel to examine the spatial distribution of close kin (IWC, 2010, p.420). Using a panel of 16 microsatellite loci, 22 potential parent-offspring pairs were identified using the likelihood-based approach described in Tiedemann *et al.* (2014). Some of the samples representing putative parent-offspring pairs were obtained from areas considered to represent different stocks under Hypothesis 3, including matches made between whales in the O-E and O-W regions. Given that this work was not yet ready to be submitted as a paper for this meeting, the SDWG was not able to provide a technical evaluation of the analysis, and the SDWG recommended that a paper be submitted for review at SC/67a. The importance of evaluating the potential for false positive and false negative detections of parent-offspring pairs (Tiedemann *et al.*, 2014) was emphasised, and the SDWG encouraged the authors to explore different approaches (e.g. software) to conduct kinship-based analyses. In addition, it was recommended that validating the identified matches by genotyping the samples at additional loci (microsatellites or SNPs) would be valuable and would allow a decrease in the probability of detecting false positive at a given statistical power. Pastene noted that development of a SNP panel for North Pacific minke whales is already underway (see discussion of SC/66b/DNA02 in Annex O, item 5) in response to one of the recommendations provided by the 2016 Expert Panel (SC/66b/Rep06, item 4.4.3.1) and, once genotyping is completed, could be utilised to verify the parent-offspring pairs identified.

The SDWG also discussed the analysis of the catch-at-age data presented in (Kitakado and Maeda, 2016). While the data collected indicated that all age groups were represented within the 'Ow' stock, in the 'Oe' stock the youngest whales, as well as females under the age of 20, were under-represented in comparison to males of the same age class. While these results were interpreted as evidence that the 'Oe' stock was

unlikely to represent a discrete stock, the SDWG noted that alternative explanations for this pattern may be available. In particular, if the migration of putative 'Oe' stock whales is segregated by age and/or sex, then many adult females with calves may already be north of the catch area when whaling effort begins in the offshore area (detailed in Appendix 3). If, as has been hypothesised by some, the migration of putative 'Ow' stock whales terminates just north of Hokkaido, then mature females and calves of that stock would still be available when whaling effort commences. This further demonstrates the difficulties in evaluating stock structure in cases where the available samples are restricted to areas considered migratory corridors or feeding grounds, where whales from different breeding stocks could potentially be mixing.

One recommendation of the 2016 Expert Panel was that 'all inferences regarding 'randomness' of observations (e.g. as assigned for common minke whales) should be substantiated by a statistical assessment of the presumed 'randomness' (SC/66b/Rep06, item 4.4.3.2). This recommendation relates to addressing the extent to which 'purging' of samples that do not demonstrate strong assignment to either the 'O' or the 'J' stock (based on the Bayesian clustering program STRUCTURE) is appropriate, which is an issue that has arisen repeatedly in past discussions (see references in Appendices 3, 4, and 5). Various levels of purging have been conducted as part of past analyses and this process has been shown to influence analysis results. In light of continued uncertainty about the best way to deal with purging of samples, the SDWG suggested that including the results of analyses conducted on both purged (at various levels) and non-purged samples would be valuable in the future, as conducted in Gaggiotti and Gascuel (2011).

The SDWG also suggested that additional exploration of the relationship between departures from Hardy-Weinberg equilibrium and F_{ST} values for different loci be conducted with the expanded dataset. Waples reported that a paper (Waples, 2015) that includes a more rigorous derivation of the theoretical basis for this method was published last year. In addition, simulations exploring the sensitivity of this approach to sampling error and different mixing fractions have been conducted, and a paper describing these results is currently in review. The SDWG welcomed this information and looks forward to discussing these results at SC/67a.

As described in Appendix 5, the SDWG agreed that it is important to discriminate between evidence supporting or rejecting the finding of heterogeneity within the 'O' stock and evidence that pertains to evaluating a specific geographically explicit hypothesis. A finding that results are not consistent with a specific two-stock hypothesis does not necessarily contradict the finding that additional heterogeneity, which may be caused by stock structure or due to other factors such as age structure or temporal changes, is present within the stock.

In summary, the SDWG thanked the proponents for presenting this new information. While the SDWG agreed that these results were important and interesting, they noted that at this stage further analyses are needed before conclusions can be drawn with respect to whether the number of stock structure hypotheses under consideration should be increased or decreased.

3.2.2.2 WESTERN NORTH PACIFIC BRYDE'S WHALES

The IWC Scientific Committee completed the most recent RMP *Implementation* for western North Pacific Bryde's whales in 2007 (IWC, 2008, p.9). During the *Implementation* two sub-areas (IWC, 2009, p.7), and four stock structure hypotheses (IWC, 2007a, p.8), were considered. Since that

time, a substantial number of additional genetic samples have accumulated, increasing both the number and distribution of samples. Using this expanded set of samples, additional genetic analyses have been conducted to further investigate the plausibility of the stock structure hypotheses under consideration. Results of these analyses were presented to the 2016 Expert Panel of the Final Review on the Japanese Whale Research Program under Special Permit in the Western North Pacific (JARPN II), and at the request of the SDWG Pastene briefly summarised the results of one of the reviewed papers (Pastene *et al.*, 2016d) that has implications for the stock structure of Bryde's whales. This paper utilised mtDNA control region sequencing (299bp) and genotypes from 17 microsatellite loci to examine the extent of stock sub-division between the two Bryde's whale sub-areas (sub-areas 1 and 2) as well as within sub-area 1 (eastern and western sectors). Significant genetic heterogeneity was found between the two sub-areas. However, no significant genetic heterogeneity was detected between eastern and western sectors of sub-area 1, although the statistical power of the data to detect structure was estimated to be high.

After reviewing these results, the Expert Panel **recommended** that the presence of multiple stocks within sample partitions should be assessed using ordination-based methods such as STRUCTURE and DAPC (SC/66b/Rep06, item 4.4.3.2). In response to this recommendation, SC/66b/SD01 was submitted to the Scientific Committee for evaluation by the SDWG.

SC/66b/SD01 responded to one of the three short-term recommendations from the JARPN II Review Workshop that the presence of multiple stocks within sample partitions should be assessed employing STRUCTURE for Bryde's and sei whales. Posterior probabilities for K were estimated for ten independent runs for each value of $K=1-5$ for Bryde's and $K=1-3$ for sei whales. In the case of Bryde's whale, Bayesian clustering analyses conducted for sub-area 1W, 1E, 2 and all sub-areas combined presented the highest likelihood probability at $K=1$ in all cases. Therefore the results of the STRUCTURE analyses conducted were not consistent with the results of hypothesis testing in Pastene *et al.* (2016d) of significant genetic heterogeneity between sub-areas 1 and 2. According to the authors, this reflects the well-documented difficulty that STRUCTURE has in detecting weakly differentiated populations. In the case of sei whales, results of the STRUCTURE presented the highest likelihood probability at $K=1$. In this case, results of the STRUCTURE and hypothesis testing were similar, and they support the existence of a single stock in the pelagic region of the North Pacific.

Following the presentation, Pastene commented that while the STRUCTURE analyses had been completed, due to time constraints additional ordination-based methods, such as DAPC, had not been conducted. The SDWG noted that STRUCTURE has little power to detect clusters when F_{ST} is low and only weak levels of differentiation are present. Thus it is not surprising that while contingency table analyses identified significant nuclear differences between sub-area 1 and sub-area 2 ($F_{ST}=0.004$, $p<0.001$), STRUCTURE was not able to identify multiple clusters within the dataset. Given this result, it is plausible that weak but potentially biologically important heterogeneity could exist within sub-area 1, but would not be detected by STRUCTURE unless it was at a level similar to or greater than that seen between the two sub-areas.

The SDWG further commented that, in comparison to STRUCTURE, other ordination-based methods (e.g., PCA, DAPC) may be better at discriminating clusters when

stocks are weakly differentiated, although the power of such methods to detect structure when effect size is small has not been tested (see Item 5). Detecting structure within western North Pacific Bryde's whales is complicated by the lack of samples from breeding areas, which is a common issue for several whales species assessed in the Scientific Committee. All of the analysed samples were collected in areas used by migrating whales and thus could represent a mixture of animals from different breeding stocks, as represented in Hypothesis 4. In addition, the boundary between the sectors within sub-area 1 is not based on biological differences and, if multiple stocks are present, this boundary could be incorrectly placed and thus confound the detection of any existing differences. As such, the SDWG emphasised the importance of utilising methods that do not require *a priori* stratification of samples when analysing this dataset.

It was noted that Hypothesis 4 was initially included as a medium-plausibility hypothesis based on whaling data because of age structure differences between sub-area 1W and the combination of sub-area 1E and area 2. However, concerns regarding the quality and reliability of this age data have been raised (IWC, 2007b, pp.413-14). These samples are no longer available for additional analysis or review, and this pattern has not been identified in more recently collected data. However, the SDWG noted that until additional information is available, a change in the plausibility status of Hypothesis 4 was not warranted.

The SDWG considered whether kinship-based analysis (Økland *et al.*, 2010) could be useful in evaluating the stock structure hypotheses under consideration. The proportion of sampled whales is small in comparison to the estimated abundance of Bryde's whales in the western North Pacific, and thus the probability of detecting parent-offspring pairs is low. Such approaches have, however, been useful in informing stock structure hypotheses in North Atlantic fin whales, in which only a small proportion of whales have been sampled (Elvarsson, 2014; Pampoulie *et al.*, 2012). The value of this approach may also be limited when evaluating hypotheses that assume mixing of multiple stocks within the same geographic area. Thus the utility of kinship-based approaches to evaluate stock structure hypotheses in North Pacific Bryde's whales needs further evaluation.

The SDWG thanked the authors for their efforts to address the recommendation of the Expert Panel. They concluded that the results presented in Pastene *et al.* (2016d) and SC/66b/SD01, showing significant genetic differentiation between sub-area 1 and 2, are not consistent with Hypothesis 1, although they cautioned that this does not necessarily confirm that the boundary between the two sub-areas is drawn correctly. While the results were not considered to be informative with respect to evaluating the plausibility of Hypotheses 3 and 4, it was noted that if more than two stocks are present in the western North Pacific, the level of differentiation between sectors within sub-area 1 must be low as it was not detected in the contingency table analysis. The SDWG **recommended** that the proponents also conduct an analysis using DAPC or other ordination-based methods that do not require *a priori* stratification of samples and can potentially discriminate between groups with low levels of differentiation.

3.2.2.3 NORTH PACIFIC SEI WHALES

SC/66b/SD01 also addressed the recommendation by the Expert Panel to conduct ordination-based analysis to further evaluate potential stock structure within North Pacific sei whales. The results presented in Pastene *et al.* (2016a) included a more extensive sample set than previous analysis

(Kanda *et al.*, 2009; 2013), but contingency table analyses did not detect genetic heterogeneity when samples collected in the eastern and western sector of the North Pacific were compared. The results of the STRUCTURE analyses presented in SC/66b/SD01 were consistent with the results of the previously conducted heterogeneity test.

In discussion, the SDWG noted that, similar to the case with western North Pacific Bryde's whales and several other whales assessed by the IWC SC, the lack of sei whale samples from breeding areas makes discriminating between stock structure hypotheses difficult. In addition, all of the sei whale samples analysed were collected within the North Pacific pelagic area, and no genetic data has been generated to represent the other four regions that have been proposed to represent distinct stocks on the basis of mark-recapture data (Mizroch *et al.*, 2015). In response to a question about whether genetic samples from areas outside of the North Pacific pelagic stock's range exist, it was noted that baleen plates from whales in the northeastern Pacific were housed at the Smithsonian at one time, but their location within that collection has not been confirmed. Although it is not currently possible to test the validity of the multi-stock hypothesis using genetic analysis, the SDWG **agreed** that the genetic and mark-recapture data currently available are consistent with a single stock in the pelagic region of the North Pacific.

4. TERMINOLOGY APPROPRIATE TO STOCK DEFINITION, UNIT-TO-CONSERVE AND 'VIABLE' POPULATION

Following a recommendation arising in 2012 (IWC, 2012, p.219), the SDWG began working on compiling a 'go-to' glossary of stock related terms, with the aim of encouraging consistent use of stock related terms within Scientific Committee reports and in papers submitted to the Scientific Committee. Initial work on this glossary focused on defining terms most commonly used in assessments of baleen whales. At SC/65b and SC/66a, joint sessions of the SDWG and the Small Cetaceans (SM) sub-committee were held to evaluate how the terms in this glossary aligned with terminology used in the SM sub-committee discussions (IWC, 2015, p.231; 2016, p.290). Several concerns were raised regarding the application of these terms to small cetaceans, largely due to differences in the behaviour and life history of small cetaceans relative to baleen whales.

No new items were discussed on this topic during SC/66b. In order to make progress on this item next year, the SDWG decided that the intersessional e-mail group formed under Lang at SC/66a should be continued (see Item 6.3 and Annex V). This e-mail group will continue to work on the development of a 'Rosetta Stone' of stock structure related terms used by the different sub-committees and working groups as well as by relevant outside groups (e.g. the IUCN) that would identify equivalencies between terms. Once compiled, this 'Rosetta Stone' would highlight where changes in terminology might be made to improve consistency of usage.

5. SIMULATION-BASED APPROACHES TO EVALUATE STOCK STRUCTURE, INCLUDING TOSSM (TESTING OF SPATIAL STRUCTURE MODELS)

TOSSM was developed with the intent of testing the performance of genetic analytical methods in a management context using simulated genetic datasets (Martien *et al.*,

2009), and more recently the TOSSM dataset generation model has been used to create simulated datasets to allow the plausibility of different stock structure hypotheses to be tested (Archer *et al.*, 2010; Lang and Martien, 2012). The SDWG noted that TOSSM has been particularly valuable in informing the interpretation of results of stock structure related analyses, but it has not been widely utilised outside of the IWC SC.

At SC/66a, Archer reported that he had attended a recent workshop focused on creating an R package that will guide users through the workflow of implementing simulations in population genetic questions. This R package would integrate Rmetasim (Strand, 2002), the package that creates the density-dependent individual-based model used for generating simulated datasets in TOSSM, into a more user-friendly package and would have some overlap with the functionality of TOSSM. At SC/66b, it was reported that the code underlying this new package has been completed and is expected to be available soon. A paper describing the functionality of the package has been submitted and will be published in the next year. The SDWG expressed their appreciation for this effort, which may allow the TOSSM framework to be utilised by a wider audience, and looks forward to reviewing this work in the future.

In discussion of the use of ordination-based methods (e.g. DAPC, PCA) to elucidate structure in North Pacific minke and Bryde's whales, the SDWG noted that there has been little effort to evaluate how well these methods work in cases where differentiation between groups is weak. Testing of these methods using a simulation-based approach, such as the TOSSM framework, would allow an evaluation of the utility of these approaches in identifying heterogeneity when effect size is small. The SDWG **encourages** such testing in light of the recommendations that have been made.

As in past years, the SDWG noted that a wide-range of simulation-based software is currently available and may have utility to the group. This year, the SDWG's TOSSM agenda item was expanded to include review of a wider range of simulation tools. As such, the SDWG looks forward to reviewing papers demonstrating the utility of simulation-based approaches to inform stock structure questions in future sessions.

6. WORK PLAN

6.1 Genetic analysis guidelines

The genetic analysis guidelines are anticipated to be completed intersessionally (convened under Waples; see Annex V) and should be ready to circulate within the Scientific Committee at SC/67a.

6.2 DNA quality guidelines

An intersessional e-mail group was formed to discuss updating the DNA quality guidelines to include data, including SNPs, produced using next generation sequencing (NGS) approaches. Topics to be addressed include analytical procedures to process the raw NGS data (trimming, filter settings, etc.) as well as issues arising from biological phenomena related to the markers of choice (e.g. linkage, selection vs neutrality, locus orthology). The group was convened under Tiedemann (see Annex V for members and Terms of Reference).

6.3 Stock definition terminology

An intersessional e-mail group was formed to identify stock-related terms used by different sub-committees and working

Table 1
Summary of the work plan for the Working Group on Stock Definition.

Item	Intersessional 2016/17	2017 Annual Meeting (SC/67a)
2.1 Guidelines for genetic data analysis	Finalise guidelines.	Prepare guidelines for inclusion on IWC website; discuss publication possibilities.
2.2 DNA quality guidelines	Intersessional e-mail group to discuss updating guidelines to include data produced using next generation sequencing approaches.	Review intersessional progress.
3. Statistical and genetic issues relating to stock definition		Review relevant papers and provide advice as requested.
4. Terminology	Intersessional e-mail group to discuss: (1) terminology used by various groups; and (2) identification of 'equivalencies' and suggestion for consistency.	Review intersessional progress.
5. Simulation-based approaches relevant to stock structure		Review new information on use of TOSSM or other simulation-based tools that provide insight into stock structure.

groups as well as by relevant outside groups (e.g. the IUCN). Once a list of relevant terms has been compiled, equivalencies between terms can be identified and modifications to better align terminology used by the different sub-committees and working groups can be proposed. The group was convened under Lang (see Annex V for members and Terms of Reference) and will report on progress at SC/67a.

8. ADOPTION OF REPORT

This report was adopted at 20:24 hrs on 15 June 2016.

REFERENCES

- Alexander, A., Steel, D., Hoekzema, K., Mesnick, S., Engelhaupt, D., Kerr, I., Payne, R. and Baker, S. 2016. What influences the worldwide genetic structure of sperm whales (*Physeter macrocephalus*)? *Mol. Ecol.* 25(12): 2754-72.
- Amaral, J., Loo, H., Jaris, C., Olavarria, D., Thiele, P., Ensor, A., Aguayo, H. and Rosenbaum, C. 2016. Population genetic structure among feeding aggregations of humpback whales in the Southern Ocean. *Mar. Biol.* 163: 132pp.
- Archer, F.I., Martien, K.K., Taylor, B.L., LeDuc, R.G., Ripley, B.J., Givens, G.H. and George, J.C. 2010. A simulation-based approach to evaluating population structure in non-equilibrium populations. *J. Cetacean Res. Manage.* 11(2): 101-14.
- Attard, C.R.M., Beheregaray, L.B. and Möller, L.M. 2016. Towards population-level conservation in the critically endangered Antarctic blue whale: the number and distribution of their populations. *Sci. Rep.* 6: 22291.
- Bando, T. and Hakamada, T. 2016. Morphometric analysis on stock structure of the O stock common minke whale in the western North Pacific. Paper SC/F16/JR41 presented to the Expert Panel Workshop of the Final Review on the Western North Pacific Japanese Special Permit Programme (JARPN II), 22-26 February 2016, Tokyo, Japan (unpublished). 11pp. [Paper available from the Office of this Journal].
- Carroll, E., Patenaude, N., Alexander, A., Steel, D., Harcourt, R., Childerhouse, S., Smith, S., Bannister, J., Constantine, R. and Baker, C.S. 2011. Population structure and individual movement of southern right whales around New Zealand and Australia. *Mar. Ecol. Prog. Ser.* 432: 257-68.
- Carroll, E.L., Baker, C.S., Watson, M., Alderman, R., Bannister, J., Gaggiotti, O.E., Grocke, D.R., Patenaude, N. and Harcourt, R. 2015. Cultural traditions across a migratory network shape the genetic structure of southern right whales around Australia and New Zealand. *Nat. Sci. Rep.* 5: 16182.
- Carroll, E.L., Childerhouse, S.J., Fewster, R.M., Patenaude, N.J., Steel, D., Dunshea, G., Boren, L. and Baker, C.S. 2013. Accounting for female reproductive cycles in a superpopulation capture-recapture framework. *Ecol. Appl.* 23: 1677-90.
- Elvarsson, B. 2014. Evaluating stock structure hypotheses using genetically determined close relatives: a simulation study on North Atlantic fin whales. *ICES J. Mar. Sci.*
- Gaggiotti, O.E. and Gascuel, F. 2011. Stock structure of North Pacific minke whales as revealed by Principal Component Analyses of microsatellite data. Paper SC/63/RMP23 presented to the IWC Scientific Committee, June 2011, Tromsø, Norway (unpublished). 7pp. [Paper available from the Office of this Journal].
- International Whaling Commission. 2007a. Report of the Scientific Committee. *J. Cetacean Res. Manage. (Suppl.)* 9:1-73.
- International Whaling Commission. 2007b. Western North Pacific Bryde's *Implementation*: Report of the First Intersessional Workshop, 25-29 October 2005, Shizuoka, Japan. *J. Cetacean Res. Manage. (Suppl.)* 9:407-27.
- International Whaling Commission. 2008. Report of the Scientific Committee. *J. Cetacean Res. Manage. (Suppl.)* 10:1-74.
- International Whaling Commission. 2009. Report of the Scientific Committee. *J. Cetacean Res. Manage. (Suppl.)* 11:1-74.
- International Whaling Commission. 2010. Report of the Expert Workshop to Review the Ongoing JARPN II Programme, 26-30 January 2009, Yokohama, Japan. *J. Cetacean Res. Manage. (Suppl.)* 11(2):405-50.
- International Whaling Commission. 2011. Report of the Scientific Committee. Annex F. Report of the Sub-Committee on Bowhead, Right and Gray Whales. *J. Cetacean Res. Manage. (Suppl.)* 12:168-84.
- International Whaling Commission. 2012. Report of the Scientific Committee. Annex I. Report of the Working Group on Stock Definition. *J. Cetacean Res. Manage. (Suppl.)* 13:217-20.
- International Whaling Commission. 2015. Report of the Scientific Committee. Annex I. Report of the Working Group on Stock Definition. *J. Cetacean Res. Manage. (Suppl.)* 16:222-35.
- International Whaling Commission. 2016. Report of the Scientific Committee. Annex I. Report of the Working Group on Stock Definition. *J. Cetacean Res. Manage. (Suppl.)* 17:283-92.
- Jackson, J.A., Carroll, E.L., Smith, T.D., Zerbini, A.N., Patenaude, N.J. and Baker, C.S. 2016. An integrated approach to historical population assessment of the great whales: case of the New Zealand southern right whale. *Royal Soc. Open Sci.* 3: 150669.
- Kanda, N., Goto, M., Yoshida, H. and Pastene, L.A. 2009. Stock structure of sei whales in the North Pacific as revealed by microsatellite and mitochondrial DNA analyses. Paper SC/J09/JR32 presented to the Expert Workshop to Review Results of JARPN II, 26-30 January 2009, Tokyo, Japan (unpublished). 14pp. [Paper available from the Office of this Journal].
- Kanda, N., Matsuoka, K., Yoshida, H. and Pastene, L.A. 2013. Microsatellite DNA analysis of sei whales obtained from the 2010-2012 IWC-POWER. Paper SC/65a/IA05 presented to the IWC Scientific Committee, June 2013, Jeju Island, Republic of Korea (unpublished). 6pp. [Paper available from the Office of this Journal].
- Kitakado, T. and Maeda, H. 2016. Fitting to catch-at-age data for North Pacific common minke whales in the Pacific side of Japan. Paper SC/F16/JR43 presented to the Expert Panel Workshop of the Final Review on the Western North Pacific Japanese Special Permit Programme (JARPN II), 22-26 February 2016, Tokyo, Japan (unpublished). 16pp. [Paper available from the Office of this Journal].
- Lang, A.R. and Martien, K.K. 2012. Update on the use a simulation-based approach to evaluate plausible levels of recruitment into the Pacific Coast Feeding Group of gray whales. Paper SC/64/AWMP4 presented to the IWC Scientific Committee, June 2012, Panama City (unpublished). 34pp. [Paper available from the Office of this Journal].
- Martien, K.K., Gregovich, D., Bravington, M.V., Punt, A.E., Strand, A.E., Tallmon, D.A. and Taylor, B.L. 2009. TOSSM: an R package for assessing performance of genetic analytical methods in a management context. *Mol. Ecol. Res.* 9(6): 1,456-59.
- Meschersky, I., Kuleshova, M., Litovka, D., Burkanov, V.N., Andrews, R., Tsidulko, G.A., Rozhnov, V.V. and Ilyashenko, V.Y. 2015. Occurrence and distribution of mitochondrial lineages of gray whales (*Eschrichtius robustus*) in Russian Far Eastern seas. *Biology Bulletin* 42: 34-42.
- Mizroch, S.A., Conn, P.B. and Rice, D.W. 2015. The mysterious sei whale: its distribution, movements and population decline in the North Pacific revealed by whaling data and recoveries of Discovery-type marks. Paper SC/66a/IA14 presented to the IWC Scientific Committee, May 2015, San Diego, CA, USA (unpublished). 113pp. [Paper available from the Office of this Journal].

- Morin, P.A., Pease, V.L., Hancock, B.L., Robertson, K.M., Antolik, C.W. and Huebinger, R.M. 2010. Characterization of 42 SNP markers for the bowhead whale (*Balaena mysticetus*) for use in discriminating populations. *Mar. Mamm. Sci.* 26: 716-32.
- Økland, J.-M., Haaland, Ø.A. and Skaug, H.J. 2010. A method for defining management units based on genetically determined close relatives. *ICES J. Mar. Sci.* 67: 551-58.
- Pampoulie, C., Ólafsdóttir, G., Hauksdóttir, S., Skirinsdóttir, S., Ólafsson, K., Magnúsdóttir, S., Chosson, V., Halldorson, S.D., Ólafsdóttir, D., Gunnlaugsson, T., Danielsdóttir, A.K. and Víkingsson, G.A. 2012. A note on a mother-foetus pair and alleged father match in the Atlantic fin whale (*Balaenoptera physalus*) off Iceland. *J. Cetacean Res. Manage* 12(2): 341-43.
- Pastene, L.A., Goto, M., Taguchi, M. and Kitakado, T. 2016a. Genetic analyses based on mtDNA control region sequencing and microsatellite DNA confirmed the occurrence of a single stock of sei whales in oceanic regions of the North Pacific. Paper SC/F16/JR46 presented to the Expert Panel Workshop of the Final Review on the Western North Pacific Japanese Special Permit Programme (JARPN II), 22-26 February 2016, Tokyo, Japan (unpublished). 11pp. [Paper available from the Office of this Journal].
- Pastene, L.A., Goto, M., Taguchi, M. and Kitakado, T. 2016b. Temporal and spatial distribution of the 'J' and 'O' stocks of common minke whale in waters around Japan based on microsatellite DNA. Paper SC/F16/JR38 presented to the Expert Panel Workshop of the Final Review on the Western North Pacific Japanese Special Permit Programme (JARPN II), 22-26 February 2016, Tokyo, Japan (unpublished). 15pp. [Paper available from the Office of this Journal].
- Pastene, L.A., Goto, M., Taguchi, M. and Kitakado, T. 2016c. Updated genetic analyses based on mitochondrial and microsatellite DNA indicated no sub-structure of the 'O' stock common minke whale in the western North Pacific. Paper SC/F16/JR40 presented to the Expert Panel Workshop of the Final Review on the Western North Pacific Japanese Special Permit Programme (JARPN II), 22-26 February 2016, Tokyo, Japan (unpublished). 20pp. [Paper available from the Office of this Journal].
- Puechmaille, S.J. 2016. The program STRUCTURE does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. *Mol. Ecol. Res.* 16: 608-27.
- Strand, A.E. 2002. METASIM 1.0: an individual-based environment for simulating population genetics of complex population dynamics. *Mol. Ecol. Notes* 3: 373-76.
- Tiedemann, R., Tiedemann, M.R., Gunnlaugsson, T., Pampoulie, C. and Víkingsson, G. 2014. Finding relatives among North Atlantic common minke whales (*Balaenoptera acutorostrata*) based on microsatellite data: the relationship between false discovery rate (FDR) and detection power. Paper SC/65b/RMP05 presented to the IWC Scientific Committee, May 2014, Bled, Slovenia (unpublished). 8pp. [Paper available from the Office of this Journal].
- Valenzuela, L.O., Sironi, M., Rowntree, V.J. and Seger, J. 2009. Isotopic and genetic evidence for culturally inherited site fidelity to feeding grounds in southern right whales (*Eubalaena australis*). *Mol. Ecol.* 18(5): 782-79.
- Waples, R.S. 2015. Testing for Hardy-Weinberg proportions: have we lost the plot? *J. Hered.* 106(1): 1-19.
- Whitehead, H., Antunes, R., Gero, S., Wong, S.N.P., Engelhaupt, D. and Rendell, L. 2012. Multilevel societies of female sperm whales (*Physeter macrocephalus*) in the Atlantic and Pacific: why are they so different? *Int. J. Primat.* 33: 1142-64.

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 and methods for genetic studies and DNA data quality
 - 2.1 Guidelines for genetic data analysis
 - 2.2 DNA quality
 - 2.3 Other issues
3. Statistical and genetic issues relating to stock definition
 - 3.1 Population structuring and migration rates
 - 3.1.1 Bowhead, right, and gray whales
 - 3.1.2 Other Southern Hemisphere whale stocks
 - 3.1.2.1 Right whales
 - 3.1.3 In-depth Assessment
 - 3.1.3.1 Sperm whales
 - 3.2 Population assignment and mixing
 - 3.2.1 Other Southern Hemisphere whale stocks
 - 3.2.1.1 Blue whales
 - 3.2.1.2 Humpback whales
 - 3.2.2 Western North Pacific Japanese Scientific Permit Programme (JARPN II)
 - 3.2.2.1 North Pacific minke whales
 - 3.2.2.2 North Pacific Bryde's whales
 - 3.2.2.3 North Pacific sei whales
4. Terminology appropriate to stock definition, unit-to- conserve and 'viable' population
5. Simulation-based approaches to evaluate stock structure, including TOSSM (Testing of Spatial Structure Models)
6. Work plan
 - 6.1 Genetic analysis guidelines
 - 6.2 DNA quality guidelines
 - 6.3 Stock definition terminology

Appendix 2

RMP SUB-COMMITTEE REQUEST: REVIEW OF NEW INFORMATION ON NORTH PACIFIC COMMON MINKE WHALES AND BRYDE'S WHALES

[The Working Group on Stock Definition is requested to] Evaluate the new information on stock structure for North Pacific common minke whales (and Bryde's whales) to advise on the information and analyses provided including whether or not they are sufficient to warrant a revision of current hypotheses (either by narrowing or increasing

the number of hypotheses or changing boundaries). The evaluation may include suggestions for additional work and should include comments on the view of the Expert Panel as well as the papers presented to the Workshop or the present meeting.

Appendix 3

A SUMMARY OF THE NEW INFORMATION ON STOCK STRUCTURE IN THE WESTERN NORTH PACIFIC COMMON MINKE WHALES PRESENTED TO THE JARPN II FINAL REVIEW

Luis A. Pastene

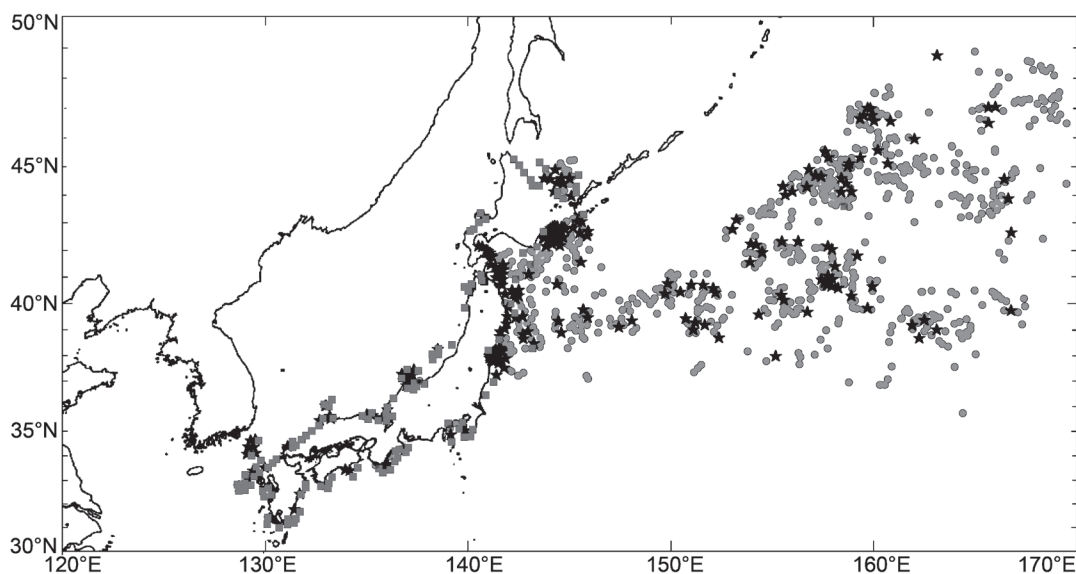


Fig. 1a. Locations of the common minke whales that were assigned to O stock (grey circles), J stock (grey squares), and unknown (black stars), based on STRUCTURE.

The relevant documents presented to the review meeting were Pastene *et al.* (2016a), Pastene *et al.* (2016b), Bando and Hakamada (2016) and Kitakado and Maeda (2016).

Pastene *et al.* (2016a) examined a total of 4,275 western North Pacific common minke whales with a set of 16 microsatellite DNA loci and the program STRUCTURE to assign individual to either 'J' or 'O' stocks. The relevant information in this paper for the discussion in the SDWG was on the unassigned individuals in the STRUCTURE analyses. A simple simulation exercise showed that the number of unassigned individuals decreased with the increase in the number of microsatellite loci used, and they were widely distributed geographically (Fig. 1). They concluded that the unassigned individuals are not related with the occurrence of additional stock structure. Based on these results, the authors considered that only the animals assigned to the 'O' stock with assignment probability greater than 90% could be used to investigate additional structure with the 'O' stock using alternatively analytical approaches.

Pastene *et al.* (2016b) examined the genetic population structure of 'O' stock common minke whale in the western North Pacific based on mitochondrial DNA control region sequencing (487bp) and microsatellite DNA (16 loci). Samples used in the tests of homogeneity were obtained during the surveys of the JARPN and JARPN II in sub-areas of the Pacific side of Japan between 1994 and 2014 ($n=2,071$ for microsatellite; $n=2,070$ for mtDNA). Whales were assigned to the 'O' stock by the analysis of STRUCTURE presented in Pastene *et al.* (2016a). Tests based on both genetic markers and different grouping of the samples showed no evidence of sub-structuring in the 'O' stock common minke whale in the Pacific side of Japan. A simulation exercise showed that the statistical power of the homogeneity test was high. In addition, a Discriminant Analysis of Principal Components (DAPC) based on the total samples used in Pastene *et al.* (2016a) showed clear differentiation between 'J' and 'O' stock whales but no evidence of sub-structuring within the 'O' stock samples. Consequently the results of this study suggested a low plausibility for the hypothesis of sub-division of the 'O' stock common minke whale into 'Ow' and 'Oe'.

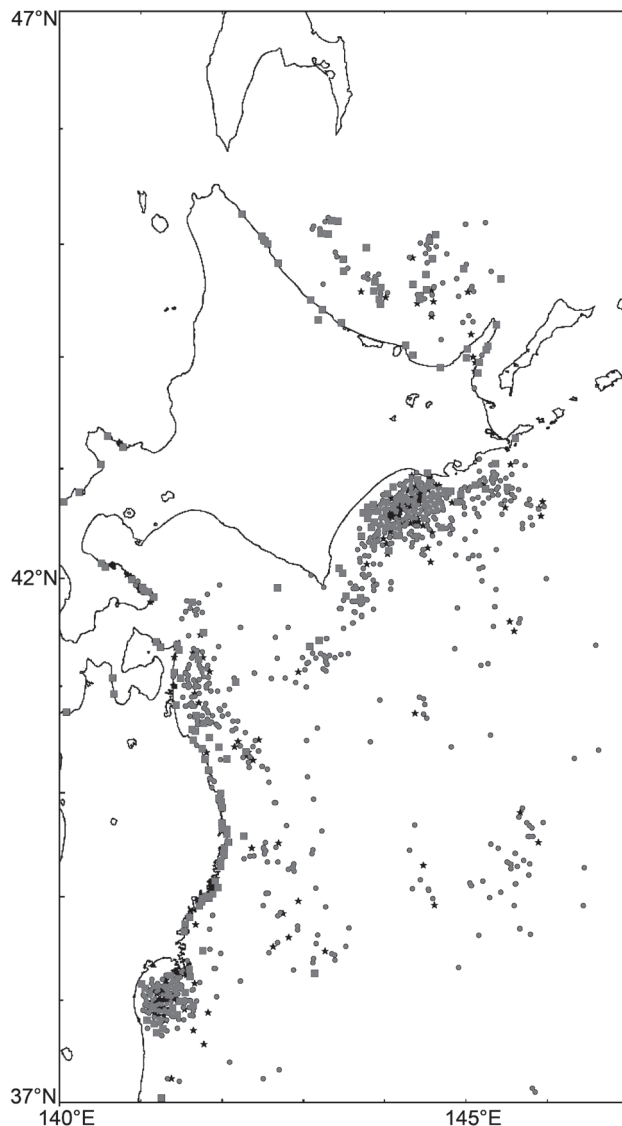


Fig. 1b. Zoomed in to show locations in the coastal area shown in Fig. 1a.

Bando and Hakamada (2016) conducted a morphometric analyses to examine stock structure of western North Pacific common minke whales by using external measurement data collected during 1994 and 2014 JARPN and JARPN II surveys. External measurements of mature males were first compared between 'O' and 'J' stock animals assigned by the microsatellite DNA analysis. Then only 'O' stock animals were compared among sub-areas. The analytical procedures used were the Analysis of Covariance (ANCOVA) and Discriminant Analysis (DA). Significant differences were detected between 'O' and 'J' stock whales. 'J' stock animals had longer head region compared to 'O' stock animals. No significant differences were detected in 'O' stock animals among sub-areas. The results of the present morphometric analyses provided no evidences for sub-structuring of the 'O' stock into 'Ow' and 'Oe' as proposed in one of the hypotheses used in the RMP *Implementation*, as common minke whales from coastal and offshore sub-areas did not differ in morphometric characters.

Kitakado and Maeda (2016) used catch-at-age data for common minke whales in the western North Pacific provided by the JARPN/JARPNII program to refine existing RMP *Implementation Simulation Trials (ISTs)* in a simple way, so as to investigate the relative plausibility of the single- and two (Ow and Oe) stock hypotheses for the 'O' stock whales in the Pacific side of Japan. While the single stock scenario seems consistent with these age data, it is difficult to reconcile the two stock hypothesis with these data because of the relative absence of particularly younger whales in a supposedly separate discrete 'Oe' stock. The analysis demonstrates the importance for management purposes of obtaining age data for the minke whales in the western North Pacific, which in turn necessitates lethal sampling. Such age data need to be incorporated in the conditioning of revised RMP *ISTs* for common minke whales in this region.

During the JARPN II final review meeting the proponents informed on preliminary results on kinship analyses. Preliminary results of the analysis of the total samples of over 4,000 animals, found a total of 22 parent-offspring pairs

for the 'O' stock. Half of the pairs showed one in the coastal and the other in the offshore area. This work is in progress and a paper will be prepared for future meeting

It is considered that a substantial amount of new information on stock structure of common minke whale has been accumulated since the last *Implementation Review*, which was based on data collected till 2007. In particular they noted the larger number of new samples (around 1,700), new analytical procedure (DAPCA, kinship, statistical power of the heterogeneity test) and the availability of age data.

Most of the analyses in the documents summarised above responded to recommendations made during the 2009 JARPN II mid-term review. According to them, a parsimonious interpretation of all different analyses and results point out to a single 'O' stock distributed from the Japanese coast till approximately 170°E.

REFERENCES

- Bando, T. and Hakamada, T. 2016. Morphometric analysis on stock structure of the O stock common minke whale in the western North Pacific. Paper SC/F16/JR41 presented to the Expert Panel Workshop of the Final Review on the Western North Pacific Japanese Special Permit Programme (JARPN II), 22-26 February 2016, Tokyo, Japan (unpublished). 11pp. [Paper available from the Office of this Journal].
- Kitakado, T. and Maeda, H. 2016. Fitting to catch-at-age data for North Pacific common minke whales in the Pacific side of Japan. Paper SC/F16/JR43 presented to the Expert Panel Workshop of the Final Review on the Western North Pacific Japanese Special Permit Programme (JARPN II), 22-26 February 2016, Tokyo, Japan (unpublished). 16pp. [Paper available from the Office of this Journal].
- Pastene, L.A., Goto, M., Taguchi, M. and Kitakado, T. 2016a. Temporal and spatial distribution of the 'J' and 'O' stocks of common minke whale in waters around Japan based on microsatellite DNA. Paper SC/F16/JR38 presented to the Expert Panel Workshop of the Final Review on the Western North Pacific Japanese Special Permit Programme (JARPN II), 22-26 February 2016, Tokyo, Japan (unpublished). 15pp. [Paper available from the Office of this Journal].
- Pastene, L.A., Goto, M., Taguchi, M. and Kitakado, T. 2016b. Updated genetic analyses based on mitochondrial and microsatellite DNA indicated no sub-structure of the 'O' stock common minke whale in the western North Pacific. Paper SC/F16/JR40 presented to the Expert Panel Workshop of the Final Review on the Western North Pacific Japanese Special Permit Programme (JARPN II), 22-26 February 2016, Tokyo, Japan (unpublished). 20pp. [Paper available from the Office of this Journal].

Appendix 4

RESPONSE TO SC/F16/JR40 REGARDING NEW INFORMATION ON NORTH PACIFIC MINKE WHALE POPULATION STRUCTURE

P.R. Wade and C.S. Baker

Pastene *et al.* (2016b) summarised genetic analyses presented to the JARPN II Expert Panel Review. The authors suggest these analyses show there is no sub-structure within 'O' type minke whales. However, our view is that these results are consistent with results previously presented by Pastene and colleagues at the last *Implementation* that also showed no sub-structure within 'O' type minke whales. In contrast, alternative analyses presented at the last *Implementation* (summarised in Wade and Baker, 2012) did show sub-structure within 'O' type minke whales; no such alternative analyses were presented to the Expert Panel.

Here we summarise a major issue (in our view) regarding handling of the data, and briefly comment on the genetic analyses and the spatial distribution of age classes.

1. Data 'purging'

In Pastene *et al.* (2016b), as in previous analyses presented by Pastene and colleagues at the last *Implementation*, the

genetic analyses showing no structure within 'O' type whales have been carried out on a data set that has been 'purged' of individuals assigned to 'J' type in an analysis using the program STRUCTURE, but also of individuals that did not assign with high probability to either type. The unassigned (discarded) samples represent ~10% of the total data set. It can be seen in Pastene *et al.* (2016a, fig. 3a) that what appears to be the majority of the unassigned (discarded) samples are exactly in the area where the putative 'Ow' stock occurs (along the east coast of Honshu and Hokkaido) (see Appendix 3, Fig. 1). The STRUCTURE analysis has identified the largest signal in the data, which is the large difference between 'J' type and 'O' type minke whales. It is our view that the unassigned samples potentially represent important heterogeneity within 'O' type whales, and that these samples should not be discarded from the analysis. If only individuals assigned to 'J' type were discarded (the 'Without-J' data set), the unassigned samples would

represent ~15% of the 'Without-J' data set, and would represent a much higher percent of the available samples in the putative 'Ow' stock area. Therefore, these discarded samples could be highly influential to the analysis. This is the explanation for why one set of analyses shows no structure, while a different set of analyses does show structure (see below), and why there are differing views on what one can conclude from the data.

2. Hypothesis testing

In Pastene *et al.* (2016b), the hypothesis test showing no structure within 'O' type whales test (comparing sub-area 7 to the west with sub-areas 8/9 to the east) was conducted on the 'O-only' data set that has been 'purged' of unassigned individuals. We note that the summary in Wade and Baker (2012) included analyses of 'unpurged' data set (undertaken independently through a Data Availability Agreement with IWC) that do show significant heterogeneity within 'O' type whales.

3. Spatial analyses

In Pastene *et al.* (2016b) a Discriminant Analysis of Principle Components DAPC was applied to the 'purged' data set ('O-only'), where individuals unassigned to either type were discarded from the analysis. In the previous *Implementation*, Gaggiotti and Gascuel (2011) found evidence for sub-structure within 'O' type whales using the 'Without-J' data set, so again the different result is due to using a different data set. Another difference is that Gaggiotti and Gascuel (2011) used a Principle Components Analysis (PCA), not a Discriminant Analysis of Principle Components (DAPC) as has been used in Pastene *et al.* (2016b), so the analysis of Gaggiotti and Gascuel has not been replicated.

4. Statistical-Catch-At-Age (SCAA) analysis

Kitakado and Maeda (2016) have results from a SCAA calculation that suggests an age structure consistent with a 'complete' population was sampled in the 'Ow' area, but an apparent partial deficit of the youngest whales and adult females (up to age 20) was sampled in the 'Oe' area, and they interpret this as evidence against population structure. However, the authors themselves note that this '...might in part be explained by some mature females having entered the Okhotsk Sea before the JARPN/JARPN II sampling further to the southeast took place,...'. In fact, at the beginning of the last *Implementation* Pastene *et al.* (2010) provided a narrative description of the migration patterns of 'O' type minke whales in which they state 'Mature females enter the Okhotsk Sea in April and May and then move further to the middle and northern Okhotsk Sea.' We roughly calculate that only ~15% of the sampled whales were taken prior to June during JARPN II, suggesting one would expect a deficit of adult females (and dependent calves, the youngest whales). We hypothesised that with two 'O' type stocks the 'Oe' whales migrate further north (into the Sea of Okhotsk) than 'Ow' whales, which are thought to move only into waters just north of Hokkaido (see fig. 2 in Wade and Baker, 2012). Therefore, coastal sampling in summer (in the 'Ow' area), which includes waters north of Hokkaido, would find adult

females, whereas offshore sampling in summer (in the 'Oe' area) would not. Therefore, it is entirely plausible that adult female and young whales have moved further north prior to JARPN/JARPN II sampling in the offshore area, which would make the observed data entirely compatible with a hypothesis of two 'O' stocks. This again highlights the difficulties of only sampling populations while on migration, rather than on their breeding or feeding grounds.

Conclusion

The genetic analyses in Pastene *et al.* (2016b) were conducted on the purged data set ('O-only'). At the last *Implementation*, analyses conducted on the 'O-only' data set similarly showed no structure, so the results presented at the JARPA II Expert Panel Review do not represent new results or new information. It will be necessary to examine alternative analyses, such as analysis of the 'Without-J' data set, as was done at the previous *Implementation*, before reaching any final conclusion on stock structure.

We reiterate that finding conclusive evidence to fully resolve North Pacific minke whale stock structure is difficult given that no samples have been collected on the putative breeding grounds in winter when presumably 'pure' stocks would exist. Instead, the primary information on population structure comes from genetic data collected from year-round coastal bycatch and commercial and scientific hunting during migration when stocks are potentially mixed, substantially complicating analyses and interpretation.

REFERENCES

- Gaggiotti, O.E. and Gascuel, F. 2011. Stock structure of North Pacific minke whales as revealed by Principal Component Analyses of microsatellite data. Paper SC/63/RMP23 presented to the IWC Scientific Committee, June 2011, Tromsø, Norway (unpublished). 7pp. [Paper available from the Office of this Journal].
- Kitakado, T. and Maeda, H. 2016. Fitting to catch-at-age data for North Pacific common minke whales in the Pacific side of Japan. Paper SC/F16/JR43 presented to the Expert Panel Workshop of the Final Review on the Western North Pacific Japanese Special Permit Programme (JARPN II), 22-26 February 2016, Tokyo, Japan (unpublished). 16pp. [Paper available from the Office of this Journal].
- Pastene, L.A., Goto, M. and Kanda, N. 2010. Specifications of plausible stock structure hypotheses for western North Pacific common minke whales. Paper SC/S10/NPM1 presented to the Preparatory Meeting for the First Intersessional Workshop for Western North Pacific Common Minke Whales, 25-27 September 2010, Tokyo, Japan (unpublished). 11pp. [Paper available from the Office of this Journal].
- Pastene, L.A., Goto, M., Taguchi, M. and Kitakado, T. 2016a. Temporal and spatial distribution of the 'J' and 'O' stocks of common minke whale in waters around Japan based on microsatellite DNA. Paper SC/F16/JR38 presented to the Expert Panel Workshop of the Final Review on the Western North Pacific Japanese Special Permit Programme (JARPN II), 22-26 February 2016, Tokyo, Japan (unpublished). 15pp. [Paper available from the Office of this Journal].
- Pastene, L.A., Goto, M., Taguchi, M. and Kitakado, T. 2016b. Updated genetic analyses based on mitochondrial and microsatellite DNA indicated no sub-structure of the 'O' stock common minke whale in the western North Pacific. Paper SC/F16/JR40 presented to the Expert Panel Workshop of the Final Review on the Western North Pacific Japanese Special Permit Programme (JARPN II), 22-26 February 2016, Tokyo, Japan (unpublished). 20pp. [Paper available from the Office of this Journal].
- Wade, P.R. and Baker, C.S. 2012. High plausibility of Hypothesis III for stock structure of the western North Pacific minke. Paper SC/64/NPM11 presented to the IWC Scientific Committee, June 2012, Panama City (unpublished). 7pp. [Paper available from the Office of this Journal].

Appendix 5

NORTH PACIFIC MINKE WHALE STOCK STRUCTURE REDUX

Robin Waples and Rus Hoelzel

Before commenting on the new analyses related to North Pacific minke whale stock-structure hypotheses, a couple of points should be made. First, the following statement, which appears in Pastene *et al.* (2016): ‘their [the five geneticists] evaluation was based on the available genetic information only despite plenty of non-genetic information was available for the *IR*’, is misleading. It is true that Hoelzel *et al.* (2013b) is based only on consideration of genetic data; this was prepared in response to a request from the Scientific Committee for a table like this. But that does not mean that non-genetic information was ignored; that information was summarised by others in the *Implementation Review*. Furthermore, Hoelzel *et al.* (2013a), which was prepared by a subset of the five geneticists, explicitly considered morphological and life history information but did not find any that was relevant to evaluating heterogeneity within ‘O’ stock.

Second, it is important to keep in mind the distinction between a finding of heterogeneity in ‘O stock’ whales, as reported in Gaggiotti and Gascuel (2011) and Gaggiotti and Durand (2010) and an evaluation of a specific version of a two ‘O’ stock hypothesis, as was conducted in Pastene *et al.* (2016). Even if a specific two-stock hypothesis is rejected, that does not by itself invalidate the finding of more heterogeneity within ‘O’ stock individuals than can be explained by sampling from a single panmictic population.

COMMENTS ON PAPERS PRESENTED TO THE JARPN II REVIEW PANEL

These papers were very useful and responsive to recommendations from previous review panels and workshops for additional types of analyses to consider.

Bando and Hakamada (2016) found no differences in morphometric characters between different sub-areas of ‘O’ stock, or between the putative ‘Oe’ and ‘Ow’ stocks. This is useful information. However, given that if two ‘O’ stocks exist they must be genetically very similar, it is not clear whether any detectable morphometric differences would be expected.

Kitakado and Maeda (2016) used catch-at-age data to show that younger whales are significantly under-represented in the ‘Oe’ area, which according to one hypothesis is occupied by a discrete stock. The relevance of this result for that particular hypothesis was discussed in a working paper by Wade and Baker. It should be noted, however, that a two ‘O’ stock scenario in which young whales of both stocks migrate closer to Japan and older whales of one stock migrate farther offshore would be consistent with the observed results. Whether this is biologically plausible is a separate issue.

The power analyses in Pastene *et al.* (2016) are very informative and show that the experimental design had high power to detect genetic differences even if the effect size was very small ($F_{ST}=0.005$ or even a bit lower). This means that the many pairwise comparisons they did of samples from different sub-areas should have shown significant differences if: (a) the areas being compared contained pure ‘Oe’ and ‘Ow’ stocks; and (b) the parametric differences between them were $>$ about $F_{ST}=0.005$. In turn, the failure to find significant differences means that if two ‘Oe’ and ‘Ow’ stocks exist, that either: (a) they don’t occur exclusively in any particular sub-area; or (b) if they do, they are separated

by a parametric $F_{ST}<$ about 0.005. If ‘Oe’ and ‘Ow’ actually overlap substantially in geographic range, then pairwise comparisons like this might yield non-significant results, even if pure ‘Oe’ and ‘Ow’ are separated by $F_{ST}>0.005$.

Using DAPC, the authors could clearly distinguish between ‘O’ and ‘J’ stock when all individuals were used (except those not assigned to ‘O’ or ‘J’ at 90% or higher). When putative ‘J’ stock individuals were removed, the results for $k=2$ did not produce a result in which the two groups represented geographically contiguous samples; instead each potential cluster contained individuals from different geographic areas. On the other hand, for the PC analysis, all combinations of PCs shown in fig. 6 of Pastene *et al.* (2016) except 3 vs 4 showed 2 or more essentially separate clusters of individuals. It seems that this result might be consistent with 2 or more ‘O’-like stocks that had strongly overlapping geographic ranges but not with a scenario in which two populations are restricted to separate geographic areas.

When provided with two populations to which most individuals can be assigned with high probability, STRUCTURE could theoretically assign individuals belonging an additional, less well differentiated stock to one or other of the primary stocks, but with lower confidence. Given a threshold resolution of approximately $F_{ST}>0.005$, even for comparisons within ‘O’ stock alone, STRUCTURE may not have the power to identify those samples. However, DAPC may be able to identify these samples as distinct (possible from both ‘J’ stock and the remainder of ‘O’ stock, or the other way around). Therefore, while there is good justification for trying to control for ‘J’ stock animals when investigating possible substructure in ‘O’ stock, having done so, it would be useful to include all samples (no pruning) for both the comparison between ‘J’ and ‘O’ stocks, and for the comparison among all individuals from the ‘O’ stock geographic region, regardless of how they assign in STRUCTURE, using the DAPC method.

Some features of DAPC are worth noting. First, the maximum number of discriminant functions for k groups is $k-1$. This means that DAPC cannot be used to directly test $k=1$ (panmixia). This can only be done indirectly by showing that results for $k>1$ are not credible. It has also been shown through a simulation study that DAPC has the potential to over-cluster under certain conditions when the true k is one (IWC, 2015). We therefore commend the continued use of alternative ordination methods, such as PCA, in addition to DAPC.

Summary

Collectively, the new analyses are very useful in helping to refine our understanding of stock structure in North Pacific minke whales. The analyses appear to be inconsistent with some specific hypotheses about the distribution of whales in space and time. On the other hand, these analyses also appear to be potentially consistent with a scenario involving two ‘O’ stocks that have very similar distributions. We do not believe the new analyses invalidate the previous findings, based on PCA, of additional heterogeneity in ‘O’-like whales. Whether that heterogeneity is caused by presence (perhaps intermittently) of additional stock(s), or is due to other factors such as age structure, temporal changes, etc., remains an open question.

REFERENCES

- Bando, T. and Hakamada, T. 2016. Morphometric analysis on stock structure of the O stock common minke whale in the western North Pacific. Paper SC/F16/JR41 presented to the Expert Panel Workshop of the Final Review on the Western North Pacific Japanese Special Permit Programme (JARPN II), 22-26 February 2016, Tokyo, Japan (unpublished). 11pp. [Paper available from the Office of this Journal].
- Gaggiotti, O.E. and Durand, E. 2010. Update analyses of the genetic structure of western North Pacific minke whales. Paper SC/62/NPM30 presented to the IWC Scientific Committee, June 2010, Agadir, Morocco (unpublished). 30pp. [Paper available from the Office of this Journal].
- Gaggiotti, O.E. and Gascuel, F. 2011. Stock structure of North Pacific minke whales as revealed by Principal Component Analyses of microsatellite data. Paper SC/63/RMP23 presented to the IWC Scientific Committee, June 2011, Tromsø, Norway (unpublished). 7pp. [Paper available from the Office of this Journal].
- Hoelzel, R., Waples, R. and Gaggiotti, O. 2013a. Report of the Scientific Committee. Annex D1. Report of the Working Group on the *Implementation Review* for Western North Pacific Common Minke Whales. Appendix 6. Summary table showing sources of support from three data categories for different hypotheses. *J. Cetacean Res. Manage. (Suppl.)* 14: 133.
- Hoelzel, R., Waples, R., Gaggiotti, O., Palsbøll, P. and Tiedemann, R. 2013b. Report of the Scientific Committee. Annex D1. Report of the Working Group on the *Implementation Review* for Western North Pacific Common Minke Whales. Appendix 8. Consideration of stock plausibility relevant to Hypothesis B and C based only on genetic data. *J. Cetacean Res. Manage. (Suppl.)* 14: 135.
- International Whaling Commission. 2015. Report of the AWMP/RMP Joint Workshop on the Stock Structure of North Atlantic Common Minke Whales, 14-17 April 2014, Copenhagen, Denmark. *J. Cetacean Res. Manage. (Suppl.)* 16:543-58.
- Kitakado, T. and Maeda, H. 2016. Fitting to catch-at-age data for North Pacific common minke whales in the Pacific side of Japan. Paper SC/F16/JR43 presented to the Expert Panel Workshop of the Final Review on the Western North Pacific Japanese Special Permit Programme (JARPN II), 22-26 February 2016, Tokyo, Japan (unpublished). 16pp. [Paper available from the Office of this Journal].
- Pastene, L.A., Goto, M., Taguchi, M. and Kitakado, T. 2016. Updated genetic analyses based on mitochondrial and microsatellite DNA indicated no sub-structure of the 'O' stock common minke whale in the western North Pacific. Paper SC/F16/JR40 presented to the Expert Panel Workshop of the Final Review on the Western North Pacific Japanese Special Permit Programme (JARPN II), 22-26 February 2016, Tokyo, Japan (unpublished). 20pp. [Paper available from the Office of this Journal].