Annex I

Report of the Working Group on Stock Definition and DNA Testing

Members: Lang, Tiedemann (co-Convenors): Aoki, Archer, Baba, Baker, Buss, Butterworth, Castro, Cipriano, Debrah, Diallo, Donovan, Goto, Hoelzel, Hosoda, Jackson, Kishiro, Kitakado, Lee, Lent, Lundquist, Mallette, Morita, Moronuki, Nelson, Øien, Pastene, Punt, Reeves, Robbins, Simmonds, Širović, Suydam, Suzuki, Taguchi, Walters, Weller, Wilson, Yasokawa, Yoshida, Zerbini.

1. INTRODUCTORY ITEMS

1.1 Convenor's opening remarks

Lang and Tiedemann welcomed participants.

1.2 Election of Chair and appointment of Rapporteurs

Lang and Tiedemann were appointed as co-Chairs, and Cipriano acted as rapporteur.

1.3 Adoption of Agenda

The adopted Agenda is given in Appendix 1. Items 2.1, 2.3, and 2.4 of the Agenda are in response to requirements placed on the Scientific Committee by IWC Resolution 1999-8 (IWC, 2000), which called for annual reports on progress in the following areas.

(1) Genetic methods for species, stocks and individual identification.
(2) Collection and archiving of tissue samples from catches and bycatch.
(3) Status of and conditions for access to reference databases of DNA sequences or microsatellite profiles derived from directed catches, bycatch, frozen stockpiles and products impounded or seized because of suspected infractions.

1.4 Review of documents

The documents identified as containing information relevant to the Stock Definition and DNA Testing Working Group (hereafter, the Working Group) were: SC/68A/SDDNA/01-02; SC/68A/ASI/07; SC/68A/Rep04; SC/68A/SH/02, SC/68A/SH/05-06, SC/68A/SH/08; SC/68A/SP/05; Huijser *et al.* (2018), Martien *et al.* (2017), Archer *et al.* (2017b) and Pastene *et al.* (2020).

2. DNA TESTING

2.1 Genetic methods for species, stocks and individual identification

Diagnosability is a measure of the ability to correctly determine the taxon of a specimen of unknown origin based on a set of distinguishing characteristics. This has historically been a central measure in the delimitation of both species and subspecies for taxonomy. As discussed in Martien *et al.* (2017) and Archer *et al.* (2017b), diagnosability differs from other measures used to distinguish populations as it is focused on the distinctiveness of individuals rather than degree of differentiation of groups. Evaluations of diagnosability are appropriate for multiple types of data (e.g. genetics, morphology, acoustics), however it is also important to evaluate the evolutionary trajectory of the groups under consideration. Finally, estimates of diagnosability should be accompanied with measures of classification uncertainty as well as individual assignment probabilities.

The Working Group thanked Archer for providing this overview. It was noted that an example of using morphometric data to evaluate diagnosability among putative subspecies is included in Pastene *et al.* (In press [see Annex H, Item 3.2.1]), which focusses on delineating subspecies of blue whales in the Southern Hemisphere.

In discussion, the Working Group noted that using small and/or biased samples may be problematic when evaluating diagnosability, because such sample sets may not adequately reflect the diversity found in the strata. The number of samples needed will depend on both the effective population size and the underlying degree of divergence between groups.

It was further noted that use of the diagnosability criterion requires that individuals are placed *a priori* into strata representing the putative taxa based on an independent measure. In the case of genetic data, this stratification is often based on morphology and/or geography. If the data used to evaluate diagnosability is informative, then an individual of unknown origin can be assigned probabilistically to a strata or taxon. However, these individuals should only be included as a 'test' and should not be used to evaluate the diagnosability of the taxa.

As noted above, the use of diagnosability alone is insufficient to delineate subspecies. In genetic studies with large numbers of loci, for example, it could be possible to distinguish clusters of closely related individuals or family groups. A second independent criterion (e.g. morphology, unlinked genetic markers) is needed to provide evidence that the diagnosable units are on different evolutionary trajectories.

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

2.2 'Amendments' of sequences deposited in *GenBank*

Genbank is a valuable resource for taxonomic, population, and forensic research. However, it is essentially an uncurated database, and inconsistencies and/or out-dated information in the metadata (e.g. taxonomic status, geographic location, locus mis-assignment) exist. At the 2005 meeting of the Scientific Committee, it was agreed that validation of DNA sequences in *GenBank* and other such repositories should be carried out routinely (IWC, 2006, Item 15.1, p.45). Subsequently, two rounds of sequence assessment were carried out in 2008 and 2010. These assessments identified some inconsistencies that appeared to be due to a lag in

the taxonomy recognised by *GenBank* or uncertainty in taxonomic distinctions under investigation at that time (e.g. the number of species and appropriate names for recently described species of 'Bryde's whales'). In *GenBank*, the sequence data and metadata can only be amended by the original submitter and not by third parties. To resolve these discrepancies, the Working Group took multiple approaches, including contacting submitters directly to encourage them to make the relevant amendments and working with *GenBank* staff to attempt to identify a mechanism by which inconsistencies could be corrected. While a small number of corrections were made (IWC, 2009, p.345; 2010, p.346), progress was limited and a straightforward solution was not identified.

Given this lack of progress, the Working Group had agreed that the revised DNA quality guidelines (see Item 3.1) would contain a section discussing the precautions that should be used when including *GenBank* sequences in a study (IWC, 2012b, p.303). Although experienced users may be aware that additional sequence validation may be needed when using GenBank sequences, the concern is that less experienced users will be unaware of the associated caveats and may inadvertently worsen the problem by utilising sequences that have been erroneously assigned to a locus or taxon. At SC/68A, text was drafted and reviewed detailing the precautions that should be taken into account when using *GenBank*, and the Working Group **agreed** to include this text in the updated version of the DNA quality guidelines (see Item 3.1 below).

2.3 Collection and archiving of tissue samples from catches and bycatches

The Committee previously endorsed a new standard format for the updates of national DNA registers to assist with the review of such updates (IWC, 2018a, p.228), and the new format worked well the last years. This year the update of the DNA registers by Japan, Norway and Iceland were based again on this new format.

Japan reported on the status of their register (see Appendix 2). The collection of samples is from scientific whaling in the North Pacific (JARPN-JARPNII, 1994-2016, NEWREP-NP, 2017-18) and the Antarctic (JARPA-JARPAII, 1987/88-2013/14 and NEWREP-A, 2015/16-2017/18), and from bycatch (2001-18).

Norway reported on the status of their register (see Appendix 3). The collection of samples of North Atlantic common minke whale is from commercial catches for the period 1997 to 2017; information on the catches for 2018 was not yet available.

No member from Iceland was able to attend this meeting, so Tiedemann reported on the status of the Icelandic register (see Appendix 4), which includes samples from scientific whaling (2003-07) and commercial catches (2006-18).

2.4 Reference databases and standards for diagnostic DNA registries

An update of the Japanese register is shown in Appendix 2. 100% of the samples collected from North Pacific minke whales (n=170) and North Pacific sei whales (n=134) under NEWREP-NP in 2018 have been analysed for both mtDNA and microsatellites. MtDNA and microsatellite analyses are also complete (100%) for the North Pacific minke whales (n=87) and the single North Pacific humpback whale that were bycaught in 2018. No bycatch of North Pacific Bryde's, sei, right, fin, or sperm whales occurred during 2018. MtDNA and microsatellite analyses are complete (100%) for all Antarctic minke whales (n=333) sampled under NEWREP-A in 2017/18.

In discussion, the Working Group asked whether Japan intended to continue to use microsatellite loci to genotype samples collected in the future, or whether they were considering shifting to the use of SNP panels as Norway has done. Japan confirmed that they plan to continue to sequence the mtDNA control region and to use the current microsatellite panel to genotype samples.

The status of the Norwegian DNA registry through 2017 is shown in Appendix 3. None of the North Atlantic minke whales that were taken by Norway during commercial whaling operations in 2018 have been analysed yet and thus no details were reported. As noted at SC/67a (IWC, 2018a), Norway has discontinued mtDNA typing of samples and substituted SNP genotyping.

An update of the Icelandic registry is shown in Appendix 4. The North Atlantic minke whales caught by commercial whaling in 2018 (n=6) have not yet been screened for either mtDNA or microsatellites. For the North Atlantic fin whales (n=146) that were taken during commercial whaling efforts in 2018, all (100%) have been analysed for mtDNA, while 98% of the loci were successfully genotyped. Of note, two hybrid blue-fin whales were caught in 2018.

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

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

This Agenda item relates to two sets of guidelines that the Scientific Committee has requested the Working Group to develop for reference in the Committee's discussions of stock structure. The DNA data quality guidelines are currently being updated (see Item 3.1 below), while the guidelines for genetic data analysis were recently published in *J. Cetacean Res. Manage.* (IWC, 2012a, p.53).

In discussion, it was noted that while the DNA data quality guidelines are available on the IWC website, they are included as a link from within the Scientific Committee Handbook. The guidelines are thus difficult to find on the website.

3.1 Update DNA quality guidelines to include discussion of NGS data

The DNA data quality control guidelines are already available as a 'living document' on the IWC website (*http://iwc.int/scientific-committee-handbook#ten*). In recent meetings, data derived from next generation sequencing (NGS) approaches, including SNPs, have been utilised to address stock structure questions. In light of these developments, the Working Group agreed during SC/67b that it would be timely to update the DNA data quality control guidelines to cover these types of data (IWC, 2019, p.241). At SC/68A, the Working Group reviewed draft text summarising DNA quality guidelines specific to SNPs.

In discussion, it was noted that the Working Group has had little experience working with low-coverage whole genome sequencing. This approach can be a cost-effective and efficient means of assessing population-scale genome data (Waples *et al.*, 2018), although this method

is not appropriate for addressing questions that require individual genotypes to be called with confidence (e.g. kinship analyses). While it is not necessary to include a thorough assessment of the benefits and cautions of using low-coverage sequencing approaches in the current round of revisions to the Guidelines, the Working Group agreed that this topic should be revisited in the future, particularly if someone with the appropriate expertise can be brought into the discussion.

For SC/68B, the group will complete their review of the updated SNP genotyping section so that a revised version can be posted on the IWC website next year. The intersessional group formed during SC/68A will continue to work on this task intersessionally (see work plan, Item 6).

Attention: SC
*The Committee **emphasises** the importance of keeping its guidelines related to genetic data quality and analyses up to date. It therefore: (1) **reiterates** the need to update these guidelines to incorporate the discussion of data quality measures used for Next Generation Sequencing approaches; (2) **agrees** to continue the intersessional e-mail group to review revised sections of the DNA data quality guidelines that apply to data generated from next generation sequencing platforms, including SNPs and whole genome sequencing; and (3) **recommends** that the guidelines be made available on the main Scientific Committee webpage to ensure that they can be easily found by researchers.*

3.2 Further applications of DNA techniques

At SC/67b, an intersessional e-mail group was convened to provide recommendations on genomic approaches to maximise the utility and minimise the depletion of tissue samples. This discussion has arisen, at least in part, due to requests of multiple research groups for access to blue whale samples collected on IWC research cruises (e.g. SOWER, POWER).

Intersessionally, information was compiled on the general advantages and disadvantages associated with three broad categories of high throughput sequencing approaches, including: (1) whole genome sequencing (WGS), in which the full genome is sequenced to varying read depths (Therkildsen and Palumbi, 2016); (2) reduced-representation sequencing (reviewed in Fuentes-Pardo and Ruzzante, 2017), in which restriction enzymes are used to select segments of the genome for sequencing (RRS, e.g. RADseq and related approaches; Baird *et al.*, 2008; Elshire *et al.*, 2011; Petersen *et al.*, 2012); and (3) high-throughput targeted capture, in which preselected genomic regions of interest are enriched and sequenced (reviewed in Jones and Good, 2016; Meek and Larson, 2019). A primary advantage of RRS is that it allows for the discovery and genotyping of a large number of SNPs without requiring that genome sequence data is available *a priori*. However, caution must be taken to ensure consistent sets of genotyped SNPs across sequencing runs and laboratories and typically the number of shared SNPs genotyped in a high proportion of individuals declines over time as samples are added to the project. Targeted sequencing has the advantage of high consistency between runs and laboratories, but requires that established reference genomes are available in order to design capture probes/baits. Several methods allow these two approaches to be combined, such that RRS is used with a subset of samples to identify SNP loci for the design of locus-specific genotyping (e.g. via microchips, high-throughput qPCR, or capture baits) that can then be applied across a large number of samples with high repeatability (Andrews *et al.*, 2014). These combined approaches may also have utility for genotyping samples with degraded and/or low-quality DNA (e.g., Ali *et al.*, 2016; Hoffberg *et al.*, 2016). Although the cost of WGS is currently higher than that of the other approaches, it produces the most complete account of individual genomic variation and is likely to become the standard for genetic studies of natural populations in the future (e.g., Suchan *et al.*, 2016). Preferably, techniques should be applied that allow both alleles of a SNP to be called and thus reveal intra-individual genetic variation. Of note, while all three approaches have been used with historical/ancient DNA (Ekblom and Wolf, 2014; Ellegren, 2014), DNA quality can affect the success of all three approaches.

The Working Group concluded that the best approach to avoid sample depletion in the future would be to conduct WGS of valuable samples. Ideally, the resulting sequence data would be held by the Secretariat and submitted to a public database (e.g. *GenBank*), and interested parties could then request use of the data rather than use of the tissue sample. Notably, however, such an approach would require sufficient funding. The Working Group identified three possible mechanisms to identify potential funds for this initiative: (1) approaching genomics facilities or funding agencies to find interested collaborators; (2) including provisions in the sample request evaluation process to prioritise and/or require projects to use WGS, meeting an agreed upon set of standards, if samples in danger of depletion are requested; and (3) exploring the possibility of integrating WGS costs into cruise budgets. The Working Group also noted that, while WGS could provide genome sequences that would be valuable in addressing a wide range of questions, it is also important to preserve some tissue for use with other emerging technologies (e.g. epigenetics). Thus if support to conduct WGS is identified, initial efforts should focus on only those samples with the largest amounts of tissue available.

In addition, the Working Group discussed considerations for evaluating sample requests that are submitted in the future. Currently, sample requests that are approved by the IWC are forwarded to the Southwest Fisheries Science Center (SWFSC), where the IWC samples are archived, to be fulfilled. In the past, SWFSC has not supplied sample requesters with tissue if the request would deplete the sample to a critical level. However, additional screening of requests to identify the optimal tissues (i.e. those least likely to be depleted) may be warranted. It was further noted that while most requests thus far have been for skin tissue for genetic analyses, requests to use blubber for hormone or contaminant studies should be subject to similar considerations. Finally, the Working Group suggested that a mechanism should be identified to ensure that the data derived from any request is made publicly available in the future within a reasonable timeframe (3 years from receipt of samples), even if the proposed research is never published.

An e-mail correspondence group was formed to make progress with these recommendations intersessionally (see work plan, Item 6).

Attention: SC
*In reviewing the results of stock structure analyses of Southern Hemisphere whale stocks, the Committee **reiterates** its concern regarding the depletion of tissue samples in existing collections (including those collected during the IWC SOWER surveys). Given recent advances in high throughput sequencing technology, the Committee*

***agrees** that: (1) sample depletion should be avoided, such that sample requests will be fulfilled only with those samples for which substantial tissue remains; and (2) whole genome sequencing (WGS) is the best approach to maximise the value and avoid depletion of tissue samples, and requests for projects using this approach (WGS) shall be prioritised. In addition, the Committee **agreed** that the intersessional working group formed at SC/67b should continue its work to provide recommendations on genomic approaches to maximise the utility of these samples for future studies.*

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

4.1 North Pacific whale stocks

4.1.1 Western North Pacific common minke whales

The First Intersessional Workshop on the *Implementation Review* of North Pacific minke whales was held in Tokyo, Japan, from 25 February to 1 March 2019 (SC/68A/Rep04). Workshop participants reviewed the results of new analyses pertaining to the stock structure of western North Pacific minke whales. At the conclusion of the Workshop, it was agreed that three stock structure hypotheses would be taken forward: (1) there is a single J-stock distributed in sub-areas 1W, 1E, 2C, 5, 6W, 6E, 7CS, 7CN, 10W, 10E, 11 and 12SW, and a single O-stock in sub-areas 2C, 2R, 3, 4, 7CS, 7CN, 7WR, 7E, 8, 9, 9N, 10E, 11, 12SW, 12NE and 13 (referred to as hypothesis A); (2) as for hypothesis (A), but there is a third stock (Y-stock) which resides that resides in sub-areas 1W, 5, and 6W and overlaps with J-stock in the southern part of sub-area 6W (referred to as hypothesis B); and (3) there are four stocks, referred to Y, J, P (Purple), and O, two of which (Y and J) occur in the Sea of Japan, and three of which (J, P, and O) are found to the east of Japan (referred to as hypothesis E). Stock P is a coastal stock. Hypothesis E is based on genetic assignment of individuals to clusters taking spatial occurrence into account (as implemented in the software GENELAND; SC/68A/Rep04).

It was noted that for *Implementation Simulation Trials*, the default assumption is that identified stocks are independent of each other, such that no exchange occurs between stocks.

The objective of this study was to test continuity of putative parent-offspring (PO) pairs among putative GENELAND populations. These analyses were requested at the first intersessional implementation review on NPMW, after initial analyses excluded putative offspring (in keeping with assumptions of the model run in GENELAND). The initial run included all samples and set K=3, after a recommendation at the intersessional about combining putative nearshore populations, here referred to as the 'Pacific coastal' (PC) population (elsewhere referred to as 'purple'). The K=3 run retained the PC population (now with individuals from the two earlier coastal clusters grouped together) together with the putative J-stock and O-stock clusters. An additional run was completed that excluded putative parents, so that a run excluding offspring and separate run excluding parents could be used to test the assignments from the K=3 run. This run identified the same four putative populations as for the earlier runs excluding offspring. A total of 52 putative PO pairs were tested (omitting a 53rd that represented a cow-foetus pair). Of these, 36 showed a match by area for the putative PO pairs. Of those 36, 31 could be confirmed by the repeat GENELAND analyses (85% - consistent with earlier estimated error rates). Sixteen suggested a mismatch by area, but only 7 of these could be confirmed by repeat GENELAND analyses (44%). The lower confirmation rate may suggest that these samples that mismatch by location of origin were more difficult for the program to assign. We also consider potential errors associated with the putative PO assignments. The original paper (SC/67A/SDDNA/01) suggested that this could be about 10% for the 48 pairs assessed at 26 loci, and as high as 46% for the 4 samples assessed at 16 loci. Analyses testing for contemporary geneflow (in SC/F19/WNPM02) also indicate a non-zero level, though a precise estimate is not available. In general, mismatches could result from movement reflecting genetic migration, error associated with GENELAND assignments, and error associated with the identification of parent-offspring pairs, among other factors. The authors concluded that the resolution is insufficient for strong inference, and that it is likely that apparent location mismatches are due to analysis error together with a level of gene flow that is consistent with the levels estimated in the population genetic analyses presented in SC/F19/WNPM02.

SC/68A/SDDNA/02 presented the results of comprehensive analyses performed using genetic and non-genetic data to assist the discussions on plausibility for western North Pacific common minke whales stock structure Hypotheses A and E. The results of *HWE* tests with F_{IS} suggested that the 'purple' group (putative coastal stock under Hypothesis E) consisted of whales from multiple breeding stocks, but this signal disappeared when the samples were analysed dividing the 'purple' group into STRUCTURE-'J' and STRUCTURE-'O' stocks. These findings suggested that it was unlikely that an independent stock represented by the 'purple' group exists in the coastal areas of the Pacific side of Japan and southern Okhotsk Sea. This inference is also favoured by the results of the pattern of genetic differentiations. Some parent-offspring pairs were found between GENELAND groups which again, is not consistent with the view that the 'purple' group is an independent stock. Furthermore, the body length distribution analyses and the geographical distribution of genetic samples showed that the 'purple' group significantly lacked mature individuals, which is inconsistent with the postulation of this group as a resident stock. Main conception date of the 'purple' and 'O' stock overlapped, and two types of colouration patterns also similar between the 'purple' and 'O' stock. These implied a possible mixture of samples from the 'J' and 'O' stocks in the 'purple' groups. In conclusion, results of genetic and non-genetic data examined in this paper provided strong support to the existence of two stocks (J and O) with temporal and spatial mixing (Hypothesis A). Those results provided little support for the existence of an additional coastal stock ('purple' group) as postulated under the Hypothesis E.

A response to SC/68A/SDDNA/02 is included in Appendix 5.

The Working Group thanked Hoelzel, Taguchi, and their colleagues for conducting this work intersessionally and presenting it to the group.

Discussion of SC/68A/SDDNA/01 focused on the parent-offspring (PO) pairs (n=16) in which the parent and offspring were assigned to different GENELAND clusters. It was noted that multiple sources of error could be present. Within the GENELAND analysis, errors (i.e. mismatches due to a lack of consistency) were identified by conducting replicate runs of the analysis and identifying individuals that were assigned to different clusters across replicates. Another source of error is that two unrelated (or otherwise related) whales may have been erroneously identified as a parent-offspring (PO) pair (i.e. false positives), as described

in detail in Tiedemann *et al.* (2017). SC/68A/SDDNA/01 notes that temporary movements of individuals without reproduction could contribute to PO matches among GENELAND clusters. In discussion, it was noted that this could be related to GENELAND taking spatial information into account for cluster assignment. Therefore, the Working Group concluded that such errors are already accounted for in GENELAND mis-assignments.

The 16 PO pairs that were identified as belonging to different GENELAND clusters were placed into three categories based on the consistency of their assignments across replicate GENELAND runs. Seven of the 16 PO pairs were assigned consistently across runs, while two of the assignments were ambiguous and seven were inconsistent. Appendix 6 shows the spatial distribution of those PO pairs colour-coded by the consistency of their GENELAND assignments across replicate runs. For four of the seven pairs that were consistently assigned across GENELAND runs, one individual was sampled in a coastal area and the second in an offshore area. All of those pairs that were either ambiguously assigned or inconsistently assigned to GENELAND clusters were matches between coastal areas. In addition, Appendix 7 explores whether the 16 PO pairs that were assigned to different GENELAND clusters were more likely to have been erroneously assigned as PO pairs. The LOD scores of these pairs did not differ from the general distribution, indicating that the rate of erroneous inference is the same among the True Positives inferred across GENELAND clusters and the inferred True Positives in general. In addition, there was no indication that the LOD score distributions differed across the three categories of GENELAND assignments, although the sample sizes for each of these categories are too small to be conclusive. Taken together, these results do not provide an indication that less confidence should be place in the PO pairs that were identified across GENELAND clusters as opposed to those identified within GENELAND clusters.

It has been suggested that the 'purple' stock could be an artefact of including the spatial information into the GENELAND analysis, in that many of the samples representing this group were collected as bycatch and were from the same very small area/same location. It was questioned whether it would be possible to test if the same patterns were observed if the dataset was 'thinned' to remove the samples collected from the same or very close to the same location. Hoelzel reported that this exercise had been conducted previously by adjusting the delta coordinates parameter, which prescribes the amount of uncertainty attached to spatial coordinates. The pattern (of identifying a coastal cluster representing the P stock) did not change, although adjusting this parameter made it more difficult to assign some of the O stock individuals. Another approach that may provide insight into the question of the validity of the purple stock is to use the coalescent isolation with migration analysis implemented in IMa3 (Burrell *et al.*, 2015). This method estimates directional migration and effective population size, and can test posterior support and identify confidence intervals for the timing of divisions between putative populations (including a lack of support for a given division point). The implementation of this approach, which is conceptually distinct from those involving assignment or mixing analyses, was encouraged at the Workshop. Hoelzel started this analysis intersessionally, but the method requires considerable computer time.

The Working Group further suggested that using a group-based assignment/exclusion approach that focuses on the relative likelihoods of offspring in the purple GENELAND cluster that have parents in either the J or O GENELAND clusters[1] could be informative. This likelihood-based approach (Group ExcLusion-Assignment Test, GELATo) was implemented in Sethuraman and Hey (2016) to evaluate the most likely source population for a small group of beluga whales inhabiting Yakutat Bay, Alaska. A key benefit of this approach is that it can also be used as an exclusion test, where low likelihoods (negative loglikelihoods) for all source populations would indicate that the test group was either of mixed origin, a nonrandom sample from one of the source populations, or from an unsampled population

At the intersessional Workshop, Hoelzel noted that work was ongoing to use GENELAND to conduct a post hoc analysis testing the relative fit of non-admixture versus admixture models. While this analysis was completed intersessionally, the results proved difficult to interpret. The output of running this model was shared with Taguchi, and both parties agreed to communicate intersessionally if progress is made in determining how to interpret the results appropriately.

A suggestion was also made to recheck the p-values associated with the tests of HW proportions, based on low and roughly equivalent F_{IS} values showing very different probabilities of deviation from HWE in table 4b of SC/68A/SDDNA/02. While it was noted that genotyping error rates (<0.01%) have already been calculated for this dataset, an additional check could be done using a jackknife approach (Morin *et al.*, 2009) implemented in strataG (Archer *et al.*, 2017a), which flags samples that contain rare homozygotes that could represent genotyping errors. In previous analyses of bowhead whales, this procedure allowed problematic samples to be identified and removed from the analysis. This approach is not meant to replace traditional error checking, as has already been completed by Japan, but rather to complement it by identifying problematic samples (perhaps due to low sample quality).

In discussion, the potential that Isolation by Distance (IBD) could create the patterns seen in the genetic data was raised. Specifically, it was questioned whether this could explain the purple stock, which appears to have some genetic connectivity with both the J and the O stock. IBD has not yet been explored using this dataset. It was noted that exploring this possibility is complicated by the fact that the samples included in this analysis were all collected from migratory routes, while IBD would be generated on the breeding grounds, though it might be apparent as isolation by temporal distance if whales from different groups are transiting through areas at different times. It was also noted that GENELAND would be more likely to generate spurious intermediate populations than identify artificial clusters at the ends of the geographic range.

As detailed above, several suggestions for additional analyses of the WNP minke whale data were received by the Working Group. In the future, however, it is important to remember that this work will no longer be conducted under the constraints of an *Implementation Review*, but rather will fall under the work done under the In-Depth Assessment sub-committee. In addition, in the discussion of NP minke whale stock structure, several issues regarding new analytical techniques, and their interpretation, have been raised. These issues are not specific to minke whales but are more broadly

[1]The GENELAND cluster containing primarily J stock whales was previously referred to as the 'green' cluster while the GENELAND cluster containing primarily O stock whales was referred to as the 'orange' cluster.

applicable to the work of SDDNA. As such, the Working Group **encourages** additional studies exploring the utility of these methods in the future.

Attention: SC
*The Committee **encouraged** additional exploration of the utility of spatially explicit genetic analyses. The Committee noted that such analyses have provided valuable information in assessing the stock structure of western North Pacific minke whales and are likely to have broad utility in elucidating stock structure in other cetaceans.*

After discussion of the new information presented, the Working Group **endorsed** the stock structure hypotheses for North Pacific Common minke whales suggested by the Workshop and detailed above. It was noted that questions remain as to the validity of the purple stock. The Working Group clarified that while Hypothesis E is being considered plausible with respect to moving forward with the simulation trials, its inclusion should not be taken as confirmation of its existence but rather as a way to further evaluate patterns in the data that are difficult to explain under Hypothesis B.

Based on Parent-Offspring (PO) relationships found both across J and P and across P and O (SC/68A/SDDNA/01) and further genetic characteristics of the inferred P-stock (especially evidence for contemporary gene flow between purple and both J-stock and O-stock consistent with genetic affinity of some P-stock individuals to J-stock, others to O-stock (de Jong and Hoelzel, 2019; Appendix 5), the Working Group further concluded that hypothesis E can only be maintained if P is not a closed stock but receives dispersal from both J-and O-stock. Deviation from HWE was detected in the putative purple stock (SC/68A/SDDNA/02), although the caveat was noted that this potential Wahlund effect is no longer supported when further GENELAND clustering and GENELAND error is taken into account (Hoelzel and de Jong, 2019; Appendix 5). Thus the remainder of the discussion focused on how the genetic data might be able to inform the simulation model regarding rates of mixing between stocks. In discussion, it was noted that many of the genetic analyses being conducted (i.e. BayesAss) are evaluating genetic exchange (i.e. the movement of gametes per generation) between groups. In the context of the simulation model, however, the key information needed is rates of demographic exchange (i.e. the movement of individuals between areas per year) between stocks. In this sense, the information derived from the PO analysis may be more informative, as in some contexts it can be considered a measure of demographic exchange.

Attention: SC
*The Committee **endorsed** the following stock structure hypotheses for western North Pacific common minke whales: (1) there is a single J-stock distributed in sub-areas 1W, 1E, 2C, 5, 6W, 6E, 7CS, 7CN, 10W, 10E, 11, and 12SW, and a single O-stock in sub-areas2C, 2R, 3, 4, 7CS, 7CN, 7WR, 7E, 8, 9, 9N, 10E, 11, 12SW, 12NE, and 13 (referred to as Hypothesis A); (2) as for hypothesis A, but there is a third stock (the Y-stock) that resides in sub-areas 1W, 5, and 6W (referred to as Hypothesis B); and (3) there are four stocks, referred to as Y, J, P (Purple), and O, two of which (Y and J) occur in the Sea of Japan and three of which (J, P, and O) that are found to the east of Japan (Hypothesis E). The Committee further **agreed** that hypothesis E can only be maintained if some demographic exchange between the P stock and both J- and O-stocks is allowed.*

The potential to use the results of PO analysis to inform mixing rates was discussed among a small group, and the approach described in Annex D (appendix 3) was put forward. For clarification, it was agreed to refer to a PO pair that assigned to different stocks as representing 'dispersal/stock transfer', further noting that stocks here were defined by clustering procedures (e.g. STRUCTURE or GENELAND) rather than spatial boundaries. In *IST* trials this mechanism is referred to as dispersal. The Working Group **agreed** that trials should be conducted under the assumption that the numbers dispersing from the P to the J stock and the P to the O stock were the same at unexploited equilibrium. It further **agreed** that initial evaluation should assume that the proportion of calves dispersing from the P to the J and O stocks is the same.

Attention: SC, CGA
*The Committee **expressed great appreciation** for the immense amount of work and high level of collaboration put toward providing the results needed to inform the Committee's decisions.*

4.1.2 Sei and Bryde's whales

The final review of JARPN II was conducted 22-26 February 2016 in Tokyo (SC/68A/Rep04). At that point, only the samples collected through 2014 had been genetically analysed. At SC/68A, the final conclusions of JARPN II were presented in SC/68A/SP/05. Appendices 6 and 7 of this report included updated genetic analyses of the stock structure of sei and Bryde's whales in the North Pacific based on the inclusion of all samples collected through 2016.

Appendix 6 of SC/68A/SP/05 presented the results of refined genetic analyses of North Pacific Bryde's whales, using mtDNA and microsatellite data from a total of 1,237 genetic samples collected during 1979-2016 from five sources: past commercial whaling, bycatch, JARPNII, POWER and Japanese dedicated sighting surveys. A total of 161 samples collected by JARPNII in 2015 and 2016 had not been analysed previously. The pairwise F_{ST} estimates and heterogeneity tests between sub-areas, i.e. 1W (135-165°E), 1E (165°E-180°) and 2 (180°-150°W), showed weak but significant genetic differentiation between sub-areas 1W/1E and 2 for both genetic markers, while AMOVA, STRUCTURE, and DAPC analyses did not show distinct genetic structuring in those sub-areas. These results suggested genetic homogeneity within sub-area 1W/1E, and genetic heterogeneity between sub-areas 1 and 2. In addition, moving average for three genetic statistics gradually changed in sub-area 1E, which suggested a possible geographical mixing of the two weakly differentiated stocks in this sub-area. These findings confirmed the conclusion presented at the JARPNII final review workshop.

Following the summary of Appendix 6, it was noted that, while one of the mtDNA comparisons made between the temporal Bryde's whale strata was statistically significant, subdividing the regions by both season and decade had resulted in one strata being represented by a small number of samples. Thus, this significance was likely an artifact of sample size.

Appendix 7 of SC/68A/SP/05 presented the results of refined genetic analyses of North Pacific sei whales, using mtDNA and microsatellite data from a total of 1,748 genetic samples collected during 1972-73 and 2002-16 from three sources: past commercial whaling, JARPNII and POWER surveys. A total of 181 samples collected by JARPNII in 2015 and 2016 had not been analysed previously. Pairwise

F_{ST} estimates between areas, i.e. Western (140-150°E), Central (150°E-180°) and Eastern (180°-130°W) areas, showed no evidence of genetic heterogeneity in North Pacific sei whales. These results were also supported by AMOVA and STRUCTURE analyses, which showed a lack of genetic structuring of this species in the oceanic areas of the North Pacific. Overall, the refined analyses confirmed this conclusion presented at the JARPNII final review workshop.

As part of the In-depth Assessment of North Pacific sei whales, two broad hypotheses regarding stock structure are being considered, one which includes only a single stock in the entire North Pacific and a second that assumes the presence of multiple stocks. One limitation in assessing the plausibility of the multi-stock hypothesis is that while a large number of samples have been collected in the pelagic sub-area, no samples are available from the other sub-areas hypothesised to represent additional stocks under the multi-stock hypothesis. In discussion, however, it was noted that the stock structure hypotheses under consideration do allow for some mixing of stocks within this pelagic region. While evidence of mixing was not detected in the analyses, it was noted that, should some limited mixing occur, the probability of detecting it would depend on the number of non-pelagic stock animals sampled and the underlying magnitude of genetic differentiation between the two stocks mixing. Thus while in principle sampling of animals from the pelagic area alone could exclude the possibility of multiple stocks, in practice the small sample size available and likely weak differentiation between the stocks precludes this.

Ultimately, the best way to address whether additional stocks are present is to collect genetic samples from areas that are expected to contain 'pure' stocks. It was noted that baleen from sei whales was collected from US whaling stations in the past and should have been sent to the Smithsonian. Efforts to relocate these samples have been unsuccessful. The Smithsonian recently hired a new Curator of Marine Mammals, however, and plans were made to contact him to ensure that he is aware of the missing samples.

The Working Group thanked Japan for presenting these results on Bryde's and sei whales in the North Pacific, which are based on a large and comprehensive dataset. They noted that the updated results are consistent with those from previous analyses (IWC, 2017) and thus do not change the conclusions reached in those discussions. However, the Working Group was happy to hear that Japan plans to use this valuable sample set to conduct kinship analyses in the future, which may provide additional insight into the stock structure of sei whales in the North Pacific.

Attention: SC

In reviewing the final report of the JARPN II surveys, the Committee ***expressed appreciation*** *to Japan for providing the results of analysis of this comprehensive dataset and* ***agreed*** *that the most recent results assessing the stock structure of North Pacific sei and Bryde's whales are consistent with those from previous analyses.*

4.2 North Atlantic sei whales

Huijser *et al.* (2018) presented the results of a genetic analyses on stock structure in the North Atlantic sei whales. Currently, three stocks of sei whales (*Balaenoptera borealis*) are defined in the North Atlantic; the Nova Scotian, Iceland/Denmark Strait and Eastern North Atlantic stocks, which are mainly based upon historical catch and sighting data. Huijser *et al.* (2018) analysed mitochondrial control region DNA (mtDNA) sequences and genotypes from 7 to 11 microsatellite loci in 87 samples from three sites in the North Atlantic; Iceland, the Gulf of Maine and the Azores, and compared against the North Pacific using 489 previously published samples. No statistically significant deviations from homogeneity were detected among the North Atlantic samples at mtDNA or microsatellite loci. The genealogy estimated from the mtDNA sequences revealed a clear division of the haplotypes into a North Atlantic and a North Pacific clade, with the exception of one haplotype detected in a single sample from the Azores, which was included in the North Pacific clade. Significant genetic divergence between the North Atlantic and North Pacific Oceans was detected (mtDNA Φ_{ST}=0.72, microsatellite Weir and Cockerham's Θ=0.20; p<0.001). The coalescent-based estimate of the population divergence time between the North Atlantic and North Pacific populations from the sequence variation among the mtDNA sequences was at 163,000 years ago. However, the inference was limited by an absence of samples from the Southern Hemisphere and uncertainty regarding mutation rates and generation times. The estimates of inter-oceanic migration rates were low (N_m at 0.007 into the North Pacific and at 0.248 in the opposite direction). Although estimates of genetic divergence among the current North Atlantic stocks were low and consistent with the extensive range of movement observed in satellite tagged sei whales, the high uncertainty of the genetic divergence estimates precludes rejection of multiple stocks in the North Atlantic.

The Working Group thanked Pastene for presenting this paper, which has implications for evaluating stock structure of sei whales in the North Atlantic.

In discussion, it was noted that the evidence for a lack of structure within the North Atlantic is based on a relatively small number of microsatellite loci (n=11), and that all of the samples were derived from areas considered to be feeding grounds or migratory corridors. Thus, as noted in the paper, the conclusions should be interpreted with caution. The Working Group agreed that analysing additional samples from a broader portion of the range would be useful in continuing to assess structure within this ocean basin.

4.3 Southern Hemisphere whale stocks

4.3.1 Fin whales

SC/68A/SH/05 presents an analysis of the addition of 37 Chilean and 107 Gulf of California (GoC) fin whale sequences to previously published fin whale control region sequences from the North Pacific, North Atlantic, and Southern Hemisphere. The study supports previous findings that GoC fin whales have much lower diversity than those in the rest of the North Pacific and other ocean basins. Although they are most closely related to other North Pacific fin whales, they show strong differentiation from all other strata. No significant differentiation was found between Chilean fin whales and those in the South Atlantic. While none of the animals in either Southern Hemisphere strata were identified as putative 'pygmy' fin whales (*Balaenoptera physalus patachonica*), data from satellite tags indicates that several of the Chilean fin whales stay in lower latitudes, matching the presumed range of pygmy fin whales.

The Working Group thanked Archer for presenting this work, which represents an update of a paper that was discussed at SC/67b.

In discussion, it was noted that the pygmy fin whale subspecies, which was described based on morphological data, has been proposed to include whales that are located

primarily in the low to mid-latitudes of the Southern Hemisphere (Clarke, 2004). The results of this work, which did not find significant differentiation between the Chilean whales and contemporary samples from the South Atlantic, do not provide support for the validity of this putative sub-species. However, some have speculated that commercial whaling in high latitude waters may have wiped out the 'true' Southern Hemisphere fin whales (*B. physalus quoyi*), such that all of the surviving whales were of the pygmy form. Conducting a comparison of the mtDNA sequence data presented in SC/68A/SH/05 with that derived from bones collected off the islands at 54°15'S, 36°45'W (Sremba *et al.*, 2015), which are thought to have been deposited prior to 1915, could provide insight into the relationship of the fin whales currently found in the Southern Hemisphere and those that existed prior to depletion by whaling.

SC/68A/SH/02 reported the results of genetic analyses on stock structure of fin whales in the Indo Pacific region of the Antarctic feeding grounds. The analyses were based on mitochondrial DNA (mtDNA) control region sequences (478bp) and genotypes at sixteen microsatellite loci, and 108 genetic samples collected in Areas III-VIW under the JARPA, JARPAII, NEWREP-A and SOWER surveys. The haplotype (0.984-1.000) and nucleotide (0.0111-0.0115) diversities were high, and similar among three sectors examined: POP1 (west of 70°E), POP2 (70°E-160°E) and POP3 (east of 160°E). Heterogeneity test based on F_{ST} and HW equilibrium failed to find significant genetic differences among the sectors. These results should be considered as preliminary and further analyses based on larger samples sizes, mainly from the Pacific sector of the Antarctic, should be conducted in the future.

The Working Group thanked the authors for presenting this work, which updates a paper (Goto *et al.*, 2014) previously reviewed by the Working Group by including additional samples.

In discussion, it was noted that a subset of the samples analysed in SC/68A/SH/02 were collected north of 60°S. Comparison of those samples with samples collected south of this boundary could provide insight into whether differences exist between fin whales found at high latitudes and those found at mid-latitudes, which could represent pygmy fin whales under the Clarke (2004) hypothesis. Japan noted that they may have the opportunity to collect biopsy samples north of 60°S in Area III while transiting to/from areas further south. Data generated from these samples could facilitate making such a comparison (S and N of 60°S degrees) in the future.

The Working Group further noted the difficulty of identifying putative strata for genetic comparisons given a lack of obvious boundaries or patterns in the data. Here the strata were tested both by comparing Areas (Area III+IV versus Area V+VI) and by examining the *longitudinal* clines of genetic variation, which identified putative boundaries at 70°E and 160°E. No genetic heterogeneity between strata was detected. As noted by the authors, however, additional samples collected over a broader geographic range should be collected and analysed before any conclusions are drawn about a lack of structure.

4.3.2 Blue whales

A pre-assessment of Southern Hemisphere blue whales was conducted by the SH sub-committee this year. As part of this discussion, Pastene *et al.* (2020) was reviewed as part of a joint session with the SH sub-committee. This paper presents the results of morphometric analyses comparing Chilean, southern Indian pygmy and Antarctic blue whales. Details of the discussion can be found in Annex H under Agenda Item 3.

4.3.3 Right whales

Southern right whale (*Eubalaena australis*) population structure can be viewed as a migratory network of winter calving/socialising and summer feeding grounds. SC/68A/SH/06 used mitochondrial DNA and nuclear microsatellite data to investigate the position of the Chile-Peru wintering ground (n=1) and the feeding ground near the islands at 54°15'S, 36°45'W (n=15) within this migratory network, including new data from Brazil (n=60) and South Africa (n=88) which was combined with published data from across the species' circumpolar distribution (nDNA=222; mtDNA=1,327). The single sample from Chile-Peru had a mtDNA haplotype previously only observed in the Indo-Pacific and had a nuclear genotype that appeared admixed between the Indo-Pacific and South Atlantic, based on genetic clustering and assignment algorithms. The feeding ground samples were clearly South Atlantic in origin, based on both genetic differentiation and clustering analyses. As a group, feeding ground samples were more similar to the southwest Atlantic wintering grounds (Brazil, Argentina) than to the South African wintering ground, and showed significant genetic differentiation from the latter. However, the weak genetic differentiation amongst the South Atlantic wintering grounds meant that population assignment methods were unable to strongly resolve the likely winter association of the feeding ground samples.

In discussion, it was noted that in the past it had been assumed that the population of SRWs on the Chile-Peru wintering ground had been extirpated during commercial whaling, and that the more recent sightings represented whales of unknown origin that had recolonised the area. Although based on a single sample, the mtDNA haplotype identified in the Chile-Peru sample suggests that this wintering ground may be more closely linked to the Indo-Pacific than to the South Atlantic.

The Chile-Peru southern right whale subpopulation is the subject of an existing Conservation Management Plan and is discussed in item 6.1.1 of Annex O. The work presented here highlights the need for additional samples to be collected from the Chile-Peru wintering ground in order to provide further insight into the origin of the whales on this wintering ground.

4.3.4 Sei whales

The mass mortality of more than 300 sei whales in the Golfo de Penas, Chile, in 2015 provided a source for the largest number of genetic samples for this species from the Southern Hemisphere. SC/68A/SH/08 presents preliminary analyses of mtDNA haplotypes (387 bp in length) from 79 bones samples in the context of other available sequences from samples collected or attributed (in the case of market samples, Baker *et al.* (2015) to the Southern Hemisphere (total of n=12), the North Pacific (n=27) and the North Atlantic (n=76). At a global scale, phylogeographic analyses showed a strong genetic differentiation between the Southern Hemisphere and both North Atlantic (phi-ST=0.69, p=0.001) and North Pacific (phi-ST=0.32 p=0.001). There was no sharing of haplotypes between oceans with a single exception of a whale sampled in the North Atlantic that clustered with haplotypes from the Southern Hemisphere. Together with other recent studies, e.g. Huijser *et al.* (2018), SC/68A/SH/08 points to a marked phylogeographic differentiation among sei whale in the North Pacific, North Atlantic and Southern Hemisphere, reflecting the existence of three major population units.

The Working Group thanked Baker for presenting this information and expressed their appreciation for the efforts of his Chilean colleagues, who collected these samples from

a remote and difficult to access region. Another sei whale mass mortality event, of smaller magnitude, occurred in southern Chile during early 2019 and will be discussed in the E sub-committee (SC/68A/E/10, see Item 4.2 of Annex K).

It was noted that the genetic divergence between the North Pacific, North Atlantic, and Southern Hemisphere was relatively high and the haplotype network indicates relatively clear separation between ocean basins. One North Atlantic haplotype was grouped with the Southern Hemisphere. This individual could represent a migrant (or descendent of a migrant) or may be the result of incomplete lineage sorting.

Taken together, these findings suggest that sei whales in different ocean basins could represent a separately evolving lineage or a subspecies, although additional samples encompassing the diversity of the species are needed to assess this possibility. While SC/68A/SH/08 incorporated the sequence data representing the North Atlantic in Huijser *et al.* (2018), the sequence data representing the North Pacific was largely derived from samples collected off Hawaii and did not include the sequence data from the North Pacific that was included in Huijser *et al.* (2018). Merging of these two datasets would allow for a more comprehensive comparison in the future. In addition, Reyes noted that a few samples of sei whales from Argentina have been collected and could potentially be added to such a combined analysis. It was further noted that sequencing the full mitogenomes of the available samples (or a subset thereof) could be informative in evaluating whether sei whales in different ocean basins are representative of subspecies.

Buss reported on progress to assess the genetic diversity of sei whales in the South Atlantic. To date, the mtDNA control region has been sequenced from 40 contemporary samples (including 13 biopsies, 3 strandings, and 24 faecal samples). The 16 skin tissue samples have also been genotyped at 13 microsatellite loci. In addition, the mtDNA control region has been sequenced from 21 historic whalebones, 15 of which were confirmed to be from sei whales, collected from the New Island whaling station, which operated during 1905-15. Buss and her colleagues are coordinating with other SH sei whale researchers analysing contemporary sei whale samples, including Marijose Perez Alvarez (University of Chile) and Ana Cypriano (Pontifical Catholic University of Rio Grande do Sul, Brazil), to ensure that the microsatellite genotypes from these studies can be combined in the future.

Of note, the single haplotype noted as shared between the Southern Hemisphere and the North Atlantic in SC/68A/SH/08 was identified in three of the samples (one biopsy and two bones). This suggests the possibility that the North Atlantic sample, which was collected in the Azores, may represent a migrant from the Southern Hemisphere or that Southern Hemisphere sei whales occasionally use the Azores.

The Working Group welcomed the opportunity to hear about this work and looks forward to hearing more about it at next year's SC meeting.

Attention: SC
*In reviewing the results of new genetic analyses of sei whales, the Committee **recognises** the importance of this work to understand stock structure of this species and **encourages** collaboration between researchers in both the Northern and Southern Hemispheres to integrate genetic datasets to allow for a more comprehensive analysis.*

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5. NEW STATISTICAL AND GENETIC ISSUES RELATING TO STOCK DEFINITION

5.1 Simulation tools for spatial structuring (e.g. TOSSM)

The Testing of Spatial Structure Models (TOSSM) approach was developed with the intent of testing the performance of genetic analytical methods in a management context using simulated genetic datasets (Pastene *et al.*, 2016a; 2016b; 2016c), 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 (Martien *et al.*, 2009). The Working Group noted that while TOSSM has been particularly valuable in informing the interpretation of results of stock structure related analyses, it has not been broadly utilised within the IWC Scientific Committee for this purpose.

A wide-range of software packages are now available for producing simulated datasets that can be used for statistical inference and/or validating statistical methods (Archer *et al.*, 2010; Lang and Martien, 2012). At SC/67b, the Working Group discussed the possibility of bringing in an Invited Participant with specialised expertise in this topic to present an overview of the applicability of this approach. Although the Working Group **agreed** that this strategy would facilitate making progress on this item, given budgetary constraints it was not feasible to address at SC/68A. However, Lang and Tiedemann plan to look into this possibility for SC/68B.

In addition, the Working Group encouraged the submission of papers utilising simulation-based approaches to inform stock structure questions to SC/68B. These approaches could include individual-based forwards-in-time genetic simulations (reviewed in Hoban *et al.*, 2012 and Hoban, 2014), as well as coalescent simulations that allow the plausibility of alternative hypotheses of population history to be evaluated (e.g. metasim, Strand, 2002). This latter method has become more commonly used in contexts relevant to the Working Group in recent years (de Jong and Hoelzel, 2019).

Attention: SC
*The Committee noted that while simulation-based approaches have been particularly valuable in informing the interpretation of results of stock structure-related analyses, they have not been broadly utilised within the Committee for this purpose. The Committee **reiterated** that bringing in invited expertise to present an overview of the applicability of such approaches would expedite progress on this Agenda item.*

5.2 PCA, DAPC, and related methods

DAPC has been used in previous analyses assessing the stock structure of North Pacific Bryde's whales (e.g. Approximate Bayesian Computation, Beaumont *et al.*, 2002; Csillery *et al.*, 2010). At SC/68A, the Working Group reviewed Appendix 6 of SC/68A/SP/05, which reran this analysis using an expanded sample set. Given that the utility of this approach as applied to North Pacific Bryde's whales was reviewed when it was originally implemented (IWC, 2018b), no further review of the utility of DAPC in evaluating Bryde's whale stock structure was conducted at SC/68A. No other papers utilising this or related approaches were submitted for review this year.

5.3 Terminology

Following a recommendation arising in 2012 (Taguchi *et al.*, 2017) the Working Group began working on compiling

Table 1
Work plan.

Topic	Intersessional 2018/19	2020 Annual Meeting (SC/68B)	Intersessional 2020/21	2021 Annual Meeting
3.1 DNA quality guidelines	Intersessional e-mail group to review recent revisions to the DNA quality guidelines that pertain to data produced using NGS approaches.	Report and finalise updated guidelines		
4.4.2 Recommendations to avoid sample depletion	Intersessional e-mail group to provide recommendations on genomic approaches to maximise the utility of tissue samples that are in danger of becoming depleted in the future.	Report and provide advice		
5.3 Terminology	Intersessional e-mail group to continue discussions of the use of stock structure-related terms within the SC.	Report	Continue as needed	Report as needed

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 (IWC, 2013). At SC/68A, the Working Group noted that some progress has been made in standardising the use of terminology within the sub-committees focusing on large whale assessments, although aligning this terminology with that used by the SM sub-committee has been more challenging (see Appendix 5, IWC, 2014).

During SC/68A, it was noted that some confusion exists regarding the correct terminology to describe gene flow versus movements of animals between areas (e.g. admixture *vs* mixing, genetic *vs* demographic exchange, dispersal). An intersessional e-mail group was formed to discuss adding definitions of these terms to the existing glossary (see work plan, Item 6)

Attention: SC
*The Committee **agrees** to continue the work of the intersessional e-mail group focused on revisiting terminology with special references to the implications of inferred stock structure in other sub-committees, particularly those dealing with large whale assessments, and to suggest revisions where appropriate for consideration at SC/68B.*

5.4 Close-kin mark-recapture

No papers were submitted that used close-kin mark recapture.

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

5.5 Epigenetic ageing

No papers were submitted that incorporated epigenetic aging.

Attention: SC
*Epigenetic ageing has the potential to be informative for many aspects of the Committee's work, and thus the Committee **encourages** the submission of papers utilising this approach in the future.*

6. WORK PLAN

6.1 Work plan

The details of the work plan are given in Table 1.

6.2 Budget requests for 2019-2020

No budget requests were received for 2019-20.

7. ADOPTION OF REPORT

The report was adopted on 18 May 2019 at 15:00.

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Appendix 1

AGENDA

1. Introductory items
   - 1.1 Convenor's opening remarks
   - 1.2 Election of Chair and appointment of rapporteurs
   - 1.3 Adoption of Agenda
   - 1.4 Review of documents
2. DNA testing
   - 2.1 Genetic methods for species, stocks and individual identification
   - 2.2 'Amendments' of sequences deposited in *GenBank*
   - 2.3 Collection and archiving of tissue samples from catches and bycatches
   - 2.4 Reference databases and standards for diagnostic DNA registries
   - 2.5 Other
3. Guidelines and methods for genetic studies and DNA data quality
   - 3.1 Update DNA quality guidelines to include discussion of NGS data
   - 3.2 Further applications of DNA techniques
4. Provide advice on stock structure to other sub-groups
   - 4.1 North Pacific whale stocks
     - 4.1.1 Western North Pacific common minke whales
     - 4.1.2 Sei and Bryde's whales
   - 4.2 North Atlantic sei whales
   - 4.3 Southern Hemisphere whale stocks
     - 4.3.1 Fin whales
     - 4.3.2 Blue whales
     - 4.3.3 Right whales
     - 4.3.4 Sei whales
5. New statistical and genetic issues relating to stock definition
   - 5.1 Simulation tools for spatial structuring (e.g. TOSSM)
   - 5.2 PCA, DAPC, and related methods
   - 5.3 Terminology
   - 5.4 Close-kin mark-recapture
   - 5.5 Epigenetic aging
6. Work plan
   - 6.1 Work plan
   - 6.2 Budget requests for 2019-20
7. Adoption of Report

Appendix 2

AN UPDATE OF THE JAPANESE DNA REGISTER FOR LARGE WHALES

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

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

Footnote no.	1	2	3	4	5	6	7	8	9	10	11	12
Species/Year	Type	No. whales	No. duplicate	No. missing	No. lab problem	No. mtDNA	% mtDNA	No. msat	%msat	Sex analysed	% sexed	Note
NP minke whale												
1994-2017	SP	2,808	0	0	8	2,800	100	2,800	100	2,808	100	
2018	SP	170	0	0	0	170	100	170	100	170	100	
2001-17	BC	2,172	0	26	2	2,172	100	2,144	99	2,142	99	
2018	BC	87	0	0	0	87	100	87	100	87	100	
NP sei whale												
2002-17	SP	1,488	0	0	4	1,484	100	1,488	100	1,488	100	
2018	SP	134	0	0	0	134	100	134	100	134	100	
NP Bryde's whale												
2000-17	SP	730	0	0	3	727	100	730	100	730	100	
2001-17	BC	5	0	0	0	5	100	4	80	4	80	Include three Omura's whale and one from the East China Sea stock
2018	BC	0	0	0	0	0	0	0	0	0	0	No BC.
NP humpback whale												
2001-17	BC	66	0	0	0	66	100	66	100	66	100	
2018	BC	1	0	0	0	1	100	1	100	1	100	
NP right whale												
2001-17	BC	3	0	1	0	3	100	2	67	2	67	Missing by the 2011 tsunami, no microsats.
2018	BC	0	0	0	0	0	0	0	0	0	0	No BC.
NP fin whale												
2001-17	BC	11	0	0	0	11	100	11	100	11	100	
2018	BC	0	0	0	0	0	0	0	0	0	0	No BC.
NP sperm whale												
2000-17	SP	56	0	0	0	56	100	56	100	56	100	
2001-17	BC	2	0	0	0	2	100	2	100	2	100	
2018	BC	0	0	0	0	0	0	0	0	0	0	No BC.
Antarctic minke whale												
1987/88-2004/05	SP	6,794	0	10	0	1,118	17	6,271	92	6,794	100	Incl. dwarf; 87/88-88/89. no microsats.
2005/06-2016/17	SP	4,550	0	549	162	3,311	73	3,839	84	4,550	100	Some missing by the 3/11 tsunami in 2011.
2017/18	SP	333	0	0	0	333	100	333	100	333	100	
Antarctic fin whale												
2005/06-2011/12	SP	18	0	0	0	18	100	18	100	18	100	

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

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Appendix 3

AN UPDATE OF THE NORWEGIAN MINKE WHALE DNA REGISTER

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Footnote no.	1	2	3	4	5	6.13	7	8	9	10	11	14		12
Species/Year	Type	No. whales	No. duplicate	No. missing	No. lab problem	No. mtDNA	% mtDNA	No. msat	%msat	Sex analysed	% sexed	SNP	%SNP	Note
NA minke whale														
1997-2017	C	11,738	112	76	2	10,652	91	11,660	99	11,660	99	1,008	9	
2018	C													

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

Appendix 4

STATUS OF THE ICELANDIC WHALE DNA REGISTER

Christophe Pampoulie and Gisli A. Víkingsson

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

Table 1 gives the present status of the registry. Samples from all the common minke whales landed as a part of the Icelandic research program (2003-07) and recent commercial catches (2008-17), as well as from commercial North Atlantic fin whale catches have been genotyped and information stored in the database (note that two hybrids blue-fin were caught in 2018).

Footnote no.	1	2	3	4	5	6	7	8	9	10	11	12
Species/Year	Type	No. whales	No. duplicate	No. missing	No. lab problem	No. mtDNA	% mtDNA	No. msat	%msat	Sex analysed	% sexed	Note
NA minke whale												
2003-07	SP	189	0	0	0	189	100	189	100	189	100	
2008-17	C	431	0	0	0	379	89	382	88	382	89	
2018	C	6	0	0	0	0	0	0	0	0	0	
NA fin whale												
2006-16	C	688	0	0	0	688	100	688	100	688	100	
2018	C	146	0	0	0	146	100	146	98	146	100	

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

Appendix 5

REPLY TO POINTS RAISED IN SC/68A/SDDNA/02

R. Hoelzel and M. de Jong

Tables 2, 3 and 4: The program Structure has relatively low power, and so those individuals that assign strongly to a cluster, fit that cluster well. The reference sample set for J- and O- stock assignments includes only those that strongly assigned, with others having been purged. Within the putative 'purple' stock, there will be a distribution of genotypes, and given evidence for ongoing gene flow with both J- and O-stock populations (Fig.1.; de Jong and Hoelzel, 2019), some will be more like J-stock, some more like O-stock, some less like either. The 'GENELAND/Structure' analyses (Tables 2c, 3c and 4c) identify those individuals from the putative purple stock that are most like J- or O-stock (having assigned to those clusters with high confidence), and then test to see if they are most like J- or O-stock. Therefore, the patterns observed in Tables 2c, 3c and 4c could be expected, but don't exclude the possibility of a purple stock with an independent evolutionary trajectory.

Tests for HWE departure: GENELAND had assigned 4 putative populations from most analyses (de Jong and Hoelzel, 2019, fig. 2), with the two (red and blue) coastal clusters later combined to generate 'purple' (following discussions at the intersessional in Tokyo). As can be seen from the insert genetic distance table in Fig. 2, the difference between 'red' and 'blue' is not the smallest in the table. Note that the possibility that red=J-stock and blue=O-stock was assessed and not supported by ABC and other analyses (see de Jong and Hoelzel, 2019). Therefore, 'purple' combines separately recognised groups, based on GENELAND. Furthermore, as illustrated in Hoelzel and de Jong (2019), there is an error rate for assignments using GENELAND of about 15%, and this is apparently higher for some comparisons than for others (especially confounding purple and each of the other two; see SC/68A/SDDNA/01). For Hoelzel and de Jong (2019) three replicates of GENELAND were compared, and only samples assigned in the same way for all three runs of the program were retained. These were then assessed for single-locus Wahlund effects, and the effect not found for within the four putative populations, while artificial mixtures did show a Wahlund effect (Fig. 3). Therefore, mixing is expected from combining separate clusters from GENELAND and due to GENELAND assignment errors, and so the indication of a Wahlund effect for the dataset shown in SC/68A/SDDNA/02 is not surprising.

Putative parent-offspring pairs: The key issues are discussed in detail in SC/68A/SDDNA/01 but summarised again briefly here. Of the 16 possible area mismatches identified based on GENELAND, only 7 could be confirmed by additional GENELAND analyses. For the 36 confirmed as being from the same area when K=3, 85% could be confirmed by additional GENELAND runs. For the 16 that were apparent mismatches from the K=3 run, only 44% could be confirmed as mismatches, suggesting that these individuals had been harder to assign.

Among the 7 that were confirmed mismatches, 5 were for PO pairs assigned based on 26 loci where the relevant paper (SC/68A/SDDNA/01) suggests the FDR may be about 10%. The other two were from 4 individuals assigned based on 16 loci, where the same paper suggests that the FDR could be nearly 50%. 10% of 48 is about 5 and 50% of 4 is 2, and 5+2=7.

Therefore, it is not possible to be confident about PO mismatches from the GENELAND analyses given the available resolution from the assignment and PO tests.

Non-genetic evidence: With respect to the presence of 'purple' during all months apart from September in 'area 2' (defined in SC/68A/SDDNA/02), fig. 4b is inconsistent with fig. 7 from de Jong and Hoelzel (2019), where individuals

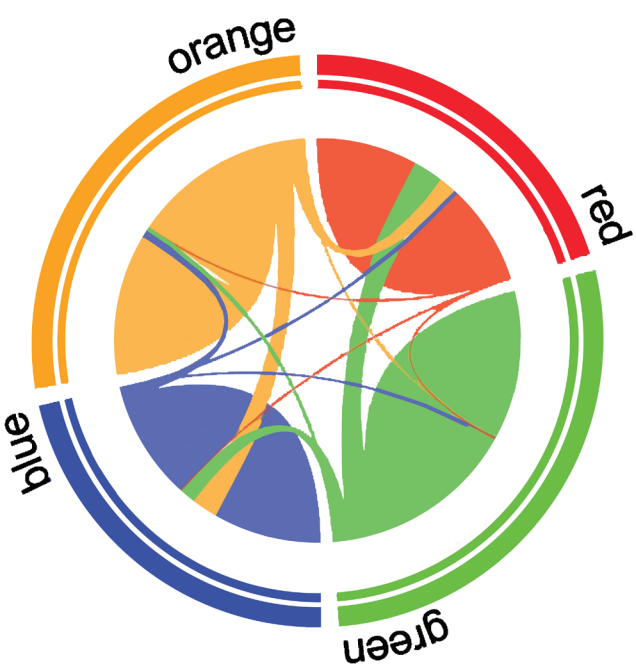

Fig. 1. Bayes Assessment of contemporary gene flow among putative populations based on the assignments found in GENELAND (see de Jong and Hoelzel, 2019).

assigned to the purple group are clearly present in area 2 during September (see Fig. 4). With respect to body length distribution, the putative J-stock is highly biased to immature animals, and it is assumed that other age classes can be found elsewhere, but without the genetic data to confirm this. For the putative purple stock, SC/68A/SDDNA/02 fig. 4 shows a broader representation of age classes (more similar to O- stock), but again the full population may not have been representatively sampled, which may be migratory or also exist in areas not sampled. For conception date, there are very few data for J-stock (mostly from bycatch), perhaps due to the nets selecting for smaller whales.

Therefore, little inference can be made for J-stock. This is the same for the putative purple stock when they are from bycatch. Conception date estimates for purple were more similar to but still significantly different from O-stock. This may suggest a difference between the two. For colouration patterns, the core problem is that we don't know the heritability or genetics of these traits, which are likely polygenic. It would be possible to generate a hypothesis consistent with the observations, that is not contradicted by the existence of a purple stock (e.g. a recent division with some continuing gene flow and mostly additive interactions for the quantitative traits, leading to a mixture of types in the purple stock). However, too little is known for any firm conclusions.

Complex model of mixing: In SC/68A/SDDNA/02 it is suggested that the apparent purple stock emerges from a complex pattern of mixing of J-stock and O-stock in that region. One problem with this interpretation is that GENELAND makes assignments based on individual genotypes and HWLE expectations for putative clusters in equilibrium. It can (and does even in this analysis) assign individuals of the 'wrong' regional stock in the same geographic area if the genetic profile is consistent with this. Hence there are multiple putative O-stock and some putative J-stock individuals assigned into the same areas as the putative purple stock whales. The problem with considering this to be simply an over-clustering artefact in GENELAND is that no matter what assumptions were made and after a number of replicate runs, putative stocks in the purple area persist (see various permutations reported in de Jong and Hoelzel (2019). Furthermore, TESS, which uses a completely different method identified essentially the same 'purple' cluster (Fig. 5).

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de Jong, M. and Hoelzel, A.R. 2019. Collaborative analysis of WNP minke whale stock structure using the Japanese microsatellite DNA database and spatially explicit population structure analyses. Paper SC/F19/WNPM02 presented to the International Whaling Commission Scientific Committee (unpublished). 26pp. [Paper available from the Office of this Journal].

Hoelzel, A.R. and de Jong, M. 2019. North Pacific minke whale putative populations as assigned by Geneland; testing for single-locus Wahlund effects. Paper SC/F19/WNPM08 presented to the IWC Scientific Committee (unpublished). 6pp. [Paper available from the Office of this Journal].

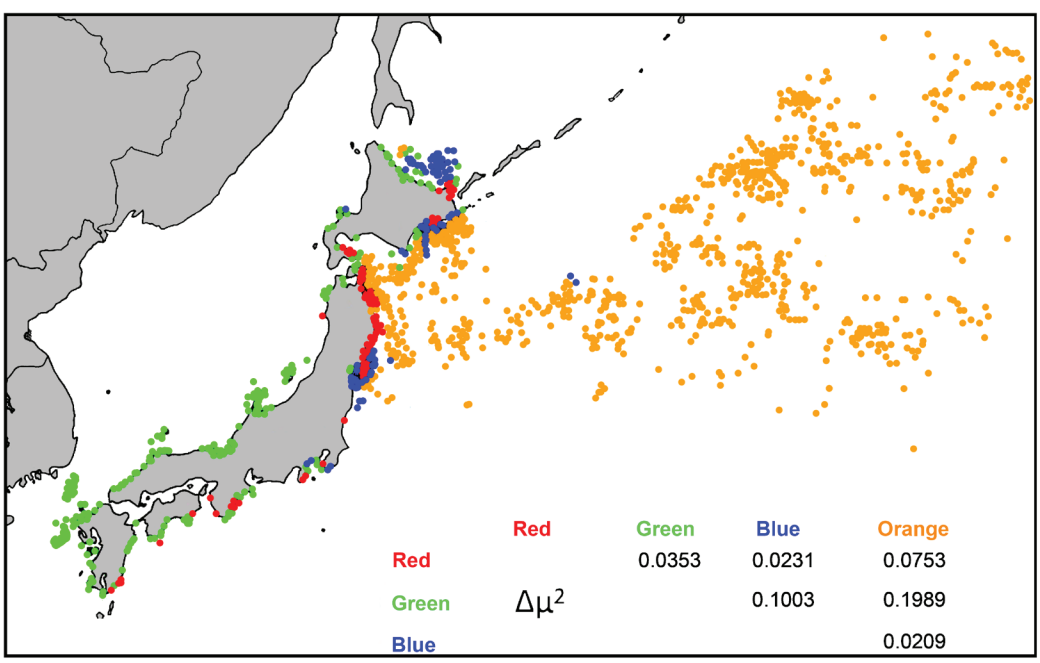

Fig. 2. Best supported clusters from GENELAND when run with 4654 samples (excluding putative offspring), 16 loci, using the correlated allele model, and allowing GENELAND to determine *K*. Inset are pairwise genetic distance measures. After de Jong and Hoelzel (2019).

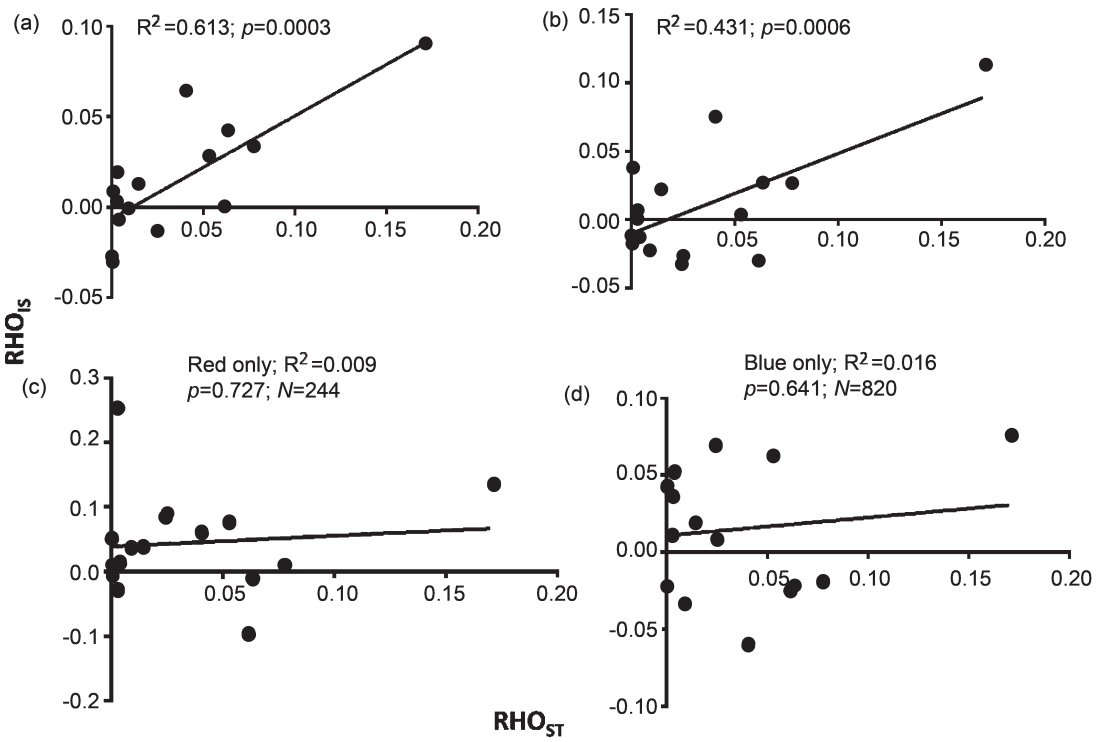

Fig. 3. Test for Wahlund effect by locus (indicated by a linear relationship) where: (a) is a mixture of 70% J-stock and 30% O-stock; (b) is a mixture of 90% J-stock and 10% O-stock; and (c) and (d) are the within population assessments for red and blue using only verified GENELAND assignments.

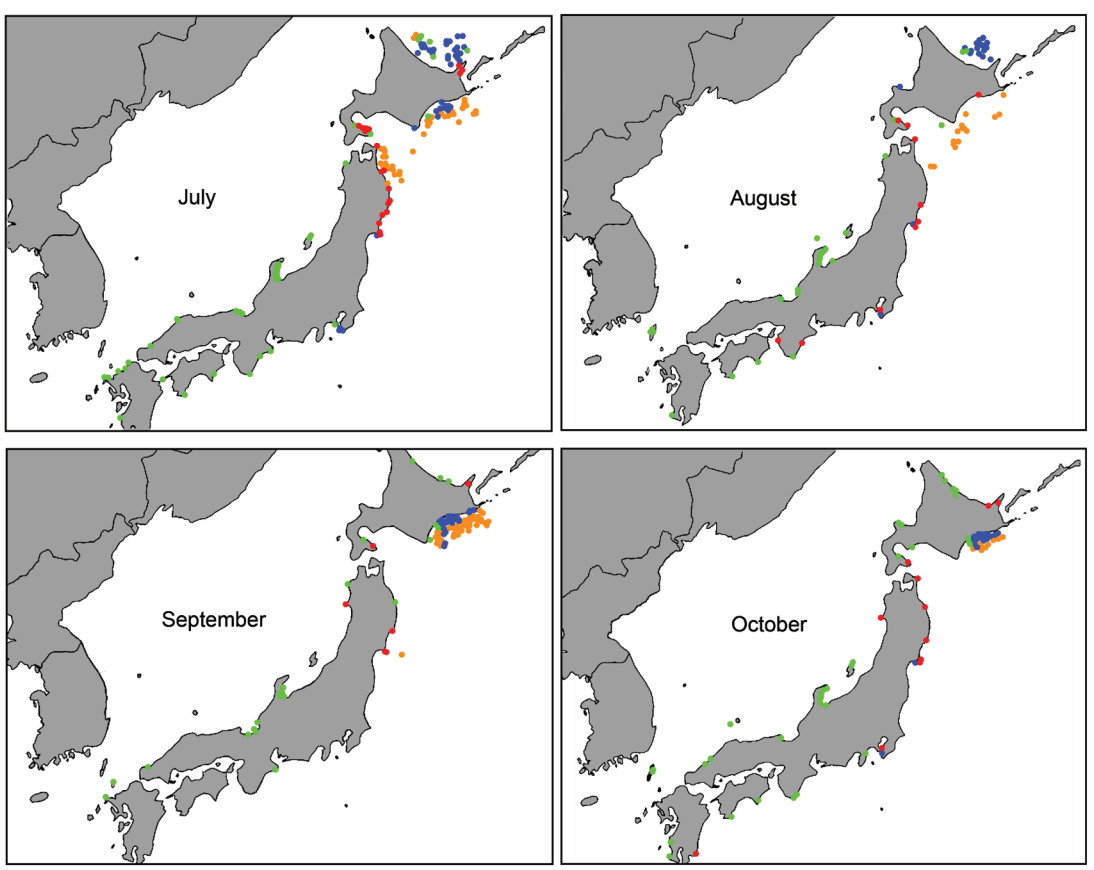

Fig. 4. Distribution of samples by assignment colour in GENELAND for four months including September. Distribution will depend on sampling effort by location and number.

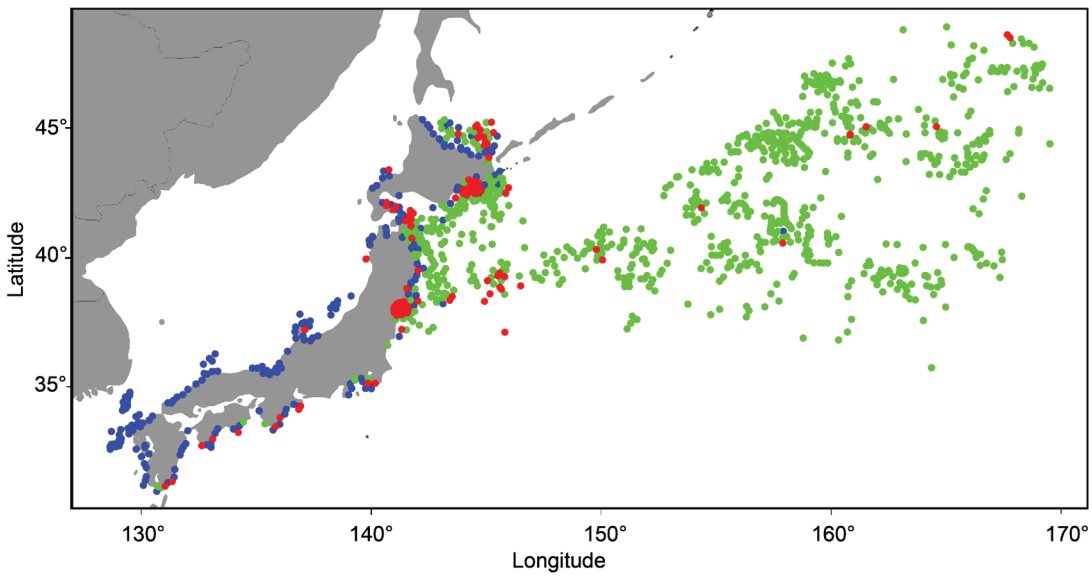

Fig. 5. Assignment of putative populations based on tessellation analysis using TESS (see de Jong and Hoelzel (2019) for further details).

Appendix 6

SPATIAL DISTRIBUTION OF PARENT-OFFSPRING PAIRS BASED ON THE GENELAND ASSIGNMENT

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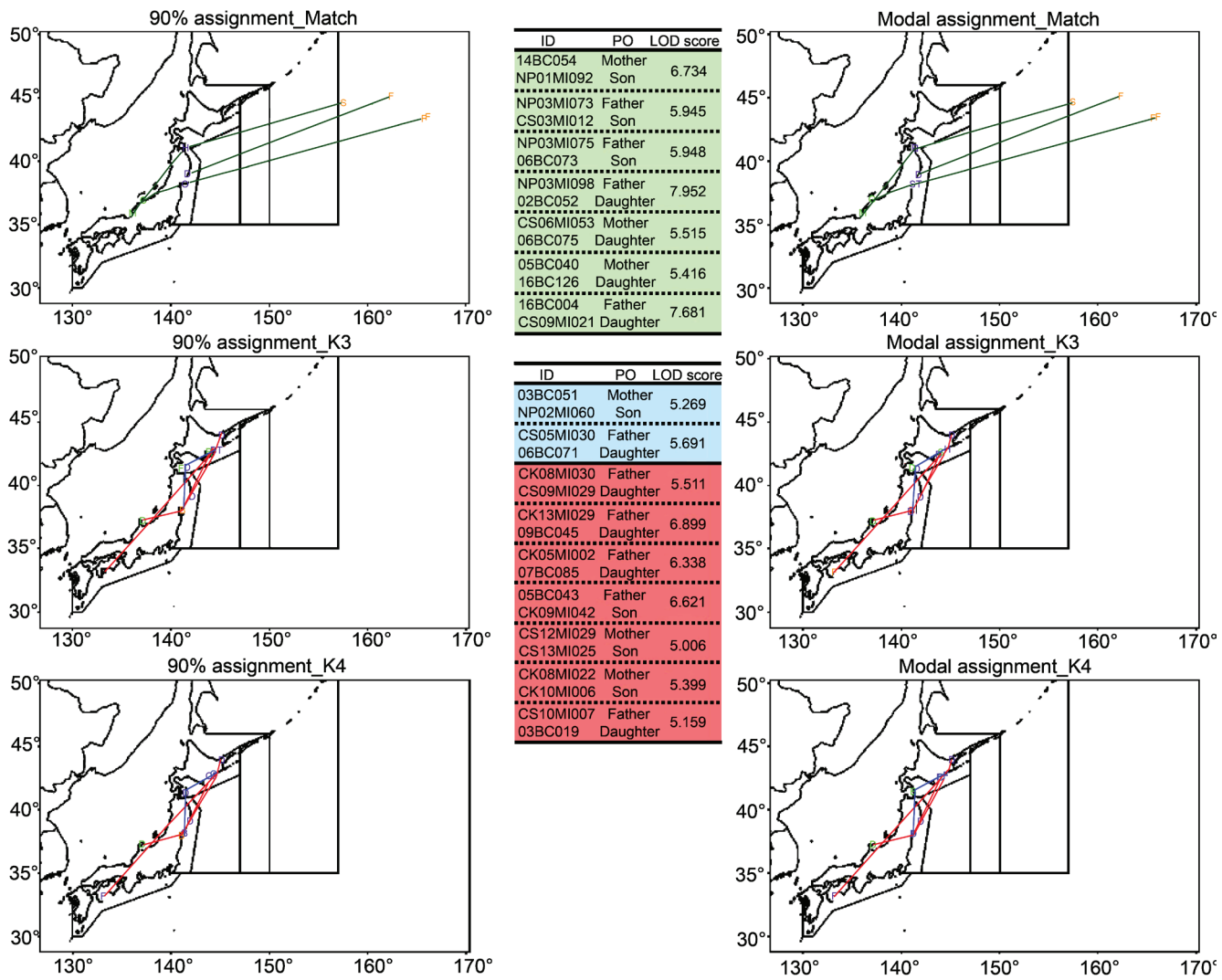
This appendix shows spatial distribution of parent-offspring (PO) pairs (n=16), based on the GENELAND assignment shown in Table 1 of SC/68A/SDDNA/01.

Main outputs of this analysis are as follows:

(1) Four of seven PO pairs were found between coastal and offshore areas, which were observed only in green shaded pairs in Table 1 of SC/68A/SDDNA/01: Mother-Son, Father-Son, Father-Son, Father-Daughter.
(2) One of the PO pairs found in offshore regions were all males.
(3) All nine PO pairs shaded with red or blue colours in Table 1 of SC/68A/SDDNA/01 were found within the coastal areas.

REFERENCE

de Jong, M. and Hoelzel, A.R. 2019. Collaborative analysis of WNP minke whale stock structure using the Japanese microsatellite DNA database and spatially explicit population structure analyses. Paper SC/F19/WNPM02 presented to the International Whaling Commission Scientific Committee (unpublished). 26pp. [Paper available from the Office of this Journal].



ID	PO	LOD score
14BC054	Mother	6.734
NP01MI092	Son	
NP03MI073	Father	5.945
CS03MI012	Son	
NP03MI075	Father	5.948
06BC073	Son	
NP03MI098	Father	7.952
02BC052	Daughter	
CS06MI053	Mother	5.515
06BC075	Daughter	
05BC040	Mother	5.416
16BC126	Daughter	
16BC004	Father	7.681
CS09MI021	Daughter	

ID	PO	LOD score
03BC051	Mother	5.269
NP02MI060	Son	
CS05MI030	Father	5.691
06BC071	Daughter	
CK08MI030	Father	5.511
CS09MI029	Daughter	
CK13MI029	Father	6.899
09BC045	Daughter	
CK05MI002	Father	6.338
07BC085	Daughter	
05BC043	Father	6.621
CK09MI042	Son	
CS12MI029	Mother	5.006
CS13MI025	Son	
CK08MI022	Mother	5.399
CK10MI006	Son	
CS10MI007	Father	5.159
03BC019	Daughter	

Fig. 1. Spatial distribution of PO pairs shown in Table 1 of SC/68A/SDDNA/01 with LOD scores for each pair extracted from SC/67A/SDDNA/01 and de Jong and Hoelzel (2019): left panel=90% threshold assignment, right panel=modal assignment. Symbol letter and colour show relationships of PO pairs and GENELAND assignment, respectively: M=mother, F=father, D=daughter and S=son. Line colour corresponds to shading colour in table 1 of SC/68A/SDDNA/01.

Appendix 7

COMPARISON OF LOD SCORES AMONG FALSE POSITIVE (FP) AND TRUE POSITIVE (TP) INFERRED PARENT-OFFSPRING (PO)-PAIRS IN NORTH PACIFIC MINKE WHALES, WITH REFERENCE TO PO-PAIRS ACROSS GENELAND CLUSTERS

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During the first Intersessional Workshop on the *Implementation Review* for western North Pacific minke whales, stock structure hypotheses have been discussed in the light of genetic clusters identified by GENELAND (IWC, 2019). Subsequently, Hoelzel and de Jong (2019) analysed inferred parent-offspring pairs (Tiedemann *et al.*, 2017; IWC, 2019) with regard to their assignment to the respective GENELAND clusters. 16 (14) of the 53 (49) inferred pairs involved individuals from different GENELAND clusters (table 1 in Hoelzel and de Jong, 2019; numbers in parenthesis are included in Tiedemann *et al*., 2017). These fell into 3 categories, i.e., 'blue': cluster assignment ambigious among different GENELAND runs; 'red': cluster assignment inconsistent among different GENELAND runs; 'green':

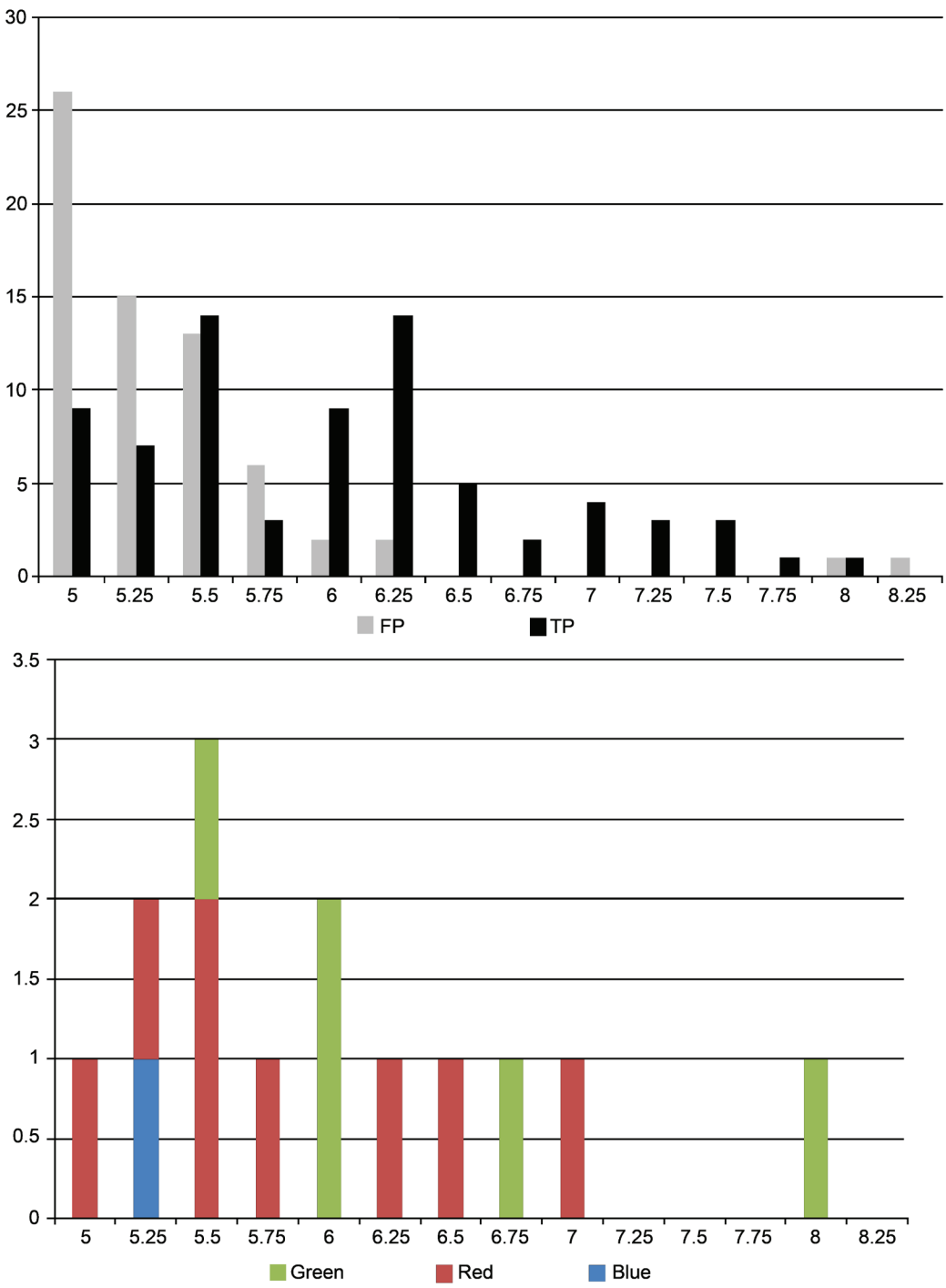

Fig. 1. LOD scores for individual PO inferences based on 26 microsatellite loci. Upper graph: Comparison among False Positives (FP) and True Positives (TP) (Tiedemann *et al*., 2017). Lower graph: True Positives (TP) found to be assigned across inferred GENELAND clusters (table 2 in Hoelzel and de Jong, 2019; for colours see text).

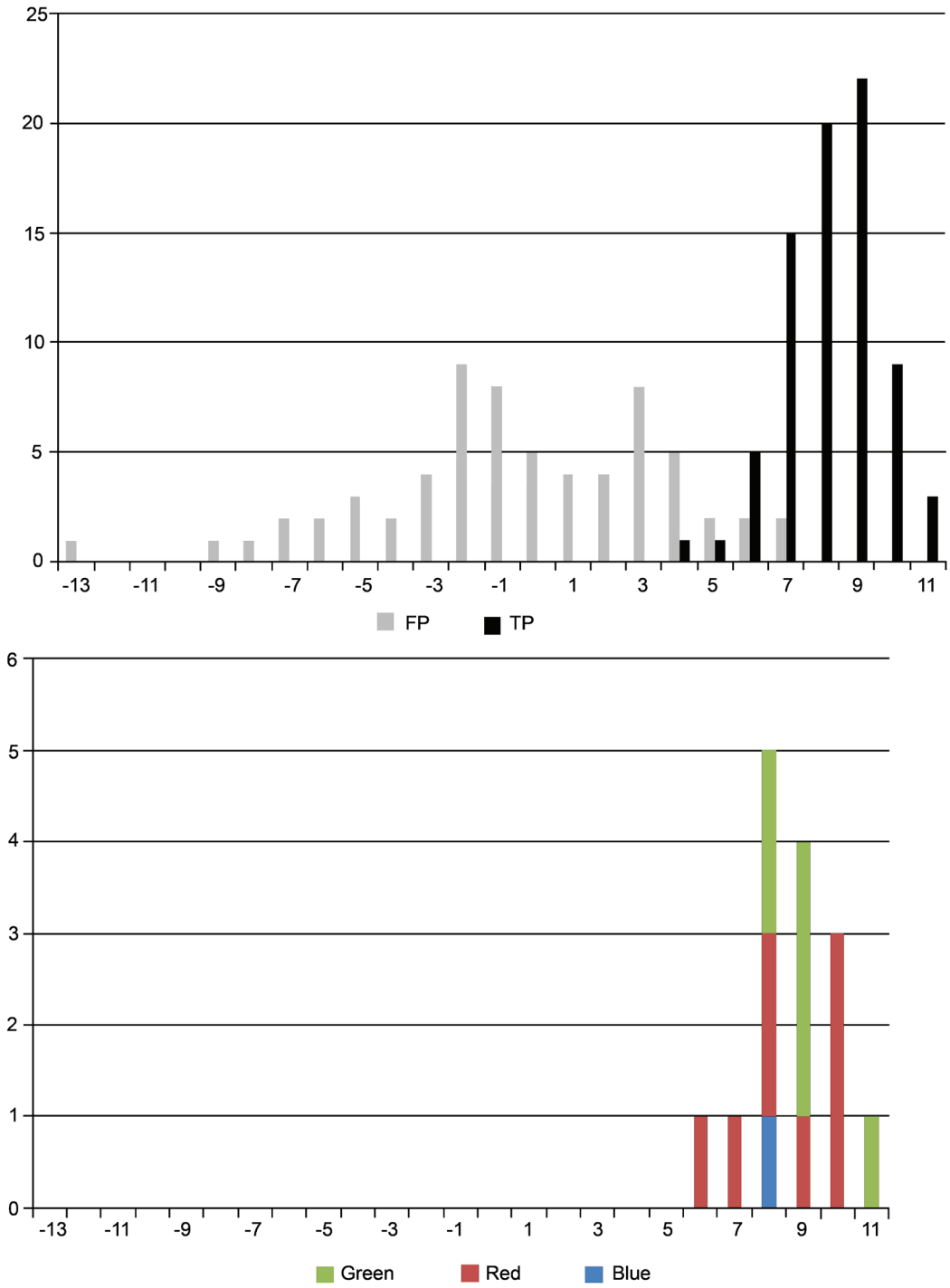

Fig. 2. LOD scores for individual PO inferences based on 26 microsatellite loci. Upper graph: Comparison among False Positives (FP) and True Positives (TP) (Tiedemann *et al*., 2017). Lower graph: True Positives (TP) found to be assigned across inferred GENELAND clusters (table 2 in Hoelzel and de Jong, 2019; for colours see text).

cluster assignment consistent among different GENELAND runs. In this appendix, I concentrate on the 14 of those PO pairs inferred by Tiedemann *et al*., 2017, omitting 2 PO-pairs inferred by subsequent analyses (the last two in table 1 in Hoelzel and de Jong, 2019). This was to ensure comparable LOD scores and having all considered PO pairs verified by 26 microsatellite loci.

False Positive (FP) means a PO pair inferred with 16 microsatellite loci (FDR 0.1), but disproved by additional analysis of 10 further microsatellites, mtDNA data (mother-offspring pair with different haplotype), or biological age information (both immature).

True Positive (TP) means a PO pair inferred with 16 microsatellite loci (FDR 0.1), confirmed by additional analysis of 10 further microsatellites, compatible with mtDNA data (mother-offspring pair with identical haplotype), and biological age information (at least one adult; if adult female, compatible with mtDNA).

Fig. 1 is based on 16 microsatellites and shows that TPs yield generally higher LOD scores than FPs, however these distributions overlap considerably.

Fig. 2 is based on 26 microsatellites. Here, the distributions of LOD scores differ substantially among FPs and TPs, with minimal overlap. No FP yielded a LOD score

beyond 7.5, while most TP had a LOD score above 7.5, with a modal value of 9 (i.e., for the interval 8.5-9.5).

Looking at those PO pairs with an assignment across GENELAND clusters, Fig. 2 shows their LOD score distribution not to differ from the general distribution for TPs. It can be hence assumed that the rate of erroneous inferences of PO-relationship (i.e. the False Discovery Rate, FDR) is the same among the inferred TPs in general and the TPs inferred across GENELAND clusters. Further, Figs 1 and 2 show that there is no indication of different LOD score distributions among the 3 categories of GENELAND assignment ('blue': ambiguous; 'red': inconsistent; 'green': consistent; see Hoelzel and de Jong, 2019 for details), but absolute numbers for these categories are small.

The observed FDR for PO pairs inferred with 26 loci was 0.1 (Tiedemann *et al*., 2017). This means that among the 49 inferred PO-pairs ranked TP, we can expect five erroneous assignments. Consequently, the 14 PO-pairs across GENELAND clusters may, at an observed FDR of 0.1 can be expected to contain 1 to 2 erroneous PO-inferences.

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