Annex O
Report of the Working Group on Stock Definition and DNA Testing

1. INTRODUCTORY ITEMS

Tiedemann welcomed participants.

1.1 Election of chairs

Tiedemann was appointed as Chair.

1.2 Appointment of rapporteurs

Cipriano and Buss agreed to rapporteur.

1.3 Adoption of agenda

The adopted agenda is given as Appendix 1.

1.4 Review of available documents

The documents identified as containing information relevant to the Stock Definition and DNA Testing Working Group, hereafter the SD-DNA WG, include SDDNA01, SDDNA02, SDDNA03 and Attard *et al.* (2024) (FI04).

2. PROVIDE ADVICE TO OTHER SUBGROUPS ON POPULATION STRUCTURE FOR REQUESTED STOCKS

2.1 North Pacific gray whale

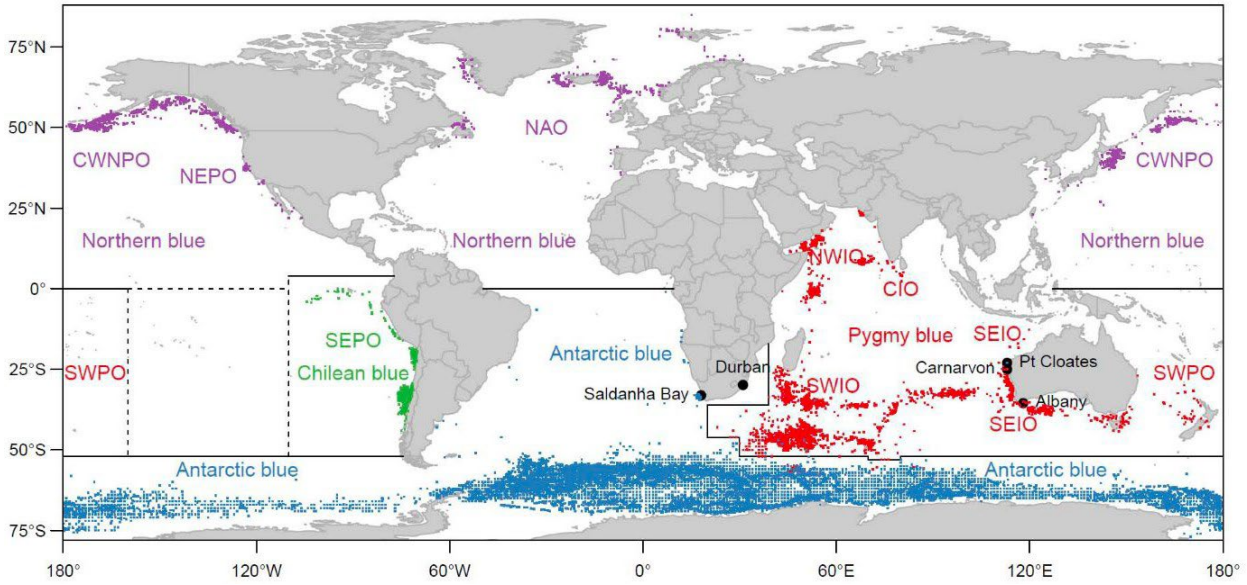
As part of the Rangewide Workshops on the Population Structure and Status of Gray Whales in the North Pacific that were held between 2014-18 (IWC, 2015; 2016; 2017a; 2018a; 2019) as well as subsequent discussion by the Committee (IWC, 2021a, b), two stock structure hypotheses (4a and 7a, see IWC, 2021b, Annex G) are currently considered high priority for inclusion in the modelling framework used to evaluate the status of North Pacific gray whales. In Hypothesis 4a, two breeding stocks of gray whales exist: an eastern breeding stock (EBS) that consists of the northern feeding group (NFG) and Pacific Coast feeding group (PCFG), both of which overwinter in the lagoons and coastal waters of Mexico; and a second breeding stock that consists of the western feeding group (WFG) of whales, which also overwinter off Mexico. Under this hypothesis, the southern Kamchatka and northern Kuril Islands feeding area (SKNK) is used by both WFG and NFG whales. In Hypothesis 7a, a third breeding stock (the Western Breeding Stock, WBS) exists that includes whales feeding on the western North Pacific feeding areas (including SKNK, which is also used by the WFG and NFG whales) and migrates to the waters off Vietnam and the South China Sea (VSC), exists. Additional hypotheses (detailed in IWC, 2021b, Annex G) are included as medium priority hypotheses and are to be considered as sensitivity tests to inform the ongoing Range-wide Review of the Status and Population Structure of gray whales.

The next Implementation Review for gray whales is scheduled for 2026. Although no new information on the stock structure of gray whales was received during SC69B, the SD-DNA Working Group reviewed past progress made by the intersessional correspondence group (ICG) that was formed at SC68B to re-evaluate the plausibility of all hypotheses under consideration and to consider issues relating to terminological distinctions being used in discussions of gray whale stock structure (IWC, 2021a). In order to prepare for the upcoming IR, the SD-DNA WG **agreed** to continue the work of this ICG, with a focus on addressing issues that were raised during SC68D (see Item 10.1.2.1 in IWC, 2023a).

2.2 Blue whales

The current understanding of stock structure in blue whales is based largely on song types (see Figure 1 and review in Sirovic and Oleson, 2022). Within the Southern Hemisphere, recognised populations include the Antarctic, the south-east Pacific Ocean (SEPO) including Galapagos to Chile; north-west Indian Ocean (NWIO) from Oman to Madagascar; central Indian Ocean (CIO) from Sri Lanka to the southern Indian Ocean; south-west Indian Ocean (SWIO) from Madagascar to Kerguelen Islands; south-east Indian Ocean (SEIO) from Tasmania westward to Indonesia; and the south-west Pacific Ocean (SWPO) from New Zealand to Tasmania. Within the North Pacific, two confirmed blue whale song types, presumed to represent two populations, exist: one in the northeastern Pacific (NEPO) and one in the central and western North Pacific (CWNPO). Within the North Atlantic (NAO), only a single song type has been recorded. Comparison of genetic and morphological data between some regions has been possible (Attard *et al*., 2010, 2012, 2016, 2018; Branch *et al*., 2007; Gilpatrick and Perryman, 2008; LeDuc *et al*., 2007, 2017; Leslie *et al*., 2020; Pastene *et al*., 2020; Torres-Florez *et al*., 2014; Sremba *et al*., 2012, 2015); where differences have been found, the results are generally consistent with the delineations derived from acoustics.

Figure 1. Modern whaling catches of blue whales showing one hypothesis for subspecies (different colours, lower case letters) and populations (upper case acronyms). Figure taken from paper SC/69A/SH/09.



Attard *et al*. (2024) (FI04) evaluates the population structure and subspecies taxonomy of blue whales using the most comprehensive dataset currently available, both in terms of genomic markers (~16,700 SNP loci and mtDNA control region sequences) and geographic coverage (n_{SNPs}=276 samples, n_{mtDNA} = 531). Clustering analysis of SNP data found support for three high-level groups, including the Antarctic, the eastern Pacific, and the Indo-western Pacific. Significant nuclear and mitochondrial genetic differentiation was found between these three high-level groups, and estimated migration rates were 1-4%, with both migrant individuals (i.e., animals moving between regions) and potential interbreeding detected. Within the high-level groups, lower but statistically significant levels of genetic differentiation were detected between the NEPO and SEPO and between the SEIO and the SWPO. Within the eastern Tropical Pacific (ETP), which was the only wintering area sampled, some samples assigned strongly to the NEPO, some to the SEPO, and others were intermediate.

The SD-DNA WG welcomed the opportunity to review Attard *et al*. (2024), noting its value in informing the ongoing in-depth assessment of Antarctic blue whales (see Annex K) as well as the pre-assessments that are currently underway for Southern Hemisphere non-Antarctic blue whales (see Annex P) and North Pacific blue whales (see Annex M).

Although previous studies suggested that population structure might be present within the Antarctic Ocean (Sremba *et al*., 2012; Attard *et al*., 2016), Attard *et al*. (2024), which used a more comprehensive dataset, did not detect genetic structure within this region. These results support assessing Antarctic blue whales as a single stock. However, it was noted that the proportion of samples that were considered migrants from other groups (3.6-4.2% from the Indo-western Pacific and 1.5-1.9% from the eastern Pacific) was in total higher than that found in the other high-level groupings. Furthermore, it was evident that there is on-going gene flow between the Antarctic and Indo-western Pacific at very low levels with several individuals displaying mixed ancestry (Attard *et al*., 2024 - Fig. 2a). Although it was agreed that the current genetic evidence suggests a single panmictic population in the Antarctic, it was noted by the SD-DNA WG that currently no genetic data were included from the higher latitudes of the South Atlantic. To further understand the Antarctic system, it was suggested that additional sampling and/or genetic sequencing from the South Atlantic is required for comparison.

The low level of divergence detected between the NEPO and SEPO, as well as the finding that some individuals sampled in the ETP have intermediate assignments to these two groups, suggest that some gene flow across the equator occurs in the eastern Pacific. Such gene flow could be mediated by overlap of the two populations at the tails of their respective breeding seasons or might also occur if some animals from one or both populations remain in the ETP year-round. However, the significant differentiation identified between the NEPO and SEPO indicate that the extent of interbreeding is likely to be low.

Attard *et al*. (2024) did not include any samples from the CWNPO. Samples from this region have been collected as part of the IWC POWER surveys and are being incorporated into ongoing analyses of population structure in the North Pacific that are being led by Sremba (Whole Genome Sequencing, WGS) and Lang (mitogenomes and ~200 SNPs).

Information regarding contemporary use of the South Atlantic by blue whales is limited. The Antarctic blue whale song type has been recorded off the west coast of South Africa between May and August (Shabangu *et al*., 2019). In addition, the SEPO song type has been recorded off the islands at 54°26'00''S, 36°33'00''W, although these records were considered to represent rare vagrants using the area (Rojas-Cerda *et al*., 2022). No other blue whale song types are associated with that region. Contemporary sightings of blue whales in the South Atlantic are rare, although it was reported that in the past several years blue whales have been sighted off Brazil and some biopsies have been collected.

Although assessments by the SC focus on demographically independent units rather than subspecies, two aspects of the Attard *et al*. (2024) results have implications for our understanding of blue whale subspecies taxonomy. Two of the three high-level groups identified by Attard *et al*. (2024) are recognised as subspecies[1] (*B. m. intermedia,* corresponding to the Antarctic grouping, and *B. m. brevicauda*, corresponding to the Indo-western Pacific grouping, with the exclusion of the samples from the northern Indian Ocean). However, the high-level grouping of blue whales from the NEPO and SEPO is inconsistent with currently recognised subspecies taxonomy for blue whales, in which NEPO whales are considered part of the Northern Hemisphere subspecies (*B. m. musculus*) and SEPO whales were proposed as a separate un-named subspecies (Branch *et al*. 2007). Further evaluation of the subspecies identity of NEPO whales would require a comparison to NAO whales. Attard *et al*. (2024) included only two individuals from the NAO (only one of which had nuclear data) and thus could provide only limited resolution on the relationship of North Atlantic and North Pacific blue whales. Additional data from the North Atlantic has recently become available. Jossey *et al*. (2024) includes whole genome and mitogenome sequences from North Atlantic blue whales, including samples from Canada (n=4 contemporary, n= 10 historic), Norway (n=7 contemporary, n=1 historic) and Iceland (n=1 contemporary, n=2 historic), and one historic sample from the South Atlantic (mitogenome only). With

[1] Committee on Taxonomy (2023). List of marine mammal species and subspecies. Society for Marine Mammalogy.

the exception of five of the Canadian samples, only mitogenome data was produced for the historic samples.

The second taxonomic consideration relates to blue whales from the northern Indian Ocean, which are currently recognised as a separate subspecies (*B. m. indica).* The northern Indian Ocean is now thought to contain at least two populations of blue whales based on song: the northwest Indian Ocean population with songs heard off Oman, Madagascar and Diego Garcia; and the central Indian Ocean population with songs heard off Sri Lanka, India, Oman, Madagascar and down to subantarctic waters. Attard *et al*. (2024) includes only a small number of samples from the northern portion of the Indian Ocean (n=1 for nuclear data and n=7 for mtDNA), and five of these are from waters off Oman. Of the three mtDNA control region haplotypes identified within the northern Indian Ocean, two are shared with the Indo-western Pacific, and the one nuclear sample clusters with the other Indo-western Pacific samples. While additional sampling, data and analyses are needed before drawing any conclusions, these results suggest that whales in the northern Indian Ocean are genetically more similar to whales in other regions of the Indo-western Pacific than would be expected for a separate subspecies.

***Attention**: SC, IA, SH, NH*

*After reviewing the results of new analyses of genetic structure of blue whales, the SD-DNA WG **agreed** that the findings are generally consistent with the stock structure being considered for assessments, in that the results support recognition of the south-west Pacific, south-west Indian, south-east Indian, south-east Pacific, north-east Pacific, and Antarctic as separate units.*

*However, little or no data was analysed from some areas known to be used by blue whales. Including data from these areas in future analyses would increase understanding of genetic structure and subspecies taxonomy. Thus, the SDDNA WG **recommends:***

1. *collaborative sharing of available tissue samples and genetic data from across the global range of blue whales, in particular from areas underrepresented in current studies (Atlantic Ocean, Northern part of Indian Ocean); and*
2. *further biopsy tissue sampling for population genetic analyses from areas underrepresented in current studies (along the South American east (Brazil) and African west coast, Northern and South Western part of Indian Ocean).*

*Furthermore, the SD-DNA WG **agrees** that including recently published data from the North Atlantic, where the nominate subspecies was described, into future analyses of subspecies taxonomy would be informative.*

2.3 North Atlantic fin whales

After reviewing new information on North Atlantic fin whales during SC69A, the Committee agreed that there was no need for additional simulation trials and agreed that the Implementation Review for North Atlantic fin whales was complete (see IWC, 2023 (69A), Annex L, Item 3). In order to prepare for the next Implementation Review, however, an ICG was formed to review existing stock structure hypotheses and to identify any additional analyses that might be used to assess the plausibility of existing hypotheses and to inform mixing parameters. Although the ICG was not able to make progress on this task intersessionally, the SD-DNA WG **agreed** that the ICG should continue its work and report on its progress at SC70 (see Table 1 Work Plan).

Previous analyses of fin whales sampled on North Atlantic feeding areas have failed to detect genetic structure in analysis of mtDNA control region sequences and genotypes at 15 microsatellite loci (Pampoulie *et al*., 2008). However, most of the genetic data made available for analyses is from

Iceland. As noted previously (IWC, 2023 SC69A, Annex O, Item 2.7), Greenland intends to contribute additional samples for future analyses, and some historic specimens are available from museums in Europe and the United States. Samples of fin whales from the Iberian Peninsula, some of which are part of the Mediterranean population, are also available. However, the lack of genetic data and/or samples from other parts of the North Atlantic fin whale range, including the waters off the U.S., Canada, and Norway, may limit the ability to resolve genetic structure.

***Attention**: SC*

*In order to facilitate future analyses of genetic structure and mixing, the SDDNA WG **reiterated** its previous recommendation (SC69A, Annex O, Item 2.7) for collaborative sharing of available tissue samples and genetic data from across the range of the North Atlantic fin whale.*

2.4 Bering-Chukchi-Beaufort Seas bowhead whales

The next Implementation Review (IR) for Bering-Chukchi-Beaufort Seas (B-C-B) bowhead whales is scheduled for 2026. The SD-DNA WG reviewed new information relevant to B-C-B bowheads and stock structure during the previous Implementation Review in 2018, after which they agreed that the results of the new genetic analyses were consistent with a lack of substructure within the B-C-B stock but that some level of historic or contemporary gene flow could exist between B-C-B and Eastern Canadian (IWC, 2018). At that time, the SD-DNA WG further agreed that it would be useful to conduct additional genetic analyses that could provide increased resolution on the genetic structure within and between the BCB and Eastern Canadian stocks prior to the next IR.

***Attention**: SC*

*In order to provide the best management advice for the 2026 Implementation Review of Bering-Chukchi-Beaufort Seas bowhead whales, the SD-DNA WG **recommends** new genetic analyses of the BCB bowheads for the 2026 Implementation Review to explore potential differentiation within and connectivity between the B-C-B and the Eastern Canadian stock as stated in the 2018 SC report. These analyses could include SNPs, mtDNA, and whole genome resequencing to make them comparable with recent studies of other bowhead stocks.*

2.5 Guiana dolphins

In 2020 (IWC, 2021d), the SD-DNA WG formed an ICG to review genetic and other lines of evidence relating to the population structure of the Guiana dolphin (*Sotalia guianensis)* and to provide advice on the 12 management unit delineations proposed by Cunha *et al*. (2020). Although the ICG did not have any progress to report this year, it was noted that during SC69A the Subcommittee on Small Cetaceans endorsed the nomination by Brazil, France, and Panamá to establish a Conservation Management Plan (CMP) for the Guiana dolphin. The proposal to consider the Guiana dolphin as a candidate for a CMP was received this year as SC69b/CMP18rev1 (Annex F).

SC69B/CMP18rev1 notes that uncertainty regarding population structure within the range of the Guiana dolphin exists and lists conducting genomic analyses of collected samples to investigate population structure, evaluate gene flow, and refine the boundaries of the management units as a high priority action. This work was encouraged by the SD-DNA WG last year (IWC, 2023a).

In recognition of the need to better understand the population structure and boundaries of the Guiana dolphin throughout its range, the SD-DNA WG ***agreed*** to continue its evaluation of available genetic and other (e.g., stable isotopes, contaminants) lines of evidence pertaining to population structure via

the intersessional correspondence group established in 2020 in order to provide advice on proposed management unit delineations.

3. DNA REGISTERS

The Committee received voluntary updates of the DNA registers from Japan (Appendix 2), Iceland (Appendix 3) and Norway (Appendix 4) that cover the period up to and including 2023. Almost all samples in the Japanese and Icelandic registers have been analysed for mitochondrial DNA (mtDNA) and a standard set of microsatellites, while almost all Norwegian samples have been genotyped for a standard set of microsatellites and, for those collected in 2016 or later, SNPs.

The Committee **thanked** Japan, Norway and Iceland for providing updates to their DNA registers using the standard format agreed in 2011 and providing the detailed information contained in their DNA registers.

4. DNA DATA QUALITY GUIDELINES

Compilation of Data Quality Guidelines for genetic studies relevant to the IWC (hereafter, the 'data quality guidelines') was initiated at SC59 (Anchorage 2007) and the guidelines have been regularly updated to follow scientific State-of-the-Art. At SC69B, the most recent version of the guidelines was revisited. The SD-DNA WG acknowledged that since the existence of these guidelines, the quality of genetic work discussed in the SC has generally increased. It was noted that the guidelines would be further improved by addressing the following aspects: (1) The quality guidelines implicitly consider aspects relevant to genotyping of samples of recent origin. Therefore, a statement was included to highlight that analysis of historical samples would necessitate further measures of scrutiny not covered here. (2) The quality guidelines implicitly consider genetic markers under study to follow patterns of neutral evolution, however, markers may be under natural selection. This aspect will be explicitly covered in the next revised version of the guidelines. (3) The most recent version of the quality guidelines includes consideration of genomic data sets, in particular genome-wide Single Nucleotide Polymorphisms (SNPs). This section was slightly revised to conform to current State-of-the-Art with respect to systematic quality control (e.g. as implemented in FAST-QC) and consideration of linkage-disequilibrium (when many loci across the genome are analysed together). (4) Genome-wide data may also reveal structural variants (e.g., chromosomal inversions) which can – if properly inferred – serve as a further category of molecular markers suitable for population inference/stock definition. It was noted that application of this marker type in IWC-related work is too rare to be currently considered in the guidelines. This may change in the future. (5) It was noted that the data quality guidelines currently do not explicitly cover mitogenomics. This will be addressed in the next revision. (6) Various editorial comments on the guidelines were considered.

It was **agreed** that – with these adjustments – the guidelines are in a form for which publication in JCRM will be targeted, after a final round of review among co-authors. This will make the guidelines a citable scientific contribution, like the IWC data analysis guidelines (Waples *et al*., 2017). Nonetheless, continuous updates will remain necessary to reflect methodological advancements. Therefore, the SD-DNA WG **agreed** that the intersessional correspondence group for DNA quality should continue its work and report on its progress at SC70 (see Table 1 Work Plan).

5. DNA DATA QUALITY AND ANALYSIS GUIDELINES CHECKLIST

The DNA data quality guidelines have just been updated (previous section). The DNA analysis (Waples *et al*., 2017) guideline is a comprehensive document. However, to facilitate both access and continuous updating, a shorter and more approachable checklist had been suggested. It was also agreed by the guideline authors that the analysis guideline document should be appended with regular updates. The SD-DNA WG discussed a first version of a DNA data analysis update and checklist, which followed the outline structure of the main document (Waples *et al*., 2017). This resulted in a document

(Appendix 5) which shall be made available attached to the original document on the IWC website. It was noted that the guidelines and checklist provide guidance both for authors of genetic work to be considered by the SC and for the SD-DNA WG to provide advice regarding contributions to the SC. As these documents need continuous updates to reflect methodological advancements, the SD-DNA WG **agreed** that the intersessional correspondence group for genetic data analysis should continue its work and report on its progress at SC70 (see Table 1 Work Plan).

6. RECOMMENDATIONS ON THE AVOIDANCE OF SAMPLE DEPLETION

The SD-DNA WG revisited the current recommendations on the avoidance of sample depletion (Appendix 6). It was re-iterated that for depleted samples, preference shall be given to Whole Genome Sequencing (WGS). In this context, it was noted that both the amount of tissue used to extract DNA (input) and the amount and quality of genomic data received (output) may vary, relative to sequencing method. For highly depleted samples, preference might be given to high quality WGS requests. To reflect methodological advancements, the SD-DNA WG **agreed** that the intersessional correspondence group on avoidance of sample depletion should continue its work and report on its progress at SC70 (see Table 1 Work Plan).

7. TERMINOLOGY USED FOR STOCK STRUCTURE-RELATED TERMS USED WITHIN THE IWC

The SD-DNA WG strives to provide at any time a consistent set of definitions for stock structure-related terms used within the IWC. It discusses potential improvements and evaluates consistent use *ad hoc* throughout its work or upon request by the SC. To reflect scientific advancement, it makes suggestions for increased consistency. As an example, it was suggested in previous years that *populations* (defined by population genetic inference) may be subdivided into smaller entities ('*stocks*', management units) for practical reasons of conservation and management, but these smaller entities must not belong to more than one population. To continuously follow scientific advancements as well as to provide a forum for *ad hoc* intersessional advice on terminology, the SD-DNA WG **agreed** that the intersessional correspondence group on terminology should continue its work and report on its progress at SC70 (see Table 1 Work Plan).

8. NEW GENETIC APPROACHES FOR USE BY THE SCIENTIFIC COMMITTEE

Among the papers discussed by the SD-DNA WG at SC69B, one (FI04) used a genomic approach, namely a reduced-representation scan for SNPs. In discussion, it was noted that Whole Genome Sequencing (WGS) is increasingly applied in population genomics, also in a conservation and management context (e.g., Celemín *et al*., 2023). While the SD-DNA WG did not receive submissions using this approach at SC69B, it encourages such contributions for SC70 to discuss the value of WGS studies to inform the work of the SD-DNA WG and the SC. It was noted that such studies may enable novel inferences potentially relevant for the SC, as, e.g., abundance estimation by close-kin-mark-recapture, inference of genetic load/inbreeding, or detection of subtle population structure by structural variants.

9. PROGRESS ON PREVIOUS RECOMMENDATIONS

Considerable progress was made on SC2252 (guidelines; see chapters 4 & 5). As these guidelines need continuous update, this recommendation shall stay active. It supersedes SC1998, SC20144, SC20145 and SC20146 which can be closed. Recommendation SC21109-SC21111 and SC2251 (on franciscana) are completed and can be closed.

10. BIENNIAL WORKPLAN

Table 1. Workplan

Topic	Intersessional 2024-25 and 2025-26	2026 Annual Meeting (SC70)

2.1 Gray whale population structure	Intersessional email group to clarify terminology associated with the gray whale stock structure hypotheses and, where needed, to further evaluate plausibility of hypothesis in preparation for the Range-wide Review of the Status and Population Structure of Gray Whales.	Report and provide advice
2.3 Fin whale population structure	Intersessional email group to review existing stock structure hypotheses and identify additional analyses that may be used to assess plausibility of existing hypotheses and to inform mixing parameters	Report and provide advice
2.4 *Sotalia guianensis* population structure	Intersessional email group to evaluate stock structure in *Sotalia guianensis*	Report and provide advice
4. DNA quality guidelines	Intersessional email group to review recent revisions to the DNA quality guidelines that pertain to data produced using HTS approaches.	Report and finalise updated guidelines
5. Analysis guidelines checklist	Intersessional email group to generate a short document providing a checklist of key aspects of the genetic data analysis guidelines and to identify aspects of the guidelines that may need updating, particularly in the context of genomic methodologies and analysis	Report and provide advice
6. Recommendations to avoid sample depletion	Intersessional email 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
7. Terminology	Intersessional email group to continue discussions of the use of stock structure-related terms within the SC	Report

Table 2. Intersessional email groups

Annex Item/Sub-committee	Type	Group short name	Terms of reference	Members
Item 2.1 SD&DNA	ICG	Gray whale population structure	To clarify the terminology used to describe the gray whale stock structure hypotheses; re-evaluate plausibility of the hypotheses, including consideration of adding new variants if needed, to inform the Range-wide Review of the Status and Population Structure of gray whales	Lang (Convenor), Bickham, Donovan, Hoelzel, Goto, Nakamura, Pampoulie, Punt, Scordino, Tiedemann, Weller.
Item 2.3 SD&DNA	ICG	North Atlantic fin whale population structure	Review existing stock structure hypotheses and identify additional analyses that may be used to assess plausibility of existing hypotheses and to inform mixing parameters	Pampoulie and Tiedemann (co-convenors), Buss, Lang, Mizroch, Palsbøll (to confirm), Witting
Item 2.4 SD&DNA/SM	ICG	Sotalia guianensis population structure	Review genetic and other evidence relating to population structure in *Sotalia guianensis*; provide advice on the proposed management unit delineations	Lang and Caballero (Co-Convenors), Archer, Baker, Briceño, Buss, Cipriano, Cunha, Domit, Fruet, Hoelzel, Lunardi, Natoli, Sousa-Lima, Tiedemann, Torres-Florez, Zerbini.

Item 4 SD&DNA	ICG	DNA quality	Review recent revisions in sections of the DNA quality guidelines that pertain to data produced using NGS approaches. Identify approaches to increase the visibility and use of the guidelines.	Tiedemann (Convenor), Archer, Baird, Baker, Bickham, Carroll, DeWoody, Hoelzel, Goto, Jackson, Lang, Palsbøll, Pampoulie, Sremba, Taguchi, Torres Florez, Waples.
Item 5 SD&DNA	ICG	Genetic data analysis	Generate a checklist of key aspects of the genetic data analysis guidelines and identify aspects of the guidelines that may need updating, particularly in the context of genomic methodologies and analysis. Identify approaches to increase the visibility and use of the guidelines.	Tiedemann (Convenor), Bickham, Buss, DeWoody, Hoelzel, Lang, Pampoulie, Sremba, Torres Florez, Waples.
tem 6 SD&DNA	ICG	Sample depletion	Discuss and provide recommendation on genomic approaches to maximise the utility of tissue samples, particularly those in danger of depletion.	Lang (Convenor), Archer, Baker, Bickham, Buss, Carroll, Harmon, Hoelzel, Goto, Jackson, Natoli, Palsbøll, Robertson, Sremba, Taguchi, Tiedemann, Torres Florez.
tem 7 SD&DNA	ICG	Terminology	Revisit the definitions that were previously put forward for stock-related terms at IWC 2014, particularly those related to large whale assessments, and revise them where necessary.	Tiedemann (Convenor), Baird, Bickham, Carroll, Cipriano, Hoelzel, Lang, Scordino.

11. ADOPTION OF REPORT

This report was adopted by consensus of the SD-DNA WG on April 26 2024.

REFERENCES

Attard, C.R.M., Beheregaray, L.B., Jenner, C., Gill, P., Jenner, M. Morrice, M., Bannister, J., Leduc R., & Möller, L. (2010). Genetic diversity and structure of blue whales (Balaenoptera musculus) in Australian feeding aggregations. *Conserv. Genet.* 11: 2437-2441.

Attard, C.R.M., Beheregaray, L.B., Burton, C.L.K., Jenner, K.C.S., Gill, P.C., Jenner, M.-N., Morrice, M.G., & Möller, L.M. (2012). Genetic identity of blue whales (*Balaenoptera musculus*) in Geographe Bay, Western Australia: Progress report. SC/64/SH27 presented to the IWC Scientific Committee, Panama, 2012. [Available from the IWC Publications Team]

Attard, C.R.M., Beheregaray, L.B., Jenner, K.C.S, Gill, P.C., Jenner, M.-N., Morrice, M.G., Robertson, K.M., & Möller, L.M. (2012). Hybridization of Southern Hemisphere blue whale subspecies and a sympatric area off Antarctica: impacts of whaling or climate change? *Mol. Ecol.* 21: 5715-5727.

Attard, C.R.M., Beheregaray, L.M., & 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. [Available at: https://doi.org/10.1038/srep22291]

Attard, C.R.M., Beheregaray, L.M., Sandoval-Castillo, J., Jenner, K.C.S, Gill, P.C., Jenner, M.-N., Morrice, M.G., & Möller, L.M. (2018). From conservation genetics to conservation genomics: a genome-wide assessment of blue whales (*Balaenoptera musculus*) in Australian feeding aggregations. *R. Soc. Open Sci.* 5: 170925.

Attard, C.R.M., Sandoval-Castillo, J., Lang, A., Vernazzani, B., Torres, L., Baldwin, R., Jenner, K., Gill, P., Burton, C., & Barceló, A. (2024). Global conservation genomics of blue whales calls into question subspecies taxonomy and refines knowledge of population structure. *Anim. Conserv*. [Available at: https://doi.org/10.1111/acv.12935]

Branch, T.A., Abubaker, E.M.N., Mkango, S., & Butterworth, D.S. (2007). Separating southern blue whale subspecies based on length frequencies of sexually mature females. *Mar. Mammal Sci.* 23: 803-833.

Celemín, E., Autenrieth, M., Roos, A., Pawliczka, I., Quintela, M., Lindstrøm, U., Benke, H., Siebert, U., Lockyer, C., Berggren, P., Özturk, A.A., Özturk, B., Lesage, V., Tiedemann, R. (in press). Evolutionary history and seascape genomics of Harbour porpoises (*Phocoena phocoena*) across environmental gradients in the North Atlantic and adjacent waters. *Mol. Ecol. Res.*

Cunha, H.A., Farro, A.P.C., & Caballero, S. (2020). Review of population structure studies for Sotalia guianensis and a proposal for Management Units. SC/68B/SDDNA/06 submitted to the IWC Scientific Committee, Virtual, 2020. [Available from the IWC Publications Team]

Gilpatrick, J.W., & Perryman, W.L. (2008). Geographic variation in external morphology of North Pacific and Southern Hemisphere blue whales (*Balaenoptera musculus*). *J. Cetacean Res. Manage.* 10: 9-21.

International Whaling Commission (2015). Report of the Workshop on the Rangewide Review of the Population Structure and Status of North Pacific Gray Whales. *J. Cetacean Res. Manage.* Suppl. 16: 489-528.

International Whaling Commission (2016). Report of the Second Workshop on the Rangewide Review of the Population Structure and Status of North Pacific Gray Whales. *J. Cetacean Res. Manage.* Suppl. 17: 567-581.

International Whaling Commission (2017a). Report of the Third Workshop on the Rangewide Review of the Population Structure and Status of North Pacific Gray Whales. *J. Cetacean Res. Manage.* Suppl. 18: 643-671.

International Whaling Commission (2017b). Report of the IWC Scientific Committee. *J. Cetacean Res. Manage.* Suppl. 18: 1-109.

International Whaling Commission (2018a). Report of the Fourth Rangewide Workshop on the Status of North Pacific Gray Whales. *J. Cetacean Res. Manage.* Suppl. 19: 521-536.

International Whaling Commission (2018b). Report of the First IWC Workshop on the Comprehensive Assessment of North Pacific Humpback Whales. *J. Cetacean Res. Manage.* Suppl. 19: 595-601.

International Whaling Commission (2019). Report of the Fifth Rangewide Workshop on the Status of North Pacific Gray Whales. *J. Cetacean Res. Manage.* Suppl. 20: 569-599.

International Whaling Commission (2021a). Report of the Scientific Committee. *J. Cetacean Res. Manage.* Suppl. 21: 1-65.

International Whaling Commission (2021b). Report of the Scientific Committee. *J. Cetacean Res. Manage.* Suppl. 23: 1-171.

International Whaling Commission (2023a). Report of the Scientific Committee (SC68D). *J. Cetacean Res. Manage.* Suppl. 24: 1-190.

International Whaling Commission (2023b). Report of the Scientific Committee (SC69A): Annex L. *J. Cetacean Res. Manage.* Suppl. 25: 1-93.

International Whaling Commission (2023b). Annex O Report of the Stock Definition and DNA Testing Working Group. [not yet in JCRM – report from SC69a].

Jossey, S., Haddrath, O., Loureiro, L., Weir, J.T., Lim, B.K., Miller, J., Scherer, S.W., Goksøyr, A., Lille-Langøy, R., Kovacs, K.M., Lydersen, C., Routti, H., & Engstrom, M.D. (2024). Population structure and history of North Atlantic Blue whales (Balaenoptera musculus musculus) inferred from whole genome sequence analysis. *Conserv. Gen.*

Leduc, R.G., Dizon, A.E., Goto, M., Pastene, L.A., Kato, H., Nishiwaki, S., Leduc, C., & Brownell Jr., R.L. (2007). Patterns of genetic variation in Southern Hemisphere blue whales and the use of assignment test to detect mixing on the feeding grounds. *J. Cetacean Res. Manage.* 9(1): 73-80. [Available at: https://doi.org/10.47536/jcrm.v9i1.694]

Leduc, R.G., Archer, F.I., Lang, A.R., Martien, K.K., Hancock-Hanser, B., Torres-Florez, J.P., Hucke-Gaete, R., Rosenbaum, H.C., Van Waerebeek, K., Brownell Jr., R.L., & Taylor, B.L. (2017). Genetic variation in blue whales in the eastern pacific: implication for taxonomy and use of common wintering grounds. *Mol. Ecol.* 26: 740-751.

Leslie, M.S., Perkins-Taylor, C.M., Durban, J.W., Moore, M.J., Miller, C.A., Chanarat, P., Bahamonde, P., Chiang, G., & Apprill, A. (2020). Body size data collected non-invasively from drone images indicate a morphologically distinct Chilean blue whale (Balaenoptera musculus) taxon. *Endanger. Species Res.* 43: 291-304.

Pampoulie, C., Daníelsdóttir, A.K., Bérubé, M., Palsbøll, P.J., Árnason, A., Gunnlaugsson, T., Ólafsdóttir, D., Øien, N., Witting, L., & Víkingsson, G.A. (2008). Lack of genetic divergence among samples of the North Atlantic Fin Whale collected at feeding grounds: congruence among microsatellite loci and mtDNA in the new Icelandic dataset. SC/60/PF/11 submitted to the IWC Scientific Committee, Chile, 2008. [Available from the IWC Publications Team]

Pastene, L.A., Acevedo, J., & Branch, T. (2020). Morphometric analysis of Chilean blue whales and implications for their taxonomy. *Mar. Mammal Sci.* 36: 116-135.

Rojas-Cerda, C., Buchan, S.J., Branch, T.A., Malige, F., Patris, J., Hucke-Gaete, R., & Staniland, I. (2022). Presence of Southeast Pacific blue whales (Balaenoptera musculus) off South Georgia in the South Atlantic Ocean. *Mar. Mammal Sci.* 38: 1425-1441.

Shabangu, F.W., Findlay, K.P., Yemane, D., Stafford, K.M., Van Den Berg, M., Blows, B., & Andrew, R.K. (2019). Seasonal occurrence and diel calling behaviour of Antarctic blue whales and fin whales in relation to environmental conditions off the west coast of South Africa. *J. Mar. Sys.* 190: 25-39.

Širović, A., & Oleson, E.M. (2022). The bioacoustics of blue whales—Global diversity and behavioral variability in a foraging specialist. *Ethology and Behavioral Ecology of Mysticetes* (pp.195-221). Springer.

Sremba, A.L., Hancock-Hanser, B., Branch, T.A., Leduc, R.L., & Baker, C.S. (2012). Circumpolar diversity and geographic differentiation of mtDNA in the Critically Endangered Antarctic blue whale (*Balaenoptera musculus intermedia*). *PLOS One* 7: e32579.

Sremba, A., Steel, D., Torres, L., Constantine, R., & Baker, C.S. (2015). Genetic identity of blue whales in the surrounding waters of New Zealand. SC/66A/SH/19 submitted to the IWC Scientific Committee, San Diego, California, 2015. [Available from the IWC Publications Team.]

Torres-Florez, J.P., Hucke-Gaete, R., Leduc, R., Lang, A., Taylor, B., Pimper, L.E., Bedriñana-Romano, L., Rosenbaum, H.C., & Figueroa, C.C. (2014). Blue whale population structure along the eastern South Pacific Ocean: evidence of more than one population. *Mol. Ecol.* 23: 5998-6010.

Waples, R.S., Hoelzel, A.R., Gaggiotti, O., Tiedemann, R., Palsbøll, P., Cipriano, F., Jackson, J., Bickham, J.W., & Lang, A.R. (2018). Guidelines for genetic data analysis. *J. Cetacean Res. Manage.* 18: 33-80. [Available at: https://doi.org/10.47536/jcrm.v18i1.421]

Appendix 1
AGENDA

1. Introductory items
    - 1.1 Election of Chairs
    - 1.2 Appointment of rapporteurs
    - 1.3 Adoption of agenda
    - 1.4 Review of available documents
2. Provide advice to other sub-groups on population structure for requested stocks
    - 2.1 North Pacific gray whales
    - 2.2 Blue whales
    - 2.3 North Atlantic fin whales
    - 2.4 Bering-Chukchi-Beaufort Seas bowhead whales
    - 2.5 Guiana dolphins
3. DNA Registers
4. DNA data quality guidelines
5. DNA data quality and analysis guidelines checklist
6. Recommendations on the avoidance of sample depletion
7. Terminology used for stock structure related terms used within the IWC
8. New genetic approaches for use by the Scientific Committee
9. Progress on previous recommendations
10. Biennial workplan

Appendix 2

An update of the Japanese DNA register for large whales

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INTRODUCTION

The technical specifications and the status of the Japanese DNA register for large whales was presented and discussed during the 2005 International Whaling Commission Scientific Committee (IWC SC) meeting (IWC, 2006). Since then, the number of genetic samples and the number of individuals analyzed and registered have been reported to the IWC SC annual meetings. The annual reports have included information of whales taken by former special scientific permits in the North Pacific (JARPN/JARPNII and NEWREP-NP) and Antarctic (JARPA/JARPAII and NEWREP-A), commercial whaling in Japan's Exclusive Economic Zone (EEZ), and bycatches along the Japanese coast.

It should be noted that the special scientific permit takes under NEWREP-A and NEWREP-NP programs were terminated in June 2019 as an effect of the withdrawal of Japan from the International Convention for the Regulation of Whaling (ICRW) on 30 June 2019. From 1 July 2019, commercial whaling within Japan's EEZ was started, and samples taken have been registered in the Japanese DNA register. The Japanese regulation on bycatches of large whales in Japan (established from 1 July 2001) requires that all animals should be registered with a DNA profile before any products derived from a bycaught animal are sold in the market.

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 the end of December in 2023 is shown in Table 1.

Table 1. The update of the Japanese DNA register for large whales till the end of December in 2023.

footnote #	1	2	3	4	5	6	7	8	9	10	11	12
Species/Year	type	# whales	# duplicate	# missing	# lab problem	#mtDNA	%mtDNA	#msat	%msat	sex analyzed	% sexed	note
NP common minke whale												
1994-2019	SP	3057	0	0	8	3049	99.7	3049	100	3057	100	
2019-2022	CW	288	0	0	0	288	100	288	100	288	100	
2023	CW	83	0	0	0	83	100	83	100	83	100	
2001-2022	BC	2575	0	26	2	2575	100	2547	99	2547	99	
2023	BC	45	0	0	0	45	100	45	100	45	100	
NP sei whale												
2002-2018	SP	1622	0	0	4	1618	99.8	1622	100	1622	100	
2019-2022	CW	100	0	0	0	100	100	100	100	100	100	
2023	CW	24	0	0	0	24	100	24	100	24	100	
2001-2022	BC	2	0	0	0	2	100	2	100	2	100	
2023	BC	0	0	0	0	0	0	0	0	0	0	No BC.
NP Bryde's whale												
2000-2017	SP	730	0	0	3	727	99.6	730	100	730	100	
2019-2022	CW	748	0	0	0	748	100	748	100	748	100	
2023	CW	187	0	0	0	187	100	187	100	187	100	
2001-2022	BC	5	0	0	0	5	100	5	100	5	100	Include three Omura's whale and one from the East China Sea stock
2023	BC	1	0	0	0	1	100	1	100	1	100	
NP humpback whale												
2001-2022	BC	73	0	0	0	73	100	73	100	73	100	
2023	BC	0	0	0	0	0	0	0	0	0	0	No BC.
NP right whale												
2001-2022	BC	4	0	1	0	4	100	3	75	3	75	One is missing by the 2011 tsunami, no microsats.
2023	BC	0	0	0	0	0	0	0	0	0	0	No BC.
NP fin whale												
2001-2022	BC	14	0	0	0	14	100	14	100	14	100	
2023	BC	2	0	0	0	2	100	2	100	2	100	
NP sperm whale												
2000-2017	SP	56	0	0	0	56	100	56	100	56	100	
2001-2022	BC	2	0	0	0	2	100	2	100	2	100	
2023	BC	0	0	0	0	0	0	0	0	0	0	No BC.
Antarctic minke whale												
1987/88-2004/05	SP	6794	0	10	0	1118	16.5	6271	92	6794	100	Incl. dwarf; 87/88-88/89. no microsats.
2005/06-2018/19	SP	5216	0	549	162	3977	76.2	4505	86	5216	100	Some missing by the 3/11 tsunami in 2011.
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, CW=commercial whaling catch, BC=bycatch.
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 analyzed for mitochondrial control region
7. % of total samples analyzed for mitochondrial control region
8. number of samples analyzed for microsatellites
9. % of total samples analyzed for microsatellites
10. number of samples analyzed for sex
11. % of total samples analyzed for sex
12. other problems or information

REFERENCES

International Whaling Commission (2006). Report of the Working Group on DNA testing. *J. Cetacean Res. Manage.* Suppl. 8: 252-258.

Kanda, N., Goto, M., Oikawa, H., & Pastene, L.A. (2014). Update of note on sampling and laboratory procedure protocols of the genetic work at the Institute of Cetacean Research (SC/65b/J27Rev). SC/65B/DNA01 presented to the IWC Scientific Committee, Bled, Slovenia, 2014. [Available

from the IWC Publications Team.]

Appendix 3

STATUS OF THE ICELANDIC WHALE DNA REGISTER

Christophe Pampoulie and Guðjón Mar Sigurðsson

Practical arrangements regarding the establishment of the Icelandic DNA register were concluded in 2007. The Marine and Freshwater Research Institute of Iceland is responsible for the establishment and maintenance of the registry that is of the same format as the Norwegian DNA registry. An ORACLE database contains all genotyped individual's information as well as information on individuals collected but not genotyped. In parallel, a DNA tissue bank has been built 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 (2008-23), as well as from commercial NA fin whale catches have been genotyped and information stored in the database.

footnote #	1	2	3	4	5	6	7	8	9	10	11	12
Species/Year	type	# whales	# duplicate	# missing	# lab problem	#mtDNA	%mtDNA	#msat	%msat	sex analyzed	% sexed	Note
NA minke whale												
2003-2007	SP	196	0	0	0	195	99	196	100	196	100	
2008-2020	C	443	0	0	0	400	90	402	91	396	89	
NA fin whale												
2006-2022	C	979	0	0	0	979	100	979	98	979	100	
2023	C	23	0	0	0	23	100	23	99	23	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 reasons other 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 samples analysed for sex

[12] other problems or information

Appendix 4

An update of the Norwegian minke whale DNA register (April 2024)

Hans J. Skaug
University of Bergen and Institute of Marine Research

footnote #	1	2	3	4	5	6.13	7	8	9	10	11	14		12
Species/Year	type	# whales	# duplicate	# missing	# lab problem	#mtDNA	%mtDNA	#msat	%msat	sex analyzed	% sexed	SNP	% SNP	note
NA minke whale														
1997-2023	C	14764	127	85	6	10652	72	14673	99	14673	99	4021	27	
2023	C	500	3	0	0	0	0	500	100	500	100	500	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 analyzed for mitochondrial control region
7. % of total samples analyzed for mitochondrial control region
8. Number of samples analyzed for microsatellites
9. % of total samples analyzed for microsatellites
10. Number of samples analyzed for sex
11. % of total samples analyzed for sex
12. Other problems or information
13. Discontinued starting from 2016
14. Started in 2016

Appendix 5
Update of DNA analysis guidelines and checklist

Hoelzel, Archer, Bickham, Carroll, DeWoody, Lang, Pampoulie, Tiedemann, Torres-Flores

The main purpose of this document is to provide a brief update of methodology that has advanced since the publication of the original guidelines document (outline below follows the original structure of Waples *et al*., 2017). This is primarily with respect to genomic methodologies. Note that the advance may come from greater resolution, or enabling analyses that wouldn't be possible without the genomic data. We recommend this to be considered for updating at each regular IWC-SC meeting.

(1) Species identification/delimitation

Issues related to alpha taxonomy come up consistently (e.g., MMS vol. 33, issue S1), especially regarding the boundary between populations and species of small cetaceans. For example, a working group in the SM sub-committee spent several years on the taxonomy of the genus *Tursiops*. This is still not fully resolved, but the identification of species and subspecies within this and other groups remains a conservation concern that is addressed by discussions at the IWC. Extensive phylogenetic reconstructions using mtDNA and multigenic nDNA markers have generated phylogenies which sometimes retain poly- and paraphyletic topologies. Phylogenies based on genomes have the power to resolve many of the issues associated with topology, and also facilitate the assessment of patterns of historical admixture and incomplete linage sorting. Taxon sampling has been an issue when a limited number of genomes were available, but the number and quality of available genomes is rapidly increasing, and the cost of generating novel genomes is decreasing. Note, however, that phylogenetic topology varies across the genome due to admixture, variation in diversity, selection, etc. Full genome phylogenies average those effects.

(2) Analysis of diversity within populations

(a) Genomic patterns of diversity give valuable detail (e.g., haplotype blocks, runs of homozygosity, genome wide H, Watterson's theta, etc.), including approaches controlling for sample size biases in these measures (e.g., rarefaction).
(b) Kinship analysis is greatly improved by including SNP data, especially from full genomes (e.g., using the relatedness2 function implemented in VCFtools).
(c) Estimates of genetic/genomic load inform about the health especially of small or bottlenecked populations and can be assessed (e.g. frequency of loss of function alleles) using genome re-sequencing when a reference genome and annotation GFF file are available (e.g., using SNPeff, see https://onlinelibrary.wiley.com/doi/full/10.1111/eva.13216; https://www.nature.com/articles/s41576-022-00448-x; https://doi.org/10.1038/s41559-024-02337-4).
(d) Within population selection can be assessed based on genomic analyses, e.g. associated with harvest, genomic characterisation of QTLs, etc. From whole genome re-sequencing (or ddRAD given a sufficient number of SNPs) a genome wide association study (GWAS) can reveal outlier regions showing greater difference or correlation between categories (e.g., habitat use) than expected by chance. These may reflect directional selection.

(3) Estimating population size, historical demography and occurrence

(a) Census size (*Nc*) can be estimated based on the indirect capture-mark-recapture of individuals through the genetic identification of close relatives using large numbers of SNP markers (to increase power).
(b) Effective population size (*Ne*), especially over historical time can use genomic scale assessments such as site frequency spectra (SFS) methods including Stairway plots, methods based on linkage disequilibrium (LD, e.g., GONE and SNeP) and coalescent methods such as PSMC. LD methods provide greater accuracy

over shorter timescales, including recent estimates, while SFS and PSMC are inaccurate for recent time periods (about the last 10K years). Note that these methods need to be considered together with caveats on implementation and interpretation (such as assumptions of panmixia etc.). Considerations of demography is important because demographic independence strongly violates the standard assumption of a single panmictic unit.
(c) Presence data can be based on metagenomic applications including e-DNA. For species identification it is often sufficient to do metabarcoding based on amplified loci (especially COI for which there is a strong database of reference sequences).

(4) Analysis of diversity among populations (aka stock structure)
It is increasingly practical and affordable to use population genomic methods, though there are caveats about quality control (QC; e.g., sequence quality scores, minimum sequencing depth and duplicate removal) and interpretation (e.g. the potential importance of LD). This includes consideration of the relative utility and merits of genome sampling (RADseq etc.), low coverage re-sequencing, and population genomics using whole genomes at reasonable depth (20X +). Note that the latter provides the best resolution and is increasingly practical. Structural variants are also likely to become more widely used for stock assessments, best assessed using high quality genomes (see https://www.nature.com/articles/s41467-020-18972-x). A consideration of hypothesis testing remains useful, such that the null should usually be panmixia with alternatives designed to test progressively more complex hypotheses (isolation-by-distance or -environment, testing for ghost populations, predefined a priori population structure, etc.).

(a) Testing for heterogeneity using large numbers of SNP markers
- (i) Putative populations defined *a priori*
- (ii) No *a priori* basis (or an uncertain basis) for grouping individuals into putative populations. In this case, the analyses are conducted on individuals rather than groups of individuals. Many SNPs increases the power to resolve cryptic populations.

(b) Describing population structure
- (i) Estimating degree of divergence using large numbers of SNP markers (though the number needed depends on the degree and duration of isolation among populations).
- (ii) Methods associated with assessing local adaptation and adaptive potential, including genome scans (GWAS), variation at candidate genes (e.g. extracted from genomes using SNPeff) and neutrality tests like Tajima's D (often in a sliding window across the genome). This is important to help identify adaptive potential.

(c) Estimating migration, including methods that estimate contemporary migration based on large numbers of SNP markers either assuming migration-drift equilibrium or based on isolation with migration models. Note the distinction between dispersal/gene flow and (seasonal) migration.
(d) High resolution admixture analysis using SNP markers (e.g., using D or f statistics).
(e) Inferring complex scenarios of population divergence based on large numbers of SNP markers (e.g. using Fast Serial SimCoal; https://doi.org/10.1093/bioinformatics/btab468).

(5) Generic/cross-cutting issues
(a) Trade-off between large numbers of SNP markers and large sample size had been an issue while generating SNPs had been relatively expensive, which is now less relevant.
(b) Resources for bioinformatic analyses are generated or modified all the time and knowledge of these tools needs to be regularly updated.
(c) It is often valuable to integrate genetic and non-genetic data, including analysing high resolution SNP datasets in the context of environmental variables using RDA, latent factor mixed models, etc., and

incorporating indigenous knowledge.

Checklist

1) Be sure to refer to the DNA quality guidelines for information on sample handling, storage and interpretation.

2) Note that processes affecting the evolution of DNA vary among DNA regions. Mitochondrial DNA is haploid in marine mammals and transmitted from mother to offspring. This means that it reflects an effective population size that is one quarter the size reflected by nuclear DNA (which is diploid and inherited bi-parentally). Also, all mtDNA loci evolve together due to strong linkage disequilibrium in the small circular genome. Across the nuclear genome, some regions may be more influenced by selection (purifying selection reducing variation, balancing selection retaining diversity or directional selection biasing and reducing diversity), some more by genetic drift, some more by DNA turnover processes (e.g., repetitive DNA like microsatellite DNA loci). When generating a reference genome, it is worth using a male individual to facilitate the comparison of paternal population structure (Y-chromosome) and female population structure (mitogenome).

3) Note that as populations evolve over time, it takes time for genetic lineages to become independent. This is referred to as 'incomplete lineage sorting' and can affect the interpretation of phylogenetic topologies as well as other analyses. Admixture can also affect interpretation of putatively distinct populations or species.

4) Population genetic analyses often assume equilibrium status with respect to Hardy Weinberg genotype proportions and linkage. Violations can affect interpretation, and so tests should be run to assess equilibrium status. Deviations from Hardy Weinberg equilibrium (HWE) may be due to inbreeding, but for microsatellite DNA loci were often associated with genotyping errors. With random mating, populations return to HWE within one or two generations. Assumptions about linkage equilibrium need to be controlled for when genomes are compared by choosing SNPs outside of the linkage disequilibrium range (which depends on Ne).

5) Small errors in genotyping can lead to misinterpretation at the population level, and so genotype quality is important. Genotyping errors will happen with next generation sequencing (genomics), but when there are very many SNPs, the effect of a small number of errors is diluted. Even so, quality control and high standards are essential. A useful tool for QC when assessing genomic data is the program fastQC (see https://rtsf.natsci.msu.edu/genomics/technical-documents/fastqc-tutorial-and-faq.aspx).

6) Demographic independence suggests divergence between stocks because it violates the assumption of panmixia. It is therefore useful to include the analysis of historical and current demographic trends (see analysis document) but note that there are important assumptions when interpreting these data, including the assumption of panmixia. For example, admixture can be misinterpreted as a population decline.

7) Various methods are available to consider the role of natural selection in differentiating populations. Methods that compare multiple loci at the population level can use deviations from neutral expectations to detect outliers that deviate from those expectations. A common method compares population diversity with Fst, but note that this is sensitive to simplifying assumptions that are often violated. Modifications of this method that compensate for these assumptions (e.g., implemented in BayesScan), still may confound strong drift with selection. At the genome level, association analyses can identify genomic

regions that are more strongly correlated or differentiated than expected (genome wide association studies – GWAS), and may indicate particular loci that can be further investigated. Note, however, that correlation is not proof of causation, and that if there are many loci of small effect involved, they may not be detected by this method.

Appendix 6
Guidelines for avoiding depletion of tissue samples
Summary of ICG progress

ICG members include: Lang (Convenor), Archer, Baker, Bickham, Buss, Carroll, Harmon, Hoelzel, Goto, Jackson, Natoli, Palsbøll, Robertson, Sremba, Taguchi, Tiedemann, Torres Florez.

Following a discussion during SC67B involving the use of tissue samples collected from Antarctic blue whales as part of the SOWER surveys, an ICG was formed to provide advice on avoiding the depletion of tissue samples (IWC, 2018). Here we summarise the discussions on this topic since that time.

The initial focus of the ICG was to compile information 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 & Palumbi, 2016); (2) reduced-representation sequencing (reviewed in Fuentes-Pardo & Ruzzante, 2017), in which restriction enzymes are used to select segments of the genome for sequencing; and (3) high-throughput targeted capture, in which preselected genomic regions of interest are enriched and sequenced (RRS, e.g., RADseq and related approaches; Baird *et al*., 2008; Elshire *et al*., 2011; Petersen *et al*., 2012). After reviewing this summary, the WG concluded that the best approach to avoid sample depletion in the future would be to conduct WGS of valuable samples (IWC, 2019).

The ICG concluded that:

- Sample depletion should be avoided, such that sample requests will be fulfilled only with those samples for which substantial tissue
- Whole genome sequencing (WGS) is the best approach to maximize the value and avoid depletion of tissue
- In most cases requests for projects using WGS will be prioritised

In addition, the ICG noted that when planning a study, consideration should be given to:

(1) Preserving some tissue for emerging genomic technologies (e.g., epigenetics, microbiome analysis) or alternative techniques (e.g., stable isotopes) that produce data outside that generated by WGS (IWC, 2021a)
(2) The amount of tissue/DNA that is required for a given approach and whether an approach that is less consumptive would be adequate to address the question (IWC, 2021b)
(3) The number of individuals included in a study. While using only a few individuals may be sufficient for making inferences about deep-time evolutionary processes, when addressing questions relevant to eco-evolutionary time scales (e.g., the most recent few generations) the tradeoff between the precision gained by sampling more genes versus including more individuals will need to be evaluated. When possible, conducting a power analysis to demonstrate the number of samples requested and the approach being used are sufficient to answer the question of interest (IWC, 2021b)
(4) Sequence quality, including the error rate, completeness (how much of genome covered), contiguity, and what portions of genome remain unresolved or incorrect. While resequencing at low depths of coverage can provide useful population-level data, not all portions of the genome will be represented and individual genotypes may be lost (IWC, 2021b).
(5) Subsampling tissues, in order to create redundancy, allow for storage in different media and/or conditions, and minimize the number of freeze/thaw cycles for each subsample (IWC, 2022).
(6) Approaches to best maximize the value of collected tissue samples prior to their use in analyses

(i.e., follow best practices for collection, preservation, curation, and archiving of tissues for omics studies, see SC68D/SDDNA3, IWC, 2022).

The ICG noted that it is important to ensure that multiple groups can make use of the data in the future, and thus the WG should identify protocols to ensure that the data is made available to others within a reasonable time frame. It further noted that technology will continue to change in the future, and so any recommendations made will need to be revisited. Given continually emerging technology, it may be valuable for all proposals for use to tissue samples collected on IWC surveys be given a technical review by SDDNA (or a subset thereof) prior to access being granted.

Table 1. The advantage and considerations of using three different approaches to generate data

Approach	Advantages	Considerations
RRS	• Relatively low cost/effort • Less DNA needed • Does not require a reference genome • Wide dispersion of markers across genome	• May require high molecular weight DNA • May have low reproducibility, particularly when genotyping large numbers of samples • Incomplete sampling of genome, limits power to detect adaptation
Targeted amplicon sequencing	• High repeatability, preferable for maintaining comparability among data sets/studies • Relatively low amount of DNA needed, has been used with low quality or degraded DNA • Cost typically somewhat higher than RR approaches • Timeinvestment/effort somewhat higher than RR due to bait design	• Can require reference genome (or prior RRS approach) • Incomplete sampling of genome (although in some cases, such as exome sequencing, it is clear what is missing).
WGS	• High marker density • Allows characterization of neutral and adaptive variation • Broadest utility - most complete account of individual genomic variation	• High cost may restrict number of individuals sequenced. • High effort - considerable • Sequencing and computing efforts needed • Some applications require high quality and amount of DNA • If resequencing, requires reference genome.

REFERENCES

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U., Cresko, W.A., & Johnson, E.A. (2008). Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLOS One* 3(10): e3376.

Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S., & Mitchell, S.E. (2011). A robust, simple Genotyping-by-Sequencing (GBS) approach for high diversity species. *PLOS One* 6: e19379.

Fuentes-Pardo, A.P., & Ruzzante, D.E. (2017). Whole-genome sequencing approaches for conservation biology: advantages, limitations, and practical recommendations. *Mol. Ecol*.

International Whaling Commission (2018). Report of the Scientific Committee (SC67B): Annex I. *J. Cetacean Res. Manage*. Suppl. 20: 1-78.

International Whaling Commission (2019). Report of the Scientific Committee (SC68A): Annex I. *J. Cetacean Res. Manage*. Suppl. 21: 1-65.

International Whaling Commission (2021a). Report of the Scientific Committee (SC68B). *J. Cetacean Res. Manage*. Suppl. 22: 1-122.

International Whaling Commission (2021b). Report of the Scientific Committee (SC68C). *J. Cetacean Res. Manage*. Suppl. 23: 1-171.

International Whaling Commission (2022). Report of the Scientific Committee (SC68D). *J. Cetacean Res. Manage*. Suppl. 24: 1-190.

Petersen, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., & Hoekstra, H.E. (2012). Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model organisms. *PLOS One* 7: e37135. [Available at: https://doi.org/10.1371/journal.pone.0037135]

Therkildsen, N.O., & Palumbi, S.R. (2016). Practical low-coverage genomewide sequencing of hundreds of individually barcoded samples for population and evolutionary genomics in nonmodel species. *Mol. Ecol. Res*. [Available at: https://doi.org/10.1111/1755-0998.12593]