

# Annex O

## Report of the Stock Definition and DNA Testing Working Group

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### 1. INTRODUCTORY ITEMS

Lang and Tiedemann welcomed participants.

#### 1.1 Election of Chairs

Lang and Tiedemann were appointed as Chair and Co-Chair.

#### 1.2 Appointment of rapporteurs

Cipriano 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 SC/69A/SDDNA/01, SC/69A/SDDNA/02, SC/69A/NH/01, SC/69A/NH/05, SC/69A/REP/01, Bierlich *et al.*, Submitted [FI05], Martinez-Loustalot *et al.*, 2023 [FI46], SC/68B/CMP26 (Martinez-Loustalot *et al.*, 2020), SC/68C/SM18 (Domit *et al.*, 2021).

### 2. ADVICE TO OTHER SUB-GROUPS ON STOCK STRUCTURE

#### 2.1 North Pacific gray whales

Two stock structure hypotheses (4a and 7a) were identified as high priority for inclusion in the modelling framework used to evaluate the status of North Pacific gray whales during the Range-wide Workshops on the Population Structure and Status of Gray Whales in the North Pacific that were held between 2014-2018 (IWC, 2015b; 2016b; 2017b; 2018c; 2019c) and in subsequent discussions (IWC, 2021b; 2021c). In both of these hypotheses, the Pacific Coast Feeding Group (PCFG) of gray whales, which is defined as the animals that are observed during the feeding season (June to November) in the region between 41°N and 52°N (northern California, United States to British Columbia, Canada) in more than one year according to photo-identification data (IWC, 2015b), is considered a feeding group that is part of the eastern Breeding Stock of gray whales. Immigration into the PCFG from the Northern Feeding Group (NFG), which includes animals that feed in the northern Bering and Chukchi Seas, occurs annually (average two whales per year) and is also modelled as a pulse of migration of 20+ individuals in each of two years (2000 and 2001). Sensitivity trials include a wider range of average immigration (between 0 and 4 whales annually and between 10 and 30 whales in the two pulse years) (IWC, 2019c).

Bierlich *et al.* (Submitted) (SC/69A/FI/05) reports on a comparison of the morphometrics (length, skull and fluke) and length-at-age growth curves between eastern North Pacific (ENP) and PCFG whales. For PCFG whales, length was derived from drone-based photogrammetry and age was estimated based on long-term photo-identification data. The ENP data, which were assumed to pertain to the Northern Feeding Group, were based on whales taken while migrating past central California during scientific whaling between 1959 and 1969. Bayesian methods were used to incorporate uncertainty associated with morphological measurements (manual and photogrammetric) and age estimates. PCFG and ENP whales were found to have similar growth rates. However, PCFG whales reach smaller asymptotic lengths, with PCFG females 0.86 m shorter than ENP females and PCFG males 0.58 m shorter than ENP males. Within each population, ENP females are 0.59 m longer than ENP males, whereas PCFG females are only 0.30 m longer than PCFG males. Small, but significant,

differences in skull and fluke morphology were also identified. Male and female PCFG whales have skull sizes that are ~2% smaller and fluke spans that are ~1% smaller than male and female ENP whales.

The SD-DNA WG thanked the authors for presenting these results. In discussion, it was noted that the morphometric differences found between PCFG and presumed NFG whales could reflect ecological divergence driven by selection for smaller body size in PCFG whales due to prey resource limitations or aspects of the foraging niche(s) used by PCFG whales. However, this pattern could also develop if whales with small body sizes are more likely to recruit into the PCFG rather than making the longer migration to the Bering Sea.

The datasets used to compare the PCFG and NFG morphology were collected during different time periods (2016-2022, aerial drone photogrammetry, SC/69A/FI/05 versus 1959-1969; measurements of whales collected during scientific whaling; Rice *et al.*, 1971) and using different methodologies (aerial photogrammetry versus examination of specimens collected during scientific whaling). While the availability of morphological data from known age whales is limited, additional data collected using aerial photogrammetry of presumed NFG whales during more comparable time periods exist and show similar mean lengths ( $12.14 \pm 0.53$  m, lactating females photographed between 2017 and 2019; Christiansen *et al.*, 2021) (mean = 12.4m, range 11.2-13.9m, females with calves photographed 1994-1998; Perryman *et al.*, 2002).

Finally, it was noted that the existing photo-identification and genetic data (Lang *et al.*, 2012; Lang *et al.*, 2014; Calambokidis *et al.*, 2019) suggest some degree of external recruitment into the PCFG, presumably from whales that previously fed in the Bering and Chukchi Seas. Thus, the morphological data collected on the PCFG range could contain a mixture of animals from either of these two groups (external versus internal recruits).

Following discussion, the SD-DNA WG **agreed** that the intersessional correspondence group (ICG) formed at SC68B (IWC, 2020) to clarify terminology and to consider the plausibility of the stock structure hypotheses in light of existing and new information be continued.

*Attention: SC*

*In the current hypotheses on stock structure of gray whales, the Pacific Coast Feeding Group (PCFG) is considered a distinct feeding group that receives some immigration of whales that feed in the Bering and Chukchi Seas. In considering new information indicating that morphological differences exist between whales feeding on the PCFG feeding ground and those that migrated past central California, the Committee noted that, given evidence of immigration into the PCFG, morphological data collected from PCFG whales may contain a mix of internally and externally recruited individuals.*

*To evaluate this possibility, the SD-DNA WG **encouraged** additional work be conducted, including:*

- (1) using simulations to generate null distributions of the morphometric data under the hypothesis that whales sampled in the PCFG comprise a single versus a mixed group and comparing that simulated data to the empirical data; and*
- (2) using multivariate analyses of the empirical data to evaluate if two cohorts can be detected.*

*After reviewing this new information, the SD-DNA WG **agreed** that the high priority stock structure hypotheses currently under consideration remain appropriate for inclusion in the modelling framework.*

## 2.2 North Atlantic humpback whales

The Comprehensive Assessment of North Atlantic humpback whales was completed in 2002 (IWC, 2002a; 2003b). Since that time, substantial new information has become available, and in 2018, the Committee agreed that it was timely to consider a range-wide In-depth assessment (IWC, 2019b, p.133). At SC69A, new information relevant to stock structure of North Atlantic humpback whales was reviewed in a joint session of the SD-DNA WG and the Sub-committee on Other Northern Hemisphere Whale Stocks.

SC/69A/NH/02 presented an assessment of the degree of divergence and inferred the rate of contemporary connectivity among humpback whale breeding populations in the North Atlantic (Cape Verde and the Antilles), based on traditional genetic markers (genotypes at 20 simple tandem repeat loci, STRs and mitochondrial control region DNA sequences) as well as genome data (10,308 SNPs across the genome and entire mitochondrial genome DNA sequences). Individual humpback whale samples were assigned to one or both of two genetic clusters identified. A single, predominantly eastern North Atlantic cluster appears to be confined to winter and summer ranges within the eastern North Atlantic and was inferred as representing the remnants of what used to be a historically isolated eastern North Atlantic breeding population. The rate of immigration of individuals of western origin into the eastern breeding population was estimated a 10-25%. The magnitude of this introgression suggested that this high level of immigration into the eastern North

Atlantic breeding grounds (Cape Verde) was a recent phenomenon, since immigration at this high rate over the long-term would have homogenised genetic diversity and erased any signal of the observed west-east genetic structure.

It was noted that the previous assessment did not take into account dispersal between breeding stocks, as genetic data were only available from the breeding stock in the Caribbean. Given that the number of individuals comprising the Cape Verde Islands (CVI) breeding stock is small, dispersal of even a small number of individuals from the larger western breeding stock into the CVI breeding stock would have a substantial demographic impact in that a non-trivial proportion of whales using CVI would be of western origin.

Most of the wintering ground samples included in the microsatellite analysis were obtained from the Greater Antilles region ( $n=91$ ), with markedly fewer samples from the Lesser Antilles ( $n=12$ ) and CVI ( $n=42$ ). Uneven sampling can result in incorrect inferences on hierarchical structure and downward-biased estimates of the number of populations (Puechmaile, 2016; Meirmans, 2018). In addition, the degree of divergence between these areas was also relatively low, which further decreases the power to detect clusters (Latch *et al.*, 2006). As a result, the precision of estimates of gene flow and assignment to clusters may have been reduced.

SC/69A/NH/05 presents a re-evaluation of the spatial structuring of the North Atlantic humpback whale population on an ocean-basin scale using an expanded photographic dataset. Analysis of photo-identification images ( $n=37,259$ ) contributed to the North Atlantic Humpback Whale Catalogue and collected between 1980 and 2015 from all North Atlantic feeding and breeding regions resulted in the identification of 9,566 individuals. Transition probabilities between all major feeding and breeding/calving areas were estimated to detect differences between breeding regions. These analyses further divided the West Indies into two distinct breeding populations: the Greater and Lesser Antilles. Breeding-to-feeding area transition probabilities were highest from the Greater Antilles to western feeding grounds (Gulf of Maine, Atlantic Canada and West Greenland), from the Lesser Antilles to eastern feeding grounds (Norway and Iceland), and from CVI to eastern feeding grounds. Migratory patterns between feeding areas and the Lesser Antilles were more similar to those from the CVI breeding segment than to the geographically closer area in the Greater Antilles, suggesting that humpbacks from these two regions within the West Indies are behaviourally distinct and may best be managed as such.

In discussion, it was noted that the results of the photo-identification and genetic analyses are broadly consistent. The genetic clustering analyses most strongly supported the presence of two genetic clusters with a strong link between the Greater Antilles breeding area and the Gulf of Maine feeding area; this pattern is also apparent in the photo-identification data where there were a high number of matches between Greater Antilles and the Gulf of Maine. In the microsatellite data clustering analysis, the Lesser Antilles appeared somewhat distinct from the other breeding areas when the number of clusters was greater than two, and the cluster apparently representing the Lesser Antilles as well as the one associated with the CVI breeding area had the strongest connection with the Norway/Barents Sea feeding area. This pattern was also apparent in the photo-identification data, where the highest number of feeding ground matches for both CVI and the Lesser Antilles was with Norway.

SC/69A/NH/05 focused on the connections between breeding areas and feeding grounds but understanding the extent of connectivity between feeding grounds and between breeding areas is also important to the assessment. It was noted that this information is available for use in the assessment.

The SD-DNA WG thanked the authors of both papers for presenting these results, which greatly increase the amount of data available to inform the assessment and highlight the value of long-term collaborative projects.

*Attention: SC*

*After reviewing substantial new information on the connectivity between feeding and breeding grounds used by humpback whales in the North Atlantic, the SD-DNA WG **encouraged** that:*

- (1) photo-identification data evaluating the connectivity between breeding grounds and between feeding grounds be made available for assessment;*
- (2) additional details of the model underlying the calculation of transition probabilities based on the photo-identification data be provided, including an evaluation of model fit;*
- (3) genetic analyses to evaluate the proportion of each breeding stock that is represented on each feeding ground (i.e., a mixed stock analysis) be conducted;*
- (4) the ICG established in 2019 (IWC, 2019a) to identify and evaluate data relevant to the Comprehensive Assessment be continued; and*
- (5) new and updated information relevant to the population structure and mixing of North Atlantic humpback whales be presented at SC69B.*

### 2.3 North Pacific humpback whales

The Comprehensive Assessment of North Pacific humpback whales began in 2016 (IWC, 2017a) with an intersessional workshop held in 2017 (IWC, 2018b). A summary of the stock structure hypotheses currently under consideration for the assessment is provided in IWC (2023, see item 8.1.1). With respect to structure on the eastern North Pacific wintering grounds, two hypotheses are being considered. Hypothesis B1 assumes that all of the whales that breed off Mexico, including those found off Baja California, are part of a single Mexico breeding stock that is separate from the Central American breeding stock (B1). Hypothesis B2 considers the whales that use the Archipiélago de Revillagigedo wintering area and the whales that winter off mainland Mexico to represent two separate breeding stocks (the offshore Mexico and mainland Mexico breeding stocks, respectively), both of which are distinct from the Central American breeding stock. For both breeding stock hypotheses, questions remain about the location of the boundary between the Central American breeding stock and the Mexico breeding stock (B1) or the Mexico coastal breeding stock (B2). The boundary region in question includes Colima, Guerrero, and Oaxaca in southern Mexico; this region is currently considered part of the Central American breeding stock's range in both breeding stock hypotheses.

Martínez-Loustalot *et al.* (2022) used photo-identification data collected from multiple locations in north, central, and southern Mexico and Central America to provide information relevant to the placement of this boundary. The movements of whales among these regions were estimated using the Interchange Index and the Movement Index. The results showed higher movements among the southern sampling areas, suggesting that most whales photographed in southern Mexico are part of the Central American breeding stock. However, the degree of interaction between the Central American breeding stock and whales that are part of other breeding stocks while on the migratory corridor is unknown and needs further study.

Martínez-Loustalot *et al.* (2020), which was previously reviewed by the Sub-committee on In-depth Assessments (see 8.1.1, p19-20, IWC, 2021d), presents the results of genetic analyses of mitochondrial DNA control region sequence data from two regions in southern Mexico (n=48 samples, Oaxaca; n=3 samples, Guerrero). Significant differences in haplotype frequencies were identified when the mtDNA data from these two regions were compared with that generated from samples (n=126) collected in Baja California, Bahía de Banderas, and the Archipiélago de Revillagigedo. In contrast, there were no significant differences between southern Mexico and data generated from Central America in Baker *et al.* (2013).

In discussion, it was noted that while a geographic boundary between breeding stocks needs to be defined for the purposes of the assessment, the photo-identification data indicates that some temporal and spatial variation exists in the proportion of Central American breeding stock whales found at different sites within the southern Mexico region. However, the results presented in Martínez-Loustalot *et al.* (2022) and in Martínez-Loustalot *et al.* (2020) are generally consistent with the current location of the boundary between the Central American breeding stock and the Mexican breeding stock (under hypothesis B1) or the Mexico coastal breeding stock (under Hypothesis B2).

*Attention: SC*

*After reviewing genetic and photo-identification data collected from sites in southern Mexico, the SD-DNA WG agreed that:*

- (1) the results were consistent with greater interchange between southern Mexico and the Central American breeding stock than with the breeding stock(s) in northern Mexico; and thus*
- (2) the stock structure hypotheses currently under consideration remain appropriate for the Comprehensive Assessment.*

### 2.4 Bering-Chukchi-Beaufort bowhead whales

The last *Implementation Review* (IR) for Bering-Chukchi-Beaufort Seas (BCB) bowhead whales was conducted in 2018. During this review, four stocks of bowhead whales were considered, including the BCB, the eastern Canadian Western Greenland (ECWG), the Okhotsk Sea (OKS), and the East Greenland-Svalbard-Barents Sea (EGSB) stocks. Following a review of the genetic data (Baird *et al.*, 2018) presented as part of that IR, the Committee agreed that the results were consistent with a lack of sub-structure within the BCB stock and that some level of historic or contemporary gene flow could exist between the BCB and ECWG stocks (p. 242, Annex I; IWC, 2019b). While not considered of immediate management concern, the Committee further agreed that additional analyses to explore potential differentiation within and connectivity between putative ECWG sub-stocks should be conducted. The additional analyses that were recommended by the WG included: (1) the use of ordination analyses, such as PCA and DAPC, to potentially discriminate between strata with low levels of differentiation; and (2) the inclusion of additional samples from the ECWG stock to increase the power to detect genetic differentiation and potentially allow whales moving between regions to be detected via genetic mark-recapture. The next *Implementation Review* for Alaskan and Chukotka bowhead whale hunts is scheduled to begin in 2025.

Paper SC/69A/SDDNA/01 incorporates additional samples collected between 2018 and 2022 from BCB whales and addresses the recommendation made during the 2018 IR to explore potential low levels of differentiation using ordination analyses. Using samples from BCB, ECWG, and OKS, standard population genetic analyses, including FST, AMOVA, and diversity indices, were conducted on both mtDNA and SNP genotype data. Results from the Principal Coordinate Analysis (PCoA) using both marker types showed large amounts of overlap between BCB and ECWG, with OKS being slightly more differentiated. In general, the BCB and ECW stocks had only low levels of differentiation (significant in some analyses and not in others), whereas the OKS stock was more distinct (significant across all analyses). The authors further noted that they are working toward obtaining more ECWG samples from Canadian collaborators to address the second recommendation made during the 2018 *Implementation Review*.

The WG thanked the authors for their presentation and for addressing the recommendation made at the 2018 *Implementation Review* (p. 241-242, IWC, 2019b). It agreed that the results are generally consistent with those from previous studies (e.g., Baird *et al.*, 2016; Baird *et al.*, 2018) indicating that while the Okhotsk and BCB bowhead whales constitute two distinct stocks, the distinction between the BCB and EC-WG stocks is less apparent, suggesting some level of historic or contemporary connectivity could exist. In discussion, it was noted that there appeared to be some heterogeneity within the samples from the Okhotsk Sea in the results of the SNP-based PCoA analysis. Running this analysis with only the Okhotsk Sea data would allow this potential pattern to be more thoroughly explored (IWC, 2019b).

*Attention: SC*

*The SD-DNA WG reviewed the results of new and updated genetic analyses of bowhead whales from the Bering-Chukchi-Beaufort Sea (BCB), Eastern Canadian, and Okhotsk Sea stocks and **agreed** that the results are consistent with those of previous analyses suggesting that while all three stocks are distinct, some level of historic and contemporary connectivity could exist between the BCB and the Eastern Canadian stock.*

## 2.5 Franciscana

The franciscana (*Pontoporia blainvillei*) is a small dolphin endemic to the Southwestern Atlantic coastal waters between central Brazil and central Argentina. It is considered the most threatened marine cetacean species in South America (Secchi *et al.*, 2003) and is listed as Vulnerable on the IUCN Red List due to fishing-related mortality at levels believed to be unsustainable (Zerbini *et al.*, 2017). In 2020, the SD-DNA WG received a paper that reviewed the available evidence relating to stock structure across the range of the franciscana and proposed that 11 management units should be recognized (Cunha *et al.*, 2020b). Given limited time to discuss these papers during the SC meeting, an intersessional correspondence group was formed and tasked with: (1) summarising the data available from genetic and other (i.e., non-genetic) lines of evidence that could be used to infer stock structure; (2) evaluating the level of support for each of the proposed subdivisions based on these combined data; and (3) providing advice on future work to address remaining questions (IWC, 2021a). Intersessionally, the ICG compiled a review of the genetic evidence pertaining to stock structure that was reviewed during a Workshop on the Review of the Status of the Franciscana that was held in April 2021 (IWC, 2021e). While the Workshop participants noted the value of the existing genetic data in informing stock structure, they agreed that a review of other (non-genetic) lines of evidence was needed before drawing any conclusions about the newly proposed management units.

Intersessionally, the ICG members drafted text summarising the data available from the non-genetic lines of evidence and its relevance to evaluating the 11 management areas proposed in Cunha *et al.* (2020b). This summary was reviewed as part of a workshop that was held in Curitiba, Brazil, in June 2022 (SC/69A/REP/01). Following the review, the Workshop participants recommended that, based on the evidence considered thus far, the 11 Franciscana Management Areas represent appropriate units for assessment and that an integrated range-wide genomic analysis be conducted.

In discussion, it was noted that the boundary between FMA III and FMA IVa, which separates the Uruguayan and Argentinian waters of the Rio de la Plata, was drawn to facilitate separate management by those two countries, although some data supporting limited movements of franciscana within FMA IVa has since become available (Wells *et al.*, 2021). However, during recent aerial surveys conducted to estimate abundance in FMA III (SC/69A/ASI/19/Rev2), franciscana were sighted near this boundary in Uruguayan waters of the Rio de la Plata. These sightings suggest that franciscana might have a continuous distribution between these two FMAs. It was noted that additional aerial surveys of this region as well as the collection and additional genetic analysis of samples from franciscana on the northern and southern portions of the Rio de la Plata are needed to determine if this boundary is appropriately placed.

It was further noted that progress is already being made to address the recommendation of Workshop participants to conduct a range-wide genomic assessment of population structure in franciscana, with efforts being made to coordinate the collection and analysis of additional samples from Brazil, Uruguay and Argentina, particularly in FMA IVd and IVe where relatively few samples have been available in the past. The SD-DNA WG looks forward to receiving an update on these efforts in the future.

The SD-DNA WG **agreed** the work of the franciscana stock structure ICG is complete, though it may be re-initiated in the future as new information becomes available.

Attention: SC, CMP

After reviewing the Report of the Workshop to Finalize the Review of the Franciscana that was held 4-5 June, 2022 in Curitiba, Brazil, the SD-DNA WG **endorsed** the following recommendations made at the Workshop:

- (1) that the 11 proposed FMAs be considered to represent separate management units for the assessment of the franciscana dolphin by the IWC;
- (2) that an integrated range-wide analysis implementing a genome-wide approach be conducted to continue refining understanding of franciscana stock structure; and
- (3) that the nomenclature 'FMA II Babitonga' be adopted in all future SC reports and assessments.

While noting that the proposed boundaries between FMAs are based on the best available information, the SD-DNA WG **agreed** that additional research to better resolve these boundaries is needed in some areas. In particular, the SD-DNA WG **encouraged** additional studies, to include aerial surveys and collection of additional samples to enable genetic analyses, be conducted to further evaluate the boundary between FMA III and FMA IVa.

## 2.6 *Sotalia guianensis*

The second small cetacean species whose stock structure is being evaluated by an intersessional correspondence group is the Guiana dolphin (*Sotalia guianensis*), which is a small coastal delphinid that ranges from Nicaragua to southern Brazil (Flores *et al.*, 2009). This species is listed as Near Threatened by the IUCN (Secchi *et al.*, 2018) and faces various anthropogenic threats throughout most of its distribution. In 2020, the Committee received a proposal to identify twelve management units within the Guiana dolphin's range (Cunha *et al.*, 2020a). Review of the support for recognition of these proposed units, including that outlined in Cunha *et al.* (2020a) is being conducted through an Intersessional Correspondence Group (ICG) formed in 2020 (IWC, 2021d). The ICG's Terms of Reference are to review the genetic and other lines of evidence relating to population structure in Guiana dolphins and to provide advice on the proposed management unit delineations. Intersessionally, the ICG updated a list of references that may have implications for understanding population structure in this species, but summaries of the relevant data have not yet been completed.

At SC69A, the SD-DNA WG reviewed the progress report on the status of current knowledge that was submitted in 2021 by the broader *Sotalia guianensis* ICG initiated in the Sub-committee on Small Cetaceans (Domit *et al.*, 2021). This progress report contains a section reviewing available data that might inform the understanding of stock structure within the range of the Guiana dolphin.

In discussion, it was noted that the twelve management units that have been proposed incorporate relatively small discrete areas, but historically Guiana dolphins were considered to be continuously distributed between Nicaragua and southern Brazil. While genetic studies have supported fine-scale structure in some areas (e.g., Hollatz *et al.*, 2011), the collection and genetic analysis of samples from throughout the range is needed to better understand if the boundaries between areas are appropriately placed. It was noted that Caballero *et al.* (2018) using mtDNA and microsatellite DNA markers suggests a clear division between the northern and southern range, but less support for sub-structure within those regions, especially along the coast of Brazil. Given that the range of the Guiana dolphin encompasses the coastal waters of multiple countries, ideally, a range-wide study could be conducted incorporating genomic approaches (e.g., SNP analyses) that facilitate collaboration across laboratories.

It was further noted, however, that obtaining biopsy samples from this species is challenging and capturing individuals for tagging and genetic sampling is risky given the potential for capture myopathy. As such, alternative methods of obtaining information relevant to stock structure should be considered. One such approach that is currently being explored in *Sotalia fluviatilis* is the use of environmental DNA (eDNA) to non-invasively collect water samples from which the species DNA may be present. Some studies of marine mammals have demonstrated the ability of eDNA to provide data contributing to the understanding of population structure (e.g., Parsons *et al.*, 2018; Székely *et al.*, 2021), and it is typically used to detect species presence (e.g., Baker *et al.*, 2018; Closek *et al.*, 2019; Alter *et al.*, 2022) which could be informative with respect to better understanding Guiana dolphin distribution and occurrence. Such studies have typically been limited to the use of relatively short sequence fragments and a few loci (see review in Suarez-Bregua *et al.*, 2022) and thus may not be appropriate for multi-locus approaches.

Stranded or bycaught individuals are an important source of genetic samples. It was noted, however, that in some areas of the range, few or no strandings have been reported.

*Attention: SC*

The SD-DNA working group **recognises** the need to better understand the population structure and boundaries of the Guiana dolphin throughout its range and **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.

Further, the SD-DNA WG **encouraged**:

- (1) the initiation of a range-wide analysis implementing a genome-wide approach be conducted to further understanding of the stock structure of the Guiana dolphin,
- (2) stranding programs throughout the range of the species to collect samples from stranded Guiana dolphins and make them available for integrated genetic analyses, and
- (3) exploration of the use of environmental DNA to inform understanding of the population structure and distribution of the species.

## 2.7 North Atlantic fin whales

The last *Implementation Review* for North Atlantic fin whales was completed in 2016 (Annex D, IWC 2017). Eight stock structure hypotheses were considered, and their respective plausibility was mostly evaluated in light of non-genetic information, given the lack of genetic structure detected using the available mtDNA and microsatellite data (Pampoulie *et al.*, 2008). Three hypotheses (IV, VII, and VIII) were ranked ‘low plausibility’ and were omitted from further consideration because they were incompatible with tagging data.

A new *Implementation Review* was conducted this year, and the SD-DNA WG reviewed the stock structure hypotheses that were previously considered. A summary of data collected since the last IR is provided in SC/69A/IST/05. Mitochondrial (mtDNA) control region sequences and genotypes at 15 microsatellite loci have been generated for samples collected in 2015, 2018 and 2022 (see Icelandic Registry Appendix 3) but have not been analysed.

In discussion, it was noted that the genetic dataset available for North Atlantic fin whales is in some respects similar to that available for the RMP/AWMP *Implementation Review* of North Atlantic minke whales that was completed in 2017 (IWC, 2018a). It was suggested that following a similar approach (IWC, 2014b; 2015a; 2016a) and incorporating both standard measures (e.g.,  $F_{ST}$ ) as well as approaches using relatedness analysis to examine kinship patterns, conducting multivariate analyses to infer mixing proportion, and examining admixture by comparing inbreeding coefficients could be helpful.

One consideration regarding using the North Atlantic fin whale sample set is that most of the available samples are from Iceland, and gaps in sample coverage in other areas limit the ability to conduct most of the analyses (e.g., evaluating mixing; relatedness analysis and even conventional analyses). Greenland has already indicated its intent to contribute further samples. Further, some historic fin whale specimens are available from museums in Europe and the United States; conducting population structure analyses from these and any other available samples, in particular from the US, Canada, and Norway, would be desirable to supplement regions of the North Atlantic where contemporary samples are limited or unavailable. This information could help inform stock structure hypotheses in future *Implementation Reviews*.

The SD-DNA WG **agreed** an intersessional correspondence group should be formed to guide additional analyses of genetic and other data that could be used to assess the plausibility of the existing hypotheses and to inform mixing parameters for the next *Implementation Review*.

*Attention: SC*

After reviewing the stock structure hypotheses considered in the previous *Implementation Review* and given that no additional data is available with which to assess their plausibility, the SD-DNA WG **agreed** that the stock structure hypotheses that were considered under the last *Implementation Review* are sufficient for use in the current *Implementation Review*.

Furthermore, the SD-DNA WG **recommended** collaborative sharing of available tissue samples and genetic data from across the range of fin whales in the North Atlantic to facilitate analyses of genetic structure and mixing.

## 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 2022. 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 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. GUIDELINES

Two sets of guidelines have been developed for reference in the Committee's discussions of stock structure: (1) the DNA quality guidelines, which provide advice on best practices for ensuring the quality of data produced for genetic analyses; (2) the genetic analyses guidelines, which provide advice on genetic analyses commonly used in the Committee's work. Both documents are intended to be 'living documents' that are updated as analytical approaches and technologies evolve. The DNA data quality guidelines are currently being updated (see 4.1 below). The guidelines for genetic data analysis were published in the *Journal of Cetacean Research & Management* (Waples *et al.*, 2018). During SC68D it was noted that some analytical approaches included in the genetic data analyses need to be updated in light of advances in genomic approaches (IWC, 2022). While some of these updates were discussed further during SC69A, no progress on this item was made due to time constraints.

##### 4.1 DNA data quality guidelines

The DNA data quality guidelines address DNA validation and systematic quality control in genetic studies and are currently available on the IWC website.<sup>1</sup> As the use of high throughput sequencing approaches to produce genetic data has become increasingly common, the need to update these guidelines to address data quality issues associated with these newer approaches has been highlighted.

In discussion, the SD-DNA WG acknowledged the challenges associated with providing advice on the use of both traditional technologies (e.g., microsatellite genotyping, Sanger sequencing) and more contemporary and emerging approaches that rely on high-throughput sequencing. Both approaches are used to address questions of interest to the Scientific Committee, and thus both remain relevant to include in the guidelines. In some cases, the cost of using high throughput sequencing approaches can be prohibitive, and it was noted that encouraging the use of such approaches, where appropriate to address questions of interest, may facilitate funding.

The SD-DNA WG **agreed** the intersessional correspondence group would continue to review and update sections of the DNA data quality guidelines that apply to high throughput sequencing data.

##### 4.2 DNA data quality and analysis guidelines checklist

During SC68C, the SD-DNA WG proposed that a relatively short 'bridge' document be generated that would: (1) provide a checklist of key aspects of data quality and analysis that could aid interpretation of documents reviewed by the Committee; and (2) update aspects of the guidelines, particularly with respect to genomic methodologies and analysis. An intersessional correspondence group (ICG) was formed to discuss and generate this document (IWC, 2021a). While no intersessional progress on the checklist was made this year, it was noted that generating such a checklist will be facilitated when the updated DNA data quality guidelines document is available.

The SD-DNA WG **agreed** to continue the work of the intersessional correspondence group to produce a short document containing a checklist of key aspects of the genetic data analysis guidelines in order to facilitate their use and **reiterated**: (1) the importance of keeping guidelines related to genetic data quality and analyses up to date; (2) the need to consider approaches to increase the visibility of both sets of guidelines as part of the terms of reference for both ICGs.

*Attention: SC, S*

*With respect to the DNA data quality guidelines, the SD-DNA WG:*

- (1) **recognised** the importance of continuing to provide advice on data produced using traditional approaches (e.g., microsatellite genotyping, Sanger sequencing); it also*
- (2) **encouraged** the use of high throughput sequencing approaches that may provide increased resolution to address questions of interest to the Committee; and*
- (3) **emphasised** the value of generating high quality reference genomes that can serve as a resource for other studies (see also item 5).*

#### 5. RECOMMENDATIONS ON THE AVOIDANCE OF SAMPLE DEPLETION

This agenda item was established based on concerns about the depletion of tissue samples collected from Antarctic blue whales during the SOWER surveys, which were subject to requests from multiple researchers for their use in a variety of

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

studies (IWC, 2019b). Since that time, discussion has primarily focused on providing advice on genomic approaches, such as Whole Genome Sequencing, that would maximise the value of the tissue used.

During SC69A, the SD-DNA working group reviewed past progress on this item. It was noted that it could be valuable to provide advice on the quantity of tissue typically needed for commonly used approaches. This would allow those evaluating sample requests to assess whether the amount of tissue being requested is reasonable for the approach being used. In addition, the WG has previously recommended that enough tissue be preserved to allow whole genome sequencing of samples, and providing quantitative guidelines would allow more direct evaluation of whether that criterion is being met. The WG also discussed how to ensure that the advice that has been compiled is useful to the groups (e.g., the POWER steering committee) that evaluate sample requests.

The SD-DNA WG **reiterates** its concern regarding the depletion of tissue samples in existing collections (including those collected during the IWC surveys). In order to increase the utility of the ICG's work to those evaluating sample requests (e.g., the POWER steering committee), the SD-DNA WG **agreed** that the ICG formed during SC67B should continue its work, with a focus on providing advice on the amount of tissue typically needed for commonly used approaches to produce genetic and genomic data.

## 6. TERMINOLOGY USED FOR STOCK STRUCTURE RELATED TERMS USED WITHIN THE IWC

Following a recommendation arising in 2012 (Taguchi *et al.*, 2017), the SD-DNA WG initiated efforts to compile 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 SC68A, the WG 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, 2014a).

Limited time was available to discuss stock structure-related terminology during SC69A. It was noted that the input of members of the SD-DNA WG could be valuable in discussion of the stock-related terms being employed as part of the Status of the Stocks Initiative; SD-DNA WG members were encouraged to contribute to those discussions.

The SD-DNA working group **agreed** to continue the work of the intersessional email 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 SC69B.

## 7. BIENNIAL WORKPLAN

For the workplan see Table 1. For details of intersessional correspondence groups, see Annex V.

Table 1  
Workplan.

Topic	Intersessional 2023/24	2024 Annual Meeting (SC/69B)
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
2.6 <i>Sotalia guianensis</i> population structure	Intersessional email group to evaluate stock structure in <i>Sotalia guianensis</i> .	Report and provide advice
2.7 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
4.1 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
4.2 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
5. 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
6. Terminology	Intersessional email group to continue discussions of the use of stock structure-related terms within the SC.	Report

## 8. ADOPTION OF REPORT

The report was adopted May 2, 2023, at 11:13 European Summer Time.

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## Appendix 1

### AGENDA

1. Introductory items
  - 1.1 Convenors' opening remarks
  - 1.2 Election of Chairs and appointment of rapporteurs
  - 1.3 Adoption of agenda
  - 1.4 Review of available documents
2. Advice to other sub-groups on stock structure
  - 2.1 North Pacific gray whales
  - 2.2 North Atlantic humpback whales
  - 2.3 North Pacific humpback whales
  - 2.4 Bering-Chukchi-Beaufort bowhead whales
  - 2.5 Franciscana
  - 2.6 *Sotalia guianensis*
  - 2.7 North Atlantic fin whales
3. DNA Registers
4. Guidelines
  - 4.1 DNA data quality guidelines
  - 4.2 DNA data quality and analysis guidelines checklist
5. Recommendations on the avoidance of sample depletion
6. Terminology used for stock structure related terms used within the IWC
7. 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 2022 is shown in Table 1 (see next page).

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Table 1

The update of the Japanese DNA register for large whales till the end of December in 2022.

footnote #	1	2	3	4	5	6	7	8	9	10	11	12
Species/Year	type	# whales	# duplicate	# missing	# lab	#mtDNA	%mtDNA	#msat	%msat	sex analyzed	% sexed	Note
NP common minke whale												
1994-2019	SP	3057	0	0	8	3049	99.7	3049	99.7	3057	100	
2019-2021	CW	230	0	0	0	230	100	230	100	230	100	
2022	CW	58	0	0	0	58	100	58	100	58	100	
2001-2021	BC	2514	0	26	2	2514	100	2486	98.9	2486	98.9	
2022	BC	61	0	0	0	61	100	61	100	61	100	
NP sei whale												
2002-2018	SP	1622	0	0	4	1618	99.8	1622	100	1622	100	
2019-2021	CW	75	0	0	0	75	100	75	100	75	100	
2022	CW	25	0	0	0	25	100	25	100	25	100	
2001-2021	BC	2	0	0	0	2	100	2	100	2	100	
2022	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	
2019-2021	CW	561	0	0	0	561	100	561	100	561	100	
2022	CW	187	0	0	0	187	100	187	100	187	100	
2001-2021	BC	5	0	0	0	5	100	4	80	4	80	Include three Omura's whale and one from the East China Sea stock
2022	BC	0	0	0	0	0	0	0	0	0	0	No BC.
NP humpback whale												
2001-2021	BC	72	0	0	0	72	100	72	100	72	100	
2022	BC	1	0	0	0	1	0	1	0	1	0	
NP right whale												
2001-2021	BC	4	0	1	0	4	100	3	75	3	75	One is missing by the 2011 tsunami, no microsats.
2022	BC	0	0	0	0	0	0	0	0	0	0	No BC.
NP fin whale												
2001-2021	BC	14	0	0	0	14	100	14	100	14	100	
2022	BC	0	0	0	0	0	0	0	0	0	0	No BC.
NP sperm whale												
2000-2017	SP	56	0	0	0	56	100	56	100	56	100	
2001-2021	BC	2	0	0	0	2	100	2	100	2	100	
2022	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	92.3	6794	100	Incl. dwarf; 87/88-88/89. no microsats.
2005/06-2018/19	SP	5216	0	549	162	3977	76.2	4505	86.4	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 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 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, 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 all genotyped individual's information as well as tissue collected ID of individuals 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 (2003-2007) and recent commercial catches (2008-2022), 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	442	0	0	0	400	90	402	91	396	89	
2021	C	1	0	0	0	0	0	0	0	0	0	
NA fin whale												
2006-2018	C	834	0	0	0	834	100	834	98	834	100	
2022	C	145	0	0	0	145	100	145	99	145	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 2023)

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Species/Year	type	# whales	# duplicate	# missing	# lab problem	#mtDNA	%mtDNA	#msat	%msat	sex analyzed	% sexed	SNP	% SNP	note
NA minke whale														
1997-2022	C	14264	124	85	6	10652	75	14173	99	14173	99	3524	25	
2022	C	576	3	3	3	0	0	570	99	570	99	573	99	SNPs not completed (estimated number)
Footnote no.	1	2	3	4	5	6	13	7	8	9	10	11	14	12

\*Lab problem refers to msats (1 or 2 loci)

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