Report of the Workshop to Review the Japanese Whale Research Programme under Special Permit for North Pacific Minke Whales

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Report of the Workshop to Review the Japanese Whale Research Programme under Special Permit for North Pacific Minke Whales (JARPN)

The Workshop was held at the Mariner's Court Hotel, Tokyo, from 7-10 February 2000. A list of participants is given as Annex A.

1. WELCOMING REMARKS

Bannister welcomed the participants, informing them that he was acting as Convenor as Smith had been unable to attend the Workshop at the last minute for reasons beyond his control. He read the Workshop a message from the Scientific Committee Chair, Zeh, in which she conveyed her warmest greetings to all participants, and expressed her confidence that those assembled would conduct a rigorous and fair review of the JARPN results. Hatanaka welcomed Bannister and the other nine participants from outside Japan. He expressed his thanks to Bannister as well as to Smith. He reminded the participants that the JARPN surveys had occurred over a six-year period, had sampled 498 minke whales, and that the resulting data had been analysed. Further, he believed that the research had been successful, and looked forward to constructive discussion and evaluation during the Workshop.

2. TERMS OF REFERENCE

The terms of reference agreed by the Scientific Committee last year (IWC, 2000b) were to:

(1) Review the methods and results of the research programme, 1994-1999.
(2) Assess the further potential of existing data for:

   (a) meeting JARPN objectives;
   (b) other objectives.

(3) Evaluate whether the main objectives have been achieved.

The main objectives of JARPN (e.g. see SC/F2K/J29) were to determine:

(1) whether or not the 'W' stock exists and if so to estimate mixing rates between the 'O' and 'W' stocks; and
(2) the feeding ecology of minke whales in the North Pacific.

The Committee expected that the report of the present Workshop would provide it with information on the plausibility of options being considered in the RMP *Implementation Simulation Trials* for North Pacific minke whales (IWC, 2000c) when those results are considered during the next Annual Meeting.

3. ELECTION OF CHAIR AND APPOINTMENT OF RAPPORTEURS

Bannister was elected Chairman. Punt, Butterworth, Haug and Taylor acted as rapporteurs.

4. MEETING ARRANGEMENTS

Bannister reviewed the arrangements for the meeting, noting with appreciation the arrangements by the hosts that simultaneous translation would be available between Japanese and English and vice versa.

5. ADOPTION OF AGENDA

The adopted Agenda is given as Annex B. It takes into account the Terms of Reference of the Workshop and the draft agenda (IWC, 2000b) developed by the Steering Group established by the Scientific Committee (IWC, 2000d).

6. REVIEW OF DOCUMENTS

The list of documents is given as Annex C.

7. REVIEW OF AVAILABLE DATA

Annex D lists the data types produced during JARPN that were made available for use during the Workshop. Annex E lists the full set of documents based on the data and material obtained during JARPN.

8. OUTLINE OF JARPN AND PAST DISCUSSIONS OF IT IN THE COMMITTEE

SC/F2K/J29 reviewed the history and objectives of JARPN. The JARPN surveys started in 1994 with the primary objective of elucidating the stock structure of minke whales in the northwestern North Pacific to assess the plausibility of working hypotheses developed by the Working Group on North Pacific Minke Whale Management Trials (IWC, 1994, pp.120-44). Originally there were three sub-objectives:

(1) to assess whether the 'W' stock exists;
(2) to provide data to estimate the mixing rate of the 'W' and 'O' stocks; and
(3) to assess the validity of the 'O' sub-stock scenario.

In 1996, the Committee agreed that the 'O' sub-stock structure scenario should be dropped from the *Implementation Simulation Trials* for North Pacific minke whales, and since then this third sub-objective has not been considered. A second objective, 'the feasibility study on the

feeding ecology of minke whales in the research ground', was added in 1996. In 1999, a sub-objective was added to the primary objective, *viz*. to estimate the mixing rate between the 'J' and O stocks.

SC/F2K/J8 outlined the JARPN research activities from 1994-1999. Surveys in 1994 and 1995 were considered to be feasibility surveys, which were followed by full-scale surveys beginning in 1996. The original research plan was for a research period lasting three to five years. The planned research area comprised sub-areas 7, 8, 9, 11 and 12 of the North Pacific (Fig. 1), which covered the area where improved stock definition information was needed. Initially, the sighting and sampling techniques were similar to those used in JARPA. However, methods were modified in 1995; this included sampling from the secondary sightings and restricting the area surveyed to waters $< 15°C$. The new survey mode continued to search and sample, with the three sighting/sampling vessels acting cooperatively, even when sea conditions were unsuitable for sighting. A special monitoring survey (SMS) was also conducted for the limited area of high minke whale abundance.

Normally three sighting/sampling vessels searched along set tracklines. When minke whales or possible minke whale schools were sighted, the vessel immediately closed on the school. Sampling was attempted after school size and species were confirmed, providing the individual was identified as a minke whale. If the school consisted of two or more individuals, the animal to be sampled was selected using random table digits as in JARPA.

The 1994 and 1995 surveys were conducted in sub-area 9. The 1996 survey was conducted in sub-areas 7, 8 and 11. All these surveys were conducted in mid-summer. The 1997 and 1998 surveys were conducted early in the migration season (May and June) following discussions by the Working Group for the North Pacific Minke Whale Trials in 1996 (IWC, 1997, pp.203-26). The 1999 survey was conducted in the west of sub-area 7 in June and in sub-area 11 in July to provide better coverage in those areas at those times.

The Workshop noted that substantial discussions of the programme had occurred in the Committee at the programme's inception, in 1994, when there was a detailed review (IWC, 1995, pp.82-5), and last year (IWC, 2000a). Discussions in the intervening years largely referred to comments in the 1994 review.

At the 1994 Committee meeting the proposal was reviewed in accordance with the Committee's agreed five sets of guidelines (A-E). For three, A (The Proposal), B (Objectives) and E (Research cooperation), the Committee agreed that the relevant guidelines had been met. For C (Methodology) there was detailed discussion, particularly of genetic analyses. In addition, the importance of abundance estimates in the context of the RMP led to confirmation of the importance of sightings estimates as well as sampling. For D (The effect of catches on the stock), after some discussion the Committee noted the difficulties it had experienced in the past in adequately providing advice on the matter, referring however to its previous advice that the effect of a small take for a short period would be negligible. It had agreed to consider the general question of how to provide such advice at its next meeting, but there was little subsequent progress in addressing the problem, despite, for example, extensive discussions at its 1997 meeting (IWC, 1998).

Last year, the Committee reviewed two options for the 1999 survey, one requiring permission from the Russian Federation for sampling in its waters. Concerns expressed then included the fact that the focus of the research whaling plans is in areas where minke whales from the 'J' stock mix with those from the 'O' stock, to estimate, *inter alia*, mixing rates of 'J' with 'O' stock animals there, while the principal

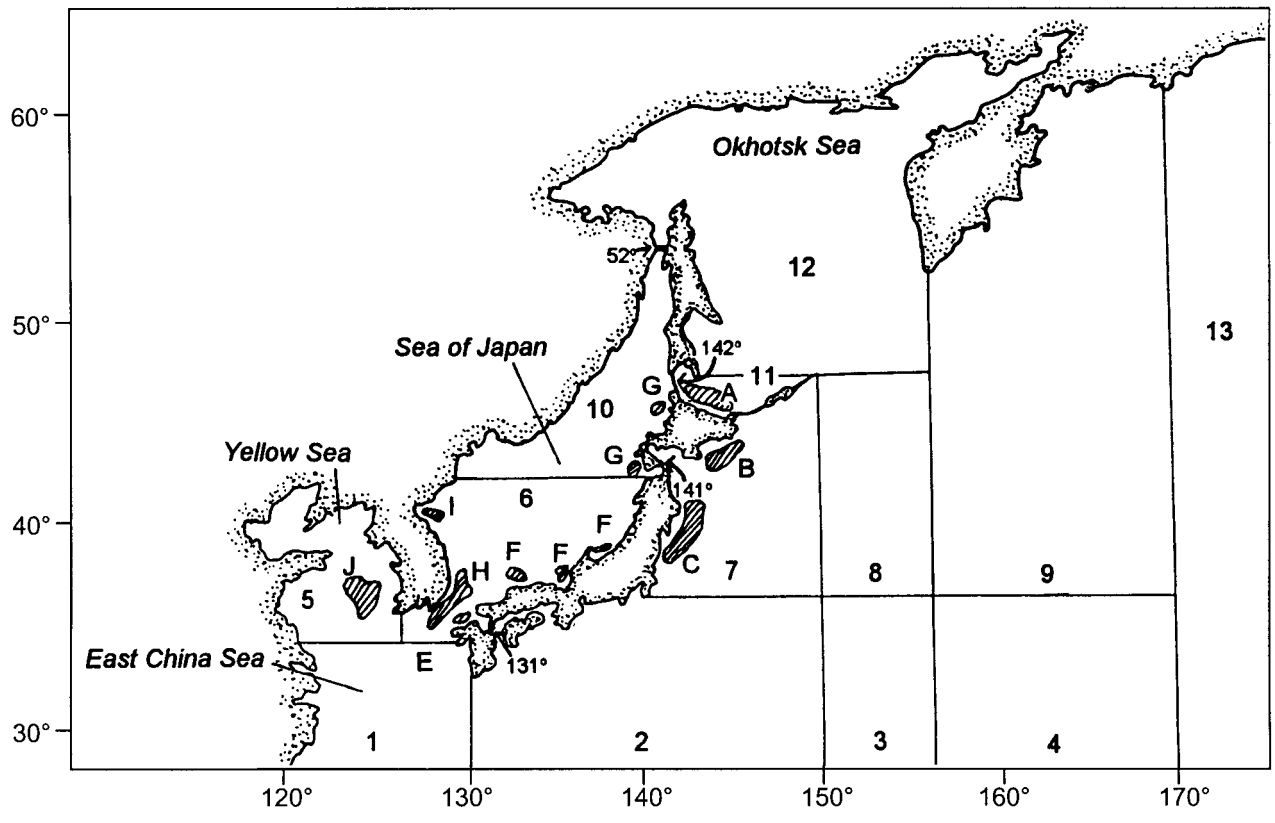

Fig. 1. Whaling grounds and the 13 sub-areas used for the *Implementation Simulation Trials* for North Pacific minke whales.

JARPN objective is to determine the mixing rate between the 'O' stock and the hypothetical 'W' stock further east. In response, it was argued that clarification of 'J'/'O' stock mixing was now an objective of the research in response to concerns raised over market sampling results. Another concern related to the take of even a small number of animals from the ' J' stock, although that was countered by the view that the present mixing rate was likely to be small and the expected catch of 'J' stock whales would be negligible compared with the annual Korean and Japanese bycatch. In the event, a majority of the Committee was unable to respond positively to a request for the Russian Federation to be urged to allow access to its waters for the proposed sampling.

In addition to those discussions in the Committee, initial results from the programme were discussed in some depth during the 1996 meeting of the Working Group on North Pacific Minke Whale Trials (IWC, 1997, pp.203-26). Information from the research was used in the process of revising the trials.

The reasons for the selection of the boundaries for the sub-areas specified for North Pacific minke whales are detailed in (IWC, 1994, p.122). The boundaries were chosen, in part, to be able to reflect possible stock and sub-stock structures under consideration at that time. They accordingly took account of the positions of historical whaling grounds, which were then argued to be perhaps indicative of site-specific sub-stocks. The southernmost boundaries of sub-areas 5-9 were chosen in the light of the absence of catches and sightings on winter surveys in lower latitudes. Other boundaries for sub-areas bordering Japan were selected to reflect the limits of the distribution of historical catches, and postulated limits to the movement of 'J' stock whales to the east of Japan and also (possibly) 'O' stock whales into the Sea of Japan. Choices of longitudinal boundaries of 150° and 157°E between sub-areas 7/8 and 8/9 respectively were largely arbitrary. The former was motivated by a (contested) view that constituted the farthest eastward that minke whales might be found when these might also be subject to exploitation by Japanese coastal whalers (IWC, 1992, p.160). The latter corresponded roughly to the southern tip of the Kamchatka peninsula to allow for possible site specificity of animals migrating northward moving to the west (i.e. into the Okhotsk Sea) and east of the peninsula.

9. OVERVIEW OF SAMPLING METHODOLOGY AND RESULTS

SC/F2K/J8 indicated that sampling methods during the first two JARPN surveys were similar to those in the JARPA surveys. However, the sampling method was then changed to that mentioned under Item 8. A total of 498 animals were collected in sub-areas 7, 8, 9 and 11. Samples were not taken in sub-area 12 since this sub-area is in the Russian Federation EEZ and permission to sample there was not given. Sampling efficiency (animals sampled per animal sighted) in 1994 was 0.49, but, after the modification to the sampling methods, sampling efficiency improved to 0.6-0.7. SC/F2K/J8 concluded that the distributions of minke whale sightings roughly matched the distribution of minke whale samples, thus indicating that the samples were collected randomly from the areas sampled.

SC/F2K/J9 presented the results of the 1999 JARPN cruise including sampling results for that year. The survey was conducted from 6 June to 26 July in sub-areas 7 west and 11 using one research mother ship, three sighting/sampling vessels (SSVs) and one dedicated sighting vessel (SV). The reason for the choice of these sub-areas is detailed under Item 8. The search covered a total of 4,459 n.miles with 293 minke whales (271 schools) sighted. As planned, 100 individuals were collected in these sub-areas (50 in sub-area 7 and 50 in sub-area 11). Biological observations and tissue sampling were conducted as in previous surveys. By-products were also produced following Article VII of the International Convention for the Regulation of Whaling. Preliminary analysis of the samples from sub-area 7 found that the composition by sex and maturity in these sub-areas was similar to those for the 1996 JARPN survey, which was conducted one month later than the 1999 survey. In sub-area 11, segregation by sex and maturity of minke whales was observed. In the western half of sub-area 11 (west of the Kitami Yamato Tai Bank), females were dominant and many were pregnant. In contrast, mature males were found predominantly in the eastern half of sub-area 11 (east of the Kitami Yamato Tai Bank). Furthermore, foetal growth curve data indicated that the females found in the western half of sub-area 11 do not all belong to the same breeding stock.

10. STOCK STRUCTURE

The Workshop had been specifically requested to comment on both the methods applied to the JARPN data and the results obtained from the analyses. It agreed to discuss and report on the methods and results simultaneously. Specific recommendations regarding methods and results are listed under Item 14.

10.1 Review of past discussions of stock structure of the North Pacific minke whale

SC/F2K/J1 reviewed studies on stock identity from 1956-1999 for minke whales in the North Pacific. Almost all studies were carried out on the western side of the North Pacific. The main approaches used were genetics, morphology/morphometry, examination of conception dates and analysis of catch distributions. Pollutant burdens and ecological markers were also used to a lesser extent. Genetic studies during the 1980s were based exclusively on allozymes; however, during the 1990s, techniques based on mtDNA and nuclear DNA were also used. Until 1994, samples were restricted to past commercial coastal whaling operations off Korea and Japan, and the analyses all related to the coastal areas of those two countries. Since 1994, samples from the offshore areas east of Japan (sub-areas 7, 8, and 9) have been collected during JARPN. The results obtained using several approaches are consistent with the view that there are different stocks ('O' and 'J') to the east and west of Japan. Analyses of genetic and conception date data suggest a temporal mixing of these two stocks in sub-area 11. 'O' stock animals move from low to higher latitude areas in spring, and the migration is characterised by a marked segregation by sex and reproductive status.

Data are available from the coast of Japan to 170°E. Several approaches have been used to investigate possible additional stock structure to the east of Japan. Genetics, morphometry and examination of conception dates, among others, it was argued, provide no evidence for additional stock structure to the east of Japan, there being no statistically significant differences found among sub-areas 7, 8 and 9.

The Committee (IWC, 1977, p.62) noted the possibility of stocks to the east and west of Japan in 1976 based on results by Omura and Sakiura (1956) and Ohsumi (1977), and, in 1977, it recognised the Okhotsk Sea/West Pacific stock and

the Sea of Japan stock (IWC, 1978, p.43). That stock structure was refined further in 1982 when two stocks (the Okhotsk Sea/West Pacific stock and the Yellow Sea/East China Sea/Sea of Japan stock) were recognised (IWC, 1983, p.98). An eastern boundary for the Okhotsk Sea/West Pacific stock was placed at 180°E at that time.

The issue of stock identity of minke whales was addressed in detail at the 1993 meeting of the Committee when a Working Group was established to develop *Implementation Simulation Trials* for North Pacific minke whales (IWC, 1994, pp.120-44). In addition to the 'O' and 'J' stocks, the trials hypothesised a western ('W') stock as well as sub-stocks within the 'O' and stocks. The *Implementation Simulation Trials* were refined during the 1996 meeting (IWC, 1997, pp.203-26) when new information obtained mainly from JARPN was considered. That led to the sub-stock structure hypothesis being abandoned although no consensus could be reached regarding the plausibility of the 'W' stock. In reviewing the matter, the Workshop noted concerns regarding the meaning of 'stock' (see Item 10.3.4).

10.2 Methods of analysis (including statistical power)

SC/F2K/J3 discussed the limitations of hypothesis testing as a means of delineating stock structure. It examined the efficacy of hypothesis-testing in determining the demographic spatial structure within a region and, in particular, how well a hypothesis-testing approach actually sets population boundaries. The performance of hypothesis-testing was evaluated using simulations where the actual population structure and dispersal rates are known and can be sampled. The average *p*-value, average degree of differentiation and the power to detect differentiation are determined as a function of the number of putative populations defined by the researcher and the accuracy of boundary placement. Statistical power and average differentiation are much higher and the average *p*-value is much lower when a region is divided too coarsely into only two putative populations than when it is divided in a way that reflects the underlying population structure accurately. There are two reasons for the inverse relationship between power and the number of putative populations defined. First, as with any real case, the number of samples per group increases as the number of groups decreases, resulting in an increase in power. Second, defining fewer groups results in more distantly related samples being placed in adjacent groups, thus increasing the degree of differentiation between adjacent groups and thereby the statistical power to detect that differentiation. The primary lesson from SC/F2K/J3 that relates to North Pacific minke whales is that initial boundary placement is important and does influence the analysis of the data. Improper initial boundary placement can compromise the power of the analysis. Even if population structure is detected, that detection does not provide support for the biological reality of the chosen boundary. The level of genetic differentiation (in statistical terms, the effect size) decreases to low levels with even a small amount of dispersal and the drop in effect size is even greater for large population sizes. This agrees completely with genetic theory on the relationship between effect size, dispersal rates and abundance.

SC/F2K/J4 described a new technique to estimate statistical power for detecting population subdivision using mtDNA data in hypothesis-testing. Case-specific simulations are used to capture the spatial relationship and abundances of the putative populations. The actual level of genetic differentiation (the effect size) between neighbouring populations varies through time because of genetic drift. This uncertainty about the level of population differentiation is captured by sampling the populations through time. For each time period a *p*-value is calculated for a series of statistics used to detect population subdivision. Statistical power is the proportion of time that the null hypothesis of panmixia is correctly rejected for a given α-level (i.e. a given probability of incorrectly rejecting the null hypothesis). Results are presented as Type I versus Type II error tradeoff curves, which do not necessitate the researcher choosing an α-level.

SC/F2K/J5 used a stepping-stone model to calculate statistical power to compare the performance of five statistics commonly used to detect population subdivision: the haplotypic statistics χ^2, H_{ST}, F_{ST}, and the sequence statistics K^*_{ST} and ϕ_{ST}. The *p*-values for all statistics were estimated using randomisation procedures. These statistics were evaluated at a high dispersal rate (1% per year) that is of interest in conservation applications. At this dispersal rate, χ^2 always performed best and haplotypic statistics always outperformed sequence statistics, which differs from previous investigations that did not examine such high rates of dispersal. The reason for the poor performance of the sequence statistics is that when dispersal is high relative to genetic drift, the phylogeographic signal is either low or non-existent. SC/F2K/J5 suggested the use of simple diagnostics, such as regressing genetic differences against geographic distance, to choose the appropriate statistic. It advises that χ^2 should be used whenever possible, but that if the situation occurs where most individuals have unique haplotypes, K^*_{ST} is preferred to ϕ_{ST} because its performance is improved by down-weighting the phylogeographic signal relative to the frequency differences.

In discussion, the Workshop welcomed these papers as they provided the first attempt at developing an approach for quantifying the effect size for use in tests of panmixia. Palsbøll suggested that the reduced power of the sequence statistic relative to the haplotype statistic observed in SC/F2K/J5 might be due to the high mutation rate (0.0001) and the few variable sites (40) employed in the simulations. For instance, in a population of 1,000 mtDNA haplotypes, a single mutation is expected in each mtDNA sequence after only 40 generations. Thus, over thousands of generations, multiple mutations are expected at many positions, degrading the phylogenetic signal captured by the sequence statistic. Genetic drift is probably so rapid in smaller populations that the effect of the mutation rate is negligible. The tests for panmixia in SC/F2K/J3, SC/F2K/J4 and SC/F2K/J5 are based only on nearest neighbour comparisons to mimic common practice. The Workshop agreed that, for application to North Pacific minke whales, potential analyses using geographic distance are not immediately pertinent because the comparisons presented to the Workshop were based on data for two rather than for many areas.

The quantitative results of SC/F2K/J3, SC/F2K/J4 and SC/F2K/J5 are dependent on the specific model and the values for its parameters. The model used was not parameterised for minke whales in the North Pacific nor was the scenario of five sub-populations arranged linearly considered directly relevant to an evaluation of JARPN. Okamura pointed out that the null hypothesis of panmixia was not strictly fulfilled in SC/F2K/J3 because only the differences between adjacent groups had been examined there. He therefore called into question the reliability of the results of SC/F2K/J3. He also drew members' attention to

the fact that hypothesis testing for North Pacific minke whales was based strictly on the null hypothesis of panmixia.

Taylor highlighted that the distributions for the effect size vary over time and reflect a balance between the rates of haplotype mutation, dispersal and genetic drift which depend on the overall sizes of the populations. In response to a query regarding the size of the assumed mutation rate, she commented that mutation rates for cetaceans were very poorly known but that the value chosen led to haplotype frequency distributions that were not too dissimilar from actual observations.

Some members questioned the inferences made in SC/F2K/J3, SC/F2K/J4 and SC/F2K/J5 regarding the management implications of dispersal rates of the order of 0.5% per year, noting that previous examinations of the performance of the RMP suggested that unintended stock depletion becomes less likely with dispersal rates of this magnitude (IWC, 1993, p.189). This is discussed further under Item 10.3.2.

10.2.1 DNA and allozyme data

In preparation for the discussion of methods and results related to DNA and allozyme data, Palsbøll (Annex F) outlined the basic concepts and statistics.

SC/F2K/J10 examined genetic population structure in western North Pacific minke whales based on eight microsatellite loci. The 496 samples collected during JARPN between 1994 and 1999 were used (sub-area 7, $n = 137$; sub-area 8, $n = 91$; sub-area 9, $n = 188$; and sub-area 11, $n = 8$). Samples from the former coastal whaling operation in Korea and from recent bycatches in the Sea of Japan (sub-area 6, $n = 39$), were used as an out-group. Significant deviations from Hardy-Weinberg equilibrium were detected in the JARPN datasets for 1996 and 1999. These were attributable to the inclusion in the analysis of samples from sub-area 11. However, no deviation was detected in sub-areas 7, 8 and 9 nor was hierarchical structure observed among sub-areas 7, 8 and 9. Homogeneity tests also revealed genetic heterogeneity in sub-area 11. SC/F2K/J10 supported previous allozyme and mtDNA analyses that mixing of 'J' and 'O' stock animals occurs in the southern part of the Sea of Okhotsk, but did not support the existence of additional stock structure to the east of Japan.

SC/F2K/J11 used restriction fragment length polymorphism (RFLP) and sequencing analyses of the mitochondrial DNA (mtDNA) control region to examine genetic stock structure of minke whales in the western North Pacific. The 418 samples (sub-area 7, $n = 139$; sub-area 8, $n = 91$; sub-area 9, $n = 188$) collected between 1994 and 1999 were used in the analysis. Samples from past coastal commercial whaling in Korea were used as an outgroup (sub-area 6, $n = 29$). Homogeneity tests were based on haplotype (F_{ST}) and sequence statistics (ϕ_{ST}) using Analysis of Molecular Variance (AMOVA). Significantly lower nucleotide diversity was estimated for sub-area 6 compared to the sub-areas of the east of Japan. The results of the homogeneity test showed that whales from sub-area 6 were genetically different from those to the east of Japan. No statistically significant differences at $\alpha = 0.05$ were found in the data for sub-areas 7, 8 and 9.

SC/F2K/J12 used data on allozymes at loci *Adh-1, Gpi* and *6Pgd* from 497 minke whales taken in the JARPN surveys to examine whether or not more than one stock of minke whales is found to the east of Japan. Although the results confirmed that substantial mixing of 'J' and 'O' stock animals occurs in sub-area 11 in July-August with the male mixing rate exceeding that of females, SC/F2K/J12 did not find any evidence for additional stock structure in sub-areas 7, 8 and 9.

SC/F2K/J32 presented the results of an additional analysis of mtDNA sequences based on JARPN samples. The purpose of the study was to understand the reasons for the low *p*-values found in the comparisons involving sub-area 9 in SC/51/RMP8, SC/F2K/J11 and SC/F2K/J6. The statistical analyses based on F_{ST} and ϕ_{ST}, indicated that a possible source of mtDNA heterogeneity can be attributed to minke whales sampled in the western sector of sub-area 9 in 1995 (west of 162°E). Sub-area 9 was divided at this longitude to maximise the difference between the east and west of sub-area 9 (Table 1). This is post-stratification. No significant genetic heterogeneity was found between western and eastern samples in sub-area 9 in 1995 using a microsatellite analysis.

Table 1

Comparison between sub-area 7 (JARPN) and sub-area 9 (JARPN) using a (randomised) χ^2 test (1,000 simulations). Values given are probabilities. The division at 162°E is based on post-stratification.

(a) Ignoring the samples from the commercial catches in sub-area 7.

	Sub-area 7 (*n*=139)	Sub-area 7 + 8 (*n*=230)
Sub-area 9 (all) (*n*=188)	0.037	0.075
Sub-area 9 (west of 162°E) (*n*=103)	0.032	0.039
Sub-area 9 (east of 162°E) (*n*=85)	0.304	0.572

(b) Including the samples from the commercial catches in sub-area 7.

	Sub-area 7 (*n*=285)	Sub-area 7 + 8 (*n*=376)
Sub-area 9 (all) (*n*=188)	0.089	0.204
Sub-area 9 (west of 162°E) (*n*=103)	0.088	0.166
Sub-area 9 (east of 162°E) (*n*=85)	0.360	0.471

Hatanaka highlighted two possible interpretations of the low *p*-values in SC/F2K/J32. The result could be a statistical artefact; such a conclusion is consistent with the lack of an indication of stock structure in sub-area 9 from, for example, nuclear DNA and allozymes. Alternatively, it may be the result of a group of animals that is genetically distinct from the 'O' stock entering the west of sub-area 9 once every few years. Such a group would probably constitute only a relatively small fraction of the total abundance in sub-area 9. Taylor expressed the view that the result in SC/F2K/J32 was not different from the recent discovery that genetically distinct stocks of Southern Hemisphere minke whales appear to be found in some areas during some years. Walløe warned of not over-interpreting the results given that the data were post-stratified to achieve the largest possible difference within sub-area 9. Punt cautioned that, although a significant result had only been found for 1995, the sample size for 1995 was larger than for 1997 and (particularly) 1994.

SC/F2K/J30 examined genetic structure between sub-areas of the western North Pacific minke whale using allozyme allele frequency data from *Adh*-1 locus. The statistical power of tests for determining whether there is a significant difference between samples from sub-areas 7 and 9 was estimated using a model for the hypothesised 'W' stock that assumed that the allele frequency for such a stock lay between those for the 'J' and 'O' stocks. SC/F2K/J30 argued that conversion of the allele frequency for the 'W'

stock compared to that for 'O' stock to a dispersal rate led to a better understanding of the results and gave a reliable guide for the relationship between gene flow and statistical power. SC/F2K/J30 concluded that it was unlikely that a stock that is genetically different from the 'O' stock is found in sub-area 9.

SC/F2K/J30 estimated the dispersal rate between sub-areas 7 and 9 from the value for F_{ST} by inverting Wright's Island formula and assuming a value for the effective population size. The Workshop agreed it would be highly desirable if distributions of dispersal rate could be derived from values of F_{ST}. Taylor outlined a modelling framework based on the simulation models similar to those that underlie SC/F2K/J3, SC/F2K/J4 and SC/F2K/J5, which can be used to determine the likelihood of different rates of dispersal given a value for F_{ST}. The approach can only utilise values of F_{ST} from analyses of mtDNA data because of the complications associated with developing a model of microsatellites as that would involve explicitly modelling breeding strategies.

The Workshop **agreed** with the conclusion of a small group (Butterworth, Okamura, Kawahara, Palsbøll, Punt, Taylor and Walløe) chaired by Walløe that the approach used in SC/F2K/J30 was probably inadequate. Reasons for this include the fact that the actual value of F_{ST} can vary substantially over time due to the impact of mutation and dispersal (Taylor *et al.*, 2000). The size of this effect can be reduced by considering multiple loci (although that is not possible for analysis of mtDNA data). Furthermore, the dispersal rate is a function of the reciprocal of F_{ST}, so that application of Equation 4 of SC/F2K/J30 will lead to a biased result. Finally, there is considerable uncertainty regarding the estimation of the effective population size for cetacean stocks (for example, the CVs for the abundance estimates for sub-area 9 range from 0.32-0.40) and the impact of the assumption that the effective population size of the two populations is the same (Taylor *et al.*, 2000). Table 3 of SC/F2K/J32 highlighted some of the uncertainty associated with this approach; the estimates F_{ST} based on data for 1994, 1995 and 1997 differ by more than an order of magnitude, as therefore would any estimate of dispersal rate.

SC/F2K/J28 examined the possibility of assigning individuals to the 'J' stock. A total of 863 mtDNA control region sequences from past commercial whaling in Japan and Korea (sub-areas 6, 7 and 11), from bycatches in the Sea of Japan (sub-area 6), and from JARPN surveys in sub-areas 7, 8, 9 and 11 were examined. Phylogenetic reconstruction of unique sequences was based on the neighbour-joining method. The tree showed a cluster containing haplotypes that occur predominantly in the Sea of Japan or share haplotypes '3' and '5' of a previous mtDNA control region RFLP analysis (Goto and Pastene, 1997). There is currently no certainty, however, that the haplotypes contained in the cluster are diagnostic markers for the 'J' stock. Nevertheless, several biological studies (SC/F2K/J13, SC/F2K/J15, SC/F2K/J17, SC/F2K/J20, SC/F2K/J25) that used this method to identify possible 'J' stock animals found significant differences between the animals identified as 'J' stock animals and other animals (assumed to be from the 'O' stock).

Using the methods of SC/F2K/J28, 2-11% of the animals in sub-areas 7, 8 and 9 were identified as being from the 'J' stock (table 2 of SC/F2K/J28). However, the percentages probably overestimate the actual number of 'J' stock animals in those sub-areas, as some 'O' stock animals may share the haplotypes used to identify 'J' stock animals. The Workshop agreed that the probability of correctly assigning animals to the 'J' stock was probably similar to that of 0.95 obtained by Congdon *et al.* (1999). However, SC/F2K/J28 is based on a larger sample of known location than SC/51/RMP20, including additional animals from sub-area 6.

SC/F2K/J6 used the randomisation version of χ^2 to reanalyse the mtDNA data gathered during JARPN. The reanalysis concentrated on the question of whether there is population structure among minke whales to the east and north of Japan. Comparisons of sub-areas 7 and 8 to sub-area 9 resulted in a *p*-value of $\approx$ 0.06-0.07 depending on whether the data for sub-areas 7 and 8 are compared separately or pooled. Given that statistical power is likely to be low for dispersal rates of interest, SC/F2K/J6 concluded that a *p*-value of 0.06 probably indicates that population subdivision is present. The power to detect population subdivision is also likely to be compromised by initial boundary placement because the boundaries between these sub-areas clearly cut through high densities of samples. SC/F2K/J6 concluded that there is no justification for asserting that these data are consistent with no population subdivision within sub-areas 7, 8 and 9, and that it is likely that the analyses of population structure have been compromised by poor initial boundary placement. The differences between stocks are not fixed differences, but rather small differences in haplotype frequencies. These small frequency differences will make it impossible to identify most individuals to their stock-origin.

SC/F2K/J7 estimated the statistical power when detecting population subdivision for a plausible stock structure for North Pacific minke whales. The analysis was limited to the question of whether two stocks exist to the east and north of Japan (i.e. within sub-areas 7, 8, 9, 11 and 12). Historical numbers were based on one of the base-case *Implementation Simulation Trials* (N1-j1g0, IWC, 1999b, pp.86-97). This trial assumes that 30% of the animals in the Sea of Okhotsk (sub-area 12) come from the 'O' stock. Power was estimated using simulations and the effect size of a dispersal rate of 0.5% between the stocks. The simulation technique was modified to use minke whale-like birth and death rates, which resulted in an average birth and death rate of 0.04 and a generation time of 19.6 years. Even for the most powerful statistic of population differentiation (χ^2), the power to detect population subdivision was only 0.49 when $\alpha = 0.05$. Using $\alpha = 0.05$ as the criterion for significance would result in making Type II errors (under-protecting minke whales) over ten times more frequently than making Type I errors (over-protecting minke whales). When Type I and Type II errors were equalised, the critical α-level was 0.23, i.e. any *p*-value less than 0.23 would result in rejecting the null hypothesis of no population structure. Even with this increased α-level, the statistical power would be only 0.77. The paper concluded by suggesting future directions for estimating statistical power for North Pacific minke whales.

Taylor indicated that one approach to handling uncertainty about boundaries was to use the data to indicate possible boundaries and then to determine the dispersal rate across each such boundary. Butterworth questioned the assertion that the current boundary at 150°E passed through an area of high density and argued that using the genetic data to identify possible boundaries was inappropriate as it involved using the data twice (once to identify the boundary and then to test hypotheses related to that boundary). This is discussed further under Item 10.2.4. SC/F2K/J7 highlighted the strategy of choosing α-levels so that the α-level equals the β-level. Butterworth considered that account also needed

to be taken of the number of tests conducted and the likely utility of the techniques on which those tests are based when interpreting results and selecting α-levels. Taylor commented that standard Bonferroni factors to correct for multiple comparisons were often inappropriate for biological questions and should not be applied blindly.

Okamura commented that the assumption of two populations and (more than) three arbitrary boundaries in the western North Pacific was appropriate when estimating statistical power. However, SC/F2K/J7 assumed two populations and two correct boundaries. Therefore, he believed that the statistical power calculated in SC/F2K/J7 was unreliable for the situation of minke whales in the western North Pacific. Finally, he commented that both the results of pooling and non-pooling of data in the western North Pacific should not be assumed to have the same statistical power.

SC/F2K/J2 described a Bayesian modelling framework that can use genotype frequency information for one or more loci to assign relative probabilities to alternative stock-structure hypotheses by means of the Bayes factor. The authors argued that this framework has advantages over maximum-likelihood estimation as it provides a basis to assign weights to alternative hypotheses. A set of prior distributions for the allele frequency probabilities for two-stock hypotheses were put forward. These range from assuming that the relative frequencies for the two stocks are independent, to assuming that they are almost perfectly correlated. When applied to the data for the *Adh-1* and *Gpi* loci for sub-areas 6, 7 and 11 for the North Pacific minke whales, the results confirm those of previous studies that there are (at least) two stocks in those sub-areas. In contrast, applications to data for sub-areas 7, 8 and 9 support the hypothesis of a single stock there, unless, *a priori*, the allele frequencies for two stocks that are adjacent spatially are likely to be very similar. The technique was not applied to mtDNA data due to the computational demands of the methods and because of the difficulties of developing appropriate priors.

It was suggested that values for the parameters of the priors needed for the approach of SC/F2K/J2 could be developed from inferences from other stocks or from mechanistic models (such as in SC/F2K/J3). However, comments were made that although high correlation in the allele frequencies among populations should be expected given the low mutation rates associated with isozyme data, it is not straightforward to use that information to provide specifications for priors for use in a Bayesian analysis. The differences in allele frequencies between stocks in some areas are high (e.g. the 'O' and 'J' minke whale stocks in the North Pacific, and the Central and NE minke whale stocks in the North Atlantic). However, they can also be negligible (e.g. for minke whales in the Southern Hemisphere) even when based on other techniques there is clear evidence for stock structure.

10.2.2 Biological (e.g. reproductive) data

SC/F2K/J13 examined some biological parameters (body length distribution, mean body length of mature animals, growth curve, maximum body length and incidence of anomalous testis) for minke whales in the western North Pacific, estimated using data collected by JARPN. The data were divided into 'O' and 'J' stock animals on the basis of the method in SC/F2K/J28 (459 and 39 individuals respectively). There are clear differences for mature females in the body length distribution, the mean body length, the maximum body length (mean body length of animals older than 14 years) and the growth curve, between 'J' and 'O' stock animals. No clear differences were found in biological parameters among 'O' stock animals collected from sub-areas 7, 8, 9 and 11. SC/F2K/J13 provided additional information about the temporal and spatial aspects of sexual and reproductive segregation. There is no case that all components (immature males, mature males, immature females and mature females) of the population are found in the same sub-area. Consequently, it was argued that it is unlikely that an independent 'W' stock exists.

In discussion, it was noted that there is a preponderance of males in sub-areas 7, 8 and 9 (SC/F2K/J13). Recent JARPN data in sub-areas 8 and 9 have increased the temporal coverage in those sub-areas. Although the proportions of females there are also low, they are similar to that in sub-area 7. Possibly, mature females in sub-areas 8 and 9 are further to the north (in the unsampled Russian EEZ) as is the case in the west, closer to Japan. It is not clear whether or not the data on juvenile proportions continue to support the argument that low proportions of juveniles in offshore areas are inconsistent with the hypothesis of more than one stock. There are confounding effects of differing sample sizes per month and sub-area. The Workshop **recommends** a multivariate statistical analysis of the data to clarify the issue.

SC/F2K/J14 examined stock structure hypotheses for the area to the east of Japan using data on conception dates from JARPN. Kato (1992) found two foetus cohorts that differ in terms of their peak conception dates. 'O' stock animals conceive in winter while 'J' stock animals conceive in autumn. Three hypotheses were developed regarding stock structure in sub-area 9: 'O' stock only, 'W' stock only and a model that allowed for mixing of 'O' and 'W' stock animals in sub-area 9. The 'O'-stock-only model gained support among the three on the basis of Akaike Information Criteria (AIC). SC/F2K/J14 also examined the statistical power to identify a 'W' stock using conception date information based on the assumption of a difference in peak conception dates of one month between the 'O' and a hypothesised 'W' stock. The results of the simulations combined with the AIC result led SC/F2K/J14 to argue that there is no 'W' stock.

Taylor believed that little could be concluded about the existence or otherwise of a 'W' stock from the results of SC/F2K/J14. This is because species in other areas (e.g. North Pacific humpback whales) that can be clearly delineated into several stocks exhibit no differences in morphology or conception dates. She commented that while differences in conception dates and morphology were relevant when defining evolutionary significant units, this was not the case when attempting to identify management units. Other members commented that differences in conception dates and morphology have been found in other species and stocks, including minke whales.

10.2.3 Morphometric and morphological data

SC/F2K/J15 examined data for thirteen characteristics to examine the morphological heterogeneity of 'O' /'W' minke whales, and morphological differences between 'O' and 'J' stock minke whales. Data for 132 individuals (22 from sub-area 7, 15 from sub-area 8, 81 from sub-area 9 and 14 for sub-area 11) from JARPN were analysed. Except for one characteristic, no morphological differences were observed between sub-areas 7, 8 and 9 (i.e. the hypothesised 'W' stock was argued to be absent) based on an analysis of covariance taking body length as a covariate, an analysis of variance, a

principal component analysis and a discriminant analysis. The analyses also suggested that there are morphological differences between 'J' and 'O' stock animals for five characteristics, although the sample size for the 'J' stock was small.

Hakamada noted that work had started on re-analysing the morphological data using the strata for sub-area 9 identified in SC/F2K/J32. The Workshop **recommended** that the work be continued and the results presented to the next meeting of the Committee.

10.2.4 Geographical distribution

SC/F2K/J16 summarised the information on distribution from JARPN. The authors believed that this information can be used to assess stock identity and to select stock boundaries. Based on the data for 1994-1999, the distribution pattern of minke whale sightings from April-September was mapped. Minke whales were widely distributed north of 40°N between the Japanese coast and the eastern boundary of sub-area 9 (170°E) from May-August (where surface temperature ranged from 3°C to 26°C). The main distribution area moved northward from 38°N to 45°N from May-July in the sub-areas to the east of Japan, which coincides with the known northward seasonal migration of the species. Blue, fin, sei, humpback and northern right whales were generally seen north of 38°N in sub-areas 8 and 9. Bryde's whales were sighted mainly in the eastern part of sub-area 7. Sperm whales were widely distributed to the east of Japan. The minke whale distribution pattern was therefore not observed for the other large whales. From the viewpoint of stock identity, SC/F2K/J16 found no clear evidence for areas of low density that might imply two stocks to the east of Japan.

Fig. 2, tabled at the meeting, shows the relative density (sightings per unit effort) by degree of longitude throughout the JARPN sub-areas. It indicates a drop in density at 147°E related to the boundary of the EEZ of the Russian Federation. The Workshop was unable to agree whether the current boundaries of sub-areas 7, 8 and 9 passed through areas of high density. It therefore **recommends** that, to further investigate this issue, the sightings data be analysed using a Generalised Linear Model that includes the covariates of year, month, Beaufort Sea state and temperature (see also Item 12).

10.2.5 Pollutant burdens

From the viewpoint of identifying stock structure, SC/F2K/J17 examined the levels of persistent organic pollutants such as PCBs and organochlorine pesticides in 76 minke whales collected during 1996 and 1999. A principal component analysis (PCA) suggested two groups. One consists of four individuals collected from sub-area 11 in 1996 which show higher concentrations of DDTs and hexachloro-cyclohexanes (HCHs). As the background levels of these contaminants are greater in the Sea of Japan than in the western North Pacific, SC/F2K/J17 suggested that these four individuals originated in the Sea of Japan and mixed with the 'O' stock. The study also suggested the use of persistent environmental pollutants as chemical tracers to examine the ecology of the North Pacific minke whales.

SC/F2K/J18 presented an attempt to identify stock-structure using ecological tracers based on accumulation levels of iron (Fe), mercury (Hg) and cadmium (Cd) in liver, and of polychlorinated biphenyls (PCBs), DDTs, chlordane compounds (CHLs), HCHs and hexachlorobenzene (HCB) in blubber, collected by the JARPN surveys in 1994-1999. Some minke whales in sub-area 11 had higher concentration levels of DDTs, especially of p,p'-DDT and PCBs. These whales also showed a relatively lower concentration of Hg. SC/F2K/J18 suggested that they might belong to the 'J' stock. The opposite pattern was found in the rest of the whales in sub-area 11, which suggests that they could be 'O' stock animals. Genetic results (SC/F2K/J28) and occurrence of scars on the skin surface also supported these conclusions. Principal component and discriminant analyses of these chemical accumulations revealed that the 'J' and 'O' groups can be clearly discriminated. SC/F2K/J18 did not find any statistically significant differences among sub-areas 7, 8 and 9 and hence save no support for the hypothetical 'W' stock.

Hester commented on the differences in degree of scarring noted on 'J' and 'O' stock animals in tables 2 and 3 of SC/F2K/J18. The scars are likely the result of bites by small mesopelagic sharks of the genus *Isistius*, which are found in tropical oceanic waters. The fact that these marks are much more prevalent on 'O' stock animals suggests that the 'J' and 'O' stocks are isolated not only by a difference in the breeding season as noted in SC/F2K/J14, but also by spatial separation of the breeding areas; 'O' stock animals using

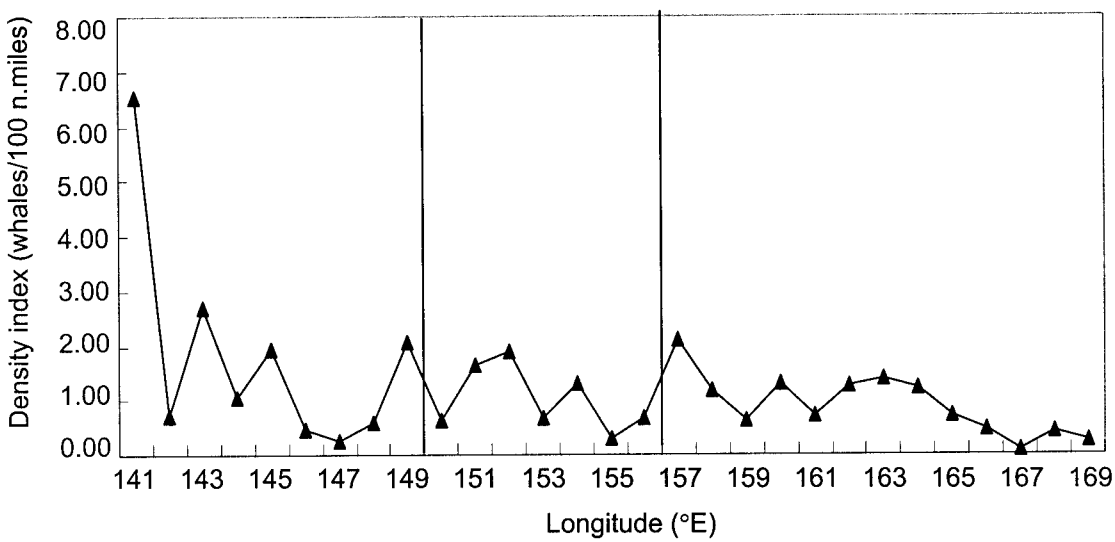

Fig. 2. Relative density by longitude across the JARPN sub-areas.

tropical oceanic waters where they are attacked by *Isistius*, whereas 'J' stock animals might breed in more coastal waters where these sharks are less likely to be encountered. He further commented that information on pollutants in cetaceans was of general interest to the Commission and this is discussed further under Item 12.

10.2.6 Parasite loads

SC/F2K/J19 summarised parasite and epizoite studies during JARPN to identify 'J' stock whales in sub-area 11 and 'W' stock animals in sub-areas 7, 8 and 9. Three species of parasites: *Lecithodesmus goliath* (Digenea), *Anisakis simplex* (Nematoda) and *Penella balaenoptera* (Copepoda) were used for the analyses because they showed rough regional differences in prevalence. Host-age related changes in prevalence were ruled out by using mature males for the analyses. The method described in SC/F2K/J28 was used to divide the animals in sub-area 11 into 'O' and 'J' stock animals. Putative 'J' stock whales were characterised by a low prevalence of *P. balaenoptera*. Although several regional differences were found in the prevalence of *L. goliath* and *P. balaenoptera* in sub-areas 7, 8 and 9, no statistically significant difference was found among these sub-areas when the frequency distributions of the intensities of both parasites were compared. Therefore, SC/F2K/J19 concluded that the 'W' stock could not be differentiated from the 'O' stock because no area-specific parasite was found, and because regional differences in prevalence and intensity were insufficiently large to identify a 'W' stock.

The Workshop discussed the possible causes for the statistically significant differences among sub-areas 7, 8 and 9 in the prevalence of *L. goliath* and *P. balaenoptera*. These included that there was some stock structure, that there is an age-effect in the parasite load with age being correlated with increasing longitude, and that the parasite load is primarily determined by feeding and migration behaviour. The Workshop **agreed** that interpretation of the results of any analyses based on parasite loads required information on the likely residence time of the parasite (noting that *P. balaenoptera* is an external rather than an internal parasite). It was noted that the likelihood of invasion by parasites depended on body condition as well as on feeding behaviour and age. For external parasites, the residence time may be particularly short if, as seems likely for *P. balaenoptera*, the parasite is lost once the animal enters cold water.

10.2.7 Other data

SC/F2K/J20 examined carbon and nitrogen stable isotope ratios in baleen, muscle and liver (21, 44 and 44 individuals respectively) of mature male minke whales to estimate recent diet and to examine stock structure. One or two δ^{15}N-depletion peaks were formed in the baleen of the whales caught in sub-areas 7 and 8 and they were considered to be formed in early summer. The growth rate of baleen was estimated to be about 130mm/year. It was therefore assumed that approximately 1.5 years of diet record would remain in the baleen plates of mature minke whales. The isotopic values in δ^{13}C-δ^{15}N maps differed between sub-areas 7+8 and 11 in the three tissues although there were no differences between sub-areas 7 and 8. Some of the whales, identified as being from the 'O' stock from genetic analyses (SC/F2K/J28), showed isotopic values characteristic of sub-areas 7 and 8 in spite of its being sampled in the sub-area 11, and some whales sampled in sub-areas 7 and 8 showed isotopic values characteristic of sub-area 11. This suggests that some of the 'O' stock animals migrate among sub-areas 7, 8 and 11.

Punt commented that basing stock structure for North Pacific minke whales on stable isotope ratios could lead to large Type I errors as these ratios may represent recent feeding behaviour rather than stock structure.

Tamura indicated that the information on condition factor ('fatness') in SC/F2K/J25 provided some data that may be relevant to assessing stock structure for minke whales in the North Pacific. Punt commented that care should be taken when interpreting differences in condition factor among sub-areas as this may be a result of differences in food availability for different areas rather than in stock structure.

10.3 Synthesis and Conclusions

10.3.1 The existence of the 'W' stock (objective 1)

Discussion of this issue was based on preliminary views summarised by Hatanaka (Annex G) and Taylor (Annex H) prior to discussion of the agenda item by the Workshop. The report as set out below does not repeat material in those summaries, but lists only points raised in discussion and conclusions reached, using the same sub-headings as in the Annexes.

10.3.1.1 DNA ANALYSIS

In the light of points raised during the Workshop, the mtDNA analyses were repeated using the χ^2 statistic, and with data from the commercial fishery in sub-area 7 both excluded and included. A significant effect (at the 5% level) between sub-areas 7 and 8 on the one hand, and 9 on the other was noted in the former case; when commercial data are included, the *p*-values, although above 5%, remain sufficiently small to be suggestive of some effect (see Table 1). The effect seems to arise from samples taken in the western part of sub-area 9 in 1995 (SC/F2K/J32).

The Workshop **agreed** that further analyses of these and other data collected during JARPN should be carried out to explore this finding further, using stratifications differing from those adopted for the sub-areas for the RMP trials, although taking care not to use identical data for both hypothesis generation and testing. Specific suggestions for these further analyses are detailed in Annex I.

The Workshop **agreed** that in the light of these results, the possibility of the existence of some group of minke whales to the east of Japan that differed from the 'O' stock could not be ruled out, but that the data nevertheless provided a basis to restrict the number of 'W' stock hypotheses that need to be considered in the RMP trials, as discussed further below.

10.3.1.2 ALLOZYME ANALYSIS

The Workshop noted that in theory allozymes should provide less resolution of stock structure than mtDNA and microsatellites. However, although this was often born out in practice, there is at least one case where the reverse holds, e.g. in the North Atlantic. The Workshop **emphasised** the need to consider results of various genetic analyses in combination.

10.3.1.3 BIOLOGICAL PARAMETERS

Discussion of sex and length data collected during JARPN is reported under Item 10.2.2 above. The Workshop **agreed** that while differences in mean conception dates comprised strong evidence for more than one stock, it is much less certain what inferences can be drawn in the reverse situation. While it **agreed** that the reverse situation could increase

probabilities assigned to a one stock hypothesis prior to such information being available, it would be difficult to quantify the extent of the change.

10.3.1.4 MORPHOLOGICAL AND MORPHOMETRIC ANALYSES

The Workshop **agreed** that discussions of the implications of these analyses were complicated by the fact that though some differences significant at the 5% level had been found, many tests for differences had been conducted.

10.3.1.5 POLLUTANTS, PARASITES, STABLE ISOTOPES AND OTHER ANALYSES

The Workshop **agreed** that the pertinence or otherwise of information under these headings to stock structure determination depended particularly on residence time and accumulation effects. More information was needed on these factors before any significant differences detected under these headings can be interpreted in the context of stock differentiation.

10.3.1.6 IN SUMMARY

The Workshop **agreed** that some of the difficulties experienced in discussing stock structure arose from lack of clarity in the Committee as to what constituted a 'stock'. This needed to be expressed in terms of likely dispersal rates between 'stocks', where 'dispersal' refers to geneflow. In the context of trials, the Workshop recognised that 'dispersal' is modelled as permanent transfer from one breeding population to another. For example, established differences between the 'J' and 'O' stocks were sufficiently large that any such dispersal rate must be negligible on the time scale relevant to demographics and management. Hester, Ohsumi and Kawahara believed that there is no evidence for the existence of a 'W' stock in the sense used by the Committee in 1993 and 1996. It was clear from analyses presented that any differences between the 'O' and hypothesised 'W' stock would have to be much smaller than those between the 'O' and 'J' stocks. In these circumstances, the associated 'O' and 'W' stock dispersal rate might be sufficiently large to notably affect demographics. The present assumption in the *Implementation Simulation Trials* of setting this dispersal rate to zero would then be inappropriate.

In particular, what has been unclear in the Committee's use of the word 'stock' is whether it is referring to 'evolutionarily significant units' or to 'management units' (e.g. see Donovan, 1991; IWC, 1999a). It was certainly not clear that the level of difference evident between the 'J' and 'O' stocks (which are clearly evolutionarily significant units) should necessarily serve as the norm for defining separate management units. On the one hand, for example, humpback stocks with no differences in biological measures (e.g. conception dates), but known from photo-identification and mtDNA studies to be demographically isolated, were cited. On the other hand, North Atlantic minke whale stocks had been differentiated by allozyme data, and in the absence of geographical features, which in the case of the J-'O' stock (the re-opening of the Sea of Japan some 10,000 years ago) rendered the development of differences easier to understand.

The Workshop noted that the Committee has established a Working Group to examine general issues of stock identity in a management context.

Given difficulties that had arisen in interpreting results from multiple applications of significance tests, the Workshop **recommends** that further methods of analysis, such as multivariate analysis, be pursued to attempt to avoid this problem.

10.3.2 Estimation of mixing rates between 'O' and 'W' stocks (objective 1)

In the RMP trials 'mixing' has been taken to refer to the extent of overlap of whales from different stocks, particularly in feeding areas. Such information is needed to interpret catches and estimates from sightings surveys of abundance on a per-stock basis. It is quite distinct from permanent transfer of animals between breeding stocks, referred to above as 'dispersal' (or 'leakage' in RMP trial terminology). Estimates of mixing determined from genetic studies (e.g. SC/F2K/J2) will be less precise the smaller the genetic difference between the overlapping stocks.

In the context of the hypothesised 'W' stock, 'mixing' relates to the sub-areas (and times) where animals from this stock might be present. The Workshop **agreed** that it would be premature to draw conclusions on the extent of the possible presence of 'W' stock animals west of sub-area 9, prior to completion of the further analyses detailed in Annex I. However, it also **agreed** that if such analyses provided no evidence to change the existing sub-area stratification from a stock structure identification perspective, then sub-areas 7 and 8 need not be distinguished for that purpose. Furthermore, current hypotheses placing 'W' stock animals in sub-area 7 and/or 8 could then be rejected. The Workshop **recommends** that the results of these further analyses are reported to the next Committee meeting for consideration.

Existing genetic analyses suggested that possible 'W' stock animals might be present in only part of sub-area 9, and then only in some years (SC/F2K/J32). Such information might be used to estimate maximal abundance for the 'W' stock, but the Workshop noted that the precision of any such estimate would be low given that sub-area 9 has been sampled only twice at most in any one month.

Regarding dispersal, the Workshop agreed that if there was a 'W' stock, there had also to be a non-negligible level of dispersal between this and the 'O' stock, for the reasons discussed above. This in turn likely meant that such dispersal effects needed to be included in the RMP trials. A sub-group of Butterworth, Kawahara, Okamura, Punt, Taylor and Walløe was appointed to advise what further computations were required to assist in determining ranges of dispersal rates appropriate for consideration in the trials. Following their advice, the Workshop **recommends** that Taylor's program (Taylor *et al*., 2000) should be used for this purpose and that the sub-group should monitor the progress of the associated computations and present a report to the next Committee meeting. Initially, dispersal rates of 0.005 and 0.0005 per annum would be examined, with sampling rates equal to and double those assumed by Taylor in SC/F2K/J7, and with distributions of the actual and sampled values of the standard genetic statistics reported. If necessary the sub-group can specify possible further calculations in the light of the results of the initial computations, if there is sufficient time to undertake the computationally extensive exercises.

10.3.3 Estimation of mixing rates between 'J' and 'O' stocks

SC/F2K/J27 presented estimates of the proportion of 'J' stock animals in sub-area 11, by month and sex. Three molecular markers were used: mtDNA control region RFLP haplotype frequencies, mtDNA control region sequencing

haplotype frequencies and microsatellite allele frequencies. The data on which the analyses were based (720 samples) were obtained from JARPN surveys and from past coastal whaling operations off Korea and Japan. Samples from sub-area 6 were used as the baseline for the 'J' stock and samples from sub-area 7 were used as the baseline for the 'O' stock. Estimates obtained using mtDNA RFLP and sequencing data were similar. These analyses were cross-checked by assuming sub-area 9 samples to reflect the 'O' stock. The mtDNA and microsatellite data suggested that a substantial fraction of the females found in sub-area 11 in April are from the 'J' stock. The estimate of the mixing proportion obtained using the mtDNA data was, however, higher though not significantly so. Furthermore, a relatively high mixing proportion of 'J' stock males in sub-area 11 was obtained using the mtDNA analyses for August. The microsatellite analysis resulted in a high but imprecise estimate for that proportion.

Noting that some of the mixing rate estimates reported in SC/F2K/J27 were based on the assumption that all samples taken from sub-area 9 were from the 'O' stock, the Workshop **recommends** that the sensitivity of these results to omission of the samples for the west of sub-area 9 (i.e. west of 162°E) in 1995 be checked as this area may contain some 'W' stock animals (SC/F2K/J32). The results should be reported to the Committee to take into account further refinement of the *Implementation Simulation Trials*.

10.3.4 Implications for Implementation Simulation Trials

The Workshop noted that the discussion and decisions reflected under Items 10.3.2 and 10.3.3 are also relevant to this agenda item.

One key aspect of the trials to which those discussions do not refer, is the variety of assumptions about the proportion of animals in sub-area 12 (the Okhotsk Sea) that may originate from the hypothesised 'W' stock. There are no data available from JARPN for this sub-area (or portions of sub-areas 7, 8 and 9 in the Russian EEZ) to perhaps shed light on this aspect. However, it may be possible to draw further inferences about the relative likelihood of alternative hypotheses about the 'W' stock in sub-area 12 on the basis of the results of the further 'W' stock-related computations recommended (see Item 10.3.1 and Annex I). The Workshop **recommends** that further genetic samples from particularly sub-areas 12, 9 and possibly 8, be obtained to facilitate clearer discrimination among alternative 'W' stock hypotheses.

11. FEEDING ECOLOGY

11.1 Background

When the JARPN feasibility studies started in 1994, the main objective was to elucidate the stock structure of western North Pacific minke whales. When those activities were upgraded from feasibility studies to full-scale studies in 1996, it was considered necessary also to assess the ecological role of the minke whales in the highly variable ecosystem of the western North Pacific. Therefore, an additional objective 'the feasibility study on the feeding ecology of minke whales in the research ground' was added to the research plan. Although some stomach contents had been collected in 1994 and 1995, dedicated feasibility studies to meet the new objective of ecological studies were not initiated until 1996, whereafter they were continued in all subsequent years.

SC/F2K/J31 gave a brief review of studies of minke whale feeding ecology conducted in the western North Pacific prior to the JARPN surveys. Those studies, which included stomach analyses of whales taken in commercial catches, had suggested geographical, seasonal and annual changes of minke whale prey species in the area. The whales were assessed as being opportunistic feeders with a broad diet and flexible feeding habits. They fed on swarming zooplankton and a number of schooling fish species, and exhibited the ability to pursue single prey species aggregations. Observed annual variation in minke whale prey species probably reflect changes in prey availability in the areas investigated. For example, in the west of sub-area 7, the change of minke whale prey species from chub mackerel (*Scomber japonicus*) to Japanese pilchard (*Sardinops melanosticus*) in 1977 corresponded with a shift in dominance of species taken by commercial fisheries in the same area in 1976. Despite previous attempts to obtain information from commercial catches, knowledge of the feeding activity and food consumption of minke whales in the western North Pacific remained fragmentary and insufficient until the commencement of the JARPN surveys.

11.2 Methods and results

SC/F2K/J22 described the geographical and seasonal changes of minke whale prey species as observed in the western North Pacific during the 1994-1999 JARPN surveys. The study used contents from 498 whale stomachs, sampled from May-September. Sixteen prey species (10 fish, 1 squid, 4 euphausiid and 1 copepod) were identified. 'Swallowing' feeding behaviour was confirmed, and it was shown that the whales had fed on swarming zooplankton and schooling fishes. Clearly, most of the whales had pursued single prey species aggregations. The results revealed both geographical and temporal changes in feeding behaviour. To the east of Japan, Japanese anchovy (*Engraulis japonicus*) was the most important prey species in May and June, while Pacific saury (*Cololabis saira*) was most important in July and August. Krill species (*Euphausia pacifica*, *Thysanoessa inermis*, *T. inspinata* and *T. longipes*) constituted the most important prey group in September. In coastal waters over the continental shelf, walleye pollock (*Theragra chalcogramma*) was also an important prey species during June and September. In sub-area 11, krill dominated among the prey species in July and August. These observed changes in minke whale prey species throughout the research area most probably reflect changes in the availability of the prey species.

The Workshop welcomed the data presented. Given its nature (a feasibility study) and less than optimal sampling regime (actually designed for the purpose of stock identity elucidation), it agreed that the work was a useful addition to the knowledge of the feeding habits of minke whales in the area. Haug raised the question why the observed temporal and geographical shifts in diets might occur. The Workshop **agreed** that present limited information about the biology, distribution and abundance of the prey species in the research area prevented any firm conclusions being drawn. Concurrent mapping of prey abundance, undertaken along with the whaling activities for the first time in 1999, might be a first step to resolving the question.

Diurnal changes in the feeding activity of minke whales in the western North Pacific were described in SC/F2K/J23. The study examined the 498 stomach samples obtained in the 1994-1999 JARPN surveys, and classified each whale according to one of four dominant prey species: Japanese

anchovy, Pacific saury, walleye pollock and krill in the sub-areas to the east of Japan, and krill in sub-area 11. The results suggested little diurnal change in feeding activity.

The lack of diurnal patterns in krill consumption differed from observations of minke whales feeding near the ice-edge in the Antarctic where there was usually a morning peak in feeding activity (Ohsumi, 1979). Kato suggested that the feeding areas surveyed might be sub-optimal with respect to krill availability, and that more Antarctic-like behaviour might take place closer to the ice-edge, for example in sub-area 12. Observations similar to those in the western North Pacific had been made in the Norwegian ecological studies of minke whales in the North Atlantic (Haug *et al.*, 1995).

SC/F2K/J24 estimated the daily and seasonal food consumption based on contents from the 498 stomachs, sampled from May-September in the 1994-1999 JARPN surveys and estimates of abundance by sub-area from the sightings surveys. Daily food consumption was estimated using two independent methods: (1) diurnal change in the forestomach and fundus content weight; and (2) energetic requirements. Estimates obtained by method (1) were 3.9-5.7 and 2.6-3.9% of body weight if the proportion of food remaining in the stomachs after eight hours of digestion was assumed to be 5 and 20%, respectively. Estimates obtained by method (2) ranged from 1.8-5.2% of body weight. The values were comparable with estimates made for northeast Atlantic minke whales and Antarctic minke whales. The estimated total consumption of prey species by minke whales occurring to the east of Japan (sub-areas 7, 8 and 9) during August and September was 2.1-4.0 $\times$ 10^4 tons of krill, 0.4-0.6 $\times$ 10^4 tons of Japanese anchovy, 2.8-6.2 $\times$ 10^4 tons of Pacific saury and 0.3-0.4 $\times$ 10^4 tons of walleye pollock. Total consumption of krill by minke whales occurring in sub-area 11 during August and September was calculated as 1.4-2.2 $\times$ 10^4 tons by the two methods.

The Workshop recognised the attempts to estimate daily and seasonal food consumption using two different and independent methods, but considered that both needed refinement. In method (1), the assumption of an average passage time of eight hours for all food items was made. Since no information is yet available on food passage time for minke whale stomachs, the assumption is subject to considerable uncertainty. The Workshop **agreed** that if this method, originally designed to calculate consumption rates in fish, is to be used in future minke whale studies, some of the assumptions should be refined using empirical data. It noted that, with the logistics applied in the JARPN surveys (including the use of a large mother vessel), experiments addressing some of these questions might well be carried out in the field. With respect to method (2), it noted that the energetic costs of blubber deposition and visceral fat deposition had not been taken into consideration in the calculations. Haug suggested that this be done, and that future calculations of food consumption using method (2) follow the approach described by Folkow *et al.* (2000) in their calculations of minke whale food consumption in the northeast Atlantic.

The Workshop noted that the consumption calculations were performed only for August and September. With the sampling design used in JARPN so far, a quantitative measure of temporal and geographical changes in minke whale diets could not be obtained. Thus, extrapolations to calculate the annual consumption of the entire population found in the research areas could not be performed. It **agreed** that if surveys are to be performed in the future, the sampling design should permit such calculations.

Results from examination of body fat condition of western North Pacific minke whales were presented in SC/F2K/J25. Data were obtained from the 498 whales collected in the 1994-1999 JARPN surveys from May to September. The properties of three different body fat condition indices were tested, and it was observed that the patterns of body fat condition differed by sex and maturity stage. The preferred index ($f(3) = W/L^b$) was applied with b, as determined by regression, being 2.0 and 2.5 for sexually mature males and females, respectively. W is total weight in kg and L the total length in metres. Relative blubber thickness and girth were also used as indicators of body fat condition. It was concluded that girth was the better measure of body fatness. Sexually mature females were slightly fatter than males. Furthermore, the body fat condition of pregnant whales increased throughout the gestation period until the foetus had attained a length of approximately 120cm. After this, either body fat condition increase ceased, or the even fatter pregnant females may have left the surveyed feeding grounds. The results indicate that there are wintering whales occurring in the research area and that most individuals of the assumed 'O' stock move gradually into the surveyed feeding grounds from breeding grounds elsewhere. After July, fat whales may either leave the surveyed feeding grounds, or may remain there for further deposit of fat reserves. The maximum figure of $f(3)$ was about 1.8 times the minimum figure. The average rate of increase in $f(3)$ from May to July was about 7% in the research area. Annual variation in body fat condition was not observed in the research area during the years 1994-1999, and the observed results may indicate that the migration pattern of animals belonging to the 'J' stock differs from that of the 'O' stock.

The Workshop was informed that the condition factor calculations use the total weight of the animals less the weight of stomach contents and the weight of any foetus. The weight was, however, not adjusted for blood lost between killing and weighing. Hester commended the usefulness of data of this type, and pointed out their importance with respect to potential future environmental change studies.

11.3 Synthesis and conclusions

The Workshop noted that the feeding ecology investigations under JARPN were only a feasibility study. The primary objective of JARPN was to obtain data necessary to address questions related to stock identity, and this implied a sampling design that was less than optimal for the ecological studies. The latter were conducted using well-established and appropriate methods, and the Workshop considered the study to be successful within those limitations.

The Workshop **agreed** that if ecological studies of minke whales are to be conducted in the area, the sampling regime must be designed to allow for a more quantitative estimation of temporal and geographical variation in diet. That would eventually permit estimation of the total annual consumption of fish and crustaceans by minke whales. Given the migration patterns of minke whales in the area, it is also of the utmost importance for future ecological studies that access be obtained to the unsurveyed feeding grounds in the Russian EEZ. They include the northern parts of sub-areas 8 and 9 and all of sub-area 12. The Workshop **agreed** that surveys in those areas would give a more complete picture of both the ecology and more general biology of the whole population, including, particularly, mature females.

The Workshop also **agreed** that it is necessary to obtain an improved understanding of the distribution and abundance of relevant prey species to better understand the dynamics of

minke whale food choice and consumption. It therefore **recommends** that acoustic and trawl surveys, designed to address such questions, should be conducted concurrently with future whale surveys, if possible.

12. OTHER STUDIES

SC/F2K/J26 overviewed the oceanographic conditions in the western North Pacific using information collected during JARPN. Two major frontal systems (the Sub-Arctic Front and the Sub-Arctic Boundary) were observed to the east of Japan. The location of the Sub-Arctic Boundary exhibits strong inter-annual variability, which leads to large anomalies in temperature. The peak-to-peak value of the year-to-year variations of Sea Surface Temperature (SST) in the front region reached about 3°C. In the Sea of Okhotsk, a strong 'temperature minimum layer' was found below 1°C. South of Hokkaido, there are three major components: the Tsugaru Warm Current, the Oyashio, and the Warm Core Ring. A preliminary analysis of the relationship between oceanographic conditions and the distribution of minke whale sightings to the east of Japan indicated a strong correlation between SST and the northward migration of minke whales.

Taylor highlighted the importance of collecting information on habitat and prey species for use in analyses of sightings data. She warned at over-interpreting the result that a correlation between the temperature and minke whale abundance had been found because the tracklines were not placed randomly to obtain an even coverage of the region. Butterworth and Fujise noted that, at the start of JARPN, the tracklines had been placed randomly. However, after it was found that no sightings were made in water with SST >15°C, it was decided not to conduct surveys in water warmer than 15°C. Fujise noted that an echo sounder had been installed on one vessel in 1999 and that it could be used in a study of prey species of minke whales.

Hester noted that SC/F2K/J17, SC/F2K/J18 and SC/F2K/J25, whilst directly addressing questions of stock identity, also address other priority issues identified by the Commission on the effects of pollution and environmental change on whales. These, and a number of other reports JARPN has produced (Annex E), constitute a valuable source of comparative information for baleen whales of the North and South Pacific (JARPA).

13. COMMISSION RESOLUTION 1999-2

The Workshop noted that under this Resolution the Scientific Committee was asked to advise the Commission on whether the information sought in research programmes under Special Permit was: (a) required for management; and (b) could be obtained by non-lethal means.

Bannister conveyed the views of the Scientific Committee Chair, Zeh, to the Workshop. She believed that while the item should be on the Workshop agenda, there should not be a long discussion of it. Rather, any results relating to this topic that emerged during the Workshop should be noted under this agenda item for presentation to the full Committee at its forthcoming Annual Meeting in Adelaide. Full discussions should occur during the Annual Meeting, when all Committee members can participate.

In that context, the Workshop noted that it had not discussed matters relevant to item (b) above, but that in relation to item (a), information obtained during JARPN had been and will continue to be used in the refinement of *Implementation Simulation Trials* for North Pacific minke whales, and consequently was relevant to their management.

14. RECOMMENDATIONS

Following discussion of Items 1-13, two additional recommendations were identified:

(1) Research potentially employing new technology should be undertaken to find the breeding grounds, recognising that the most definitive stock structure data will likely come from such grounds.
(2) The age-composition data collected during JARPN should be analysed further to provide information for use in conditioning of *Implementation Simulation Trials.*

Table 2 outlines all the Workshops recommendations.

In response to the suggestion that research be conducted in sub-area 13, the Workshop **recommends** that a full proposal be presented for consideration at the next meeting of the Committee.

15. ADOPTION OF REPORT

The report was adopted at 16:50 on 10 February 2000.

The Workshop expressed its appreciation to the Japanese scientists for the clear presentation of their analyses, which greatly facilitated evaluation of the data collected during JARPN. Hatanaka expressed appreciation to Bannister for his guidance during the meeting. Bannister thanked all

Table 2

Workshop recommendations.

Item	Recommendation
(a) Tasks to be completed by the next meeting of the Committee, if possible	
10.2.2	Multivariate analysis of sex and maturity data
10.2.3	Progress analysis of morphological data using alternative stratification
10.2.4	GLM analysis of sightings data
10.3.2	Completion of further analyses of genetic data with different stratifications
10.3.2	Computation of genetic statistic distributions for different dispersal and sampling rates
10.3.3	Re-estimation of mixing proportions using alternative stratification
14	Analysis of age-composition data
Longer-term tasks	
10.3.1	Use of methods that avoid multiple applications of significance tests
10.3.4	Obtain further genetic samples from sub-areas 12, 9 and possibly 8
11.5	Use of acoustic and trawl surveys concurrently with whale surveys for dynamics of minke whale predation
14	Identification of minke whale breeding grounds

participants for undertaking a vigorous and fair review; he expressed the appreciation of all present to the Government of Japan and the Institute of Cetacean Research for their hospitality, meeting arrangements and provision of facilities, and in particular, to the hard working Secretariat personnel. He thanked the rapporteurs for their unstinting contributions. He also thanked the interpreters for their cheerful hard work throughout the Workshop, noting how much the simultaneous translation had assisted the Workshop's discussions.

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Annex A

List of Participants

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Annex B

Agenda

1. Welcoming remarks
2. Terms of Reference
3. Election of Chair and appointment of rapporteurs
4. Meeting arrangements
5. Adoption of Agenda
6. Review of documents
7. Review of available data
8. Outline of JARPN and past discussions of it in the Committee
9. Overview of sampling methodology and results
10. Stock structure
    - 10.1 Review of past discussions of stock structure of the North Pacific minke whale
    - 10.2 Methods of analysis including statistical power
        - 10.2.1 DNA and allozyme data
        - 10.2.2 Biological data (e.g. reproductive)
        - 10.2.3 Morphometric and morphological data
        - 10.2.4 Geographic distribution
        - 10.2.5 Pollutant burdens
        - 10.2.6 Parasite loads
        - 10.2.7 Other data
    - 10.3 Synthesis and conclusions
        - 10.3.1 The existence of the 'W' stock (objective 1)
        - 10.3.2 Estimation of mixing rates between 'O' and 'W' stocks (objective 1)
        - 10.3.3 Estimation of mixing rates between 'J' and 'O' stocks
        - 10.3.4 Implications for *Implementation Simulation Trials*
11. Feeding ecology
    - 11.1 Background
    - 11.2 Methodology of data collection
    - 11.3 Methods of analysis
    - 11.4 Results
    - 11.5 Synthesis and conclusions
        - 11.5.1 Determination of feeding ecology (objective 2)
12. Other studies (e.g. oceanography)
13. Commission Resolution 1999-2
14. Recommendations
15. Adoption of report

Annex C

List of Scientific Documents

SC/F2K/J1. PASTENE, L.A., GOTO, M. and FUJISE, Y. Review of the studies on stock identity in the minke whale *Balaenoptera acutorostrata* from the North Pacific.

SC/F2K/J2. PUNT, A.E., BUTTERWORTH, D.S. and WADA, S. On the use of allele frequency data within a Bayesian framework to evaluate the relative probabilities of alternative stock structure hypotheses for the North Pacific minke whales.

SC/F2K/J3. MARTIEN, K.K. and TAYLOR, B.L. The limitations of hypothesis testing as a means of demographically delineating independent units.

SC/F2K/J4. TAYLOR, B.L., CHIVERS, S.J. and DIZON, A.E. Estimating the statistical power to detect population subdivision using mitochondrial DNA.

SC/F2K/J5. TAYLOR, B.L. and CHIVERS, S.J. Evaluating the performance of different statistics to detect population subdivision.

SC/F2K/J6. TAYLOR, B.L. Genetic population structure in the western North Pacific minke whale: an analysis of mtDNA data.

SC/F2K/J7. TAYLOR, B.L. and CHIVERS, S.J. An example of the calculation of the statistical power to detect population sub-division in North Pacific minke whales.

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SC/F2K/J15. HAKAMADA, T. and FUJISE, Y. Preliminary examination of the heterogeneity of external measurements of minke whales in the western part of the North Pacific, using data collected during 1994-1999 JARPN surveys.

SC/F2K/J16. MATSUOKA, K., HAKAMADA, T., FUJISE, Y. and MIYASHITA, T. Distribution pattern of minke whales based on sighting data during the JARPN 1994-1999.

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SC/F2K/J32. GOTO, M., ABE, H. and PASTENE, L.A. Additional analyses of mtDNA control region sequences in the western North Pacific minke whales using JARPN samples.

Annex D

Datasets Produced by JARPN

[Table 1 on following pages]

Table 1

Datasets produced by JARPN.

Categories:

A = Data already submitted to the IWC (original or coded); Aa: Data already submitted to the IWC. Free access for the Scientific Committee members (the reviewers); Ab: Data already submitted to the IWC. The users should have an agreement with ICR.

B = Studies using the data have already been published or reported to the IWC/SC meeting as a SC document. (Data have not yet been submitted to the IWC secretariat); Ba: Data holders have already agreed (or permitted) to open the data for the JARPN review; Bb: Users should have permission from the data holder.

C = Neither the original data nor a study using the data have been submitted to the IWC.

Stage of data analysis (SDA); 1 = Data not used for analysis yet; 2 = Data analysis in progress; 3 = Data analysis finished.

Stage of data coding (SDC); 1 = Data coding in preparation; 2 = Data coding in progress (including validation); 3 = Data coding and validation finished.

	1994			1995			1996			1997			1998			1999			Sample
	CA	SDA	SDC	CA	SDA	SDC	CA	SDA	SDC	CA	SDA	SDC	CA	SDA	SDC	CA	SDA	SDC	size
I Biological data																			
Baleen plate set mouth cavity	C	2	2	C	2	2	C	2	2	C	2	2	C	2	2	C	1	2	498
Baleen plates, length of plate series	C	1	3	C	1	3	C	1	3	C	1	3	C	1	3	C	1	2	498
Blubber thickness (14 points)	C	2	3	C	2	3	C	2	3	C	2	3	C	2	3	C	2	3	134
Blubber thickness (3 points)	C	2	3	C	2	3	C	2	3	C	2	3	C	2	3	C	2	3	498
Blubber thickness (11 points)	-	-	-	-	-	-	C	2	3	C	2	3	C	2	3	C	2	3	377
Body length (BIWS)	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	498
Body proportion	Bb	3	3	Bb	3	3	C	2	2	C	2	2	C	2	2	C	2	2	498
Body weight	Bb	2	3	Bb	2	3	Bb	2	3	C	1	3	C	1	3	C	1	3	498
Brain weight	C	1	2	C	1	2	C	1	2	C	1	3	C	1	3	C	1	2	270
Catching date (BIWS)	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	498
Catching location (BIWS)	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	498
Corpora albicantia and lutea (number)	Bb	2	3	Bb	2	3	Bb	2	3	C	1	3	C	1	2	C	1	2	79
Discovery-type marks recovery	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	498
Epididymis weight	C	1	2	C	1	2	C	1	2	C	1	2	C	1	2	C	1	2	419
Foetus, body length (BIWS)	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	38
Foetus, body proportion	C	2	2	C	2	2	C	2	2	C	2	2	C	2	2	C	1	2	30
Foetus, body weight	C	2	2	C	2	2	C	2	2	C	2	2	C	2	2	C	1	3	38
Foetus, number (BIWS)	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	39
Foetus, sex (BIWS)	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	39
Jacobson's organ shape	C	1	1	C	1	1	C	1	1	C	1	1	C	1	1	C	1	1	498
Lactation condition	Bb	1	3	Bb	1	3	Bb	1	3	Bb	1	3	Bb	1	3	C	1	2	79
Mammary gland measurements	C	1	1	C	1	1	C	1	1	C	1	1	C	1	1	C	1	1	79
Maturity stage	Bb	2	3	Bb	2	3	Bb	2	3	Bb	2	3	Bb	2	2	C	2	2	498
Allozyme	Bb	3	3	Bb	3	3	Bb	3	3	C	1	1	C	1	1	C	1	1	298+
Mitochondrial DNA control region RFLP	Bb	3	3	Bb	3	3	Bb	3	3	Bb	3	3	C	3	3	C	2	2	498
Mitochondrial DNA control region sequences	Bb	3	3	Bb	3	3	Bb	3	3*	Bb	3	3	Bb	3	3	C	2	2	498
Nuclear DNA microsatellite	Bb	3	3	Bb	3	3	Bb	3	3*	Bb	3	3	C	3	3	C	2	2	498
Organ weights	C	2	3	C	2	3	C	2	3	C	2	3	C	2	3	C	1	2	135
Parasites, external occurrence record	Bb	2	3	Bb	2	3	Bb	2	3	C	1	1	C	1	1	C	1	1	498
Parasites, internal occurrence record	Bb	2	3	Bb	2	3	Bb	2	3	C	1	1	C	1	1	C	1	1	498
Ribs (number)	C	1	2	C	1	2	C	1	2	C	1	2	C	1	2	C	1	1	498
Sex (BIWS)	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	498
Skeleton (whole skeleton measurement)	C	1	1	C	1	1	C	1	1	-	-	-	-	-	-	-	-	-	12
Skull (length and breadth)	C	1	2	C	1	2	C	1	2	C	1	2	C	1	2	C	1	2	490

cont...

Table 1 continued.

	1994			1995			1996			1997			1998			1999			Sample
	CA	SDA	SDC	CA	SDA	SDC	CA	SDA	SDC	CA	SDA	SDC	CA	SDA	SDC	CA	SDA	SDC	size
Detailed measurements of skull	C	2	2	C	2	2	C	2	2	-	-	-	C	1	1	C	1	1	38
Stomach contents (BIWS format)	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	Aa	-	-	498
Stomach contents weight, first stomach (excl. liquid)	Bb	2	3	Bb	2	3	Bb	2	3	C	2	3	C	2	3	C	2	3	482
Stomach contents weight, (incl. liquid)	C	2	3	C	2	3	C	2	3	C	2	3	C	2	3	C	2	3	497
Stomach contents weight, second-fourth stomachs (excl. liquid)	C	2	3	C	*2*	3	C	2	3	C	2	3	C	2	3	C	2	3	494
Stomach contents volume, first stomach	-	-	-	-	-	-	C	2	3	C	2	3	C	2	3	C	2	3	356
Stomach contents volume, second-fourth stomachs	-	-	-	-	-	-	C	2	3	C	2	3	C	2	3	C	2	3	374
Tail notch shape	C	1	1	C	*1*	*1*	C	1	1	C	1	1	C	1	1	C	1	1	498
Testis weight	Bb	2	3	Bb	*2*	*3*	Bb	2	3	Bb	2	3	Bb	2	3	C	2	3	419
Uterine horn (breadth)	C	1	1	C	1	1	C	1	1	C	1	1	C	1	1	C	1	1	79
Ventral grooves (number)	C	1	1	C	1	1	C	1	1	C	1	1	C	1	1	C	1	1	498
Diatom film record	C	1	1	C	1	1	C	1	1	C	1	1	C	1	1	C	1	1	498
Photographic record of foetus	C	1	1	C	1	1	C	1	1	C	1	1	C	1	1	C	1	2	39
II Environmental data																			
Heavy metals	Bb	2	3	Bb	2	3	C	2	2	C	2	2	C	2	2	C	2	2	
Organochlorine	Bb	2	3	Bb	2	3	C	2	2	C	2	2	C	2	2	C	2	2	
Temperature (XBT survey)	C	2	3	C	2	3	C	2	3	C	2	3	C	2	3	C	1	3	
Marine debris	C	1	1	C	1	1	C	1	1	C	1	1	C	1	1	C	1	1	
III Sightings data																			
Angle and distance experiment data	Ab	2	3	Ab	2	3	C	1	3	C	1	2	C	1	2	C	1	2	
Photo-ID, other species than minke whale	C	2	2	C	2	2	C	2	2	C	2	2	C	2	2	C	1	2	
Sighting data	Ab	3	3	Ab	3	3	C	1	2	C	1	2	C	1	3	C	1	3	
Survey effort data	Ab	3	3	Ab	3	3	C	1	3	C	1	2	C	1	3	C	1	3	
Weather data	Ab	1	3	Ab	1	3	C	1	3	C	1	2	C	1	3	C	1	3	

Annex E

List of Scientific Papers based on Data and Material Obtained during JARPN

1. Abe, H., Goto, M., Palsbøll, P.J. and Pastene, L.A. 1997. Preliminary microsatellite analyses of western North Pacific minke whales, *Balaenoptera acutorostrata*. Paper SC/49/NP12 presented to the IWC Scientific Committee, September 1997. 11pp.
2. Abe, H., Goto, M. and Pastene, L.A. 1998. Further microsatellite analysis in the western North Pacific minke whale, *Balaenoptera acutorostrata*. Paper SC/50/RMP8 presented to the IWC Scientific Committee, April 1998. 10pp.
3. Abe, H., Goto M., Katsumata, Y., Mizutani, M. and Pastene L.A. 1999. Preliminary microsatellite DNA analysis to investigate stock structure in the Antarctic minke whales (*Balaenoptera acutorostrata*). Paper SC/51/CAWS9 presented to the IWC Scientific Committee, May 1999. 12pp.
4. Aono, S., Tanabe, S., Fujise, Y. and Tatsukawa, R. 1996. Specific accumulation of persistent organochlorines in minke whale (*Balaenoptera acutorostrata*) and their prey species from the Antarctic and the North Pacific. Paper SC/48/O22 presented to the IWC Scientific Committee, June 1996. 10pp.
5. Aono, S., Tanabe, S., Fujise, Y., Kato, H. and Tatsukawa, R. 1996. Persistent organochlorines in minke whale (*Balaenoptera acutorostrata*) and their prey species from the Antarctic and the North Pacific. *Environmental Pollution.* 98: 81-89.
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10. Fujise, Y. 1995. On the first-year survey of the Japanese Whale Research Program under Special Permit in the North Pacific. *Geiken Tsushin* 385:1-8 [In Japanese].
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15. Fujise, Y. 1996. On the second-year survey of the Japanese Whale Research Program under Special Permit in the North Pacific in 1995. *Geiken Tsushin* 390:1-14 [In Japanese].
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23. Goto, M. and Pastene, L.A. 1997. Population structure of western North Pacific minke whale based on an RFLP analysis of mtDNA control region. *Rep. int. Whal. Commn* 47: 531-537.
24. Goto, M. and Pastene, L.A. 1997. RFLP analysis of the mitochondrial DNA control region in minke whales sampled during the 1996 JARPN. Paper SC/49/NP10 presented to the IWC Scientific Committee, September 1997. 10pp.
25. Goto, M. and Pastene, L.A. 1998. Population structure in the North Pacific minke whale as revealed by RFLP and sequencing analyses of the mtDNA control region. Paper SC/50/RMP7 presented to the IWC Scientific Committee, April 1998. 15pp.
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29. Government of Japan. 1995. The 1995 research plan for the Japanese Whale Research Program under Special Permit in the Northwestern part of the North Pacific - continuation of the feasibility study. Paper SC/47/NP1 presented to the IWC Scientific Committee, May 1995. 8pp. + 13pp Appendix.
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63. Zenitani, R. 1999 Distribution of minke whales in sub-areas 7E and 8 from spring to early summer season. (Summary of the 1998 JARPN survey.) *Geiken Tsushin* 404:1-12 [In Japanese].
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Annex F

The JARPN 2000 Workshop Crash-Course in Cetacean Genetics

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LOCATION OF GENETIC MATERIAL AND MODES OF INHERITANCE

Most eukaryote cells have their genetic material (the **DNA**, deoxyribonucleic acid) located in at least two cellular compartments: the cell nucleus and the mitochondrion. Plant cells have an additional cellular compartment, the chloroplast, which also has its own genome. This paper focuses exclusively on mammals.

Inheritance of nuclear DNA

The cell nucleus is **diploid**, i.e. it contains two full complements of chromosomes, one inherited from each parent. Each such **haploid** set of chromosomes consists of a sex chromosome (the Y- or the X-chromosome) as well as a full set of autosomal chromosomes. This kind of inheritance is termed **bi-parental** or **Mendelian** transmission.

During the formation (**meiosis**) of the germ cells (the sperm in males and oocyte in females) each identical pair of chromosomes will align and exchange segments of DNA, a process termed **recombination**. After recombination, the two 'new' haploid sets of chromosomes are separated and allocated to each germ cell, which then contains a haploid set of chromosomes. In this manner the genetic material is reshuffled between sister chromosomes every generation. The more distant the two different DNA sequences (**loci**) are located on a chromosome the higher the likelihood they will segregate to different **sister chromosomes** (the two homologous chromosomes in a diploid organism) during recombination. Such loci are thus not linked and segregate independently. Loci in close proximity to each other, on the other hand, may be linked to some variable degree and may thus segregate as a single unit, rather than independent units.

The importance of linkage is often ignored in population genetic analyses. Population genetic analysis is in essence based upon estimation of genetic relatedness, from which inferences are drawn. The data collected from each locus constitutes an independent estimate of, for example, the degree of divergence among sub-populations. However, if two loci are linked they *do not* represent two independent estimates of divergence, which, if linkage is ignored and few loci analysed, may bias the outcome.

Basic terms

A specific segment of DNA (e.g. a large section or just a single nucleotide position) is termed a **locus**. Due to combined action of mutation and recombination, the actual DNA sequence at the same locus will accumulate differences among sister chromosomes over time. Each such unique variant at a specific locus is termed a **haplotype** or an **allele**. For loci located on autosomal chromosomes in a diploid nucleus, there are two copies of each locus, which may have identical (**homozyogote**) or different (**heterozygote**) haplotypes. Identical haplotypes may be identical by descent (**autozygote**) or by state, but not descent (**allozygote**). The term *allele* and *copy* are often used interchangeably and the actual meaning has to be deduced from the context. In this paper, however, allele has the same meaning as haplotype, i.e. each unique variant at a locus. The **genotype** refers to the combination of the two copies at a locus. The **homozygosity** is the probability of sampling two copies of the same haplotype, i.e. $H_O = \sum_{i=1}^{j} p_i^2$, where p_i is the population frequency of the i^{th} haplotype. The **heterozygosity** is the probability of sampling two copies of different haplotypes, i.e. $1\text{-}H_O$. In many instances the **observed** and the **expected** hetero/homozygosity is estimated, the former simply being the proportion of homo/heterozygote individuals in the sample, and the latter the expected number estimated from the observed haplotype frequencies (assuming panmixis, see below).

Inheritance of mitochondrial DNA

The mitochondrion is the organelle responsible for cellular respiration. The mitochondrion resembles a prokaryote in many ways and probably represents an ancient symbiosis between an eukaryote and prokaryote cell. Over time, most of the genes in the mitochondrial genome have been transferred and incorporated into the nuclear genome, and today the mitochondrion in eukaryote cells is not an independent unit. In cetaceans the mitochondrion contains a single circular chromosome, approximately 16,500 nucleotides long (Arnason and Gullberg, 1993). Although there is only one 'chromosome' this may exist in several thousand copies in each mitochondrion. In most instances all the mitochondrial DNA molecules in a single organism appear to have identical nucleotide sequences, although an increasing number of cases are being reported where more than one mitochondrial haplotype has been detected (**heteroplasmy**, e.g. Baker *et al.*, 1990). Contrary to nuclear chromosomes (usually denoted nuclear DNA – **ncDNA**) the mitochondrial DNA (**mtDNA**) is inherited in an **uni-parental** fashion, more precisely from the mother, i.e. **maternally** transmitted (e.g. Gyllensten *et al.*, 1985). The sperm cell does not appear to contribute any mitocondria at the fusion with the oocyte during fertilisation. Paternal leakage of mtDNA has been detected (e.g. mice, Gyllensten *et al.*, 1985) and some non-mammalian species, such as mussels, have been shown to have bi-parental inheritance of mtDNA (Zouros *et al.*, 1992). The general rule for mammals, howevef, is a strict maternal inheritance of mtDNA. Since all chromosomes contained in a mitochondrion have an identical nucleotide sequence, recombination would not have any effect even if it occurred (recent reports suggests that mtDNA may recombine). Thus, mtDNA is inherited not

only in a maternal but also a **clonal** fashion, and all loci in the mtDNA linked. This implies that mutations are not exchanged between different evolutionary lineages contrary to ncDNA.

STRUCTURE AND CHANGES IN DNA SEQUENCES

Basic structure of DNA

A chromosome consists of two opposite directed and complementary strands. Each strand is composed of four different deoxyribonucleic acids: the pyrimidines, thymine (**T**) and cytosine (**C**), as well as the purines, adenine (**A**) and guanine (**G**). The complementary nature of the two-strand configuration has the advantage that each strand acts as a template for the other. During DNA synthesis (i.e. at replication or repair), the DNA polymerase simply uses the complementary strand as the template and adds a G if the complementary strand has an A in that position and vice versa, or a T if the complementary strand has a C (and vice versa).

Changes in the DNA sequences

The sequence of nucleotides changes over time due to mutations. Mutations are introduced by extrinsic factors such as radiation and oxidation, as well as intrinsic factors, such as errors during replication. Mutations can be divided into two kinds, **substitutions** and **insertions**/**deletions** (also nicknamed **indels**) which either might involve single nucleotides only or larger sections of DNA. Substitutions are usually limited to a single or few nucleotides and generally occur at relatively low rates (in the order of 10^{-9}). Indels, on the others hand often involve larger segments of DNA and in some instances at high rates (e.g. 10^{-5}-10^{-4} at microsatellite loci, Edwards *et al.*, 1992).

The mode of mutation depends to a large extent upon the nucleotide sequence itself, i.e. whether it is a repetitive sequence or not. A commonly analysed class of loci is **simple tandem repeats** (**STRs**) also called **microsatellite loci** (Tautz, 1989; Edwards *et al.*, 1991). A microsatellite locus consists of a short DNA sequence of less than six nucleotides in tandem repeat (see Fig. 1).

Microsatellite loci predominantly mutate by insertion or deletion of repeats presumably caused by single-strand slippage facilitated by the repetitive nature of the nucleotide sequence (Levinson and Gutman, 1987; Schlötterer and Tautz, 1992). During DNA replication the two complementary strands may slip and subsequently mis-align (due to the repetitive nature of the sequence), which implies that one or more repeats are added or lost depending on the direction of the slippage (see Fig. 2).

In contrast, nucleotide substitutions usually involve only a single nucleotide as illustrated in Fig. 3. Such mutations are often referred to as SNPs (Single Nucleotide Polymorphism's, pronounced 'snips'). These kinds of mutations are typically observed in non-repetitive DNA sequences (see Fig. 3).

Apart from the actual mode of mutation, the region/nucleotide position also in part determines the rate of mutation. Put simply, parts of the genome are likely to be subjected to various degrees of selection pressure (i.e. affecting the fitness of the individual), whereas other regions are not. Our current knowledge of which nucleotide sequences are under selection pressure and which are not is very limited. In addition, it is likely that selection pressures vary with context (e.g. abiotic factors, stage of development, amino acid composition etc.) making predictions difficult.

Nc- and mtDNA nucleotide sequences at which mutations supposedly do not affect the phenotype are thus assumed to be **selectively neutral** (or **neutral**) (Kimura, 1985). The rate

```
3'-tacctgacgtggAGAGAGAGAGAGAGAGAGAGAGtaccggcagc - 5'  (AG)11
5'-cgttcagtacaaGAGAGAGAGAGAGAGAGAGAGAcgttaatgat - 3'  (GA)11
```

Fig. 1. Tandem repeated array or microsatellite.

```
3'-tacctgacgtggAGAGAGAGAGAGAGAGAGAGAGtaccggcagc - 5'
5'-cgttcagtacaaGAGAGAGAGAGA - 3' [DNA Polymerase->]
                  ||
                  AG          [Single stranded loop due to slippage]
```

Fig. 2a. Slippage creates a loop.

```
3'-tacctgacgtggAGAGAGAGAGAGAGAGAGAGAGtaccggcagc - 5'GA)11
5'-cgttcagtacaaGAGAGAGAGAGAGAGAGAGAGAcgttaatgat - 3'(GA)12
                    ||
                    AG
```

Fig. 2b. DNA polymerase continues strand synthesis in effect adding an extra GA-repeat.

```
3'-tacctgacgtggAGAGAGAGAGAGAGAGAGAGAGtaccggcagc - 5'(GA)12
5'-cgttcagtacaaGAGAGAGAGAGAGAGAGAGAGAcgttaatgat - 3'(GA)12
```

Fig. 2c. In subsequent replications one daughter strand will give rise to a sequence with an additional GA-repeat.

Fig. 2. Single-strand slippage at microsatellite locus during replication.

Original nucleotide sequence

3′- tacgacgattgcagaagctctgcacataagagttcgc - 5′

5′- cgtagtagccatgaggatctcatgtgcggagacctat - 3′

Mutation in one strand, e.g. generated during replication.

3′- tacgacgattgcagaagctctgcacataagagttcgc - 5′

5′- cgtagtagccatgag**A**atctcatgtgcggagacctat - 3′

All subsequent daughter strands will contain the 'new' base.

3′- tacgacgattgcagaGgctctgcacataagagttcgc - 5′

5′- cgtagtagccatgag**A**atctcatgtgcggagacctat - 3′

Fig. 3. Single nucleotide substitutions.

of mutation at such nucleotide sequences is presumably equal to the 'base line' mutation rate. DNA sequences under selection may, for instance, be sequences that encode for enzymes. A mutation in the DNA sequence at such a locus may result in a change of the amino acid encoded at that position (a **non-synonymous mutation**) which in turn may change the function/efficiency of the enzyme (or render it non-functional). Nucleotide sequences that are subject to selection introduce additional variables that subsequently have to be considered in the data analysis, i.e. only certain mutations are viable and thus selection might introduce an increased possibility of multiple identical mutations at the same nucleotide position (**convergence** or **homoplasy**). Not every amino acid substitution alters the functionality of the enzyme and not all nucleotide substitutions in an encoding DNA sequence yield an amino acid change (**synonymous**/**silent mutations**) and therefore might be selectively neutral.

Detecting genetic variation

Isozyme electrophoresis

There are many different biochemical techniques by which genetic variation can be detected. From the mid-1960s to the late-1970s, isozyme or allozyme electrophoresis was the predominant method. Isozyme electrophoresis detects differences in overall electrical charge of enzymes and is a fast and low-cost technique.

The genetic variation uncovered by isozyme electrophoresis are those mutations that generate a change in the amino acid sequence, i.e. limited to DNA positions that encode for enzymes and likely to be subject to selection. Despite this latter aspect, isozyme analyses have yielded much insight into the evolution and population structure of a host of organisms. The real possibility of selection is often dealt with by discarding loci that deviate from expectations, which harbours a risk of introducing biased results (i.e. primarily due to circular data partition). Although still used in many studies and organisms, the vast majority of genetic analyses aimed at cetaceans today employ DNA-based techniques (see below).

RFLP analysis

In the late 1970s technical advances meant that mutations could be detected directly at the level of the DNA itself and not indirectly through isozyme electrophoresis. The initial population studies involved RFLP analyses (**Restriction Fragment Length Polymorphisms**, pronounced 'reflips' according to Dr. Andy Dizon). The method employs restriction endonucleases (REs), enzymes that *in vitro* 'cut' the DNA strand at a specific nucleotide sequence (typically between four to six nucleotides in length). A mutation (be it a substitution or an indel) in the recognition sequence of a particular RE will alter the nucleotide sequence and the RE will subsequently fail to recognise the site and not cut the strand. The result is detected as a single long DNA fragment rather than two (see Fig. 4). The advantage of RFLP analyses is its simplicity, speed and the fact (in relation to isozyme analyses) that any portion of the genome in principle can be analysed. Its main disadvantage is that the nature of the mutation is unknown, i.e. if it is a substitution, an indel or even multiple mutations.

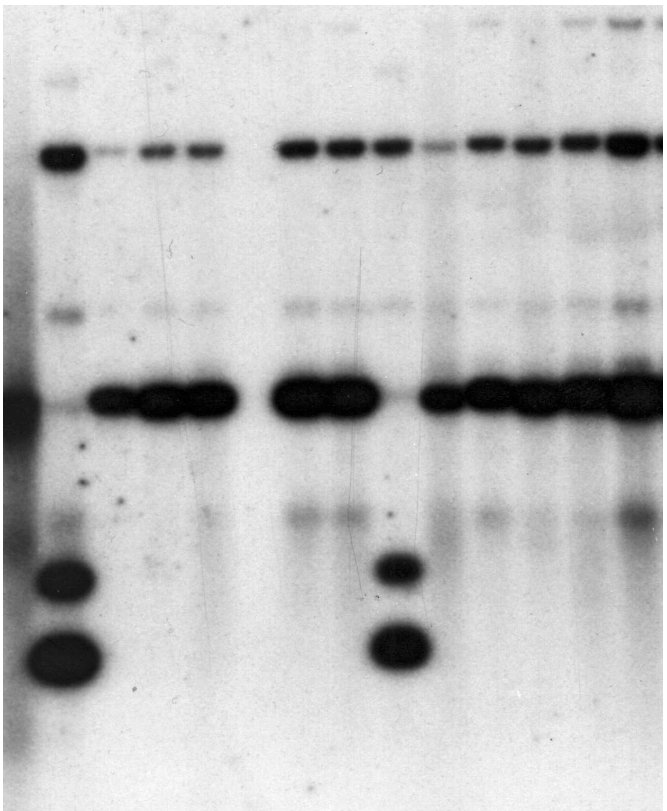

Fig. 4. Autoradiogram of mitochondrial RFLPs.

Nucleotide sequencing

The most precise characterisation of any locus is obtained by determining the actual sequence of nucleotides. Initially, nucleotide sequencing of a specific DNA segment was technically challenging, time consuming and expensive.

However, thanks to the development of the **PCR** technique (**Polymerase Chain Reaction** – *in vitro* amplification of a specific segment of DNA thereby generating billions of identical copies, a pre-requisite for nucleotide sequencing; Mullis and Falooma, 1987), fluorescent labelling and automated detection of DNA fragments, nucleotide sequencing is today a trivial, off-the-shelf exercise. For a detailed record of the data, see Fig. 5 [this figure is not reproduced here – colour copies are available from the Office of this Journal, the legend reads: Fig. 5. Output (chromatogram) of nucleotide sequence obtained by from an 'automated' ABI Prism 377Ö DNA sequencer]. The main drawback of nucleotide sequencing directly by PCR is when it comes to diploid loci (i.e. ncDNA in mammals). The difficulty arises because both copies of the locus are amplified and sequenced at the same time. Any SNP along the nucleotide sequence is evident as two peaks/bands at the same position indicating the presence of two different nucleotides. Hence, while it is possible to detect all SNPs and the kind of nucleotides at polymorphic site it is not possible to assign nucleotides to haplotype (i.e. which of the two copies each nucleotide 'belong' to). This aspect currently constitutes the single main obstacle of using ncDNA SNPs for population genetic analyses. The situation can be partially or fully resolved by various methods, such as cloning of single haplotypes with subsequent isolation and sequencing of individual clones. However, these manipulations are time consuming and other errors are introduced during the process greatly increasing the experimental effort.

Due to the haploid nature of mtDNA, each individual only contains a single haplotype and thus any mtDNA locus is easily sequenced directly.

Micro- and minisatellite analyses

As explained above, repetitive nucleotide sequences, such as microsatellite and minisatellite (longer repeats, Jeffreys *et al.*, 1985) loci, generally evolve by changes in the number of repeats, i.e. in the length of the repetitive region. Microsatellite alleles can thus be distinguished by differences in length of the repetitive region. The DNA sequence amplified is the repeat array plus part of each flanking sequence, to which the PCR olgi-nucleotide primers anneal. The amplified fragments will therefore differ in length if they differ in the number of repeats (see Fig. 6). Contrary to ncDNA SNPs, the haplotype (i.e. the length) of each copy at a diploid locus is readily discernable, which make microsatellite loci ideal markers for diploid genomes (although their mode and rate of mutation add other uncertainties, see below).

RAPDs, AFLPs and multi-locus fingerprinting

Each of these methods detects variation at multiple loci in a single experiment. The data looks much like a bar code with multiple bands each representing a haplotype of an unknown locus. The advantage of these methods is that multiple variable loci are detected in a single analysis. There are some technical issues with some of the methods, such as RAPDs (**randomly amplified polymorphic DNA**), which are highly sensitive to the experimental conditions and reproducibility is thus a major issue. Common for RAPDs and multi-locus fingerprinting is that the bands are resolved by electrophoresis through an agarose rather than a high-density polyacrylamide matrix. This implies that it is difficult to ascertain the size of each fragment (band) with confidence, which subsequently makes it difficult to compare data across different experiments and laboratories (this is not the case with microsatellite or sequence data). In terms of data analysis, the multi-locus approach has the disadvantage that alleles cannot be assigned to specific loci, which implies that some standard population genetic parameters (i.e. panmixis, linkage etc.) are difficult or impossible to estimate. In brief, data are scored as pre/absence of bands, i.e. equivalent to a dominant genetic marker (however, some fragments might represent the same locus).

The amount of genetic variation within populations

The amount of genetic variation within a population basically depends upon the mutation rate and the effective population size (the number of individuals that are breeding). The amount of genetic variation is captured in the composite parameter θ. For a diploid Mendelian inherited genome:

$$\theta = 4N_e\mu$$

where N_e denotes the effective population size and μ the mutation rate. Intuitively, it makes sense that these two parameters determine the level of variation in a population. The more copies of a locus a population contains (the effective population size, N_e), the more haplotypes possible, which in turn is positively correlated with the rate by which new haplotypes are generated (the mutation rate, μ).

The degree of variation in a sample (and thus θ) can be estimated from a number of variables, such as the number of haplotypes, the **heterozygosity** (defined above), the number of polymorphic positions, the **nucleotide diversity** (defined below) as well as the variance in number of repeats (for microsatellite loci). The expected value and variance of θ when estimated from the above variables has been derived for several different evolutionary models. Some of the standard demographic assumptions in such models are: panmixis, discrete generations, negligible migration and constant population size (N_e). The mode of mutation constitutes a central aspect in such models. The three most commonly employed mutation models are: the **infinite allele model** (every mutation generates a new haplotype), the **infinite site model** (every mutation happens at a new nucleotide position) and the **stepwise mutation model** (basically the manner in which microsatellite loci are believed to evolve, i.e. mutations move alleles back and forth between different allelic states, Fig. 7).

An estimate of θ can be obtained from the heterozygosity, assuming an infinite allele model, by numerically solving the equation below:

$$\hat{\theta}^3+(7-t)\hat{\theta}^2+(8-5t)\hat{\theta}-6t=0$$

where $t = H/(1 - H)$, and $H = 1-\sum_i^i x_i^2$, where x_i is the frequency of the i^{th} haplotype (Chakraborty and Wiess, 1991).

θ may also be estimated from the variance in repeat number at microsatellite loci assuming a stepwise mutation model (Di Rienzo *et al.*, 1994):

$$\hat{\theta} = \frac{2}{(n-1)}\sum_i (j_i - \bar{j})^2$$

where n is the number of chromosomes sampled, j_i the number of repeats detected at the i^{th} copy and the mean number of repeats for all sampled chromosomes.

```
1          11         21         31         41         51         61
GACACAGAGA TGTAGAAGGA Ggcaaggcag caaagcagga aaggagagga ccaagaggtg agtgagtgat
---------- >
   Forward primer

71         81         91         101        111        121        131
gcagagggat gatagtgaga tagatacaca gatacacaga tgatagatac atagatagat gataaatgga

141        151        161        171        181        191        201
tgataaatgg gtaatagatG ATAGATAGAT AGATAGATAG ATAGATAGAT AGATAgacag acagacagac
                      -------- microsatellite repeat ---------

211        221        231        241        251        261        271
agatGGGTCT CCTGCCACTG CCAATtttgg gacgttctgg gttttatttt atttgagaag gtggattatt
    < ----------
         Reverse primer

281        291        301        311        321        331
gtccccttt acagatgacc atcctgagaa cattggaata cttttggctg caaa
```

Fig. 6a. The nucleotide sequence at microsatellite locus GATA053 in humpback whale (*Megaptera novaeangliae*) and the position of the oligo-nucleotide primers employed in the PCR amplification of the locus.

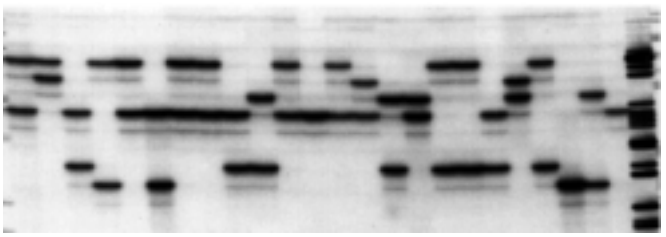

Fig. 6b. Autoradiogram of the genotypes obtained at the tetra-nucleotide microsatellite locus GATA053 in humpback whale.

Fig. 6. Microsatellite data.

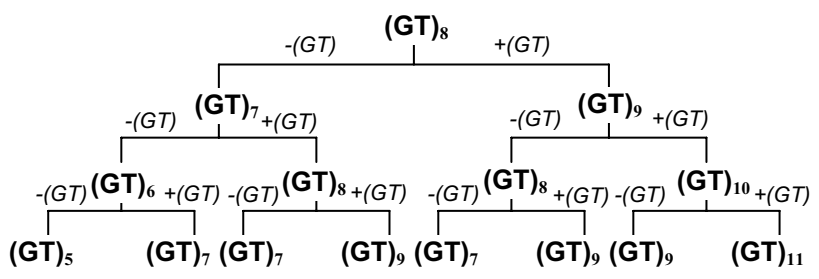

Fig. 7. The step-wise mode of mutation at microsatellite loci by loss and gain of repeats.

Estimates of θ can be utilised for many purposes, e.g. estimates of θ from different populations but at the same loci (i.e. the same mutation rate across all populations) provide relative estimates of N_e. Comparing estimates of θ at different loci from the same set of individuals in a single population yields an estimate of the relative difference in mutation rates (μ) between loci. It should be noted, however, that the variance of estimates of θ is often considerable.

Perhaps a more interesting use of θ lies in detecting deviations from the model expectations. In principle, estimates of θ from different variables should yield similar results, e.g. number of haplotypes, heterozygosity etc. Since the variables might be sensitive to different aspects of the underlying model, any large discrepancies in estimates of θ obtained from different variables is indicative of violations of the underlying model. This approach has been used to detect fluctuations in population size from microsatellite genotype data.

The distribution of variation among individuals

A single population (or sometimes sub-population) is generally defined as a **panmictic** (i.e. random mating) population in a genetic sense. Random mating in this context *does not* imply that individuals choose mating partners indiscriminately, but simply that the loci that are analysed are not traits that are involved in the selection of mate (which is likely to be true for most loci). The equilibrium genotype frequencies for Mendelian inherited loci under panmixis are known as the **Hardy-Weinberg** (and Castle, in some instances, Hardy, 1908) equilibrium. Under panmixis the frequency of each genotype is simply the probability of sampling those two copies, i.e. the haplotype frequencies in the sample. For instance, in the case of a locus with two different haplotypes, the probability of sampling a genotype with two identical and two different haplotypes is and $2p_Aq_B$, respectively, where p_A is the sample frequency of haplotype *A* and q_B of haplotype *B*.

Comparing observed genotype with the expected Hardy-Weinberg frequencies is a common way to test for deviations from panmixis, i.e. possible population structure within a sample. Traditionally this is done by a G- or χ^2-test, which is somewhat less powerful than randomisation tests. A relatively powerful statistic for multiple loci is the average heterozygosity per individual. The observed value is obtained from the data and the expected distribution by randomising the data within each locus among individuals.

Probability of identity

Genetic data are also used to identify individuals. The **probability of identity** (*I*) (Paetkau and Strobeck, 1994), i.e. the probability that two unrelated individuals from the same panmictic population have identical genotypes, is the probability of collecting the same genotype twice, i.e. $\sum_{i-1}^{j} p_i^4 + \sum_{i \neq j} p_i q_j$. For multiple loci the overall probability of identity is obtained as the product of *I* for each locus. The probability of identity varies with the degree of genetic variation in the population *as well as the degree of relatedness*, i.e. the more closely related the higher the probability of having identical genotypes. One fact to keep in mind in this regard is that the number of pairs of related individuals is inversely correlated to the degree of relatedness for most relevant population models, e.g. even though half-siblings have a higher probability than unrelated individuals of having identical multi-locus genotypes any random population sample will contain much fewer pairs of half-siblings than pairs of unrelated individuals. Thus, the absolute contribution of 'false' matches will be similar or lower than that from unrelated individuals despite the higher probability of identity.

Genetic drift

An important evolutionary process is **random genetic drift** or just **genetic drift**. Genetic drift simply denotes the sampling error in haplotype frequencies across generations. In essence, the offspring generation is a random sample of haplotype copies collected from the parent generation. As with any other finite sample this process is subject to stochastic variance, which in turn yields variation in the population haplotype frequencies. In the absence of the introduction of 'new' copies/haplotypes either by mutation or gene flow from other sub-populations and assuming constant population size, any neutral locus would eventually become monomorphic, i.e. all individuals possess the same haplotype. The rate by which the population becomes monomorphic (in the absence of mutation and gene flow) or the rate of **fixation** is inversely correlated with the size of the effective population. The effect of genetic drift upon genetic diversity implies that a severe reduction in effective population size holds the potential of reducing the degree of genetic variation. However, there are two points to keep in mind in this regard; the effective population size (in a population genetic sense) is a harmonic mean, which implies N_e has to be reduced for many generations in order to have an effect upon the level of genetic diversity. Each individual contains two copies of each locus, which implies that a sample, for instance, of 50 individuals will contain 100 copies at any given autosomal locus and thus contain most of the variation contained in the population. These two facts imply that in theory a bottleneck will have to be severe and prolonged in order to result in any measurable reduction in genetic diversity.

In the presence of mutation/migration, a point of equilibrium is reached when the rate of loss of haplotypes (by genetic drift) equals the introduction of new haplotypes (by mutation/migration). Because genetic drift is a random process, two isolated populations, initially part of the same population, will diverge increasingly over time with regard to haplotype frequencies or reach a state of equilibrium depending upon the rate of gene flow and mutation.

If loci are under selection, the sampling across generations is not random and the effects of genetic drift are either accelerated or decelerated.

GENETIC DIVERGENCE AMONG POPULATIONS

Population genetic structure evolves under **assortive mating**, i.e. when mating is non-random. Many different variables can cause population genetic structure, e.g., spatial or temporal segregation. Assortive mating implies individuals are more closely related within sub-populations than between sub-populations, or, in other words, the ancestors of an individual are likely to originate from the same sub-population. The likelihood that descendants and ancestors are part of the same sub-population decreases with the amount of dispersal (**gene flow**) among sub-populations. Thus, if gene flow is high relative to the rate of genetic drift and mutation then population genetic structure is unlikely. How high a level of gene flow will prevent population genetic structure depends upon the mutation rate and effective population size. It is commonly assumed that more than ten migrants per generation are sufficient to prevent

significant levels of divergence in haplotype frequencies among sub-populations.

The statistics developed to measure the degree of population structure are, in principle, based upon the effect of assortive mating, i.e. if population genetic structure exists, individuals within sub-populations will be more closely related to each other than to individuals from other sub-populations. The majority of measures of population genetic divergence estimate the *reduction in genetic variation due to population structure*. In essence, $divergence = 1 - \frac{\overline{\theta}_{SUB}}{\theta_{TOT}}$, where $\overline{\theta}_{SUB}$ is the average genetic variation within a sub-population and θ_{TOT} is the genetic variation in the total sample. It follows that the degree of population structure can thus be estimated from the same variables that θ (see above) is estimated from, e.g. the expected heterozygosity, number of alleles, nucleotide diversity, number of polymorphic positions as well as variance in repeat number.

Haplotype data are basically of two kinds: haplotype only or haplotype as well as the relative degree of relatedness among haplotypes (i.e. a measure of the number of mutations between haplotypes). The statistics used for these kinds of data are often referred to as haplotype and sequence statistics, respectively. Personally, I prefer the terms frequency (only) and distance (as well) statistics.

Haplotype/frequency data

The relative degree of relatedness within and among sub-populations is estimated by the decrease in heterozygosity (= increased homozygosity) or variance due to population structure. This approach was originally formulated by Sewall Wright (1969) who proposed a number of inbreeding coefficients, called *F* statistics. If two samples of individuals are collected from two different populations, the difference in haplotype frequencies due to genetic drift implies that the heterozygosity will be lower within each population than in the overall sample. The more divergent haplotype frequencies, the greater the decrease in heterozygosity. An example of such a statistic is Wright's F_{ST}, which is defined as:

$$F_{ST} = \frac{H_T - \overline{H}_S}{H_T}$$

where $\overline{H}_S$ is the average H_S among all sub-populations;

$$H_S = 1 - \sum_{i=1}^{j} p_{i,s}^2$$

i.e. the expected heterozygosity in sub-populations; and

$$H_T = 1 - \sum_{i=1}^{j} \overline{p}_i^2$$

i.e. the expected heterozygosity of the entire sample. The term p_i denotes the frequency of the i^{th} haplotype of j haplotypes in total.

There are other statistics, which are similar in principle, such as Hudson's H_{ST} (Hudson *et al.*, 1992). The main difference between F_{ST} and H_{ST} is that the latter weigh the contribution of H_S from each sub-population by the sample size (reflecting the uncertainty in the estimation) in the same manner as K_{ST} (see below). F_{ST} and H_{ST} take values between 0 and 1.0. Weir's θ is a F_{ST} statistic as well, but estimates the partition of variance (Weir and Cockerham, 1984). There are other measures of genetic divergence, such as Nei's D (Nei, 1987), which can take any value from 0 to infinity. Nei's *D* is defined as:

$$D = -1\mathrm{n}(I)$$

where $I = \frac{J_{XY}}{\sqrt{J_{XX} J_{YY}}}$, where J_{XX} and J_{YY} denote the estimate of homozygosity (defined above) in population *X* and *Y*, respectively. The term J_{XY} denotes the probability of choosing one copy from population *X* and one copy from population *Y* of identical haplotype.

Sequence/distance statistics

Genetic analyses may often not only determine what haplotype a given sampled copy is, but also the relative degree of genetic divergence among different haplotypes. In the case of nucleotide sequence data, the degree of divergence (at the intra-specific level, i.e. among closely related haplotypes) is commonly expressed as the number of nucleotide substitutions per nucleotide position. This extra level of information provides extra power when there are many different haplotypes, i.e. when haplotype frequencies are low. There are several statistics which estimate the degree of divergence among copies within and between populations. An example is K_{ST} suggested by Hudson *et al.* (1992). K_{ST} estimates the reduction in nucleotide diversity due to population structure. The definition of K_{ST} by Hudson *et al.* (1992) is:

$$K_{ST} = 1 - (K_S / K_T), \text{ when}$$
$$K_S = wK_1 + (1 - w)K_2, \text{ where } w = n_1/(n_1 + n_2)$$

and

$$K_i = \frac{\sum_{j=1}^{n_i - 1} \sum_{k=j+1}^{n_i} d_{ij,ik}}{\binom{n_i}{2}}, i = 1,2$$

and

$$K_T = \frac{\sum_{i=1}^{2} \sum_{j=1}^{n_i - 1} \sum_{k=j+1}^{n_i} d_{ij,ik} + \sum_{j=1}^{n_1} \sum_{k=1}^{n_2} d_{1j,2k}}{\binom{n_1 + n_2}{2}}$$

The term n_i denotes the number of sequence copies collected from locality *i*, and $d_{ij,lk}$ the number of single nucleotide substitutions between the j^{th} sequence from locality *i* and the k^{th} sequence from locality *l*.

Microsatellite statistics

As explained above, microsatellite loci evolve in a stepwise manner, i.e. a mutation results in the addition or deletion of otherwise identical repeats. Thus, the mode of mutation does not fit an infinite allele model, in that a new mutation event has a high probability of generating a haplotype already present in the population (Fig. 7). This implies that for loci composed of repetitive sequences there is likely to be a considerable degree of homoplasy, especially when mutation rates are high, as is the case for many microsatellite loci. To accommodate these aspects microsatellite-specific

estimators of genetic divergence among populations have been developed. The variable used to estimate the degree of divergence is either: the variance in repeat number (Slatkin, 1995), or the mean number of repeats per copy (Goldstein *et al.*, 1995). Taking the mutation mode (basically a linear random walk) into account introduces additional and considerable amounts of variance in the estimation of divergence and thus a large number of loci will need to be analysed in order to obtain a reasonable precise estimate of the degree of genetic divergence. This is true for any class of loci, but even more so for loci that evolve rapidly in a stepwise manner. If the taxa under study are likely to have diverged recently the level of homoplasy might be at a level when a stepwise mutation model can be ignored and infinite allele model-based statistics used instead (which has a lower variance). It has been found, however, that the desirable linear relationship between the degree of genetic divergence and time since divergence becomes eroded quite quickly at microsatellite loci. For instance, Paetkau *et al.* (1998) found that the linearity between divergence time and genetic divergence ceased at less than 20,000 years.

Assessing the significance of an estimate of divergence

Even if samples were collected from the same panmictic population, the sampling variance is likely to yield an estimate of divergence between the samples that is larger than zero. Hence, before estimating the level of genetic divergence, a homogeneity test is usually performed. Any appropriate estimator of genetic divergence can be used as a test statistic in addition to more conventional statistics, such as χ^2 or *G*. The most powerful manner by which to obtain the probability of the observed value under the null-hypothesis (that samples were collected from the same population) appears to be Monte Carlo permutations from which the null-distribution of the test statistic is estimated (e.g. Roff and Bentzen, 1992).

Likelihood methods

The above presented estimators are all relatively simple, moment estimators. Genetic divergence can also be estimated in a traditional or Baysian likelihood framework, although these approaches are often computationally intensive, which is likely to become less of a problem as computers become more powerful (Nielsen *et al.*, 1998).

Model assumptions

It is important to keep in mind that the above estimators are based upon a number of assumptions, e.g. the mode of mutation. The change in haplotype frequencies due to genetic drift is negatively correlated with the effective population size, and thus the rate of genetic divergence depends upon population size. This in turn implies that if populations differ radically in terms of effective population sizes, the degree of genetic divergence cannot necessarily be compared directly from one inter-population comparison to another. There are distance estimators that are less sensitive to differences in population sizes, e.g. Cavelli-Sforza's Chord distance (Cavalli-Sforza and Edwards, 1967), but these are rarely used. Fluctuations in the effective population size, e.g. population bottlenecks or expansions, will thus also change the rate of divergence.

The validity of the standard assumptions can often be tested, although if significant deviations are detected there might be no obvious solutions in terms of how to scale the degree of divergence. Often such tests are based upon estimation of θ from different aspects of the data, such as heterozygosity and number of haplotypes, or variance in repeat number (microsatellite loci). An estimate of the same parameter (in this case θ) but from different aspects of the data should generate similar observed values. If not, this might indicate a violation of the model assumptions. This approach (the ratio of estimates θ from different aspects of the data) has proven useful for detecting exponential decrease or increase in the effective population size, e.g. at microsatellite loci estimating θ from the heterozygosity and variance in repeat number (e.g. Kimmel *et al.*, 1998).

Other, more advanced likelihood methods have been developed to obtain an estimate of θ for the most likely rate of population growth/decrease (Kuhner *et al.*, 1998).

INDIVIDUAL-BASED APPROACHES

Detection of individuals and/or parent-offspring pairs

The ease and speed by which microsatellite loci are analysed implies that it is relatively straightforward to obtain genotypes from multiple loci from the sampled individuals. If a sufficient number of loci have been analysed individuals can reliably be identified, and with additional loci, parent-offspring relations as well. The distribution of individuals or parent-offspring relations in time and space can be utilised to obtain estimates of current rates of dispersal and (i.e. population structure) as opposed to the evolutionary measures mentioned above. The temporal scale of such dispersal estimates may be more relevant to management issues. However, in order for a sample to contain a useful number of parent-offspring relations or re-sampled individuals, the sampled proportion of each population must be relatively large. While this approach holds a lot of promise it is still in its initial stages.

Assignment tests

F_{ST} statistics do not effectively combine the additional information that is contained in multiple genotypes, e.g. if the genotype has been determined at several microsatellite loci for each individual. With basis in the expected Hardy-Weinberg genotype frequencies the likelihood of each single-locus and thus also the combined multi-locus genotype observed in each individual is readily estimated (Paetkau *et al.*, 1995). This aspect has long been used to estimate the population origin of individual samples in mixed-stock fisheries. However, the same approach can in principle be utilised to detect population differentiation. The typical procedure is to estimate the likelihood of each individual multi-locus genotype in each sub-population (after removing the individual from the sample) and assign the individual to the sub-population its multi-locus genotype is most likely to originate from. The higher proportion of individuals assigned to the sub-population they were sampled from, the higher the degree of differentiation. In some procedures individuals will only be assigned to a sub-population if the likelihood of the multi-locus genotype is significantly higher for one sub-population than the remaining sub-populations. No formalised statistics have yet been developed using this approach to estimate the degree of genetic differentiation. There are several problems, e.g. how to deal with uneven sample sizes and what is a 'significant' proportion of assignment.

POSTSCRIPT

Inferring population/stock structure and dispersal rates from population genetic data is basically an estimation process. Although a wealth of genetic markers and techniques have

become available, the resolution and reliability of any such study hinges critically upon sample sizes (both in terms of samples and genetic markers), as is true for any estimation. Hence, drawing affirmative conclusions from small sample sizes, few loci, or based upon a poorly understood population/evolutionary/mutation model harbours a real risk of misinterpretation. Population genetic analyses contain an additional level of variance due to the sampling of genes across time (i.e. generations) apart from the sampling variance due individuals and loci, which is why results from single loci should be viewed with utmost caution.

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Annex G

Comprehensive Summary of the 'W' Stock Hypothesis[1]

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INTRODUCTION

The hypothesis of two stocks of minke whale, the Sea of Japan-Yellow Sea-East China Sea stock ('J' stock) and the Okhotsk Sea-West Pacific stock ('O' stock), was commonly used for minke whales in the western North Pacific. The IWC Scientific Committee conducted a Comprehensive Assessment of North Pacific minke whales in 1991, and agreed that there were at least two stocks (IWC, 1992). The eastern boundary of the 'O' stock for the run of HITTER/FITTER models was set at 157°or 150°E, because there were no available data east of 157°E (IWC, 1992). New stock structure hypotheses were introduced by the Working Group on North Pacific Minke Whale Management Trials in 1993, i.e. three sub-stocks in the 'J' stock, four sub-stocks in the 'O' stock and an additional stock (the 'W' stock) in the central region (157-170°E or 150-170°E) (IWC, 1994b).

These new hypotheses were developed because there was no direct evidence to rule them out, although there was no evidence to support them either (Hatanaka *et al.*, 1994). Recognising this, the Scientific Committee has pointed out the desirability of improving information on the stock structure in the area (IWC, 1994a). Accordingly, the Japanese Government started a research programme under special permit (JARPN) in 1994. These hypotheses were re-examined in the Working Group on North Pacific Minke Whale Trials in 1996, based on the information obtained from JARPN and re-analyses of old data (IWC, 1997b). The group agreed that the available data and information were generally inconsistent with there being sub-stocks and the sub-stock structure was dropped in the trials (IWC, 1997a). However, while the group agreed that no evidence was presented to support the hypothesis for the 'W' stock, the group could not conclude on the plausibility of the 'W' stock. The Scientific Committee agreed that a comprehensive review of JARPN should be held in 2000 to evaluate whether the objectives have been achieved. This paper summarises the results of the 'W' stock hypothesis obtained through the JARPN programme until 1999.

DIRECT INFORMATION TO SUPPORT THE 'W' STOCK HYPOTHESIS OBTAINED THROUGH JARPN

DNA analyses

SC/F2K/J11 described RFLP analysis of a whole mtDNA control region and sequencing of a 487 base-pair segment of the control region using 418 samples obtained through JARPN and 29 samples taken in Korea and the Sea of Japan. Results showed that whales from sub-area 6 could be discriminated from whales from the Pacific side of Japan. However, no significant statistical differences were found among sub-areas 7, 8 and 9, thus the 'W' stock hypothesis was not actively supported.

SC/F2K/J10 described microsatellite analysis for the samples obtained by JARPN. They investigated eight microsatellite loci, and showed that samples from sub-areas 7, 8 and 9 followed the Hardy-Weinberg equilibrium. The authors concluded that their results did not support the existence of the 'W' stock in the offshore waters of the North Pacific but supported the mixing of 'J' and 'O' stocks in sub-area 11.

Allozyme analysis

SC/F2K/J12 described an allozyme analysis, using 497 specimens obtained through JARPN from 1994-1999. The results showed that the existence of stocks other than 'O' stock was not supported in sub-areas 7, 8 and 9 and that the mixing of 'J' and 'O' stocks occurred in sub-area 11.

Biological parameters

SC/F2K/J13 described analyses on biological parameters. After identification to either the 'J' or 'O' stock or an individual basis based upon mtDNA analysis (SC/F2K/J11), the authors found significant differences in the mean body length of physically mature animals, conception date, growth and maximum body length between 'J' and 'O' stocks. No differences were found in these biological markers between sub-areas 7, 8 and 9. However, the sex ratio and size composition were different to various degrees between these sub-areas, suggesting segregation by sex and maturation within one stock.

SC/F2K/J14 examined the possibility of the existence of 'W' stock minke whales in sub-area 9 by means of hypothesised models based on conception date in the samples obtained by JARPN. A model for which only the 'O' stock existed in sub-area 9 received most support on the basis of AIC, and the statistical power of the result was discussed.

Morphological and morphometric analyses

SC/F2K/J15 examined the data of external measurements collected during JARPN in order to detect the existence of a hypothesised 'W' stock. From the analysis of covariance using 12 measurements of body proportion, no significant difference was observed among sub-areas 7, 8 and 9. The

[1] Originally presented as SC/F2K/J21.

results did not support the 'W' stock hypothesis. However, significant differences were found between 'J' and 'O' stocks by means of analysis of variance (AMOVA) and discriminant analysis.

Pollutant burden

SC/F2K/J17 investigated persistent organic pollutants such as PCBs and organochlorine pesticides in the blubber of 76 animals collected by JARPN. Two groups were identified; one of them (including four individuals) collected from the Sea of Okhotsk was estimated to be derived from the Sea of Japan. Little evidence for the 'W' stock hypothesis was found.

SC/F2K/J18 described a study of heavy metals and organochlorines accumulated in minke whale tissues. The discriminant analysis revealed no significant differences among the Pacific sub-areas (sub-areas 7, 8 and 9) and they also found no evidence to support the 'W' stock hypothesis.

Parasites

SC/F2K/J19 analysed nine species of parasites and two species of epizoites. No theoretical 'W' stock could be differentiated from 'O' stock animals because no area-specific parasite was found and regional differences in prevalence and intensity of parasite were large enough to identify 'W' stock. However, 'J' stock whales were characterised by a low prevalence of *Pennella balaenoptera* compared with 'O' stock animals.

Stable isotopes

SC/F2K/J20 investigated the stable isotope ratio of carbon and nitrogen on 44 samples taken from sub-areas 7, 8 and 11. The values of stable isotopes showed different patterns between the Pacific (sub-areas 7 and 8) and Okhotsk Sea (sub-area 11), but not within the Pacific (between sub-areas 7 and 8). This result coincided with the population structure revealed by genetic analyses, and did not support the 'W' stock hypothesis.

Others

SC/F2K/J16 described seasonal variations in the distributions of minke whales based on sightings made during the JARPN surveys. Although not all areas and seasons were covered, the authors estimated that minke whales are distributed continuously from coastal waters to offshore waters in the western North Pacific.

SC/F2K/J25 investigated body fatness of minke whales sampled by the JARPN programme. No difference was observed between the 'O' stock (sub-area 7) and the hypothesised 'W' stock (sub-area 9), while it was observed between 'J' and 'O' stocks.

CONSISTENCY BETWEEN RESULTS OBTAINED BY JARPN AND PAST KNOWLEDGE

Results obtained from the JARPN Programme revealed clear differences between the 'J' and 'O' stocks but no difference between the 'O' and 'W' stocks. Differences between 'J' and 'O' stocks were found in mtDNA (SC/F2K/J11), nuclear DNA (SC/F2K/J10), allozyme (SC/F2K/J12), maximum body length, growth curve and conception date (SC/F2K/J13), pollutants (SC/F2K/J18 and SC/F2K/J17), parasites (SC/F2K/J19) and stable isotopes (SC/F2K/J20). These results are consistent with the established theory that there are two stocks of minke whale in the western North Pacific (Omura and Sakiura, 1956; IWC, 1983; Ohsumi, 1983; Hatanaka, 1997; SC/F2K/J1).

The JARPN Programme provided data on sex ratio and maturity rate by sub-areas. These data were tested to see whether they are consistent with hypotheses on segregation and migration. The length compositions by sex and by sub-areas were examined in SC/F2K/J13. They found that females were relatively abundant in the Okhotsk Sea (sub-area 11), the maturity rate (rate of mature animals) was relatively lower in coastal waters (sub-area 7) and mature males were dominant in offshore waters (sub-area 9). SC/F2K/J20 estimated that some animals caught in the Okhotsk Sea (sub-area 11) migrated from the West Pacific (sub-areas 7 and 8), based on stable isotope analysis. This information is consistent with the hypothesis on migration and segregation of the 'O' stock given by Hatanaka and Miyashita (1997). At the same time, segregation by sex and maturity, especially in sub-area 9, indicates that animals in this sub-area can not comprise a complete stock, but rather that they are a part of a stock.

DISCUSSION

The Working Group on North Pacific Minke Whale Trials held in 1996 agreed that no evidence was presented to support the hypothesis for the 'W' stock, although some members considered that the information did not exclude the possibility of its existence. The reasons were: (1) insufficient data to detect genetic differences; (2) insufficient seasonal coverage of sub-area 9 (not included April to May, and size and sex compositions of animals in April-June are important); (3) insufficient spatial coverage of sub-area 9 (especially not the northern coastal area); and (4) large portions of sub-areas 8 and 12 had not been sampled.

Through the six years of the JARPN Programme, most of these points have been addressed. The number of samples used in mtDNA analyses, for example, increased from 121 to 502 including four biopsy samples. Eighty-one samples (including size and sex data) were obtained in May-June in sub-area 9, and 91 samples were from sub-area 8. SC/F2K/J11 pointed out that genetic analyses based on JARPN samples used large sample sizes, at least larger than those used in most genetic studies on marine mammals.

However, samples were not obtained from the northern coastal area of sub-area 9 and from sub-area 12, because the permission to sample in Russian waters could not be obtained. Data from sub-area 12, such as sex ratio and size composition, would be useful for examination of the segregation and migration of the 'O' stock, and samples from this sub-area are essential for detecting the mixing rate of 'J' and 'O' stocks. However, information from sub-area 12 would not provide direct evidence with respect to the 'W' stock hypothesis, because animals in sub-area 9 (pure 'W' stock) show no difference from 'O' stock. As for the northern coastal area of sub-area 9, length and sex data from Russian catches are available (Ivashin, 1992). If abnormally large animals are ignored, the sex ratio and male length distribution are similar to those in sub-area 12, and length composition of females is similar to those in sub-area 11 (Hatanaka and Miyashita, 1997). This means that small animals (smaller than 6m or so) are relatively few in this area, and small animals of 'W' stock are still unknown in this case.

The necessity of showing statistical power when presenting the results of analyses has been pointed out. However, it is difficult to set the appropriate alternative

hypothesis in the case of the 'O'/'W' stock situation, because no significant differences were observed between them. However, SC/F2K/J30 tried to estimate statistical power in the analysis of conception date, assuming that mean difference of conception date was one month between the 'O' and 'W' stocks. In this case, the 13 available samples from sub-area 9 had a power of about 70%. SC/F2K/J14 conducted a simulation for estimating statistical power using allele frequency data from the *Adh-1* locus. It was estimated that the present sample size in sub-area 9 (178 individuals) had a good statistical power in the case of two stocks, one was 'O' stock in sub-area 9 and the other was an assumed stock in which 80% of 'O' stock and 20% of 'J' stock were mixed. The authors also estimated a dispersal rate of 0.33 between sub-areas 7 and 9 and pointed out that there was no meaning in distinguishing such a slight difference for management objectives. In addition, SC/F2K/J11 estimated that the dispersal among sub-areas 7, 8 and 9 was 131.2 females per generation. These high dispersion rates suggested that those animals were effectively the same population.

Some may say that the JARPN Programme cannot provide any definitive evidence to prove that 'O' and 'W' were the same stock, even though it provided results that suggested no substantial differences between them. However, the 'W' stock hypothesis was not based on supportive information, but rather because there were no available data in offshore waters in 1993. However, at this time a large amount of data and samples are available through the JARPN Programme, none of which support a 'W' stock hypothesis. I believe, therefore, that we have no more reason to maintain it. In other words, if the present information was available at the meeting of the Working Group on North Pacific Minke Whale Management Trials in 1993, the 'W' stock hypothesis would not have been established.

In summary, a fairly large amount of data has been collected through the JARPN Programme and a wide variety of analyses have been conducted. Despite this, no supportive information on the existence of a 'W' stock has emerged. The JARPN Programme has revealed various differences between the 'J' and 'O' stocks. This indicates that the various methods applied to detect the existence of different stocks could give positive results when more than one stock is present.

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Annex H

An Alternative Summary of the Results Relating to the 'W' Stock Hypothesis

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Annex G presents a summary of JARPN results on the question of whether data collected provides evidence for more than one stock within the JARPN study area. This working paper presents an alternate interpretation of the same data under the same headings.

DNA analysis

Mitochondrial DNA data are analysed in SC/F2K/J6, SC/F2K/J7, SC/F2KJ11, SC/F2K/J32 and Goto and Pastene (1999). All these analyses reveal low *p*-values comparing either 7 to 9, 8 to 9, or 7 and 8 together to 9. SC/F2K/J32 shows clearly significant differences when the data are re-stratified between sub-area 9W and other areas. These results are consistent with more than one stock utilising the area and are similar to results for Southern Hemisphere minke whales where additional stock structure was accepted (summarised in Pastene and Goto, 1997). Although similar evidence for multiple stocks was not found when microsatellites were used, this is not an uncommon result in cetacean research. There are two possible explanations: (1) power is lower because the effective population size is approximately four times as large for nuclear DNA as for mtDNA, which effectively halves the effect size; or (2) there is more dispersal among males.

Allozyme analysis

Allozymes generally provide less resolution on stock structure because of their lower mutation rate. For example, no differences were found using allozymes for minke whales in the Antarctic and yet differences were found using mtDNA. Allozymes are commonly used when differences on an evolutionary scale are expected. This is consistent with finding differences between the 'J' and 'O' stock which probably diverged hundreds of generations ago.

Biological parameters

Although it is good to look for potential differences in body length and conception dates because they can be expected between populations that differ on an evolutionary time scale, not finding differences does not constitute evidence against there being stock structure. The timing of breeding and body size are related to the environment that animals encounter. It is likely that female minke whales across the open North Pacific time reproductive events to match with maximum productivity along the ice edges, which may not differ markedly across the northern part of the ocean basin. The reproductive timing of other stocks in the open North Pacific basin is the same within a species for other whales, such as gray, white and humpback whales, and yet multiple stocks are known to exist. Because JARPN was only able to examine minke whales in the southern part of their summering grounds, and minke whales are known to segregate latitudinally by sex and age, it is clear that sub-areas 7, 8 and 9 do not completely contain any stock. Adult females are known to be in more northern waters. Therefore, while one can state that sub-area 7 does not contain an entire stock, one cannot state that the lack of certain sex and age groups in sub-area 9 constitutes evidence against an offshore stock because essentially the same condition exists across this latitudinal range.

Morphological and morphometric analyses

Although *p*-values were not presented when > 0.05 in SC/F2K/J15, the following differences were found: Table 2: V11 $p > 0.01$, v5% $p < 0.05$, v10%, $p < 0.01$, v11% $p < 0.01$.

Pollutant burden

Differences ($p < 0.05$) were found between years but these were attributed to 'J' and 'O' stock differences.

Parasites

Differences were found in parasite prevalence but not for intensity within sub-areas 7, 8 and 9.

Stable isotopes

Stable isotopes did not show differences within sub-areas 7, 8 and 9 but participants agreed that such studies are used to see what animals have consumed on the time-scale of weeks and that therefore this technique was not appropriate for studies of stock structure.

Others

SC/F2K/J25 compared body fatness between sub-areas 7 and 9 and obtained the following *p*-values by month: May = 0.07, June = 0.12, August = 0.12.

SUMMARY

SC/F2K/J21 summarised the JARPN results as presenting no evidence for stock structure within sub-areas 7, 8 and 9. The above summary shows that there are many indications that more than one stock may utilise these waters. Differences

between management units or stocks can be expected to be much smaller than differences between populations that differ at a level sufficient for evolution to take place. A commonly used rule of thumb is that populations' evolutionary differences can accumulate if there are fewer than one disperser per generation. The Sea of Japan stock differs from other North Pacific minke whales at this evolutionary level and is inappropriate as a standard against which to measure the 'W' stock hypothesis. It is also inappropriate to only consider data of management significance when $p < 0.05$. Such a standard could result in a very unsatisfactory type 1 to type 2 error ratio.

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Annex I

Suggestions for Alternate Stratification and Analyses of Genetic Data

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In addition to homogeneity tests between sample partitions under different stratification schemes, estimating possible correlations across space and/or time with genetic distances between partitions could be performed (e.g. Mantel test). Such tests may be more appropriate in situations where gene flow is high. To avoid putting potentially heterogeneous samples within the same partition, partitions should be kept relatively narrow in terms of space and time.

Regarding stratification (for the above kind and homogeneity tests) we suggest that sample partition be based on additional parameters. Oceanographic features such as water temperature and currents, topography, etc. could be considered. In addition, new stratification analyses should include the recommendation (Item 10.2.4) to analyse sightings data using a Generalised Linear Model that includes (among other variables) the covariates of year, month, Beaufort Sea state and temperature.

The above factors could be considered in addition to or in combination with biological parameters, fat content, putative migration routes and prey preference.