# Research proposal accompanying management variant 2 of the RMP *Implementation* for western North Pacific Bryde's whale

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**ABSTRACT**

The Scientific Committee concluded that three of the four management variants used during the RMP *Implementation* for western North Pacific Bryde's whale performed acceptably from a conservation perspective and recommended that those variants could be implemented without a research program. It also agreed that variant 2 was not acceptable without research. Conservation performance for variant 2 was 'unacceptable' for three 'medium' weight trials associated with sub-stock structure in sub-area 1 (stock structure scenario 4). This paper presents a research proposal focused to elucidate whether or not sub-stocks occur in sub-area 1. The research proposal is written following the pro-forma agreed by the Committee in 2007 for research programmes associated with the 'variant with research option'. It also considers most of the specific suggestions offered by the Committee in 2007 on a previous draft of the proposal. The ultimate objective of the research program is to be able to provide information to the Committee so that it could modify (or confirm) its decisions regarding the appropriate plausibility level for the trials on which variant 2 performed 'unacceptably'.

KEYWORDS: MANAGEMENT PROCEDURE, PACIFIC OCEAN, BRYDE'S WHALE, GENETICS

Note: The proposal made below assume initiation of commercial whaling under the RMP in 2009. If this schedule is delayed, the proposals modify in obvious ways.

## RESEARCH PROPOSAL

### (1) Hypothesis to be evaluated or parameters to be estimated

During the RMP *Implementation* for western North Pacific Bryde's whale four management variants were considered:

(1) Variant 1: Sub-areas 1W, 1E and 2 are *Small Areas*;
(2) Variant 2: Sub-area 2 is taken to be a *Small Area* and the complete sub-area 1 is treated as a *Small Area*. For this management option, all of the future catches in sub-area 1 are taken from sub-area 1W;
(3) Variant 3: Sub-area 2 is taken as a *Small Area* and sub-area 1 is a *Combination Area*. Sub-areas 1W and 1E are *Small Areas*, with catch-cascading applied; and
(4) Variant 4: Sub-areas 1 and 2 (combined) are a *Combination Area*, and sub-areas 1W, 1E and 2 are *Small Areas*, with catch-cascading applied.

During its 2007 annual meeting the Scientific Committee agreed that variants 1, 3 and 4 all performed acceptably from a conservation perspective and recommended that these variants could be implemented without a research program. The Committee also agreed that variant 2 was not acceptable without research (IWC, 2008a).

Variant 2 had acceptable performance for all 'high' weight trials. However, the conservation performance was 'unacceptable' for the 'medium' weight trials BR13, BR15 and BR17 (see list of trials in Appendix 1). All these trials are related to the hypothesis of two sub-stocks in sub-area 1, which mix to each other across the boundary of the 1W and 1E sub-areas (stock structure hypothesis 4, see Figure 1).

The only evidence supporting the sub-stock scenario in sub-area 1 comes from analyses of age distribution data, which were conducted during the First Intersessional Workshop on western North Pacific *Implementation* (FIW). Results indicted some differences in age distribution between whales in sub-areas 1W and 1E+2, and one of the explanations given by the FIW to explain such result is that sub-stock structure occurs in sub-area 1. Other explanations given by the FIW to explain differences in age distribution were: i) differences are related to age reading and/or sampling issues in the commercial data, ii) differences are real and reflect age-segregated distribution within a population (IWC, 2007).

We present here a research program focused to elucidate whether or not sub-stocks occur in sub-area 1 (stock structure scenario 4). The ultimate objective of the research program is to be able to provide information to the Committee so that it could modify (or confirm) its decisions regarding the appropriate plausibility level for the trials on which variant 2 performed 'unacceptably'.

## (2) Methods-Data Collection

The primary technique to be used to investigate sub-stock structure in sub-area 1 will be the genetics, which has been so far the main source of information to delineate the stock structure hypotheses used in the trials. In applying this technique several previous recommendations from the SC will be considered. The research will involve the use of secondary techniques, e.g. satellite tags and examination of age distribution.

*Genetics*: genetic samples will be collected in sub-areas 1W and 1E (divided at 155°E) in the western North Pacific using commercial catches under IWC RMP, and biopsy samples. An increase of the sample sizes will increase the resolution of the genetic analysis to detect differences between sub-areas. Catches and biopsies will be taken in spring-summer months.

The total annual sample size is given by the catch quota derived from using Variant 2 and Trial Br2 (n=109) (IWC, 2008b). A total of 55 animals will be taken in sub-area 1W and 54 in 1E. Additionally a total of 10 biopsy samples will be taken in each of these sub-areas each year. These samples will be analyzed in conjunction with the total genetic samples from JARPN II surveys and past commercial whaling. The previous genetic analysis in sub-areas 1W/1E involved samples sizes of 261/140 and 260/125 for mtDNA and microsatellite analyses, respectively (Pastene *et al*., 2004). In addition there is a total of 112 and 88 genetic samples collected by JARPN II in these two sub-areas between 2004-07, which will be incorporated into the analysis.

Biopsy samples will be collected from free ranging whales using crossbows. Skin samples from caught whales as well as biopsy samples will be stored in 95% ethanol. Laboratory work will be conducted to obtain mtDNA control region sequences (300bp) and microsatellite profiles at 17 loci (Kanda *et al*., 2007). Laboratory work will be carried out at the genetic laboratory of the Institute of Cetacean Research. Laboratory procedures for mtDNA sequencing and microsatellites can be found in Pastene *et al*. (2007) and Kanda *et al*. (2007), respectively.

*Satellite tags*: one of the assumptions of stock structure hypothesis 4 is that there are two breeding grounds (containing two separated sub-stocks) in low latitude waters. Whales from these breeding grounds migrate in summer to high latitude feeding grounds of sub-areas 1W and 1E. Genetic differences between sub-areas are assumed to be difficult to detect because whales from the two breeding grounds are partially mixed in the feeding grounds. Experiments on satellite tags will be conducted in sub-areas 1W and 1E to elucidate pattern of migration and the location of the breeding grounds. Satellite telemetry experiments will be conducted at the end of the catch season (coinciding with the last part of the feeding period), in conjunction with biopsy sampling. In this way the location of breeding grounds will be elucidated and the genetic analysis will be possible by using the biopsy samples collected from the same marked animal. A total of 5 satellite telemetry experiments will be conducted annually in each of sub-area 1W and 1E.

The satellite mark Argos transmitter (Telonic, INC, USA) shown in Figure 2 will be used. Technical details of this mark were given in Nishiwaki (2002). The mark will be delivered from the catcher boat using the air gun described by Kasamatsu *et al*. (1991).

*Biological parameters*: the pattern of age distribution in whales from sub-areas 1W and 1E will be investigated by the use of newly collected age data (collected by JARPN II and new commercial operations), where the bias inherent to past commercial whaling are less relevant. If the differences found previously in age composition between sub-areas are real, these differences should be revealed through the analysis of newly collected age data and new ageing protocol.

Earplugs will be collected from each animal caught in sub-areas 1W (n=55) and 1E (n=54) using the same procedure used in JARPN II surveys. The protocols for age reading, along with the relevant data collected from each animal correspond to those used by the Laboratory of Marine Biology, Department of Ocean Science, Faculty of Marine Science, Tokyo University of Marine Science and Technology (see Appendix 2). Age reading will be conducted at this laboratory.

**(3) Methods-Analytical**

*Genetics*: as explained above samples will be collected in sub-areas 1W (65 including biopsies) and 1E (64 including biopsies).

For the genetic analyses standard statistical tests such as randomized chi-square and Fst will be used to evaluate genetic differences between sub-areas 1W and 1E (same as in Pastene *et al*. 2004). In addition assignment test will be conducted using microsatellite data. These analyses will be conducted annually, as new samples are accumulated, as well for the 5-year period and the final set of these data (completed 10 years). Genetic data obtained from past commercial whaling operations and JARPN II surveys will be also used in these analyses.

After the 2009 whaling season a total of 177 and 152 genetic samples will be available in sub-areas 1W and 1E, respectively (new catches in 2009+ new biopsies in 2009+ data accumulated from JARPN II between 2004 and 2007).

The power of the analyses is one of the matters to be considered. At the Workshop on the *Pre-Implementation Assessment* (IWC, 2006), Kitakado *et al*. (2005) presented some results on the statistical power for commonly used testing methods such as the chi-square permutation test (Roff and Bentzen, 1989) and Fisher's exact test (Raymond and Rousset, 1995). In this work, however, only an equilibrium state in allele frequencies based on Dirichlet distribution was supposed for generating genetic data for the analyses. Furthermore, no suitable ranges of the population differentiation were given to help the interpretation of power curves. The Workshop offered the following two specific recommendations: (i) develop a distribution of Fst for the western North Pacific Bryde's whales to interpret the results of the power analysis; (ii) consider the feasibility of evaluating power using models that explicitly include changes over time in demographics and that can be tailored to the data for the resource under consideration (IWC, 2006; 2007). Regarding item i) soft-wares such as GenePop will be used. Alternatively, it can be computed via bootstrap or by empirical Bayes method (Kitada *et al*. 2007). On item ii), the use of individual-based models is planned, to follow the change in allele frequencies over time taking into account the population size, migration rates, mortality rates, mutation rates etc., and then take samples after some generations. The simulation protocol used in this research proposal, which is a modification of the method in Kitakado *et al*. (2005), is as follows:

1. Consider a finite island model with a couple of diploid subpopulations. The subpopulations are assumed to have a same population size and equal sex ratio. The mating system is random within each of subpopulations, and linkage is ignored when multiple loci are employed. All the loci have a same mutation rate.
2. Migration rates (or numbers of migrants) are primary parameters to be controlled for assessing the power.
3. After suitable generations, pre-specified numbers of samples are taken from the subpopulations, and then the population differentiation is tested using the two methods mentioned above.
4. The power can be assessed against the migration rates or the true Fst at the final generation for several scales of the sample size.

This procedure is achieved by using EASYPOP (Balloux, 2001), a well-developed computer program for population genetic simulation, and it is simple as well as easy to handle. However, the demography of Bryde's whale is of course more complicated. In this sense, the simulation model developed under TOSSM would be useful. Such a simulation needs a variety of information on life history parameters. Life history parameters of Bryde's whales obtained during the JARPN II research might be incorporated into the model. Investigation on the possibility to develop simulation protocol for this individual-based model is a target in this research.

In addition temporal trends in summary statistics for genetic data will be compared with environmental data to attempt to determine whether distribution patterns of putative sub-stocks have changed over time.

*Satellite tag*: experiments on satellite telemetry will be conducted in sub-areas 1W and 1E to elucidate the pattern of movement and the location of the breeding grounds. Five experiments will be conducted in each sub-area 1W and 1E at the end of the catch season.

*Biological parameters*: age distribution will be generated for whales in sub-areas 1W and 1E using the newly collected data. Age distribution between these sub-areas will be compared statistically using standard method (e.g. chi-square test as used during the FIW).

Inferences based on non-genetic approaches will be integrated with those based on genetics inferences using 'human integration'.

**(4) Timeline-Including Assessment of Feasibility**

*Genetics*: as indicated above a total of 65 and 64 annual genetics samples are expected from commercial whaling operations and biopsy sampling in sub-areas 1W and 1E, respectively. Such target is feasible. Laboratory and data analysis will be conducted annually for these samples and reported to the Committee. For example for the whaling season of 2009 the generation and analyses of DNA data will be produced after the operation in that year has been completed and the results presented to the 2010 SC meeting. This is feasible from the logistic point of view.

The simulation model to evaluate power of the genetic analysis will be presented to the 2010 SC meeting by examining the DNA data collected in 2009 (in conjunction of course with the previous DNA data). The Committee will have the opportunity to discuss and evaluate these analyses and to propose modifications of the model, if necessary. Then these modifications will be implemented using the samples taken during the 2010 whaling season and results presented to the 2011 SC meeting.

*Satellite tags*: as indicated above a total of 5 experiments will be attempted in each sub-area each year. Results of the field work will be presented annually to the SC. After the first three years, results of the satellite tags experiment will be presented to the SC (e.g. at the 2012 SC meeting). If all planned experiments are successfully completed, a maximum of 15 marked whales can be expected for each sub-area (1W and 1E). The SC will have the opportunity to discuss and evaluate these experiments and to propose modifications, if necessary. Then these modifications will be implemented using the samples taken during the 2012-2014 whaling seasons and results presented to the 2015 SC meeting.

*Biological parameters*: data from the first three years (2009-2011, n=165 and 162 for sub-areas 1W and 1E, respectively + the samples collected by JARPN II till 2007) will be used to compare the age distribution of whales in sub-areas 1W and 1E. Results will be presented to the 2012 SC meeting. If that presentation indicates that more data are required to assist draw firm conclusions, updated analyses will be presented annually to the Scientific Committee as ageing information for new samples becomes available.

A comprehensive report will be presented after completed the first five years of the research program and a final report after completed the research period (10 years).

**(5) Others**

*Data availability*: data obtained will be available to Committee members through the Data Availability Group (DAG), on an annual basis.

*Reports to the SC*: reports will be presented to the Scientific Committee as indicated in (4).

*Implication of one of the research components not succeeding*: as explained above the most relevant component of the research is the genetic analyses. If this component does not succeed, the ability to achieve the overall objective of the research plan will be seriously compromised. The other components of the research are used as complementary techniques, and are unlikely to elucidate stock structure *per se*.

*Quality control of the genetics work*: genetic analysis will be conducted at the genetic laboratory of the Institute of Cetacean Research. Analysts have all acceptable education, training and experience with cetacean genetic. Reagents and equipment are properly maintained and monitored regularly. Procedure used for mtDNA and microsatellites are generally accepted in the field. Appropriate controls are used as specified in the procedures. Portions of the whale tissue samples and DNA extracts are retained and stores in 95% ethanol or at least $20^{o}$C.

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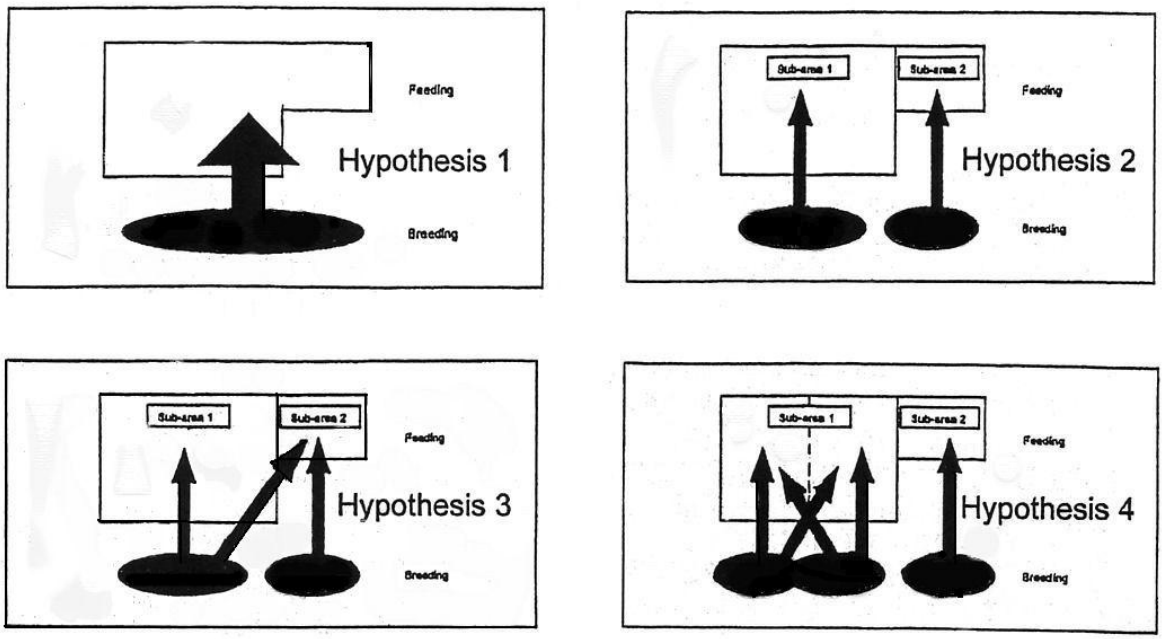


Figure 1: Stock structure hypotheses used during the RMP *Implementation* of western North Pacific Bryde's whale.

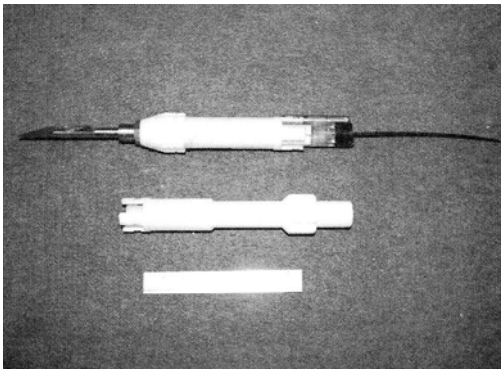

Figure 2: Satellite tags to be used in this research program (see details in Nishiwaki, 2002).

Appendix 1: List of *ISTs* used for the RMP *Implementation* of western North Pacific Bryde's whale

| Trial No. | Stocks | Sub-stocks | $MSYR_{mat}$ | Mixing matrix | Process error | Stochastic mixing in 1W/1E | Catch series | Age-dependent Mixing? | 1W / 1E boundary | Comment | Trial Weight |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Br1 | 1 | No | 1 | A | Baseline | No | Best | No | 165°E | Stock structure hypothesis 1 | M |
| Br2 | 1 | No | 4 | A | Baseline | No | Best | No | 165°E | Stock structure hypothesis 1 | H |
| Br3 | 2 | No | 1 | B | Baseline | No | Best | No | 165°E | Stock structure hypothesis 2 | M |
| Br4 | 2 | No | 4 | B | Baseline | No | Best | No | 165°E | Stock structure hypothesis 2 | H |
| Br5 | 2 | No | 1 | C | Baseline | No | Best | No | 165°E | Stock structure hypothesis 3 | M |
| Br6 | 2 | No | 4 | C | Baseline | No | Best | No | 165°E | Stock structure hypothesis 3 | H |
| Br7 | 2 | Yes | 1 | D | Baseline | No | Best | No | 155°E | Stock structure hypothesis 4 | M |
| Br8 | 2 | Yes | 4 | D | Baseline | No | Best | No | 155°E | Stock structure hypothesis 4 | M |
| Br9 | 2 | No | 1 | B | Baseline | No | Best | Yes | 165°E | B + Age-dependent mixing | M |
| Br10 | 2 | No | 4 | B | Baseline | No | Best | Yes | 165°E | B + Age-dependent mixing | H |
| Br11 | 2 | Yes | 1 | D | $\sigma_p = 0.9$ | No | Best | No | 155°E | D + Additional process error | M |
| Br12 | 2 | Yes | 4 | D | $\sigma_p = 0.9$ | No | Best | No | 155°E | D + Additional process error | M |
| Br13 | 2 | Yes | 1 | D | Baseline | Yes | Best | No | 155°E | Stochastic mixing | M |
| Br14 | 2 | Yes | 4 | D | Baseline | Yes | Best | No | 155°E | Stochastic mixing | M |
| Br15 | 2 | Yes | 1 | D | Baseline | No | Best | No | 160°E | Alternative Boundary 1 | M |
| Br16 | 2 | Yes | 4 | D | Baseline | No | Best | No | 160°E | Alternative Boundary 1 | M |
| Br17 | 2 | Yes | 1 | D | Baseline | No | Best | No | 165°E | Alternative Boundary 2 | M |
| Br18 | 2 | Yes | 4 | D | Baseline | No | Best | No | 165°E | Alternative Boundary 2 | M |
| Br19 | 2 | Yes | 1 | D | Baseline | No | Low | No | 155°E | D + Low catch series | M |
| Br20 | 2 | Yes | 4 | D | Baseline | No | Low | No | 155°E | D + Low catch series | M |
| Br21 | 2 | Yes | 1 | D | Baseline | No | High | No | 155°E | D + High catch series | M |
| Br22 | 2 | Yes | 4 | D | Baseline | No | High | No | 155°E | D + High catch series | M |
| Br23 | 2 | No | 1 | B | Baseline | No | High | No | 165°E | B + High catch series | M |
| Br24 | 2 | No | 4 | B | Baseline | No | High | No | 165°E | B + High catch series | H |
| Br25 | 2 | No | 1 | B | $\sigma_p = 0.9$ | No | Best | No | 165°E | B + Additional process error | M |
| Br26 | 2 | No | 4 | B | $\sigma_p = 0.9$ | No | Best | No | 165°E | B + Additional process error | H |
| Br27 | 2 | No | 1 | B | Baseline | No | High | Yes | 165°E | B + Age-dep mixing+high catch | M |
| Br28 | 2 | No | 4 | B | Baseline | No | High | Yes | 165°E | B + Age-dep mixing+high catch | H |

Appendix 2: Protocol for age reading in the North Pacific Bryde's whales.

Individual ages for this whale species will be determined by counting growth laminae in the core of earplugs as indicated below:

1. Earplug will be collected from external auditory meatus at left side, for principal age reading. The earplug will be collected from the right side as a back up for the cases such as damaging or missing some parts of the left earplug.

2. The collected earplugs will be preserved in 10% formalin solution.

3. Earplugs will be bisected at longitudinal axis of the earplug core.

4. The earplug surface will be smoothed by the whetstone.

5. At least two readers (hopefully three readers) will be nominated and conduct age reading after appropriate training process. Age readings will be conducted independently among the two or three age readers.

6. Individual age reading will be obtained from counting growth laminae on the core under annual deposition rate. Age readings will be firstly conducted by the order from the first sample to the final sample (the first data set, Reader A). Secondary, another independent age readings will be conducted by the order from the first sample to the final sample (the second data set, Reader A). Another independent age readings will be conducted again (the third data set, Reader A). If the three readings will be agreed, the agreed age will be assigned to be the animal age by the reader A. If two reading will be agreed, the agreed age will be again the animal age by the reader A. If the three readings will be different, median value of the readings will be the animal age by the reader A.

7. Mean values (round off at integral number) among age readings by the two or three readers will be the final animal age.

8. If the some portion of the earplug core will be missing, the value will be expressed as "n+G" in the case of missing at germinal side, "n+N" missing at neonatal side. Such counting will be not used for obtaining individual ages.

Appendix 3: Suggestions on the research proposal on western North Pacific Bryde's whale offered by the Committee in 2007 and explanation on how these suggestions were addressed

General

- The proposal needs to emphasize that the ultimate objective of the research programme is to be able to provide information to the Committee so that it could modify (or confirm) its decisions regarding the appropriate plausibility level for the trials on which variant 2 performed 'unacceptably'.

*The last paragraph in section (1) emphasizes this ultimate objective.*

- The proposal should indicate the implications of one (or more) of the components of the research program not succeeding – how will this impact the ability to achieve the overall objective.

*This is explained in the third item of section (5). The genetics is the relevant component of the research program. If this component does not succeed, the overall objective of the program will be compromised.*

- It is necessary to clarify the intension that inferences based on non-genetic approaches will be integrated with those based on genetic information using "human integration".

*This is noted in the last paragraph of item (3).*

Section 1

- The hypotheses to explain the differences in age composition between sub-areas 1E+2 and 1W should be included in this Section.

*The hypothesis was added in the second last paragraph of section (1).*

Section 2

- Plans for the collection of genetic samples and age data indicating intended spatial spread are needed.

*Number of samples and spatial distribution was explained in the third paragraph of section (2).*

- More details regarding how the satellite tags are to be attached should be provided.

*This was explained in section (2) 'Satellite tags' (second paragraph).*

- The protocols for age-reading, along with a summary of the data collected for each animal (e.g. readability, who will do the age-readings, how many times each plug is read, etc.) should be given.

*This is given in Appendix 2.*

Section 3

- The method for determining power (Kitakado *et al*., 2005) should be included as an Appendix to the proposal, and the recommendations regarding the modifications to this method from the 1st Intersessional Workshop should be listed and details of how they are to be implemented provided.

*Details of the power analysis are explained in details from the third paragraph of section (3).*

- The proposed spatial and temporal distribution of samples (where it is intended that genetic samples will be collected and animals tagged with satellite tags) needs to be documented. In particular, it is important to attach satellite tags across sub-area 1 particularly if telemetry data are to be used to estimate mixing rates.

*This was specified in section (2).*

- The proposal should make clearer that the aim of the telemetry component of the programme is to elucidate the location of the breeding grounds and to estimate mixing rates.

*To be realistic the number of marks will not be large enough for the objectives of the mixing rates. Tags could rather provide information on the movement pattern of the animals and location of breeding grounds. This is specified in several parts of the report.*

- An analysis should be conducted to show how the use of existing mark-recapture data to estimate mixing rates would be improved by data from satellite tags and the likely increase in precision (essentially a power analysis for the telemetry data); such an analysis could be conducted after some initial data have been collected (the timeline for this work would need to be included in Section 4).

*This is related to the previous comment.*

- The rationale for the genetic sample sizes is needed. It is recognized that this will necessarily be sub-optimal at the start of the program, but the proposal should indicate timelines for how the approach of Kitakado *et al*.(2005) will be modified, *inter alia* to implement the recommendations of the 1st Intersessional Workshop.

*This is explained in section (4), 'genetics'.*

- The recommendations from the 1st Intersessional Workshop regarding age-validation should be included in the proposal and details provided regarding how they will be addressed.

*The research program will not consider the use of old earplug samples because the difficulty and enormous effort to search for such samples, and because such effort is not justified when the possibility that previous results in age composition could be explained from sampling bias in the commercial data. If the differences found previously in age composition between sub-areas are real, these differences should be revealed through the analysis of newly collected age data and new ageing protocol.*

- The importance of the existing age data, relative to the additional data, needs to be emphasized because part of the support for multiple sub-stocks in sub-area 1 is the interpretation of the existing data and not a lack of age data *per se*.

*See previous comment.*

Section 4

- This section needs to indicate annual targets for data collection (including the spatial distribution of samples), and laboratory and data analyses, progress against which will then be reviewed by the Committee each year of the program. It also is recognized that consideration could be given to an 'iterative approach' whereby the sample sizes planned for each successive year depend on the results of the analyses for the preceding years. If such an 'iterative' approach is taken, the proposal would need to provide a mechanism for evaluating progress that would be used by the Committee when it conducts the annual reviews of the programme.

*This was explained in section (4), by research technique.*

- Details on how quality control (i.e. personnel, and laboratory and data storage procedures) can be assured need to be provided

*This was provided in the fourth paragraph of section (5).*