Progress report of the work conducted in response to NEWREP-A research recommendations by the IWC Scientific Committee

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Luis A. Pastene[1], Toshihide Kitakado[2] and Tsutomu Tamura[1]

[1]*Institute of Cetacean Research, Toyomi-cho 4-5, Chuo-ku, Tokyo 104-0055, Japan*
[2]*Tokyo University of Marine Science and Technology, Konan 5-7-4, Minato-ku, Tokyo 108-8477, Japan*

ABSTRACT
This paper reports progress in addressing the recommendations on NEWREP-A made by the IWC SC. The proponents started the program after completing the most important recommendations related to the need of lethal sampling and sample size (recommendations 1 and 26). Other relevant recommendations are being addressed during the implementation of the NEWREP-A, and progresses are reported in this document. A few recommendations are considered of low priority and will not be considered further.

INTRODUCTION
In accordance with Annex P, the proposal of the NEWREP-A program was reviewed firstly through the Expert Panel review workshop in February 2015 (IWC, 2016a) and subsequently by the whole International Whaling Commission Scientific Committee (IWC SC) during its 2015 annual meeting (IWC, 2016b). A number of scientific recommendations focused to improve the NEWREP-A program were endorsed by the IWC SC in 2015 (IWC, 2016b).

The NEWREP-A proponents' view on the recommendations was at first explained in GOJ (2015a):

a) Recommendations can be categorized into two in the context of the evaluation of the necessity of lethal sampling and/or reasonableness of the sample size, A= recommendations that are relevant to 'a full evaluation of whether any new lethal sampling is required' and 'issues related to sample size' (13 recommendations), and B= the other recommendations that are not relevant to such issues (16 recommendations).
b) It is not necessary to address all the recommendations 'before there is a final conclusion on lethal techniques and samples sizes'.
c) Among the recommendations that are classified in the category 'A' above, the proponents observe that those works in response to particular recommendations should be given the highest priority.
d) Recommendations related to category 'B' above can be addressed in the course of the NEWREP-A implementation.
e) Given the conclusion of the IWC SC on some particular topics, some recommendations are considered not relevant or of low priority.

GOJ (2015b) provided progress of the analytical work related to the higher priority recommendations under 'A' above (1, 11, 13 and 26). During the course of the discussion at the 2015 IWC SC, it became clear that two recommendations, 1 ('Evaluate the level of improvement that might be expected either in the SCAA or in RMP performance by improved precision in biological parameters using simulation studies including updated *Implementation Simulation Trials*') and 26 ('*Provide a thorough power analysis of sample sizes required to detect change in ASM and follow the other recommendations in this Item*') are the most relevant to consider before the start of the program. Further analyses on the two recommendations together with a progress report on the work conducted on other recommendations were presented at the 2016 IWC SC (Kitakado, 2016; GOJ, 2016a; 2016b).

During the 2016 IWC SC annual meeting, the proponents carried out further analyses on the higher priority recommendations (1 and 26) following advices from members of the Committee (Kitakado and Butterworth, 2017; Kitakado, 2017).

At the end of the 2016 IWC SC annual meeting, members expressed their opinions regarding the proponents' work on the recommendations relevant to 'a full evaluation of whether any new lethal sampling is required'. Some members 'commented that although the work required to fulfil the Committee's recommendations from last years is still in progress, these tasks remain incomplete and the results thus far have not demonstrated that

the NEWREP-A programme requires lethal sampling to achieve its stated objectives' (IWC, 2017). Other members 'commented that the proponents had responded satisfactorily to most of the recommendations of the Expert Panel, noting that some of the suggested further analyses have already been completed, while others are in progress or will be addressed within a reasonable timeframe' (IWC, 2017).

At the same meeting, the proponents stated that 'after the 2015 Scientific Committee meeting, the proponents had conducted additional analyses indicated by the Scientific Committee. They had assessed that all items pointed out by the Scientific Committee to be conducted prior to the start of NEWREP-A had been completed at a reasonable level, and had decided to implement NEWREP-A. They believe that the current results already demonstrated the utility of age data to improve the performance of CLA. While implementing NEWREP-A, the proponents stated that they will report on further progress on the additional work following the steps specified by the Advisory Group, which in their opinion go beyond the original scope of Recommendation 1, in view of the use of the collected data in the Committee's future work' (IWC, 2017).

The proponents consider therefore that they have sufficiently demonstrated that the recommendations to be addressed prior to the start of the program (1, 26) had been dully completed.

This paper reports progress on the recommendations on NEWREP-A made by the IWC SC. The proponents reserve some degree of flexibility in the timing to respond the recommendations within the implementation period of NEWREP-A. Also they will give low priority to recommendations considered not essential to address NEWREP-A research objectives.

PROGRESS REPORT

Recommendations related to need of lethal sampling and sampling size
01: Evaluate the level of improvement that might be expected either in the SCAA or in RMP performance...
10: Evaluate the effect on SCAA of assuming 'resting' females...
11: Update SCAA with respect to density-dependence following Punt et al. (2014)...
12: Identify more fully the data to be used to inform the time-varying natural mortality in the SCAA...
13: Develop metrics to evaluate the benefits of including time varying ASM data in the SCAA.
26: Provide a thorough power analysis of sample sizes required to detect changes in ASM...

The work on recommendations 1, 11 and 26 was completed to a reasonable level. See section Introduction and GOJ (2015b), GOJ (2016a), Kitakado (2016), Kitakado and Butterworth (2017), Kitakado (2017) and IWC (2017).

Recommendations 10, 12 and 13 are of lower priority in comparison with recommendations 1 and 26. Some of these recommendations were addressed to some degree (e.g GOJ, 2015b) and further analyses will be conducted during the implementation of the first half of NEWREP-A.

Recommendations related to stock structure
02: Analyses to distinguish between two stocks with mixing versus isolation by distance
03: Simulation study to examine how additional sampling

Preliminary results of analyses related to recommendation 02 above (Kitakado, personal communication 2016) showed that the effect size of the stocks in the Antarctic is too low to allow for the methods proposed by the review workshop to distinguish between the two hypotheses. The proponents consider that the hypothesis of at least two stocks with mixing in the research area is the hypothesis better supported by the genetic and non-genetic data (see explanation of the rational in Pastene and Goto, 2016). However they will complete the additional analyses suggested by the time of the 2018 IWC SC meeting.

The proponents consider that the work associated with recommendation 03 has lower priority among the remaining recommendations as this topic is not related to the main objectives of NEWREP-A. However they will present a document responding to this recommendation at the 2018 IWC SC meeting.

Recommendation related to effect of catches on stocks
27: Provide additional analyses on effect of catches upon the stocks…

The proponents had provided results based on one application of the CLA and by using the program Fitter. The NEWREP-A review workshop agreed that the conclusion that catches of the order of 333 every 2nd year form these analyses will not harm the stocks is very likely robust to the analytical method applied (IWC, 2016a). Therefore the proponents see no real need to implement this recommendation.

The NEWREP-A review workshop main rationale for this recommendation was that the application of the CLA was not based on the RMP variant that had been recommended by the Scientific Committee in 1992. The proponents' responses to this rationale are a) the recommendation regarding the use of RMP *ISTs* to assess the effect of catches has not been accepted by the IWC SC, and b) there was little information on stock structure in 1992 so that *ISTs* at that time are no longer realistic given the stock structure related data collected in the interim. The calculations already presented relate to a more realistic stock structure than that assumed for these trials, which the IWC SC has in any case not revisited for over 20 years, rendering them out of date.

Recommendations related to sighting survey design
06: Estimate g(0) for all species.
07: (1) Review survey design and methods…, (2) Work closely with the IWC Scientific Committee, (3) Ensure that future survey plans follow fully the guidelines...

Survey design and protocols with both the IO and closing modes were implemented during the dedicated sighting surveys in Area IV in 2014/15 (Matsuoka *et al*., 2015), in Area IV in 2015/16 (Isoda *et al*., 2016), and in Area V in 2016/17 (Isoda *et al*., 2017). The analysis of data collected will allow the estimation of g(0) for large whales.

Research plans including the elements in the recommendations above have been presented annually to the IWC SC (GOJ, 2015a for the survey in 2016/17; Hakamada *et al*., 2016 for the survey in 2016/17), and the research plans have been endorsed by the Committee. The plan for the survey in 2017/18 is presented to the IWC SC in Hakamada *et al*., 2017).

Therefore the proponents have responded to these recommendations by working closely with the Scientific Committee and ensuring that the surveys plan follow fully the guidelines approved by the Committee.

Recommendation related to feeding ecology and ecosystem modeling
21: Compare overlap in diet amongst fin and Antarctic minke whales using stable isotopes…
23: Investigate stable isotopes along edge of baleen plates…
25: Provide an improved outline of the proposed ecosystem and multispecies model structures…

The study related to recommendation 21 involves two steps: the first is the stable isotope analyses of the prey species (krill) samples to ensure the correct determination of stable isotope baselines; and the second is stable isotope analyses of skin samples of Antarctic minke whales and of biopsy samples of fin and humpback whales. At this juncture, the stable isotope analyses of four Antarctic krill samples and of 16 Antarctic minke whale skin samples have been conducted. The isotope value of $\delta 13C$ and $\delta 15N$ at the base of four krill samples were -26.90±0.45‰ and 3.68+0.36‰, respectively. The isotope value of $\delta 13C$ and $\delta 15N$ at the base of 16 skin samples of Antarctic minke whales were -25.04±0.43‰ and 7.26+0.61‰, respectively. Further analyses will be conducted on additional samples to obtain a correct determination of stable isotope baselines. This study is being carry out in collaboration with the Laboratory of Marine Ecosystem Change Analysis, Field Science Center for Northern Biosphere, Hokkaido University. Final results will be presented to the mid-term review meeting.

Regarding recommendation 23, stable carbon and nitrogen isotope ratios will be determined along edge of the baleen plates of 10-20 Antarctic minke whale baleen plates. Each baleen plate will be examined at around 20 places following Mitani *et al*. (2006). The analysis of baleen plates from ten Antarctic minke whales sampled by the NEWREP-A in the Ross sea in 2016 were already conducted to understand annual cycle of nitrogen and carbon, and to estimate prey species among five potential prey species: Antarctic krill, Ice krill, silver fish, Australian krill and Australian pelagic fish. The annual cycle of nitrogen in baleen plates was 7.7±2.0cm (mean±SD, range: 6.0-10.0cm), while no constant cycle was observed in carbon. The stable isotopic values at the base of baleens in carbon and nitrogen were -25.01±0.49‰ and 7.03+0.33‰, respectively. Assuming that the

trophic enrichment factor in this species is the same as that in fin whales (2.77‰), the Antarctic krill was the most likely prey as the difference in nitrogen between the baleen and prey was the minimum (3.48‰) among five potential prey species. No seasonal variation in carbon suggested that they were fasting after feeding in the Antarctic, while there were two individuals which had a nitrogen cycle of more than 12cm in baleen plates. The extended period of nitrogen cycle suggested that they remained at the Antarctic to save their energy and to accumulate the energy for their growth to prepare for the next year by skipping migration to the breeding grounds. This study is being carried out in collaboration with the Field Science Center for Northern Biosphere, Hokkaido University. Final results will be presented to the 2018 IWC SC meeting.

An update of the Mori-Butterworth Antarctic ecosystem model (recommendation 25), taking JARPA and JARPAII data into account, is well advanced by a university student for a post-graduate thesis. The decision has been made to await the results from this work to provide further basis upon which to improve the outline provided previously. A summary of results from this work will be presented to the EM sub-committee at this year's IWC SC meeting.

Recommendation related to krill survey
14: Consider the adoption of this multibeam sonar in krill surveys
15: Trial the ship and echosounder system(s) in Japan well before going to the Antarctic…
16: When both NEWREP-A and CCAMLR-type surveys are conducted, try to survey the same transects…
17: Conduct full analysis of statistical power to detect changes in krill…
18: Develop more detailed plans to consider whether comparisons between stomach contents and…
19: Ensure that sufficient time is allocated for adequate net sampling…
20: Give careful consideration to scale and design of oceanographic sampling…

Two kinds of krill surveys are being planned/implemented: Whale Sighting Vessel-Based Krill Surveys (WSKS) and CCAMLR-Type Dedicated Krill Surveys (CTKS). For the design and implementation of these two kinds of surveys, the proponents have been consulting with CCAMLR specialists. The general plan for both kind of surveys was presented to the 2015 meeting of the CCAMLR's Working Group on Ecosystem Monitoring and Management (WG-EMM) (GOJ, 2015c), and useful input from the Working Group was received (CCAMLR, 2015).

Two WSKS surveys have been already conducted, the first in Area IV in 2015/16 (Wada *et al*., 2016) and the second in Area V in 2015/16 (Wada *et al*., 2017). The preliminary results of the first WSKS were presented to the 2016 meetings of the CCAMLR's Working Group on Statistics, Assessments and Modelling (WG-SAM) and WG-EMM (Matsuoka et al., (2016). Again useful comments and suggestions were received from the specialists in both Working Groups (CCAMLR, 2016a; 2016b).

The research plan of the first CTKS will be presented to the 2017 meetings of the WG-SAM and WG-EMM, and the plan will be revised and improved by incorporating the suggestions from the specialists in both Working Groups. The first CTKS is planned for 2018/19.

In response to recommendation 15 above, calibration of the echosounder system (EK 80) has been conducted in Japan before the start of the 2015/16 and 2016/2017 WDKS. Details are provided in Wada *et al*. (2016; 2017).

All other recommendations will be addressed in consultation with CCAMLR specialists when the CTKS starts.

Recommendations related to development of new non-lethal techniques for Antarctic minke whale
04: Comprehensive biopsy sampling feasibility study
05: Comprehensive telemetry feasibility study
08: Examine feasibility of using DNA methylation ageing technique with Antarctic minke whale…
09: Examine use of hormones in blubber to detect sexual maturity
22: Develop a more powerful approach to estimating energy intake using a bioenergetics model…
24: Use 'non-lethal' techniques on all animals; develop 'condition indices'…

Explanation of the design of the biopsy sampling feasibility studies (Recommendation 04) has been included in the research plans for the dedicated sighting surveys presented annually to, and endorsed by the IWC SC (GOJ, 2015a; Hakamada *et al*., 2016). The design has considered all elements in the recommendation. Results of the feasibility study conducted in Area IV in 2015/16 were presented by Isoda *et al*. (2016), and those obtained inthe

study conducted in Area V in 2016/17 are presented by Isoda *et al*. (2017). A Generalized Linear Model (GLM) used to compare the efficiency between biopsy and lethal sampling, indicated that the success of biopsy sampling was significantly lower than the lethal sampling (Isoda *et al*., 2017). Final results will be presented to the 2018 IWC SC annual meeting.

Explanation of the design of the telemetry feasibility studies (Recommendation 05) has been included in the research plans for the dedicated sighting surveys presented annually to, and endorsed by the IWC SC (GOJ, 2015a; Hakamada *et al*., 2016). The design has considered all elements in the recommendation. Results of the feasibility study conducted in Area IV in 2015/16 were presented by Isoda *et al*. (2016), and those obtained in the study conducted in Area V in 2016/17 are presented by Isoda *et al*. (2017). The focus on the first feasibility studies was on the attachment system, which was improved in the second study. Details of the attachment system, effort, number of successful trial and tracking of the whales are shown in Isoda *et al*. (2016; 2017). Final results will be presented to the 2018 IWC SC annual meeting.

The plan for the feasibility study on DNA methylation (recommendation 08) was presented by GOJ (2015b). A total of 100 samples for which earplug readings were considered excellent or good were selected for the DNA methylation study. The selection of samples considered different years and sex. The feasibility study is being conducted using two approaches, pyrosequencing method and direct sequencing method. The former is being applied in collaboration with Riken Genesis Co. while the latter with the Laboratory of Biology of the Azabu University School of Veterinary Medicine and Graduate School of Veterinary Medicine. DNA methylation changes were identified at the Antarctic minke whale genes homologous to the humpback whales. The procedure for identification of age-related DNA methylation site (CpG) and measurement of methylation level followed previous study on humpback whale by Polanowski *et al*. (2014). DNA methylation rate of seven CpGs on three different loci were scored successfully. Furthermore regression analyses of each CpG against age (determined by earplug reading) were conducted. The regression coefficients of each CpG were lower than in the case of the humpback whale study. The next step will involve multiple linear regressions analyses for combined CpG. Final results of the feasibility study will be presented to the 2018 IWC SC annual meeting. Depending on the results and evaluation, further analyses could be conducted considering other whale species and a larger number of loci.

The plan for the feasibility study on hormone in blubber (recommendation 09) was presented by GOJ (2015b). The idea here is to estimate reproductive status in females using the concentration of progesterone in blubber, with appropriate accuracy. To this end, a total of 230 female blubber samples (157 pregnant, 17 non-pregnant and mature, and 56 immature) of Antarctic minke whales from Area IV and V sampled in the 2015/16 NEWREP-A survey will be analyzed by ELISA assay, using Crocodile mini-workstation (Titertek-Berthold). The hormone extractions from blubber samples and ELISA assay follow mainly the procedure by Nagata *et al*. (1996) and Pérez *et al*. (2011), respectively. The analytical validation and procedure are consulted with the Faculty of Agriculture, Tokyo University of Agriculture and Technology. Measurement of progesterone in the above indicated blubber samples are ongoing. These data will be compared with reproductive information (from anatomical studies on ovaries) to evaluate the feasibility of this progesterone technique to determine reproductive status. As blubber can be obtained by biopsy samples, this technique can potentially be used by non-lethal means. Final results will be presented to the 2018 IWC SC annual meeting.

Recommendations 22 and 24 involves a huge task and thus needs careful consideration. Biopsy samples which might be used for the tuning of bioenergetics model have already been collected. More time is required for the discussions to plan the details of such an exercise to take place.

Recommendations related to other topics
28: Improve mechanisms for co-operative research
29: Provide information on programme management, personnel and logistic resources

Regarding recommendation 28, the proponents have already posted a formal protocol for outside scientists to submit proposals for both field and analytical work. Expanded information on the mechanisms for co-operative research was presented in the revised research plan proposal for NEWREP-NP (GOJ, 2017), which is also valid for NEWREP-A (see section 6 and Annexes 20 and 22 of the NEWREP-NP revised research proposal).

Expanded information and explanation of the logistics and project management was presented in the revised research proposal for NEWREP-NP (GOJ, 2017), which is also valid for NEWREP-A. In particular refer to section 5 and Annex 21 of the research proposal.

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