

## **Report of the Antarctic Minke whale ear plug experiment**

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### Background

Analyses of the combined commercial and JARPA catch-at-age data have provided robust indication of trends in minke whale recruitment which have important implications for understanding of the population's dynamics. However, this key result is dependent on ageing having been carried out consistently over time, as a drift in reader performance could produce the trend in question as an artefact. The primary aim of the work proposed is to determine whether there is evidence of such a drift in reading, and, if so, to quantify it. A secondary aim is to quantify age-reading error variability (a matter which the Scientific Committee has identified as of high priority) because estimates of natural mortality rate from statistical catch-at-age analyses are sensitive to the extent of such variability. Furthermore, the JARPA review identified certain concerns about existing information which need to be resolved before results based on such data might be accepted by the Scientific Committee. This work will provide some of the information needed to resolve these concerns.

### Method

The method is provided in the protocol specified in *J. Cetacean Res. Manage.* 11 (Suppl.) 2009: Appx 4, p. 209. Left ear plugs were selected only from females. The experimental sample comprised 50 randomly taken from each of 5 sub-sets totaling 250 ear plugs in all. The sub-sets were taken from Area IV in the periods 1974/5-1976/7, 1982/3-1984/5, 1989/90-1991/2, 1997/8-1999/2000 and 2003/4-2005/6, thus encompassing a 25-year time span. The ear plugs were selected by the staff of the university laboratory in Tokyo under the supervision of Professor Kitakado.

The sample was numbered independently of all existing identifying marks for the first reading. The numbering was random for the entire set of 250 plugs, but the plugs were read in numerical order from 1-250. After completion of the first reading, the sample was reassigned new identifying numbers and re-ordered randomly. The ear plugs were then read again in numerical order 1-250. After the second reading was completed, a subset was randomly selected from the 250 set, but this time choosing 10 plugs from each time period, totaling 50 plugs in all. These were then read again.

During the reading procedure, the reader had no input or access to actual data pertaining to the sample, i.e. the plugs were read "blind".

### Experiment results

Lockyer, under contract to the IWC, travelled to Japan where she stayed from 30 November – 19 December 2009 to perform the age readings.

During the period 1-2 December, a sample of 100 specially selected ear plugs, independent of the experimental sample, were made available to Lockyer who had expressed the wish to undertake a "trial" reading of minke whale ear plugs in general. 50 ear plugs from this sample were read in order to become re-familiarised with the GLG counting methods for this species. The specimens bore their true ID numbering. However, Lockyer examined them "blind". The results of this trial – although not part of the experimental design, are included in the tabulated

results (Appendix 1, sheet 1). This trial also helped to refine the design of the proposed age recording form that includes all information originally requested in the protocol, where feasible.

The first reading began on 2 December in the afternoon, and continued each day until completion, with readings on 3, 4, 6 and 7 December, with approximately 50 plugs read each day or a maximum of 70 on any one day, with breaks every 2 hours to rest the eyes. A Nikon binocular microscope was used to examine all ear plugs with an eye objective 10xB22 and zoom magnification x0.8-x8 facility. Even at maximum magnification, it was only just possible to read all Growth Layer Groups (GLGs) at the plug base of some older animals. Five ear plugs were placed in water in separate petri dishes with individual labeling for examination at any one time. These were then replaced in sample jars before the next set of 5.

The second readings began after a 2-day break – to clear the mind! The readings were then commenced on 10 December and continued 11, 12, and 15 December. A break was then taken 16 December, before embarking on the final third reading of a sub-set of 50 ear plugs. These were completed on the 17<sup>th</sup> December.

Throughout the experimental readings, the excel data book (Appendix 1) was updated regularly (usually after reading 10 plugs) compiling all information written on the working form. This also helped to make convenient breaks between each microscope use, and avoid monotony. Lockyer was well aware that reading efficiency was greatly dependent on the degree of alertness, and on two occasions, the day's reading session was terminated because of onset of tiredness.

#### Comments on the age reading results

The oldest whale examined was >60 GLGs and the youngest had no GLG visible (young of year). The impression was that ear plug size in general was very variable, and not always correlated with age. In addition, the early-forming GLGs were the most problematic to interpret, the pattern of deposition frequently appearing distorted and irregular, especially in old animals. For this reason, the source of error in ageing in old animals is thought likely to be mainly due to problems in the early GLGs. The late-forming GLGs were much easier to interpret, despite becoming more narrowly packed together, because they were usually regular in form.

The colour of the ear plugs varied from pale ivory through tan to dark brown. Usually the young plugs were pale cream in colour while most old plugs appeared dark. However, this was not always consistent. The pale colouration frequently made it difficult to discern any GLG differentiation, and very young plugs were often problematic to be specific about GLG age. In addition, accessory laminae were sometimes present and confusing. For this reason occasionally two possible alternative readings were provided because the reader could not be certain which to choose. Normally – not in an experimental situation – one might refer to biological data to help resolve such issues.

#### Age Readings

The age readings are provided in the Appendix 1 which is in the form of an excel workbook. The age readings should be interpreted with reference to the explanations that follow in the next section below.

Specific comments on interpretation of the age readings

In order to understand the final age readings in each trial reading, those undertaking the analysis should be aware of the following notations explained below.

Specimen ID no	Age readings			Comments					
	Trial counts - given in sequence	Agreed count from trials based on weighted mean (CHL)	Best count according to Japanese method of average of counts	Plug complete? Yes or No; comment	Neonatal line present? Yes or No	Central cut? Yes or No	General appearance	Readability - Excellent; Good; Poor; Unreadable	Other

**Specimen ID no**

This refers to the experimental number provided for this reading stage of the experiment. It has been provided by Japanese scientists.

**Age readings**

In general I have used the following descriptors. When I cannot be certain about an age, the age is prefixed by ca – e.g. ca N. When part of the plug is missing, + is suffixed on the age. However, + can also be applied in young animals (range up to 6 GLGs) where a new GLG is forming at the edge but maybe incomplete. Other ways of giving this are e.g. N - N<sub>+1</sub> – in other words a range. Sometimes two possible ages are offered because of difficulties in reading. Here the ages will be e.g. N or P. Where only a minimum age is counted in difficult to read plugs, the age will be given as e.g. >N. Sometimes this notation is also used for incomplete plugs.

**Trial counts - given in sequence**

This gives the numbers of GLGs counted in sequence. The minimum number of trials is three, but may be many more depending on the confidence of the reader in what is being seen. It should be noted that before recording counts, the ear plug has been scanned several times to get a feel for the GLG patterns with rough counts made. The written counts reflect when the reader is more confident in the counting.

**Agreed count from trials based on weighted mean (CHL)**

In cases where there is no consistency of count, the mean may be weighted to the most recent count depending on the relative confidence in the reading.

**Best count according to Japanese method of average of counts**

The mean here is a simple mean and treats all readings equally.

**Comments**

**Plug complete? Yes or No; comment**

Yes denotes that all parts of the core were found, even if in two or more pieces. A comment will usually describe how many pieces or what is missing.

**Neonatal line present? Yes or No**

Yes means that at least part of the Neonatal Line (NL) has been identified.

**Central cut? Yes or No**

Yes means that the core is adequately exposed at the centre line.

**General appearance**

Information on colouration, relative size, etc. is given here. However, this has not been consistently provided, but has often been added if there has been a problem with reading. If the plug or part of it is attached to the glove finger, this is noted.

**Readability - Excellent; Good; Poor; Unreadable**

E – Excellent means very clear GLGs and little error likely in reading.

G – Good means generally quite readable with mostly clear GLGs. However, there may be some error.

P – Poor means parts of the plug are difficult to read because GLGs are obscure or irregular. A large margin of error is likely in GLGs.

U – Unreadable means that the clarity of GLGs is so poor and/or confusing, that any GLG count provided is likely to be erroneous or incomplete. **I strongly suggest that where any category includes the term "U", any age provided is ignored.**

Combinations of categories e.g. G/P mean partly good and partly poor – often which part will be specified e.g. P(top)/G (base).

**Other**

Here expanded information on readability may be given; also possible transition phase age if determined.