# Current Status and Future Plans of Age reading by Earplugs in Baleen Whales under the Scientific Permits, with special reference to Antarctic Minke Whales

Ryoko Zenitani*, Toshiya Kishiro**, Takashi Hakamada** and Hidehiro Kato***

*) *The Institute of Cetacean Research, 4-5, Toyomi-cho, Chuo-ku, Tokyo 104-0055, Japan. (e-mail; zenitani@cetacean.jp)*

**) *National Research Institute of Far Seas Fisheries, Fukuura 2-12-4, Kanazawa-ku, Yokohama 236-8648, Japan*

***) *Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-Ku, Tokyo 108-8477, Japan*

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**Abstract**

Because the differences had been pointed out in the body length of Antarctic minke whales at same age class younger than 5years between samples of commercial whaling and JARPA, the body length and age data for animals 5 years or younger (2,270individuals) collected in 1971/72-1986/87 (Areas IIIE, IV, V and VIW) were cross-checked with the original ageing notes and biological records of whales caught. Correcting coding errors and excluding the biologically unlike age readings, the differences became much smaller between of commercial and JARPA samples. We also conducted an inter-reader calibration using 100 earplug samples collected under the commercial whaling; this revealed a strong correlation with high accuracy between the commercial and JARPA data sets. Through the analyses, it was suggested that both commercial and JARPA age data sets could be used as comparable data sets or data analyses requiring systematic age data such as VPA analyses, but it was better to exclude data of age classes 5 years or younger. Current age reading manner and sample preparation was reviewed. And future plans on improvement of age readings in baleen whales were proposed with incorporating multi-readers ageing system as a short term project and obtaining new age indicators using stable isotope in baleen plates and aspartic acid racemization in the lens as middle term projects.

## 1. Introduction

It was pointed out that there were differences in mean body lengths for young age classes in Antarctic minke whales between by commercial whaling and by JARPA data (Polacheck, 2006). The difference is not seen in the mean body length of commercial whaling and JARPA in the age of six years old or older. In the young age classes, especially the individual of three years old or younger, mean body length of commercial was larger than that of JARPA. In responses, Kato and Zenitani (2006) made the following explanations for such differences:

(1) There were distinct selectivity that whaler tended to take larger whales under commercial operation.

(2) Differences in age specific readability between of commercial and of JARPA samples. Especially age specific readability of young individuals in commercial whaling samples was relatively inferior (the age in young individuals could not be read in many cases).

(3) A slower growth rate (particularly animals under the adolescent growth stage) over recent years.

They also noted some coding errors should be worth to be checked, and then we have made voluntary base study focused on validation for ages of such younger ages for the Antarctic minke whales.

According to suggestion made at the JARPA review meeting at Tokyo in 2006, we also tried re- reading of earplugs subsets collected under the 1978/79-1986/87 commercial whaling for assessing substantial ageing error between commercial and JARPA data set.

For future improvement, after brief reviewing on age-reading process by earplugs for baleen whales species collected under the Japanese scientific permit, and our future plan for earplug age reading is outlined.

## 2. Age reading of Antarctic minke whales

2.1 Validation of younger age data in Antarctic minke whales

Earplug age readings were made at the Far Seas Fisheries Laboratory under commercial whaling. For all species, age reading data were firstly recorded on the ageing filed note (AFN) which in turn was posted in the biological records of whales caught (BRWC). Data in BRWC were inputted into the WHALE MASTER TAPE (WMST) by Far Seas Fisheries Laboratory (lately the National Research Institute of Far Seas Fisheries). For the present study, we used the three sources such as AFN, BRWC and WMST for data validation.

Taking accounts of nature pointed by pervious analyses, which pointed out such differences in age of 5 years and younger especially in younger than age 4 years, thus we examined age data for animals 5 years old or younger (2,270 individuals) recorded in the WMST in 1971/72-1986/87 (Areas IIIE, IV, V and VIW) were cross-checked with BRWC and AFN. Firstly cording errors were cross-checked, we found total of 45 inappropriate coding (2.0% to the total) and those were corrected (Table 1). Secondary we checked appropriateness of their readings from biological view points with referring respective records on BRWC. Common criteria were: body length unlikely large against age recorded corpora counts and testes weight unlikely large as specified in Table 2. Through such screening by such criteria, a total 474 age readings were categorized to be “biologically unlike” and exclude from the new data set. A present study will be developed which will attempt to assess it is possible to determine whether the “biologically unlike” samples can be identified using criteria are inherent to the earplug itself (e.g. broken plug, etc). The first step in this program (which will provide a basis for identifying the next steps) will be to identify a subset of the earplugs (including those for which the age-estimates are ‘acceptable’ and ‘biologically unlike’, but with a focus on the latter to ensure adequate sample sizes) and categorize them by the assigned age and whether the plug was broken.

As a result, among the data set of 2270 individuals aged five years old or younger, 45 individuals (2.0%) and 474 individuals (20.9%) were identified or categorized to be “coding error” and “biologically unlike”, respectively. Then, coding errors were corrected, and the length distribution by age classes 1,2,3,4 and 5 in the new data set (1,754 individuals) that had excluded data with the “biologically unlike” were again re-constructed in Fig.1. Table 3 compared mean body length by age class of crude and corrected (new) data sets by commercial whaling and by JARPA. As for the mean body length of three years old or younger, differences of the mean length of new data set in JARPA data that had been seen before became smaller after the correction. Though the differences of the mean length with old data set and JARPA of three years old or younger were 1.5-1.9m (Polacheck, 2006), the difference with new data set has become considerably smaller to be 0.6 – 1.4m.

2.2 Assessment of an inter-reader calibration in ear plug reading between commercial and JARPA data sets

As for the earplugs of the past commercial whaling, in principal, only the earplugs retaining complete core without missing have been kept. The earplugs missing or broken some parts of core have not been systematically kept their whole parts even age estimations had been done data with gathering fragments or parts of core. Because the state of younger ear plugs was not especially good, most earplugs have not been kept. For present assessment, the good states of 100 earplugs were randomly chosen from well preserved earplugs collected in 1978/79-1986/87 commercial whaling and stored under good state, and resultant sample ages were 15 years old or older (Fig.2). Original age readings had been made by Kato in respective year during 1978/79 and 1986/87. Re-reading these earplugs conducted by Zenitani by the blind-test manner such that an individual re-reading was conducted without referring to previous age reading and other biological data at all.

Plots of original age (K) data as x-axis against re-reading age (new age (Z)) data as y-axis is shown in Fig.3, and scattered diagram is also shown in Fig 4. Table 4 shows frequency of age difference between the original age (K) and the new age (Z) and Fig. 5 shows frequency distribution of age difference. Table 5 shows mean age difference between the original age (K) and the new age (Z), with attaching results of paired sample t-test and Wilcoxon signed-rank test. A regression line (intercept nil) was fitted to each plots (to the original on the new age), a positive and strong correlation (R=0.996) was seen, and the slop of the regression line was 1.001.

It was confirmed that the pair readings of 50 samples (50% of the total pairs) were identical counts between the original and the new data sets, and 85% of the total pair readings were within ±3 growth layers. Mean age difference between the original age (K) and the new age (Z) was very small as being 0.010±0.220 (S.E.). Tow statistical tests such as the paired sample t-test and Wilcoxon signed –rank test were applied for assessing systematic difference between the original and the new data sets, and they revealed no significant differences between the two data sets at very high significant level (paired sample t-test: p value=0.964, Wilcoxon signed –rank test: p value=0.946).

### 2.3 Overview

In addition to coding errors, there are relatively large numbers of samples classified into biologically unlike categories. This mostly thought to be due to missing neonatal parts leading under-estimate of sample ages, subsequently leads to over-representation of mean body length of the commercial samples.

Excluding biologically unlike age readings, the new data set revealed much smaller differences in mean body length at same ages between commercial and JARPA data sets. And both data sets are potentially provide more realistic comparability. However, in spite of improvements, differences between JARPA and commercial data sets still exist among age classless of age 5 years and younger judging from Fig. 1. Such differences can be explained by difference in catching selectivity between the two data sets. Mean age at recruitment for this species was estimated about 7years under commercial operation (Harwood and Kato, 1989), this gives generally higher mean body lengths for the commercial catch than for JARPA catches. The present Inter-reader calibration revealed strong correlation with higher accuracy between two data sets the commercial and JARPA data sets, using relatively older earplug samples. Although the resultant lower age of sample distribution used was 15 years in the present calibration, this nature can be extended for younger animals than 15 years but older than 5 years.

As above, for data analyses requiring systematic age data such as VPA analyses, both commercial and JARPA age data sets can be used as comparable data sets but it is likely better to exclude age classes 5 years or younger.

## 3. Current manner of age reading by earplugs collected under JARPA and JARPN

### 3.1 Sampling and preparation of earplugs

Firstly, skull is removed from the body using a large knife and secondary the ventral side of the skill is turned up. Skull is pulled with wires at left and right sides of lower jaws. Third the external auditory canal of the skull is cut by a small knife, pulling both side of the skull with wires. Forth exposed earplugs are cut and removed very carefully using a small knife and tweezers by researcher. Sampled left and right earplugs and glove fingers preserve in each bottle with 10% formalin solution. In case of fragmented earplugs, all parts are sampled and preserve in bottle. Surface of earplug is cut by scalpel and grinded carefully by grindstone to the mid line along longitudinal axis in the laboratory.

### 3.2 Age reading process

Age of minke whale is determined by reading growth layers appearing on the bisected surface of the earplug. Individual age is determined using growth layers in earplug that are counted by a counter under a stereoscopic microscope.

Basically, a left earplug is read. A right earplug is read below of the state of a left earplug not good. When the earplug has been damaged or the formation of the growth layer is defective, the growth layer is irregularity etc. An individual age reading of earplug is conducted without refereeing to other biological data at all.

Each earplug is continuously read by three times, and the results are recorded in the recording form. The three two readings are identical, such age is taken as an individual age, however, when all three readings are not identical, in principal, a middle value is taken as an individual age. When the neonatal or/and germinal part of earplug cores are missed, age is recoded as “number of growth layer + G or +N”, and such readings don't treat as individual ages.

All of earplugs including their fragments have been completely preserved after respective age reading under the JARPA and other scientific permit programs. Current major readers are; Zenitani for Antarctic minke whales, Kishiro and Kato for common minke whales and Kato for other species.

**4. Future improvement of age reading by earplugs collected under the scientific permits.**

For improvement of age reading by earplugs, we are now considering following plans for both short-term and middle term.

(1) Short term: For obtaining more objective and independent age readings we are planning to incorporate multi-readers aging system by three readers or more for every individual. We have already started to train age reading for three tow young readers including graduate students of TUMSAT and an ICR researcher. During training process, we also plan to start exercise using fin whale earplugs for education purpose, which retains most distinctive growth lamina in the core of earplugs among baleen whales, and then extend education to other species. We will start this exercise in this year with repeating reading calibration between newly recruited readers and existing readers. Multi-readers ageing system will be planned to apply firstly for Antarctic minke whales and secondary for other species. It is also planned to conduct experiments to improve sampling rate of earplugs without missing by fixing whole parts of auditory meatus with resins.

(2) Mid-term: For obtain an independent additional age markers for calibration with earplug aging; we plan to conduct feasibility studies on stable isotope analysis such as $\delta^{15}N$ and $\delta^{13}C$ in baleen plates to develop ageing method of baleen whales with allying rationale by Vander-Zanden and Rasmussen (2001) and Jenkins *et al*. (2001). And we have also planed to try feasibility studies to apply eye lens of baleen whales using on aspartic acid racemization in the lens examined by Bada (1984), Nerini (1983) and George *et al*. (1999). Although their validities are still not known it is worth to investigate utilities of those techniques.

**5. ACKNOWLEDGMENTS**

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Table 1 Summary of the consequences of coding errors, with respect to revised age against the original age.

| | | Original age | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | total |
| Revised age | 1 | - | 0 | 0 | 0 | 0 | 0 |
| | 2 | 1 | - | 0 | 0 | 0 | 1 |
| | 3 | 0 | 1 | - | 0 | 0 | 1 |
| | 4 | 0 | 0 | 0 | - | 0 | 0 |
| | 5 | 0 | 0 | 0 | 0 | - | 0 |
| | 6+ | 0 | 2 | 0 | 1 | 1 | 4 |
| | unknown | 2 | 6 | 11 | 10 | 10 | 39 |
| | total | 3 | 9 | 11 | 11 | 11 | 45 |

Table 2. Criteria used to identify samples that are “biologically unlike” (numbers in parenthesis are number of age estimates determined to be “biologically unlike” for each reason).

| | Age 1 | Age 2 | Age 3 | Age 4 | Age 5 |
|---|---|---|---|---|---|
| Males | | | | | |
| Length | > 6.5m (22) | > 7.3m (74) | > 7.9m (74) | > 8.3 m (38) | |
| Length and Testes weight | | 7.1-7.3m >0.3kg (8) | | 8.0-8.3m >0.9kg (25) | |
| Females | | | | | |
| Length | > 6.5 m (15) | > 7.5 m (60) | > 7.9 m (79) | > 8.5 m (45) | 8.8m (34) |

Table 4 Comparison of mean body lengths by crude and corrected data set by the commercial whaling and by JARPA in respective age class 1 to 5 years.

**Male**

| | | | N | Mean | S.D. | S.E. | Mean difference | Min. | Max. |
|---|---|---|---|---|---|---|---|---|---|
| Age 1 | Commercial | crude | 29 | 7.1 | 0.63 | 0.12 | 1.8 | 5.8 | 8.1 |
| | | corrected | 6 | 6.1 | 0.23 | 0.09 | 0.9 | 5.8 | 6.4 |
| | JARPA | | 60 | 5.2 | 0.30 | 0.04 | | 4.5 | 5.8 |
| Age 2 | Commercial | crude | 141 | 7.5 | 0.55 | 0.05 | 1.9 | 6.0 | 8.7 |
| | | corrected | 55 | 6.9 | 0.31 | 0.04 | 1.4 | 6.0 | 7.3 |
| | JARPA | | 176 | 5.5 | 0.27 | 0.02 | | 4.8 | 6.2 |
| Age 3 | Commercial | crude | 273 | 7.6 | 0.50 | 0.03 | 1.6 | 6.1 | 9.0 |
| | | corrected | 196 | 7.4 | 0.37 | 0.03 | 1.3 | 6.1 | 7.9 |
| | JARPA | | 70 | 6.1 | 0.35 | 0.04 | | 5.3 | 7.0 |
| Age 4 | Commercial | crude | 316 | 7.8 | 0.45 | 0.03 | 1.1 | 6.4 | 9.5 |
| | | corrected | 247 | 7.7 | 0.36 | 0.02 | 1.0 | 6.4 | 8.3 |
| | JARPA | | 83 | 6.7 | 0.31 | 0.03 | | 6.1 | 7.4 |
| Age 5 | Commercial | crude | 346 | 8.0 | 0.47 | 0.03 | 0.7 | 6.4 | 9.2 |
| | | corrected | 343 | 8.0 | 0.48 | 0.03 | 0.7 | 6.4 | 9.2 |
| | JARPA | | 99 | 7.3 | 0.38 | 0.04 | | 6.4 | 8.0 |

**Female**

| | | | N | Mean | S.D. | S.E. | Mean difference | Min. | Max. |
|---|---|---|---|---|---|---|---|---|---|
| Age 1 | Commercial | crude | 26 | 6.9 | 0.97 | 0.19 | 1.6 | 5.1 | 8.9 |
| | | corrected | 9 | 5.9 | 0.45 | 0.15 | 0.6 | 5.1 | 6.4 |
| | JARPA | | 49 | 5.3 | 0.26 | 0.04 | | 4.7 | 5.9 |
| Age 2 | Commercial | crude | 150 | 7.3 | 0.68 | 0.06 | 1.8 | 5.5 | 9.0 |
| | | corrected | 86 | 6.9 | 0.45 | 0.05 | 1.3 | 5.5 | 7.5 |
| | JARPA | | 193 | 5.6 | 0.25 | 0.02 | | 4.9 | 6.1 |
| Age 3 | Commercial | crude | 291 | 7.6 | 0.61 | 0.04 | 1.5 | 5.7 | 9.3 |
| | | corrected | 206 | 7.3 | 0.42 | 0.03 | 1.2 | 5.7 | 7.9 |
| | JARPA | | 103 | 6.1 | 0.40 | 0.04 | | 5.2 | 7.2 |
| Age 4 | Commercial | crude | 324 | 7.9 | 0.62 | 0.03 | 1.1 | 6.0 | 9.6 |
| | | corrected | 274 | 7.7 | 0.50 | 0.03 | 1.0 | 6.0 | 8.5 |
| | JARPA | | 93 | 6.7 | 0.37 | 0.04 | | 5.9 | 7.7 |
| Age 5 | Commercial | crude | 374 | 8.1 | 0.57 | 0.03 | 0.8 | 6.4 | 9.9 |
| | | corrected | 332 | 8.0 | 0.48 | 0.03 | 0.7 | 6.4 | 8.8 |
| | JARPA | | 110 | 7.3 | 0.46 | 0.04 | | 6.3 | 8.2 |

Table 5. Differences (K - Z) in counts between the new readings by Zenitani of 100 Antarctic minke whale earplugs (Z) and the original age readings by Kato (K). See also Fig. 5

| Difference | n | % |
|---|---|---|
| -6 | 2 | 2.0 |
| -5 | 4 | 4.0 |
| -4 | | |
| -3 | 4 | 4.0 |
| -2 | 7 | 7.0 |
| -1 | 8 | 8.0 |
| 0 | 50 | 50.0 |
| 1 | 8 | 8.0 |
| 2 | 5 | 5.0 |
| 3 | 3 | 3.0 |
| 4 | 5 | 5.0 |
| 5 | 4 | 4.0 |
| 6 | | |
| Total | 100 | 100.0 |

Table 3 Mean age difference between original age (K) and new age (Z), and results of paired sample t-test and Wilcoxon signed-rank test.

| | n | Mean difference | S.D. | S.E. | p value: Paired-sample t-test | p value: Wilcoxon siged-rank test |
|---|---|---|---|---|---|---|
| Original age (K)-New age (Z) | 100 | 0.010 | 2.200 | 0.220 | 0.964 | 0.946 |

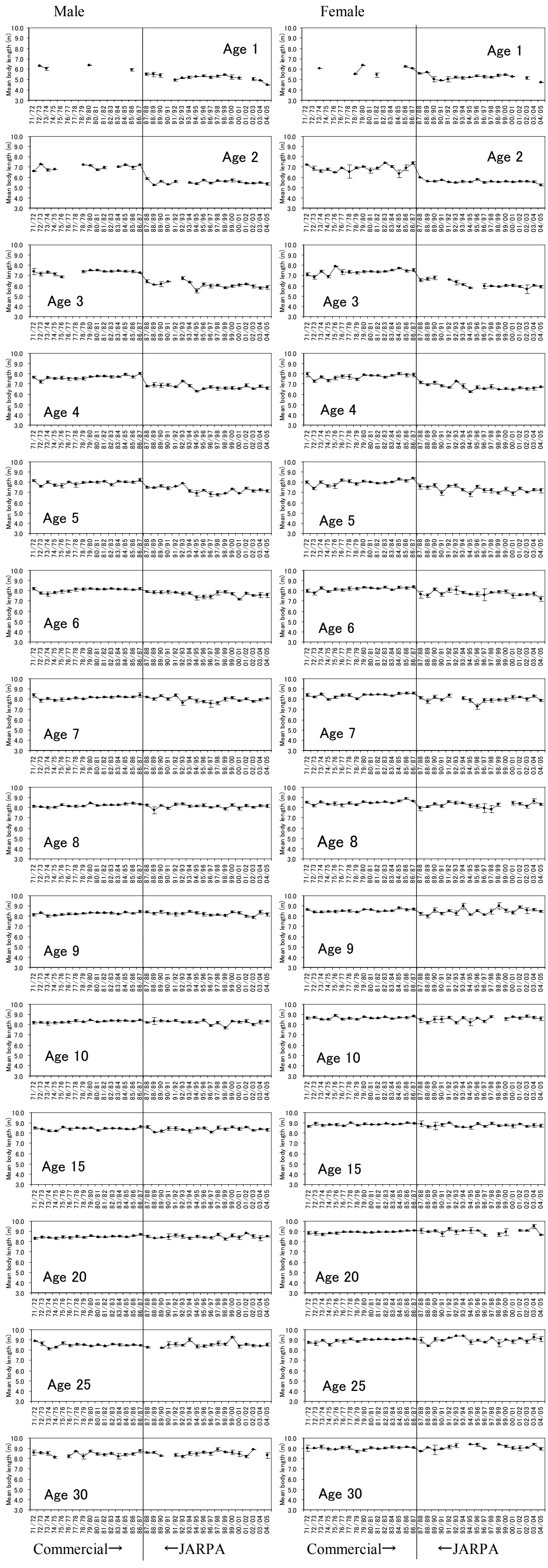


Fig.1. Mean body length and standard error by age class of Antarctic minke whales using new 1971/72-1986/87 commercial data and 1987/88-2004/05 JARPA data.

Fig.2. Current preservation status of Antarctic minke whale arplugss collected under commercial whaling. Each earplug is backed in small plastic bag with 10% formalin solution.

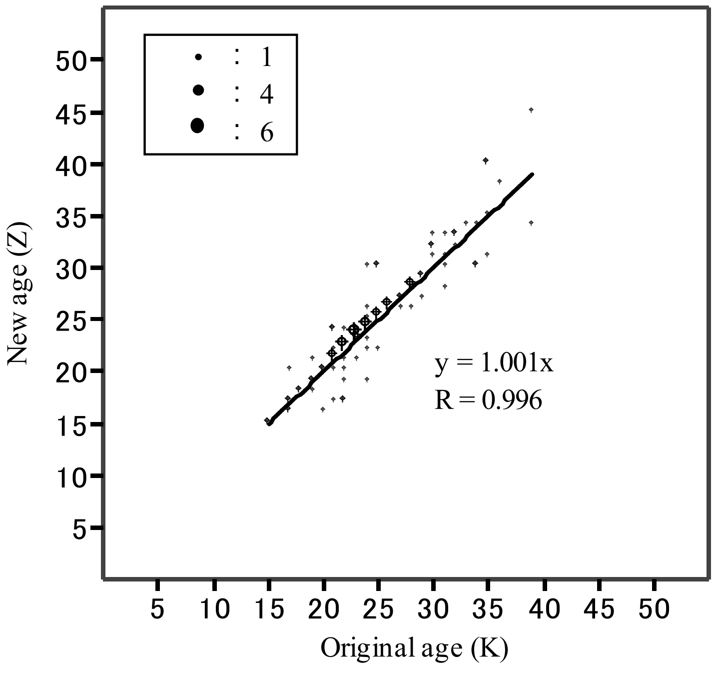


Fig.3. Plots of the new-readings by Zenitani of 100 Antarctic minke whale earplugs randomly chosen from the stock on the original age readings by

| New age (Z) \ Original age (K) | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total | 2 | 0 | 5 | 2 | 4 | 3 | 9 | 11 | 9 | 11 | 7 | 4 | 3 | 5 | 3 | 4 | 4 | 3 | 1 | 3 | 4 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 45 | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | 1 |
| 44 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 |
| 43 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 |
| 42 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 |
| 41 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 |
| 40 | | | | | | | | | | | | | | | | | | | | | 2 | | | | | | | | | | | 2 |
| 39 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 |
| 38 | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | 1 |
| 37 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 |
| 36 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 |
| 35 | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | 1 |
| 34 | | | | | | | | | | | | | | | | | | | 1 | 1 | | | | | 1 | | | | | | | 3 |
| 33 | | | | | | | | | | | | | | | | 1 | 1 | 2 | | | | | | | | | | | | | | 4 |
| 32 | | | | | | | | | | | | | | | | 2 | | 1 | | | | | | | | | | | | | | 3 |
| 31 | | | | | | | | | | | | | | | | 1 | 1 | | | | 1 | | | | | | | | | | | 3 |
| 30 | | | | | | | | | | 1 | 2 | | | | | | 1 | | | 2 | | | | | | | | | | | | 6 |
| 29 | | | | | | | | | | | | | | | 2 | | | | | | | | | | | | | | | | | 2 |
| 28 | | | | | | | | | | | | | | 4 | | | 1 | | | | | | | | | | | | | | | 5 |
| 27 | | | | | | | | | | | | | 2 | | 1 | | | | | | | | | | | | | | | | | 3 |
| 26 | | | | | | | | | | 1 | | 4 | 1 | 1 | | | | | | | | | | | | | | | | | | 7 |
| 25 | | | | | | | | | | 1 | 4 | | | | | | | | | | | | | | | | | | | | | 5 |
| 24 | | | | | | | 2 | 1 | 2 | 5 | | | | | | | | | | | | | | | | | | | | | | 10 |
| 23 | | | | | | | | | 6 | 1 | | | | | | | | | | | | | | | | | | | | | | 7 |
| 22 | | | | | | | 1 | 5 | | 1 | 1 | | | | | | | | | | | | | | | | | | | | | 8 |
| 21 | | | | | 1 | | 4 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | | | 7 |
| 20 | | | 1 | | | 2 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | | | | 5 |
| 19 | | | | | 2 | | | 1 | | 1 | | | | | | | | | | | | | | | | | | | | | | 4 |
| 18 | | | | 2 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | 3 |
| 17 | | | 2 | | | | 1 | 2 | | | | | | | | | | | | | | | | | | | | | | | | 5 |
| 16 | | | 2 | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | 3 |
| 15 | 2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 2 |

Fig. 4. Scatter diagram of the new readings by Zenitani (Z) of 100 Antarctic minke whales earplugs randomly chosen from the stock on the original readings by Kato (K) .

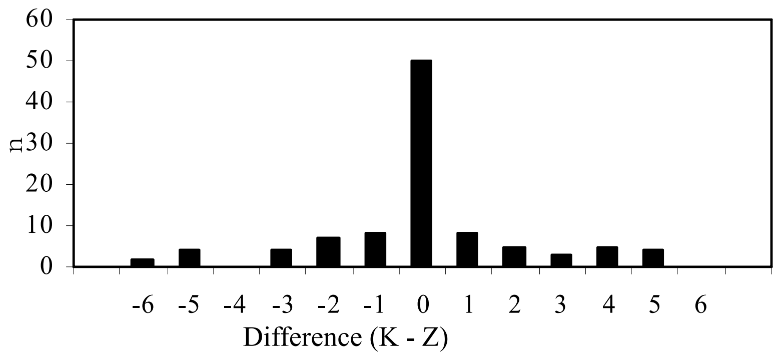


Fig.5. Differences in counts between the new readings by Zenitani of 100 Antarctic minke whale earplugs (Z) and the original age readings by Kato (K). (see also Table 3)