# PHOTOMETRIC METHOD FOR COUNTING LAMINAE IN EAR PLUG OF BALEEN WHALE*

TADAYOSHI ICHIHARA

## INTRODUCTION

Since Purves (1955) found out the ear plug as a valuable age character of the baleen whale in the course of study on sound conductivity, several scientists have researched for the relation between other age characters appreciated until then and the number of laminae in ear plug. They have concluded that the ear plug is the most valuable character for the age determination throughout the life span of baleen whales. The core of ear plug is formed from the epithelial cell of so-called glove-finger which is closely connected with tympanic ligament and it is constructed by the alternation of bright and dark lamina in the macroscopic observation. Ichihara (1959) concluded in the histological examination that these alternations result from the fatty and keratinized degeneration of the epithelial cells of glove-finger.

It is most important for determining the whale age to count accurately the laminae composed of two different components mentioned above and to avoid the interpretation favoured by individual scientists. Besides, it is desirable that the thickness of each lamina can be automatically measured and recorded. Measurement for the thickness of the most proximal lamina being now constructed is indispensable to study the annual accumulation rate of laminae. In order to attain these purposes, the photometric method for laminae counting should be devised.

My grateful acknowledgements are due to Dr. Moriso Hirata, Department of Physics, Faculty of Science, University of Tokyo, who accomplished the photometric counting apparatus and permitted me to describe its mechanism in this paper. I am also indebted to Dr. Hideo Omura, Director of the Whales Research Institute, for his encouragement throughout this work. My thanks are due to Dr. R. M. Laws, Nuffield unit of tropical animal ecology, Uganda and to Mr. G. C. Pike, Biological Station at Nanaimo, Fisheries Research Board of Canada, who kindly sent me some specimens of ear plugs for standardizing the laminae counting and informed me their counts with other biological data. The personal communications as well as the exchange of ear plug between them and me stimulated the idea for this work. Mrs. Sadako Tsumori assisted me in counting the laminae of ear plug from the fin whale in the North Pacific.

## TRIAL FOR STANDARDIZING THE LAMINAE COUNTING IN EAR PLUG

In 1958, I counted the laminae of ear plugs from 408 fin whales caught in the North

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Pacific expedition. Dissecting microscope with 12 from 8 magnifications is used for the counting. A trained assistant, Mrs. Sadako Tsumori, counted independently the laminae in the same specimens to examine the individual discrepancy in the laminae counting. The discrepancy of counts between both persons occurs for 277 of 408 specimens. The ear plug of vague laminae constitutes most of 277 plugs and its ratio corresponds to 67.9 percentage of total ear plugs examined.

In the course of laminae counting in the ear plug taken from the Antarctic fin whale in 1959/60 season, I separated the ear plugs of female whales captured by a Japanese fleet into two categories ; one is the specimens indicating clear laminae which can be counted easily, and the other is that showing vague laminae, whose counting being somewhat arbitrary. In Table 1, the number of specimens in two categories are indicated respectively in area IV and area V–VI. Area IV includes the waters to 130°E from 70°E, area V to 170°W from 130°E and area VI to 120°W from 170°W in the Antarctic Ocean.

TABLE 1. TWO CATEGORIES OF EAR PLUG FROM THE FEMALE FIN WHALE IN THE ANTARCTIC

| | | Area IV | Area V–VI | Total |
|---|---|---|---|---|
| 1) | Specimens of clear laminae | 40 | 39 | 79 |
| 2) | Specimens of vague laminae | 120 | 121 | 241 |
| | Total | 160 | 160 | 320 |
| | Percentage 2) to total | 75.0 | 75.6 | 75.3 |

Average ratio of specimens of the vague laminae to the total ear plugs examined is 75.3% in the Antarctic and the nearly same ratio is obtained both in area IV and in area V–VI.

Ear plugs in vague laminae are possibly counted at individual option. The occurrence of vague laminae is less frequent in the North Pacific than in the Antarctic as far as the present materials concern. The fact that 84.1% of total ear plugs in the North Pacific expedition in 1958 is from the male whale supports this evidence, because the alternation of laminae is more regular in the male than in the female plugs.

The occurrence of individual discrepancy in the laminae counting is examined further in Fig. 1 & 2 for the ear plugs obtained in the North Pacific expedition in 1958. With the increment of laminae, less count or over count increases for the ear plug. The percentage frequency of ear plugs in which the discrepancy in count occurs is indicated at each 5 laminae in Fig. 1. The frequency increases rapidly until 25 laminae but remains on the same level over 26 laminae. Although the different counts are obtained in most of ear plugs with many laminae, Fig. 1 indicates the evidence that the count for many laminae is coincident between individuals, if the structure of laminae is clear.

In the ear plugs of which the laminae count is lacking in agreement, the individual deviation from the mean count is expressed as follows. If one person counts 19 laminae and another person does 21 laminae for the same ear plug, the percentage deviation from the mean is

$$1/20 \times 100 = 5(\%)$$

Such a calculation is practised for each ear plug and the percentage frequency of deviation is indicated as the histograms in Fig. 2. With the increment of laminae, there is a tendency that the range of discrepancy becomes narrower but much skewness of the histograms appears positively, therefore, the mean value as expressed in the dotted line decreases gradually. The mean value is 6.70% for 1–20 laminae group, 4.11% for 21–40 group, 3.06% for 41–60 group and 3.12% for the group over 61 laminae. For the examined ear plugs, the mean discrepancy is $\pm 4.68\%$ as indicated in the bottom in Fig. 2.

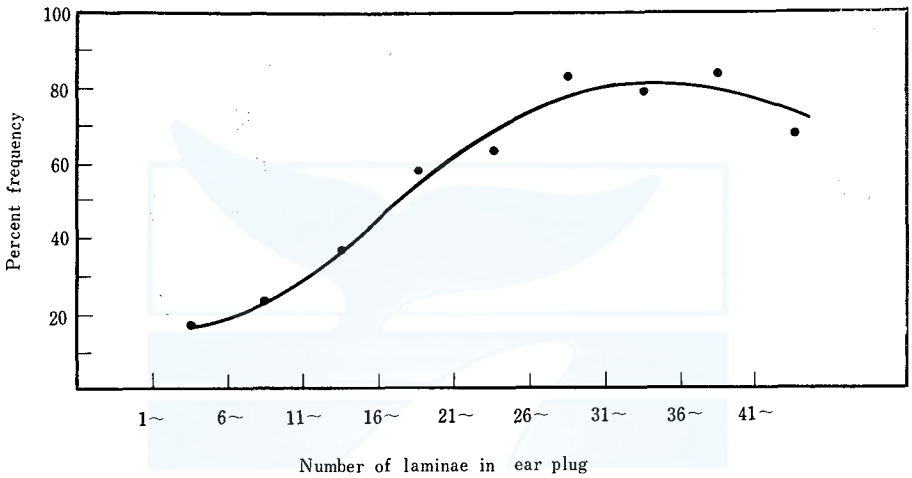


Fig. 1. Percentage frequency of discrepancy in the laminae counting of ear plugs from the North Pacific fin whales.

For standardizing the counting method of laminae, it is a way to exchange some ear plugs among scientists belonging to different countries and to count the laminae with each other. By this way, the individual interpretation can be avoid to some degree. For the advancement of whale biology, the agreement among scientists is of most improtance. In 1959, the Whales Research Institute sent ear plugs of 6 fin whales caught in the Antarctic Ocean to Dr. Laws and ear plugs of 11 fin whales captured in the North Pacific to Mr. Pike. Dr. Laws who had then the duty at National Institute of Oceanography in England, sent me 8 ear plugs from Antarctic fin whales. Accepting the program of exchange, Mr. Pike sent me ear plugs of 10 fin whales captured off Vancouver Island. The laminations of these 35 ear plugs was independently counted and checked with each other.

It is concluded from the practice of this program that in the ear plugs of fin whales, if the structure of laminae is clear, the nearly same count is obtained, while, if the structure is vague, the value of laminae count is possibly lacking in agreement. Particularly, the intermediate bright layers are often found between the dense layers in the female ear plug. The presence of intermediate laminae, of which structure is usually vague and in some case clear, results in the discrepancy in the laminae counting. It is worthy to remind that most of female ear plugs in the

Antarctic are composed of specimens showing vague laminae, as indicated in Table 1.

## PHOTOMETRIC METHOD FOR COUNTING LAMINAE

The laminae counting is eventually to count the number of alternations of bright

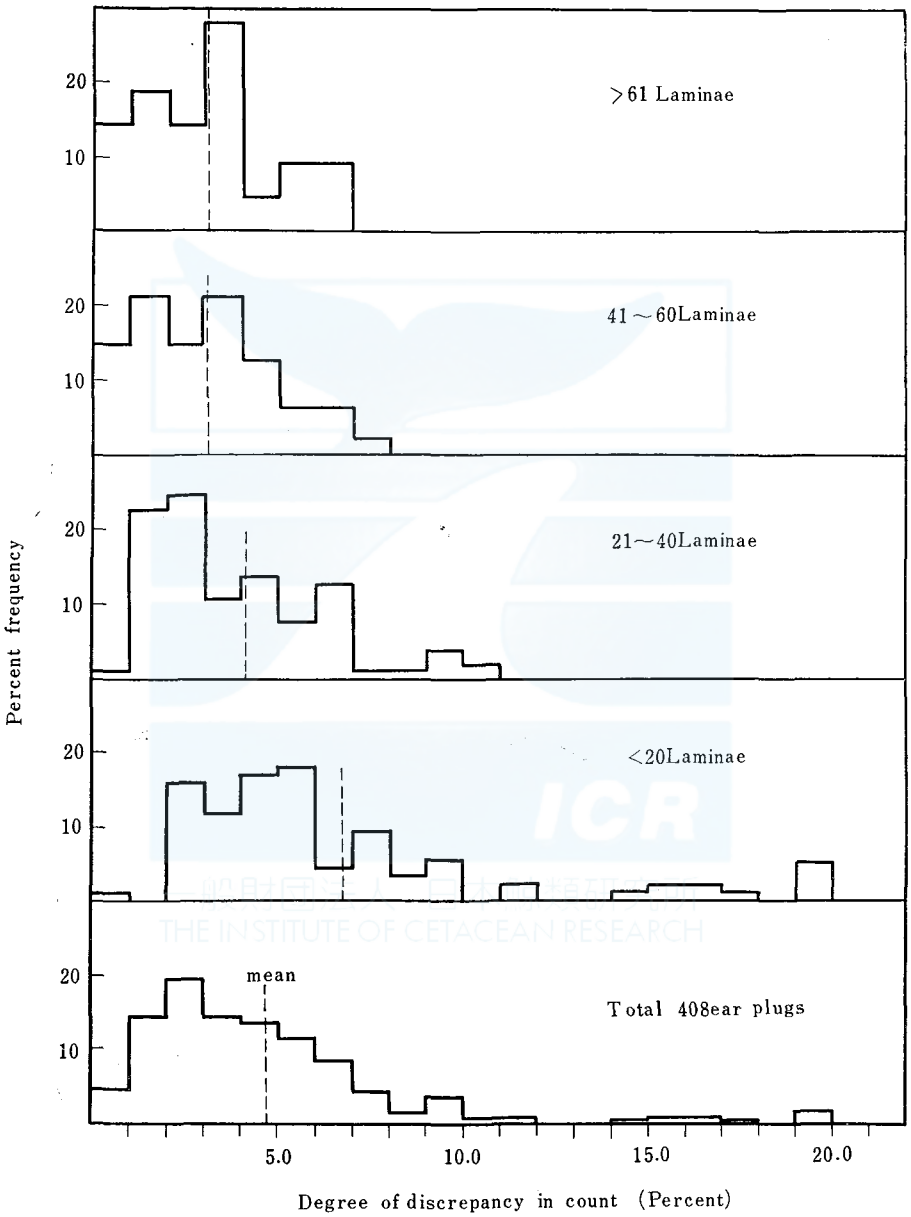


Fig. 2. Percentage deviation of individual counting from the mean count for the laminae of ear plug in the North Pacific fin whale.

and dark layers in the ear plug. The usual method of counting by naked eye observation or by means of dissecting microscope includes some ambiguity for the

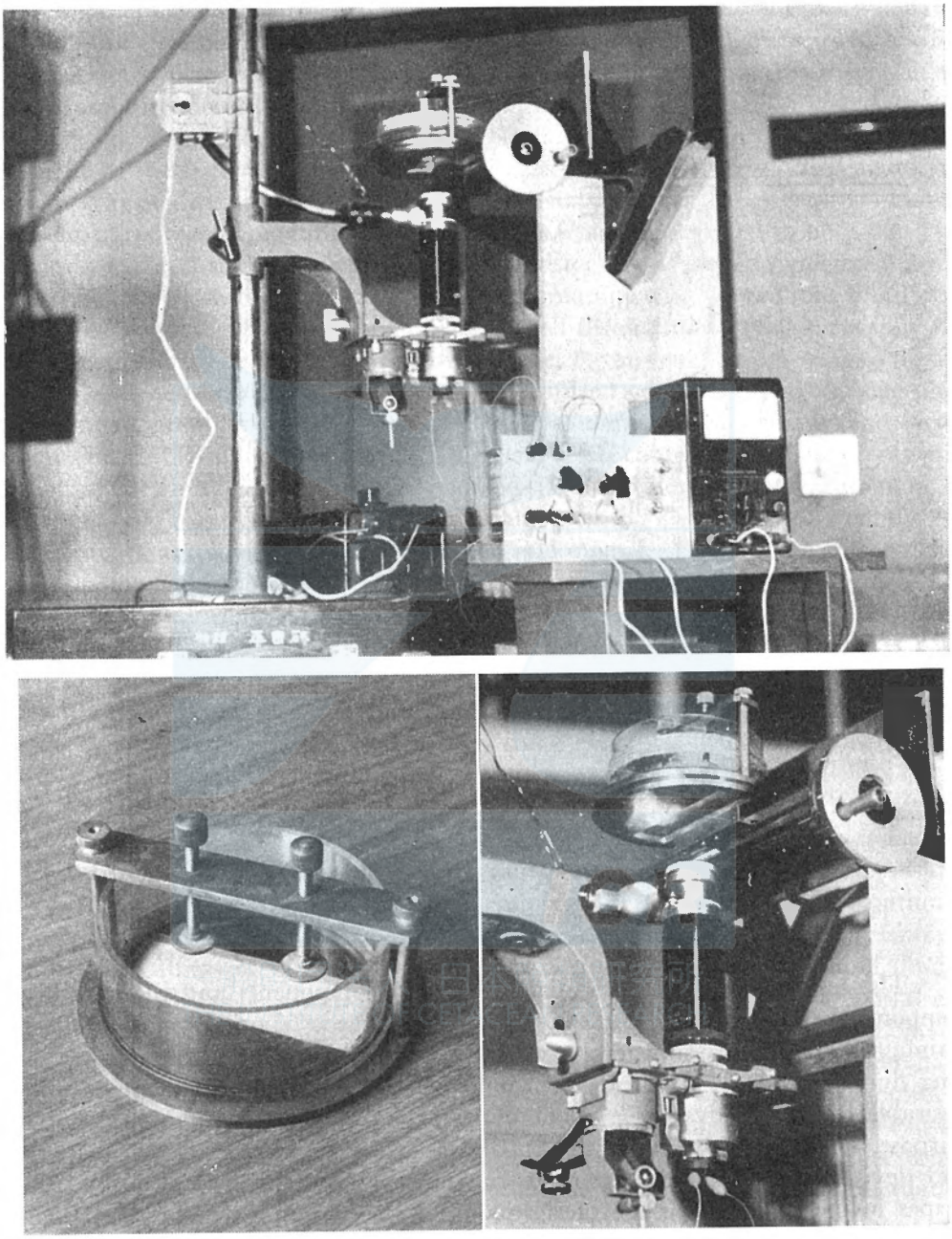

Fig. 3. Photographs of photometric counting apparatus. upper: General view, lower left: Glass vessel with the device of pressing screws, lower right: Upper half of the apparatus.

agreement among individual interpretations by different observers, especially in the case of vague laminae as pointed out in the preceding article. The judgment through our sense of sight is not always reliable.

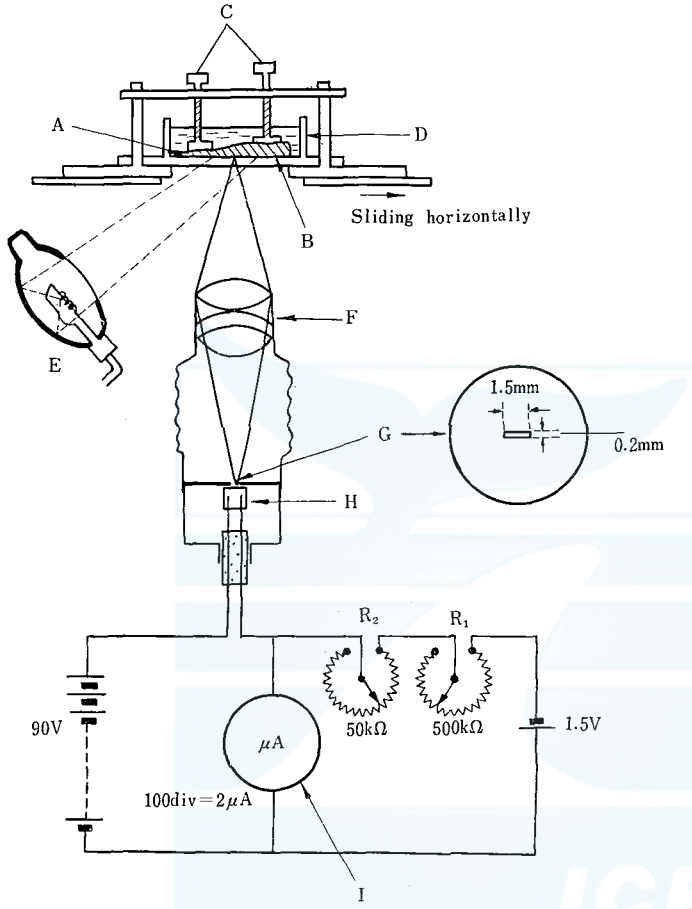


Fig. 4. Schematic diagram indicating the mechanism of photometric apparatus for counting laminae in the ear plug.

The laboratory work for staining the fatty components and the keratinized components respectively in the ear plug after slicing is laborious work, while this staining method is not sufficiently reliable for preserving the records unaltered for long duration. More accurate recordings of laminae in many ear plugs will be desirable for the analysis of age composition in the whale population. For this purpose, I have tried to utilize the apparatus, which had originally been designed and developed by Dr. M. Hirata to measure photometrically the brightness of stripes in the bisected surface of ear plug. A brief outline of this method will be given in the following lines.

Mechanism: The photograph of photometric apparatus is shown in Fig. 3, and its schematic diagram in Fig. 4.

The flat surface of longitudinally bisected ear plug (A) is smoothed with a fine whetstone. This specimen put in a glass vessel (D) containing 10% formalin solution for keeping the ear plug from drying, where the smoothed surface is faced the flat bottom glass plate (B), is pressed downwards by means of two screws (C). This glass vessel is placed on the sliding bench of the comparator and is driven in the horizontal direction by rotating. The flat surface of the plug is illuminated through the bottom glass plate by a 8 mm cine lamp (E). The magnified image of the laminae of the specimen is focused by the lens (F) on the blacked thin plate (G), which is provided with a small slit, $1.5 \times 0.2$ mm, opened at its centre. For the present purpose, the magnification ratio of the image is adjusted to about 2. Just behind the slit, a small piece of CdS photo-resistor (H) is placed, which is fed with a 90 V dry cell through a transistorized microammeter. A suitable small compensating voltage is applied from 1.5 V dry cell through the variable resistors ($R_1$, $R_2$) to the microammeter (I) as is shown in Fig. 3, thus enabling us to read the fine variation of the brightness of fatty and keratinized components in the ear plug.

For the first experimental step, the scale of the microammeter is read successively at each 0.1 mm displacement of the glass vessel containing the plug by turning the micrometric screw. It may be more disirable to develop some automatic recording apparatus by connecting the microammeter to a pen-writing system through an adequate amplifier, and by driving the micrometric screw with a tiny synchronized motor. Furthermore, it is possible to conduct the measurement by continual watching the scanning spot on the image of laminae if the optical system can be modified by inserting a thin semi-transparent glass plate below the objective lens in an inclined orientation and projecting the same image to the watching window. Construction of such an automatic recording apparatus is now being planned, but at the present stage, I will describe only some examples of data obtained by the simple apparatus mentioned above.

Records: The method is applied for four ear plugs from the Antarctic fin whales captured in 1959/60 season. The reading of the microammeter is made at each 0.1 mm sliding position of ear plug and plotted in the figure. The pulsating curve in Fig. 5 & 6 is obtained by connecting these plotted spots with continuous line. The scale of ordinate in the figures is an arbitrary one and represents the relative brightness in the surface of the bisected ear plug. The scale of abscissa indicates the distance in mm from the proximal end of ear plug to the distal end. In the fluctuation of brightness in Fig. 5 & 6, the higher brightness represents the bright layer composed of fatty components in ear plug and, on the other hand, the lower brightness indicates the dark layer composed of keratinized epithelial cells of glove-finger in ear plug. Prenatal layer (Ichihara, unpublished) exists in the distal end of inidividual plug core and appears in the extremely higher brightness. Laminae in ear plug, therefore, can be counted as the number of fluctuations recorded in the paper. Numbers of my own count also are shown in Fig. 5 & 6.

Photographs of ear plugs from the Antarctic fin whales, tested for this photometric method are indicated in Fig. 7. One of these ear plugs is from the male fin whale and the remains are from the female fin whale. The biological data of

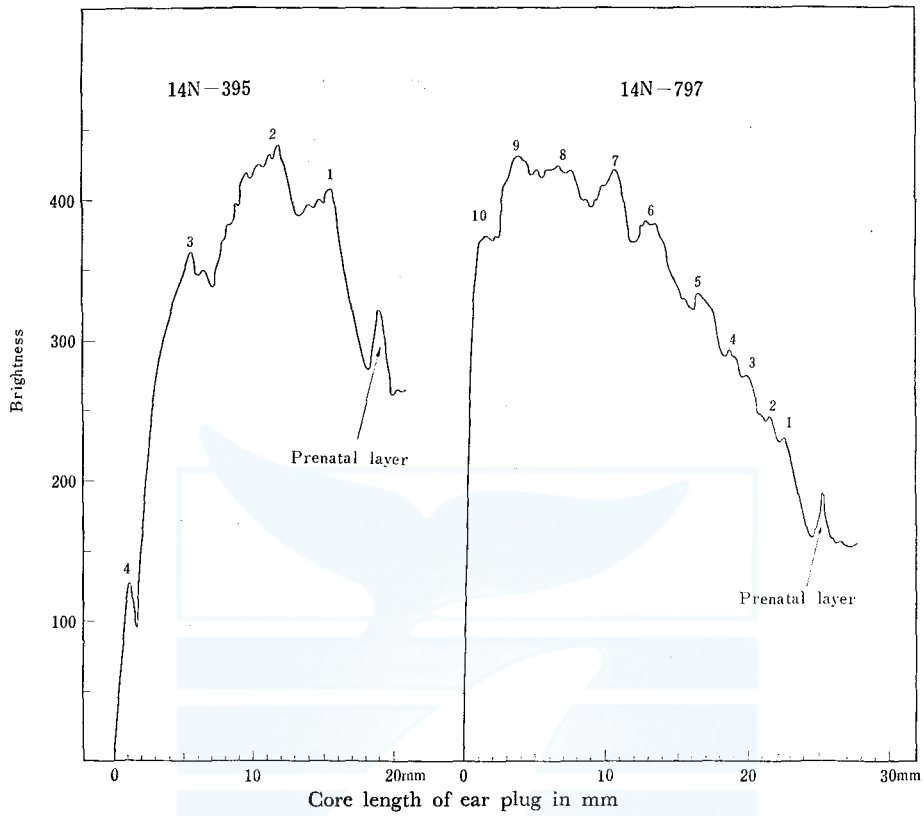


Fig. 5. Photometric records of brightness in the surfaces of bisected ear plugs which were obtained from the Antarctic two fin whales.

TABLE 2. BIOLOGICAL DATA OF FIN WHALES OF WHICH EAR PLUGS ARE USED FOR THE PHOTOMETRIC METHOD

| Serial No. | Body length in feet | Sex | Position of capture | Date of capture | Foetus length in feet | Ovary Corpora Number | Testis Weight in Kg | Laminae number in ear plug |
|---|---|---|---|---|---|---|---|---|
| 14N-395 | 61 | Female | 57-19S, 91-29E | 11. Jan. 1960 | None | 0 | — | 4 |
| 14N-797 | 68 | Male | 60-38S, 153-56E | 25. Jan. 1960 | — | — | 19.6, 19.1 | 10 |
| 14N-100 | 69 | Female | 57-43S, 96-06E | 1. Jan. 1960 | M 5-4 | Mature | — | 31 |
| 14N-1585 | 70 | Female | 62-53S, 150-19E | 7. Mar. 1960 | F 5-9 | 18 | — | 42 |

these fin whales are tabulated in Table 2 with special reference to the sexual state.

## DISCUSSION

In the laminae counting, it is of importance to observe the prenatal layer in the ear plug. In the course of bisection for the ear plug, the prenatal layer is often grinded down, however, without confirming the existence of prenatal layer the total laminae are never counted.

Photometric method records the whole alternation of bright and dark layer

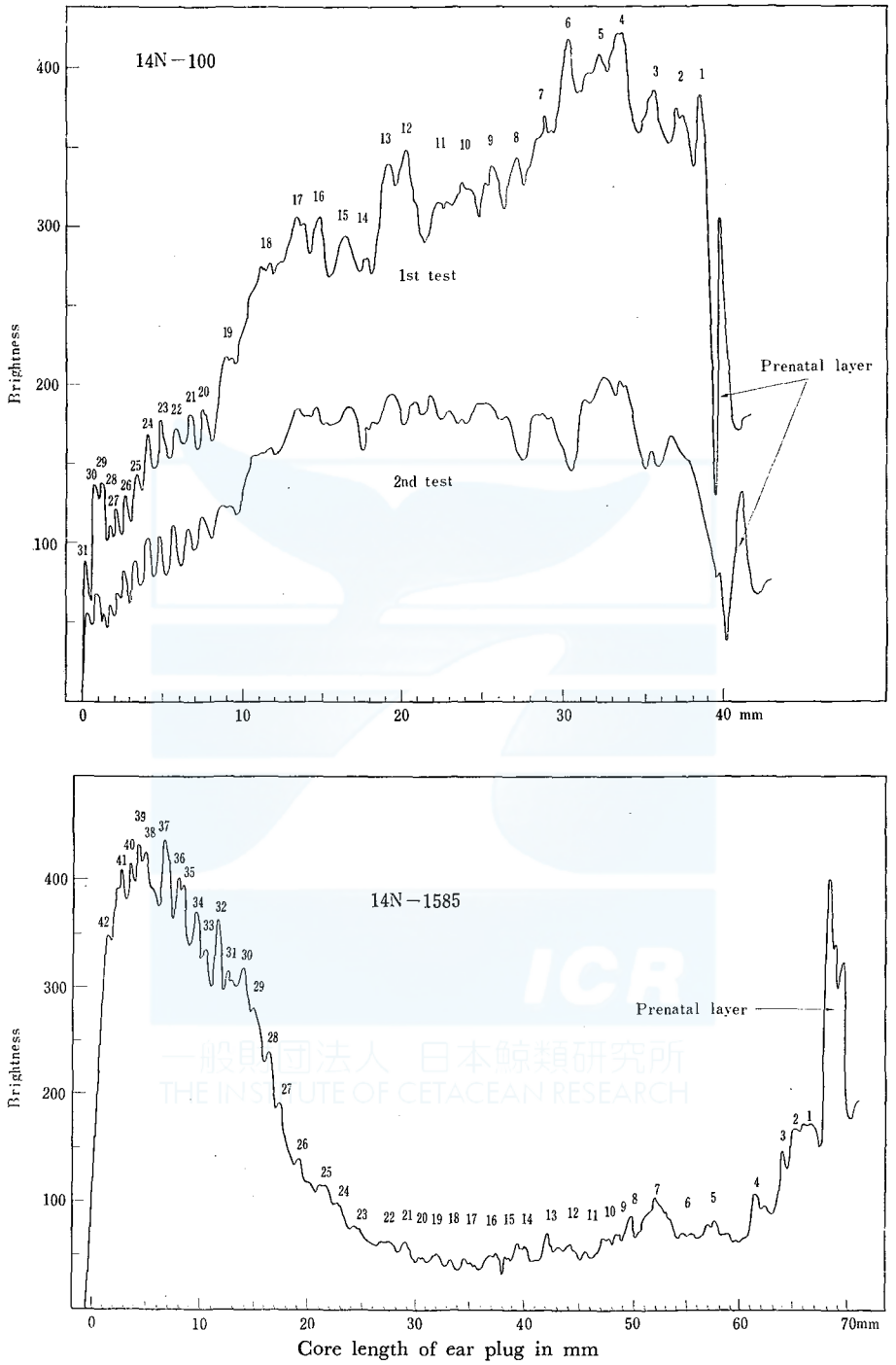


Fig. 6. Photometric records of brightness in the surfaces of bisected ear plugs which were obtained from the Antarctic two fin whales.

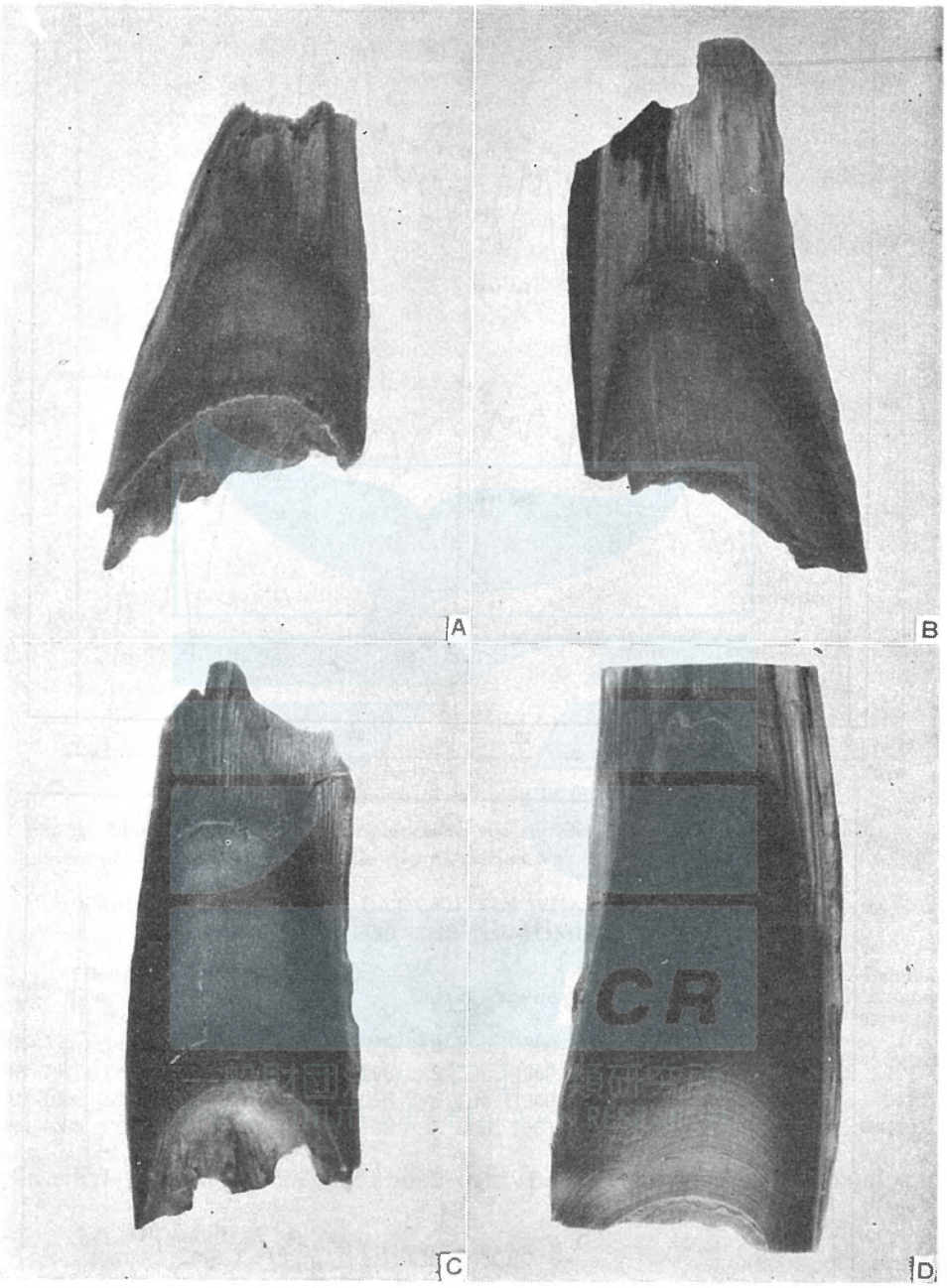


Fig. 7. Photographs of bisected ear plugs from the Antarctic four fin whales, tested for the photometric method. A ; Serial no. 14N-395, B ; 14N-797, C ; 14N-100, D ; 14N-1585.

in the ear plug. The dense layer which is supposed to be significant for determining age is possibly selected from the records of alternation. The distance between the higher and the neighbouring lower brightness is suggestive to find out the dense

layer among various fluctuations. In some cases, the dense layer is found in the periodicity in the fluctuation of brightness. On the other hand, whether or not the laminae are chear, it is noteworthy that the histological structure in the alternation of two kinds of layers is similar. The evidence that Walford's formula (1946) is not applied even for the growing layer of the ear plug indicating clear laminae suggests that there is no rigid periodicity in the thickness of each lamina. The alternation of each lamina, therefore, should be understood as the reflexion of the physiological rhythm of whale body, but further anatomical and physiological researches are needed for this study.

The general colour tone of the surface of bisected plug core is indicated in the curve of brightness in Fig. 5 & 6. In the general tendency except 14N–100 specimen, it has the highest brightness near the proximal end of plug core. In the distal half of 14N–1585 specimens in Fig. 6, the clear laminae are not recorded, as compared with the proximal half of that ear plug. This record shows the frequent presence of the intermediate laminae in the distal half of the specimens, in which the arbitrary count is probably made by individuals.

When the bisected ear plug on the glass plate (B) in Fig. 3 is slided along its longitudinal axis, it is presumed that the longitudinal bright band in the ear plug, (See Fig. 7) disturbs the measurements of brightness in the concentric laminae. To examine this disturbance, the second test is tried for 14N–100 specimen at the slight different position and also indicated in Fig. 5. The similar fluctuations of brightness are obtained between such two examinations. Photometric method is one of the most valuable to standadize the laminae counting in the ear plug.

## SUMMARY

Accurate counting of laminae in the ear plug is most necessary to determine the age of baleen whales. Interpretation of laminae favoured by individual scientist stands in the way of advancement in the whale biology. Independent counting of laminae by two persons in our institute through naked eyes or dissecting microscope observation indicates that the individual discrepancy in laminae counting is usually 10% to the total laminae number.

For standardizing the laminae counting, the exchange of ear plugs between England and Japan, besides between Canada and Japan were practised in 1959. The results that the laminae were counted independently and checked with each other indicates that the nearly same counting is made in the ear plug showing clear layer but that the discrepancy in counting occurs in the ear plug of vague layer. As two third of ear plugs from female fin whales is composed of vague laminae, the individual discrepancy in counting for them has a great effect on the age determination. To avoid the individual interpretation, the photometric method for counting was devised. By this method, the whole alternation of bright and dark layer in the ear plug can be recorded and the thickness of each layer can be measured. The objective interpretation begins with the record of permanent use.

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