## ON THE AGE CHARACTERISTICS AND ANATOMY OF THE TUSK OF *DUGONG DUGON*

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#### ABSTRACT

This study is based on 15 samples obtained at Celebes, Luzon, Mulgrave, and Thursday Islands. The dentinal growth layers in the larger tusk, possiblly the permanent 1st upper incisor, are the most useful for age determination unless the tusk is erupted and abraded. Cemental layers and the circular ridge on the surface of the tusk are usefull to a lesser degree. The formation cycle is suspected as annual from comparison with the growth of reared animals. The female seems to attain sexual maturity at about 10 years of age, and live for 45 years. Special pattern of distribution of enamel on the tusk is described.

## INTRODUCTION

The dugong, *Dugong dugon* (Müller 1776), is one of the sirenian species decreasing in numbers at the various places of its habitat, but a considerable number is still being exploited or killed incidentally in some regions (Bertram and Bertram 1968, Colin and Bertram 1970, Allen *et al.* 1976, Jones 1976). The basic biological information needed for conservation are still insufficient (Heinsohn 1972), and the establishment of a method of age determination is one of the most important studies.

Scheffer (1970) was the first to describe the growth layers in the dentine of the tusk and suggest a possible use for age determination. He distinguished two kinds of layers, namely the coarse and fine layers, and assigned them as representing lunar and daily cycles respectively. Recently Mitchell (1976 and 1978) indicated the possibility of annual or biannual formation of the former layer.

The present study aims to describe the anatomy of the tusk, to improve the method of observing the growth layers, and to have some considerations on the accumulation rate of the growth layers.

## MATERIALS AND METHOD

The present study is based on the tusks of 15 individuals. The specimen Toba-2, the youngest, was imported by the Toba Aquarium, and a tusk was presented for

•								
			TABLE 1. L	IST OF MAT	ERIALS			
1.	No. of sample	Toba-2	RMM002	RMM001	Kamiya-1	Kamiya-2	TK389-1	TK389-2
5.	Capture, date	—, May, '77	26, Aug., '75	25, July, '75	20, May, '78	20, May, '78	ĺ	1
з.	Capture, place	Luzon I.	Celebes I.	Celebes I.	Mulgrave I.	Mulgrave I.	1	1
4.	Death, date	—, May, '77	21, Oct., '75	20, Oct., '75	20, May, '78	20, May, '78		1
5.	Death, place	Luzon I.	Okinawa <sup>1)</sup>	Okinawa <sup>1)</sup>	Mulgrave I.	Mulgrave I.	Thursday I. <sup>2)</sup>	Thursday I. <sup>2)</sup>
6.	Sex	۴0	0+	0+	۴0	۴0	O†	۴0
7.	Body length (cm)	169.7	204.0	254.0	191.0	207.0		[
ω.	Body weight (kg)	80.1	To A Real Procession		I	]		
9.	Maturity	Immature	Immature	Adult	l	[	l	
10.	Straight length of tusk (mm)	37.2	94.9	172.5	88.8	119.5	210.3	164.8
11.	No. of growth ridges	ŝ	æ	19	11	I	l	]
12.	No. of dentinal layers <sup>3)</sup>	2 (1.5 cycles)	7	18	10	21 +	45	
13.	No. of regular layers <sup>3)</sup>	2	7	10	8	10 +	11	1
14.	Last layer	Thin	Thick	Thick	Thick	Thick	Thin	]
	(coarse)	stainable	stainable	stainable	unstainable	unstainable	unstainable	]
15.	Eruption	No	No	No	No	Yes	No	$\mathbf{Y}_{\mathbf{es}}$
1)	Arrived at Okinawa on 29, Sept.	., 1975. 2) Picke	d up on the beau	ch. 3) Coarse	postnatal dentina	al layers.		

this study. The two females, RMM001 and 002, were kept alive for about 20 days at the Okinawa Ocean Exposition, and the skeletons were presented to the University of the Ryukyus. Other two tusks with body length were provided by T. Kamiya who was presented the tusks by an islander of Mulgrave Island. The nine tusks without biological data (8 females and one male) were picked up by Nishiwaki on Thursday Island. Another female tusk was purchased by Nishiwaki on Celebes Island. The sex of animals without biological data was decided by the eruption and size of the tusks. The tusks which are too small to determine the sex are not included. Some of these samples, including the oldest, are shown in Table 1 and Fig. 1.



Fig. 1. The tusks of *dugong dugon* used in the present study. 1: Toba-2, 2: RMM002, 3: RMM001, 4: TK389-1, 5: TK389-2.

The body length of the animal was measured on a straight line connecting the anterior tip of the upper lip and the bottom of the tail notch. The maturity was studied by Dr T. Kamiya of the Faculty of Medical Science, University of Tokyo. Among the three specimens with biological data, only RMM001 is sexually mature with several corpora albicantia in the ovaries. RMM002 had neither corpus nor follicle in the ovaries.

Preparation of the tusk was made following the method used for the teeth of some odontoceti (Kasuya 1976, Kasuya 1977). One or two longitudinal sections of a thickness between 1.0 mm and 2.0 mm were taken from each tusk using a saw with a diamond blade. One side of the section was polished with grinder and whet stones until the center of the tusk was exposed, and the surface was glued

on a piece of clear plastic plate (1 mm in thickness) with quick adhesive synthetic resin (cyanoacrylate resin). Then the other side was polished in the same way and a ground section of a thickness between 50  $\mu$ m and 60  $\mu$ m was prepared. If the tooth section is glued on a plate which is so rigid that is not bent by the swell of the tusk during polishing in water, the preparation will separate from the plate.

Some of the thin ground sections were decalcified in 5% formic acid for a few hours, stained with Mayer's haematoxylin for 30 minutes, and mounted with permanent mounting medium. The small tusk or the small pieces of tusks were ground thinner (20 to 30  $\mu$ m) before decalcification and staining, and used for the detailed observations of the structure of the tusk. The half cut ground sections are also used for a limited purpose.

## RESULT

## Structure of the tusk

Since there is found a small rudimental tusk beneath the gum at the mesial side of the large tusk, the larger tusks listed in Table 1 will possibly correspond to the permanent first upper incisors (Peyer 1968). The size of the rudimental tusk, or the probable deciduous first upper incisor, is about 40 mm in length and 5.5 mm in diameter in the specimen RMM002, and slightly smaller in older RMM001. There was a feature indicating the resorption of the dental tissue at the distal end of the deciduous tusks of Kamiya-1, RMM001, and RMM002. This tusk is present on a young male Toba-2, but absent on the skulls with erupted large tusks (Pl. 4, Fig. 7). Possibly the deciduous tusk will be, in males, resorbed or lost soon after the eruption of the permanent tusk. Though the growth layers are present in this rudimental tusk (Pl. II, Fig. 4), they are not investigated in the present study because the layers near the tip are lost by erosion. All the following analyses are made on the larger tusks or the probable permanent first incisors.

The tip of the unerupted tusk has several points separated by grooves as if there are several cusps (Pls I and II). The enamel layer of a thickness varying from place to place from 130  $\mu$ m to 350  $\mu$ m covers the entire surface of the fetal dentine. However, it is rudimental, except for the ventro-mesial part, on the surface of the first and second postnatal dentinal growth layers of the long cycle mentioned later, and is completely absent on the tooth formed afterward. On the ventro-mesial surface of the tusk, the enamel layer is deposited on the entire length of tusk (Pl. IV, Figs 5 and 6). This restricted distribution of the enamel means that when the erupted tusk is abraded into a chisel shape (Pl. IV, Fig. 7 and Marsh *et al.* 1978 Fig. 3)the cutting edge is reinforced and sharpened with enamel.

The cementum covers almost the entire external surface of the older tusk, but is thin or partially lacking on the enamel surface at the distal tip of the tusk of young individuals.

## Dentinal growth layer

When the thin ground section is observed under transmitted light, there is found a clear translucent line near the tip of the tusk (Pl. II, Fig. 3 and Pl. III, Fig. 3). This will be the neonatal line. This neonatal line appears less clearly as an unstainable layer on the decalcified and stained section. The thickness of the fetal dentine ranges from 0.36 mm to 0.52 mm with a mean of 0.39 mm (n=4).

In the postnatal dentine, there are observed under transmitted light two kinds of growth layers, namely the coarse long cycle and fine short cycle layers. They correspond to the coarse and fine layers described by Scheffer (1970). Since the opaque layer reflects the light, it appears as a white layer under the reflected light, the translucent layer appears dark (Table 2). The opaque dentinal layer appears

# TABLE 2. COMPARISON OF THE NATURE OF THE COARSE DENTINAL GROWTH LAYERS AND THE MORPHOLOGY OF TUSK

Reflected light	White	Dark
Transmitted light	Opaque	Translucent
Haematoxylin	Stainable	Unstainable
Pulp cavity	Wide	Narrow

as a stainable layer on the decalcified and stained preparation (see the last layer in different preparations in Plates). The latter feature is same as that of *Tursiops truncatus* (Sergeant 1959) and of the fur seal (Kasuya unpublished), but opposite to the dentine of the spotted and striped dolphins (Kasuya 1976) or the nature of the skeletal and tooth layers of some other mammals (Klevezal and Kleinenberg 1967). Though the coarse growth layers are clearer on the decalcified and stained section, the thin ground section is usable for the purpose of age determination. Possibly even the half cut logitudinal ground preparations are also suitable (Mitchell 1976, 1978).

The pulp cavity opens wide when the stainable or opaque dentine is accumulated, and becomes narrow when the dentine of the opposite phase is deposited (Pl. IV, Fig. 4). Accordingly the circular ridge on the external surface of the tusk correspond to the phase when the accumulation of stainable dentine ends and that of the unstainable dentine starts, and the circular grooves to the opposite phase. Possibly the growth of the tusk will be slower in the period when the unstainable dentine is deposited. The number of the external ridges coincides, in young animals, with that of the stainable layers counted including the fetal dentine (Table 1).

The thickness of the coarse growth layer is between 2.6 mm and 3.6 mm in the the young dentine of, and between 0.5 mm and 1.0 mm in the dentine of the older age. The number of the fine growth layers in one accumulation cycle of the coarse layer ranges between 10 and 15 layers in most of the coarse layers (Pl. IV, Figs 3 and 4). However, when the extremely fine layers are counted, the number is in some part of the tusk can be 30 or more as indicated by Scheffer (1970). An improvement of the method of reading the fine layers is needed for detailed study



Fig. 2. The thickness of the coarse dentinal layers in the tusk of the dugong. The thickness is measured between the centers of the opaque layers. The thickness of fetal dentine is plotted on no. 0 layer, and the first and the last postnatal layers can be of incomplete thickness.

of their accumulation rate.

#### Cemental growth layer

There exist faint growth layers in the cementum, which can be observed on decalcified and stained sections. Though the cementum is not deposited on the tusk of Toba-2, there are observed about 16 stainable layers on RMM001 (Pl. II, Fig. 2). This suggest that the accumulation rate of the cemental growth layer is same with the coarse growth layer in dentine but the deposition of the cementum starts 1 or 2 years after birth (assuming annual deposition of the coarse dentinal layer).

## DISCUSSION

Heinsohn (1972) studied the growth of the dugong based on large number of samples killed incidentally by shark control nets. There was found no difference in body size between sexes. He considered that calves are born at Townsville in August and September at a length of about 1.1 m. They start grazing before 3 months of age, but accompany their mothers for more than one year. The mean body length at the attainment of sexual maturity was calculated as 2.53 m (male) and 2.58 m (female). By plotting the body lengths on the date of catch moved back and forth into different years, he constructed a hypothetical growth curve, which suggests that the calves attain the length of about 1.8 m in one year, 2.3 to 2.4 m in two years, and 2.5 to 2.7 m in 3 years. However, Mitchell (1976)

considered, based on the skull length at the attainment of sexual maturity obtained by Spain and Heinsohn (1974) and on the relationship between the age and skull length she obtained, that the sexual maturity is attained at the age of 10 growth layers, or presumably at 10 or 5 years, depending on the hypotheses of the annual or biannual formation of the layer. A direct estimation of the growth rate of the species is given by Jones (1976) based on the two individuals kept in captivity at Mandapan Camp. This estimate gives a growth rate much slower than that of Heinsohn (1972).

In the present study the annual growth rate of the species is calculated from the 4 known age individuals by assuming various formation cycle of the layer, the neonatal length of 1.1 m, and continuous growth from the smallest to the largest individual. Though the 169.7 cm male, Toba-2, has two opaque layers in the tusk, both of the 1st and 2nd (the last) postnatal layers are thin and is considered as incomplete. Since the sum of the thickness of the two layers is only 4.60 mm, or 154% of the mean of the thickst first or second layers of other 3 individuals, its age was assumed as 1.5 accumulation cycles.

The natural environmental changes which affect the deposition of dentine in the tusk could be daily, lunar and annual cycles (Scheffer 1970). However, as in the case of the sperm whale, there can exist a situation where two parallel minor layers composing one annual layer are counted separately and the accumulation



Fig. 3. Mean annual growth rate of the dugong. Closed circle and thick solid line indicate the growth in captivity (Jones 1976), open circle and dotted line the growth rates calculated from the growth layers (annual accumulation), and cross and thin solid line the growth rates estimated by Heinsohn (1972).

rate is considered as biannual (Klevezal and Kleinenberg 1967). As shown in Fig. 3, the growth rates calculated on the assumption of an annual formation of the coarse growth layer coincide with those of the captive individuals reported by Jones (1976). The assumption of the biannual or monthly formation of the coarse growth layer gives worse coincidence. This suggests that the formation of the coarse dentinal layer is annual. This result and seasonal change of the nature of the last incomplete layer (Table 1) suggest that the stainable dentine is deposited, in the equatorial and northern tropical waters, in the season from June to October.

It was indicated that there are usually observed 10 to 15 fine layers in one cycle of the coarse dentinal layer. Since the deposition of the latter is estimated as annual that of the former can be suspected as representing the lunar cycle or endogenous rhythm of about one month as *Berardius* (Kasuya 1977). There is observed, on RMM001 and 002, a sharp unstainable dentine near the pulp cavity, and 2 or 3 fine stainable layers are deposited inside of it (Pl. IV, Figs. 1 to 4). The extraordinary unstainable layer could be formed by physiological change of the animals soon after capture. The number of the fine layers formed after it coincides approximately with the length of life in captivity in months. This is another suggestion of the presence of about 30 days cycle in the deposition of the dentine.

Sexual maturity of the female is, in the present materials, attained by the age of 17 coarse layers but not attained at the age of 7 layers. In the latter individual, RMM002, all the seven dentinal layers show a simple regular feature. However, such regular layers are restricted, in the adut female RMM001, to earlier 10 layers, and the growth layers accumulated after this age show more irregular looking with conspicuous accessory layers between them (Pl. I). The number of the regular layers of 10 females and 1 male ranges from 8 to 12 with the mean at 9.7 layers. It is often observed on the maxillary tooth of the sperm whale that the dentinal growth layers are regular in the immature stage and it changes, in the dentine formed after the age at sexual maturity, into irregular feature with conspicuous accessory layers (for the structure of the tooth layer see Ohsumi *et al.* 1963). This is in the strong resemblance with the dentinal growth layers of the dugong tusk. Accordingly it is suspected that the sexual maturity of the female dugong will be attained at about 10 coarse growth layers, or presumably at about 10 years of age.

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## EXPLANATION OF PLATES

## PLATE I

Thin ground section of the tusk RMM001, photographed under transmitted light. Circle indicates the coarse postnatal opaque layer, and the open circle the 10th layer.

Fig. 1. A whole view of the section.

Fig. 2. Distal part of the section.

Fig. 3. Proximal part of the section.

#### PLATE II

- Fig. 1. Decalcified and stained section of the tusk RMM001, proximal portion of the same tusk shown in Pl. I. Circle indicates coarse stainable layer in dentine.
- Fig. 2. Decalcified and stained section of a part of cementum of RMM001, taken from the position corresponding to the first postnatal dentinal layer. Circle indicates the stainable growth layer, C cementum, and D dentine.
- Fig. 3. Thin ground section of the tusk Toba-2. Cross indicates enamel layer, open circle the neonatal layer in the dentine, closed circle postnatal coarse opaque layer in the dentine. This photograph is taken inserting the tooth section into the negative holder of an enlarger, and the translucent layer is shown dark.
- Fig. 4. Thin ground section of the deciduous first upper incisor of RMM002. The distal end (top) is resorbed. C indicates cementum, and D dentine. Photograph is taken in a same way as Fig. 3.

#### PLATE III

- Fig. 1. Thin ground section of the tusk TK389-1, the distal portion. Photographed under transmitted light.
- Fig. 2. The proximal portion of the same section.
- Fig. 3. Thin ground section of the tusk RMM002. Photographed under transmitted light. For Symbols see Pl. II, Fig. 3.

#### PLATE IV

- Fig. 1. Decalcified and stained section of the proximal portion of the tusk RMM 002, showing the fine dentinal growth layers. C indicates cementum, D dentine, and P pulp cavity. The open circle indicates the position of the strong unstainable layer possibly representing the dentine deposited soon after the capture.
- Fig. 2. Decalcified and stained section of the proximal portion of the tusk RMM-001. For the explanations see Fig. 1.
- Fig. 3. Lower magnification of the preparation shown in Fig. 1. C indicates cementum, P pulp cavity, and open circle the coarse stainable dentinal layer. About 12 or 13 fine layers are observed in one cycle of the coarse layer.
- Fig. 4. Lower magnification of the preparation shown in Fig. 2. E indicates the cast of decalcified enamel. For further explanations see Fig. 3.
- Fig. 5. Thin transverse ground section of the tusk TK389-2, taken from the cervix. Photographed under transmitted light. C indicates cementum, D dentine, and E enamel.
- Fig. 6. Thin transverse ground section of the tusk RMM001, taken from the midlength of the tusk. The distribution of the cementum (C), dentine (D), and enamel (E) is indicated.
- Fig. 7. Antero-dorsal view of the tusk of a probable male dugong, showing the pointed tip of the tusk.

PLATE I



PLATE II

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PLATE III





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