

# Utility of the Transition Phase in Earplugs of the North Pacific Common Minke Whale as an Indicator of Age at Sexual Maturity

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## Abstract

Whale age at sexual maturity is one of the most important biological parameters that can be used in stock management and population analysis. Earplugs have been widely used as an indicator of age among rorquals. It has also been accepted that the transition phase in the earplug can be used as an indicator of age at sexual maturity in fin whales, sei whales, and Antarctic minke whales. This study aimed to provide further insight into the utility of the transition phase as an indicator of age at sexual maturity in the North Pacific common minke whales, which has not yet been clarified. The relationship between sexual maturity and transition phase in earplugs was examined using 981 readable earplugs from common minke whales that were sampled at the JARPN and JARPN II scientific permit survey platform in the western North Pacific from 1994 to 2011. The transition phase was recognized in 53.2% of mature males and in 58.6% of mature females. Most whales in which the transition phase was recognized in the earplug were sexually mature. A significant correlation was found between the number of corpora and time after sexual maturation, as revealed by the transition phase, demonstrating that the transition phase is a valid indicator of age at sexual maturity in common minke whales. However, it was difficult to recognize the transition phase in whales that had recently attained sexual maturity because insufficient time had elapsed since its formation. To avoid potential bias, the use of earplugs as an indicator of age should be restricted to whales more than 12 years old.

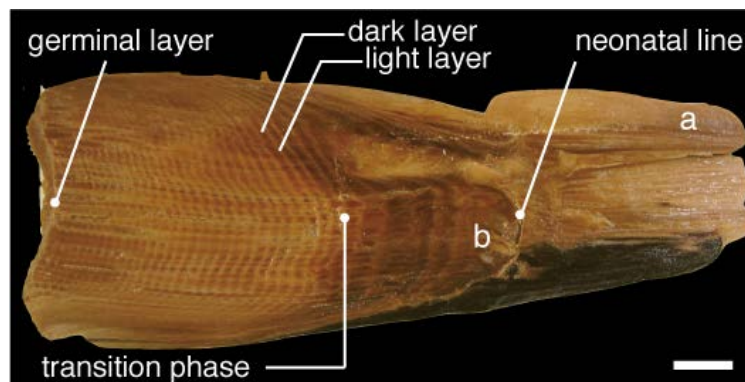
## Keywords

Common Minke Whale, *Balaenoptera acutorostrata*, Earplug, Age at Sexual Maturity, Transition Phase

## 1. Introduction

Information on whale age is of key importance for estimating life-history parameters that can be used for stock management. To obtain data on age, it is necessary to identify characteristics that represent the age of an individual. Earplugs have been widely used to determine age among rorqual species [1] [2]; characteristics of whale earplugs in relation to animal age have been discussed in detail by Purves [3]. The earplug accumulates in the external auditory meatus [4] and comprises a core and outer covering [3] [5] (Figure 1). The auditory meatus is once closed just beneath the epidermis, and the earplug is never shed throughout the whale's lifespan. The outer covering of the earplug is secreted by epithelial cells in the external auditory meatus, whereas the core, which comprises concentric light and dark laminae, is secreted by papillae on the surface of the glove finger [3]. The light and dark layers are formed during the feeding and breeding periods, respectively [6]. Histological observations of fin whale (*Balaenoptera physalus*) earplugs have indicated that fat content tends to be lower in the dark layer and higher in the light layer [6]. Many baleen whales migrate between breeding locations in low-latitude waters (winter) and feeding locations in high-latitude waters (summer); this migration occurs once every year and is reflected in earplug growth layers, with a pair of dark and light layers representing 1 year [1] [6] [7] [8] [9]. Other features of the earplug include the neonatal line, which is formed at birth [10] in the apical portion of the core (Figure 1).

However, age reading from the earplugs of the North Pacific common minke whale (*B. acutorostrata*) is generally believed to be difficult and impractical because of their softness and poor formation of growth layers. Considering the characteristics of earplugs, Maeda *et al.* [11] addressed the technical development of sampling called the gelatinized extraction method, which prevented damage to earplugs at the collection stage and was useful for improving age readability, especially in younger whales. From these studies, the readability of common minke whales was improved compared with that from commercial whaling because of careful collection and efforts in the technical development of earplug sampling. Furthermore, Maeda *et al.* [12] reported that earplugs of



**Figure 1.** Bisection of an earplug of a fin whale (age = 36 years, age at Tp = 7 years), a: outer covering; b: core with growth layers. Scale bar: 10 mm.

common minke whales in the western North Pacific were considered useful as a valid age tool.

More than a whale's age can be inferred from the earplug. Lockyer [8] examined fin whales from the southern hemisphere and observed a distinctly spaced growth layer that diminished suddenly, which was called the transition phase (Tp) (Figure 1). This Tp in the earplug, where widely spaced growth layers abruptly become much closer together, indicates the age at sexual maturity and is believed to be linked with changes in the skull's growth rate after sexual maturity [13] [14]. The presence of Tp has been reported for fin whales in the southern hemisphere [8] and Iceland [15] [16]; for sei whales (*B. borealis*) in the southern hemisphere [13], North Pacific [17], Iceland [18], and the Antarctic; and for minke whales (*B. bonaerensis*) [19] [20] [21] [22]. Tp is useful for estimating age at sexual maturity, which is one of the most important biological parameters, and it can be used to monitor changes in this parameter within whale populations [15] [16] [19] [21] [23] [24]. However, the appearance and utility of Tp have not been clarified in common minke whales. Our objective here was thus to investigate the usability of Tp as a characteristic for estimating age at sexual maturity in the North Pacific common minke whale.

## 2. Material and Methods

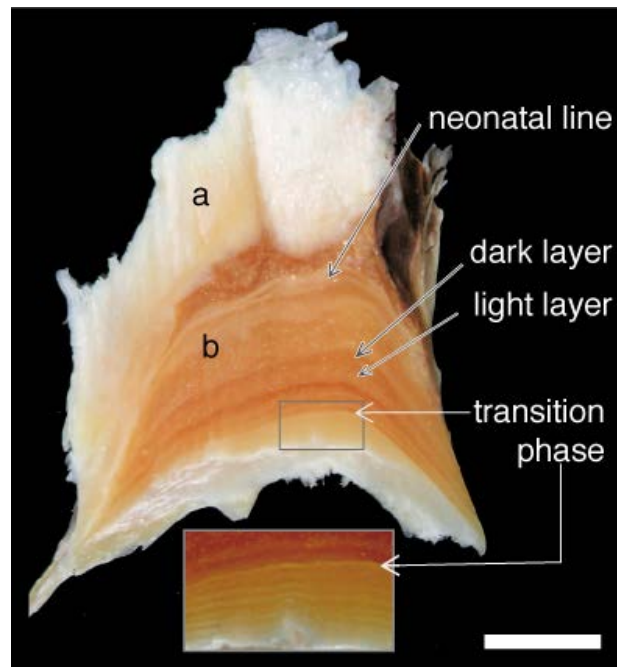
We used readable earplugs obtained from 775 male and 206 female North Pacific common minke whales collected during the Japanese Whale Research Program under Special Permit in the western North Pacific JARPN (1994-1999) and JARPN II surveys (2000-2011). Sampled earplugs were preserved in 10% neutral buffered formalin solution. In the laboratory, the earplugs were cut flat along the central axis of the core using a blade according to Lockyer [8]. Then, the earplugs were ground on a wet stone to expose the neonatal line and the growth layers and to smooth the surface. The cut surfaces of the earplugs were then examined underwater using a stereomicroscope (Olympus SZX10). Age was determined by reading the growth layers appearing on the bisected surface of the earplug, assuming annual deposition of growth layers (one pair of dark and light laminae accumulate per year). We recorded Tp when we clearly identified the part that exhibited a sudden decrease in its spacing (Figure 2).

We also used biological data on the following morphological characteristics: body length, sex, skull width (measured at the largest part of the zygomatic process of the temporal bone), weight of testis, and number of ovulations. A male with a testicle weighing more than 290 g [25] and a female with at least one corpus luteum or albicans in her ovaries [2] were regarded as sexually mature.

## 3. Results

### 3.1. Identification of Tp

A visible Tp was observed in 43.5% of male North Pacific common minke whales and in 26.2% of females (Table 1). The appearance of Tp was related to



**Figure 2.** Bisected surface of a earplug of a North Pacific common minke whale (male, body length = 7.07 m, age = 19 year, age at Tp = 6 year), a: outer covering; b: core with growth layers, and magnified view (below) of the part at the transition. Scale bar: 5 mm.

**Table 1.** Percentage of North Pacific common minke whales in which the transition phase (Tp) was observed, by sex.

	<i>n</i>	With tp	Without tp	Recognition of Tp (%)
Male	775	337	438	43.5
Female	206	54	152	26.2

**Table 2.** Percentage of sexually mature North Pacific common minke whales based on reproductive organ morphology, by sex.

	<i>n</i>	Immature	Mature	Sexual maturity rate (%)
Male	775	144	631	81.4
Female	206	118	87	42.2

maturity status; a higher proportion of females than males were immature (**Table 2**), and the majority of whales with a visible Tp were mature individuals (**Figure 3**). However, there were a few immature individuals with a visible Tp in both sexes (one male, three females). Examination of body length and age showed that the body lengths of immature whales exhibiting a Tp were larger than those of immature whales without a visible Tp (**Figure 4**), and the former tended to be older than the latter (**Figure 5**). The range of body length and age of immature whales with Tp overlapped those of mature ones. Tp was apparent in 53.2% of mature males and in 58.6% of mature females (**Table 3**). **Figure 6** shows the relationship between age and presence of Tp in mature whales. Tp was more frequently observed in older whales of both sexes. Approximately 25% of

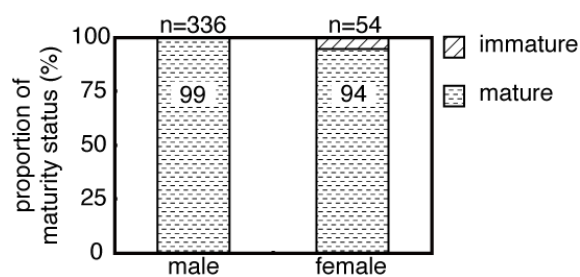
whales from 4 to 11 years old had a visible Tp, and >50% of whales more than 12 years old exhibited Tp. It was difficult to recognize Tp in mature whales that had recently attained sexual maturity.

### 3.2. Age in Relation to Number of Ovulations and Skull Width

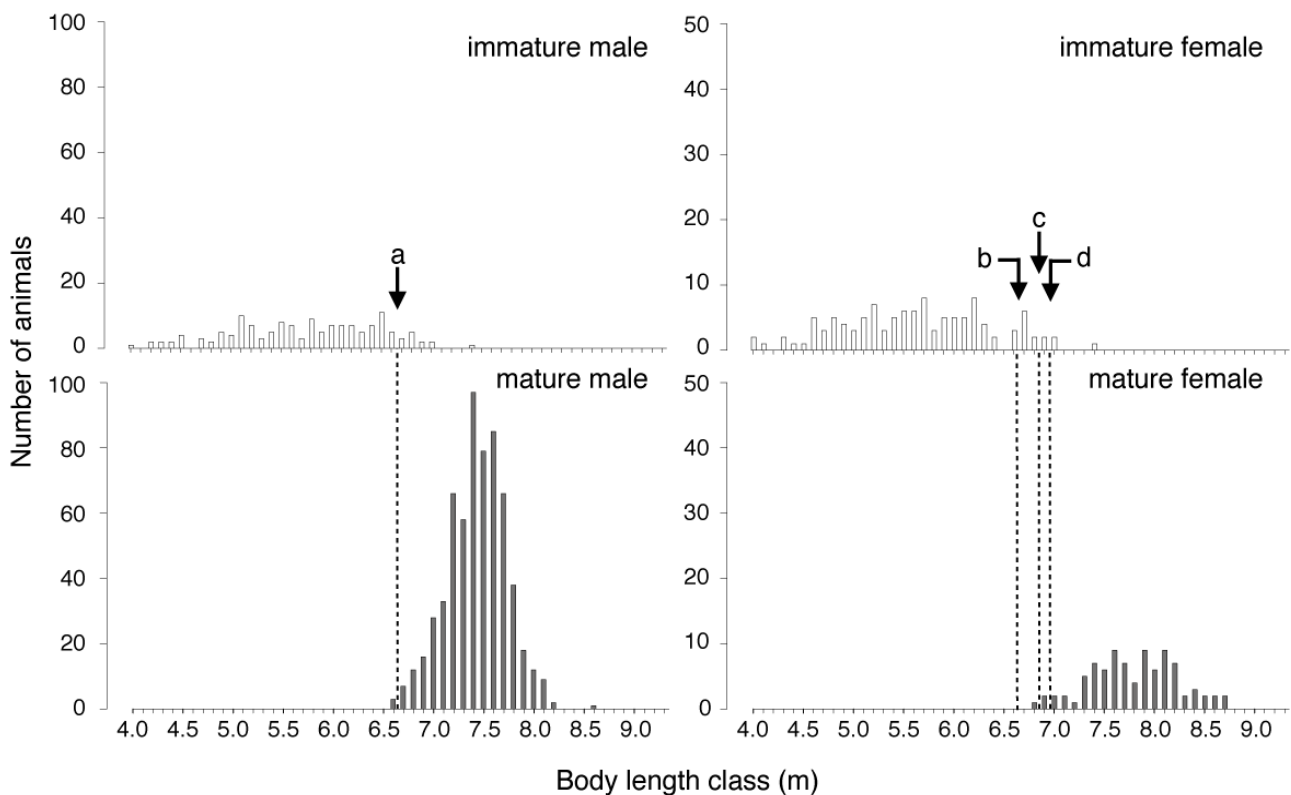
The number of ovulations clearly increased with age after sexual maturation based on Tp (Figure 7). The relationship between age after maturation ( $X$ ) and number of ovulations ( $Y$ ) was estimated by linear regression as follows:

$$Y = 0.80X + 0.96$$

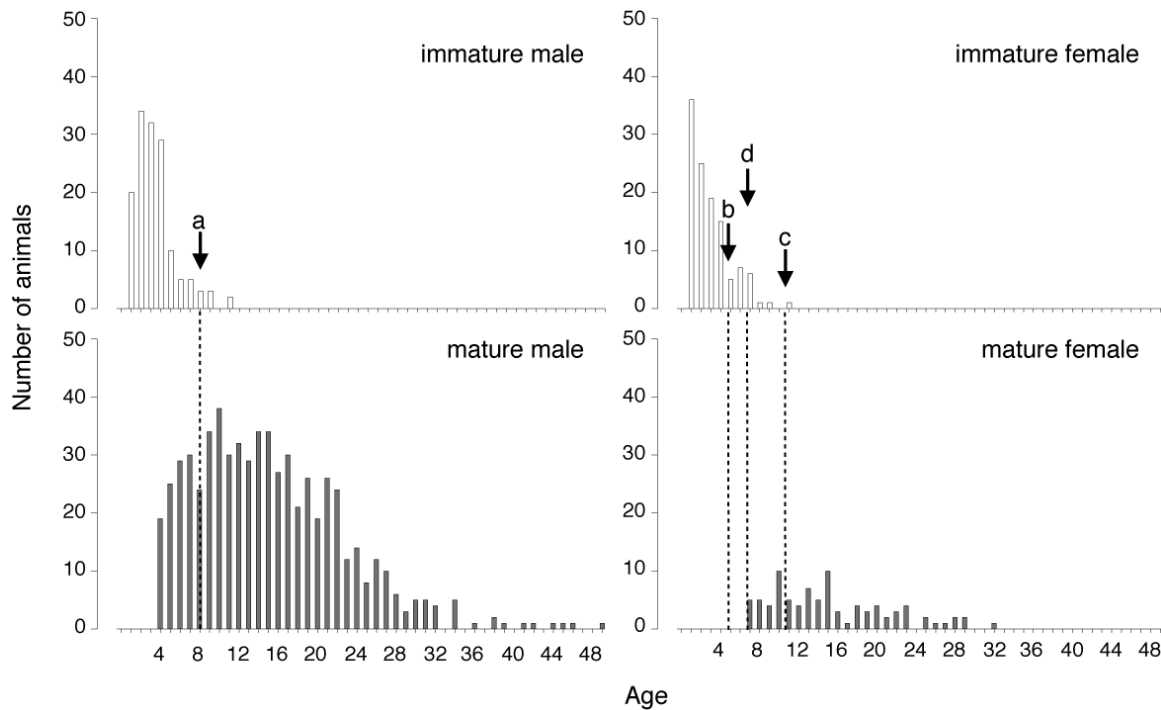
The  $X$  and  $Y$  intercepts were 0 and 1, respectively, lending further credence to the view that Tp is a valid indicator of age at sexual maturity.



**Figure 3.** Percentage of North Pacific common minke whales with the transition phase that were sexually mature, by sex.



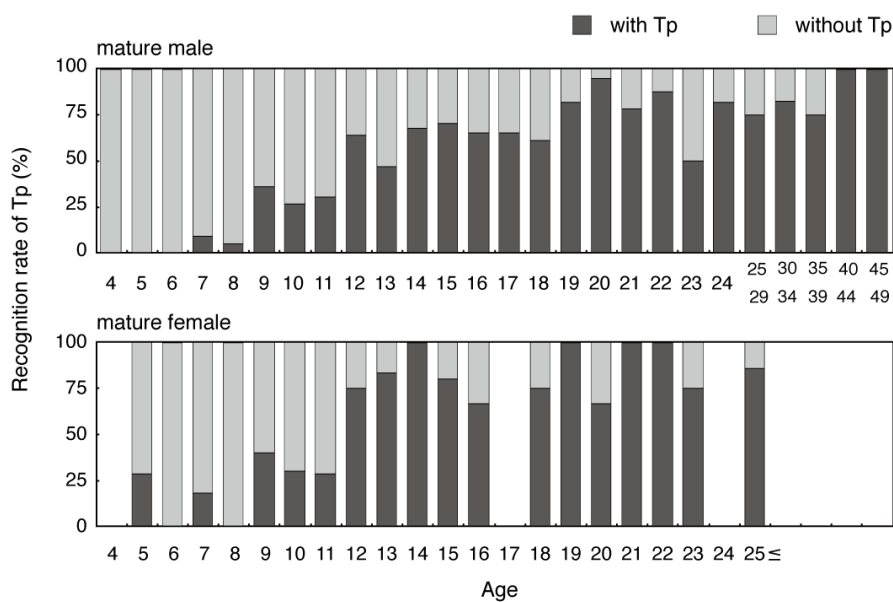
**Figure 4.** Body length of North Pacific common minke whales. Four whales had an observable transition phase (Tp); arrows and broken lines indicate these whales (a: 6.63 m; b: 6.61 m; c: 6.86 m; d: 6.94 m).



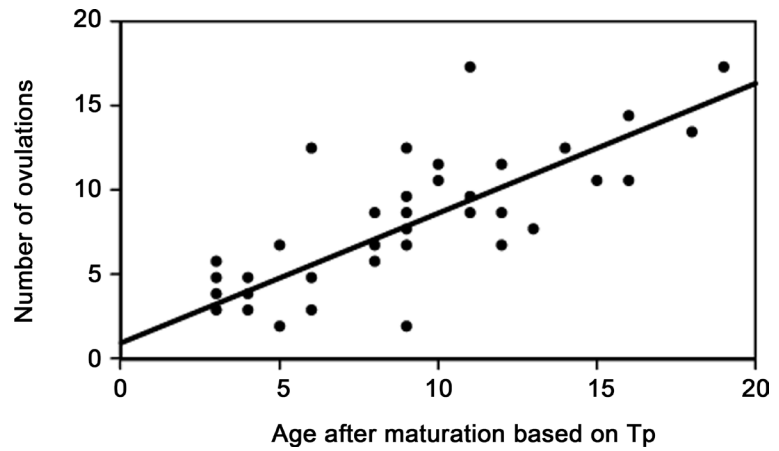
**Figure 5.** Age (years) of studied North Pacific common minke whales. Arrows and broken lines mark immature whales with an observable transition phase (a: 8; b: 5; c: 11; d: 7).

**Table 3.** Percentage of mature North Pacific common minke whales in which the transition phase (Tp) was observed, by sex.

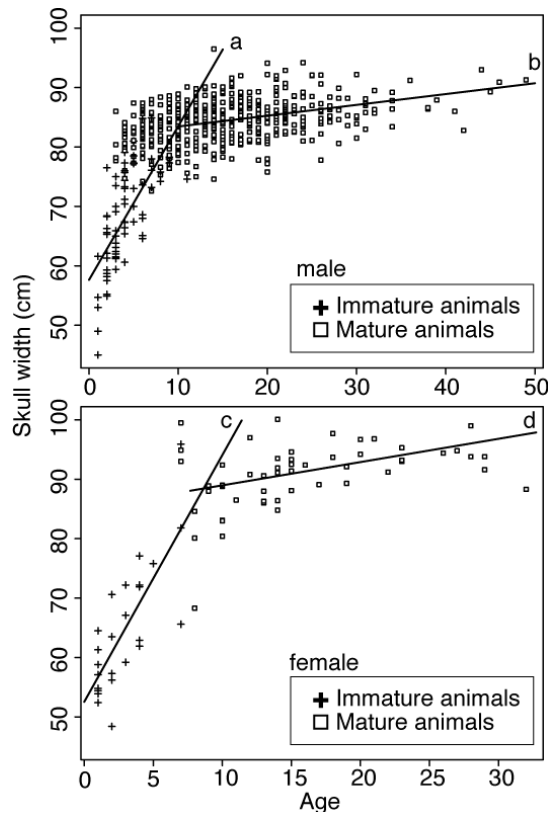
	<i>n</i>	With tp	Without tp	Recognition of Tp (%)
Male	631	336	295	53.2
Female	87	51	36	58.6



**Figure 6.** Relationship between age and visibility of transition phase (Tp) in mature male and female North Pacific common minke whales.



**Figure 7.** Number of ovulations according to age after sexual maturation based on the transition phase (Tp) ( $n = 42$ ).



**Figure 8.** Skull width in relation to age of immature North Pacific common minke whales and mature whales in both sexes (male: upper, female: lower). Regression line: a: immature male, b: mature male, c: immature female, d: mature female.

The regression relationships ( $Y = \text{skull width}$ ,  $X = \text{age}$ ) for each category were as follows (Figure 8):

Immature whales in males:  $Y = 2.86X + 56.72$  ( $R = 0.70$ )

Mature whales in males:  $Y = 0.18X + 81.62$  ( $R = 0.39$ )

Immature whales in females:  $Y = 4.15X + 52.54$  ( $R = 0.77$ )

Mature whales in females  $Y = 0.39X + 85.02$  ( $R = 0.43$ )

A significant difference was observed between the slope of the regression for immature and mature whales in both sexes (ANOVA test,  $P < 0.001$ ). Age at the crossing point of the regression line was 9.27 in males and 8.69 in females.

### 3.3. Mean Age at First Ovulation and Age at Tp

There were four females for which only one corpus luteum or albicans was observed in their ovaries; therefore, mean age at first ovulation was 8.75 years ( $SD = 1.5$ ,  $min = 7$ ,  $max = 10$ ,  $n = 4$ ). Mean age at Tp in males was 7.68 year ( $SD = 1.81$ ,  $min = 4$ ,  $max = 13$ ,  $n = 336$ ), whereas that in females was 7.82 years ( $SD = 1.58$ ,  $min = 4$ ,  $max = 11$ ,  $n = 51$ ).

## 4. Discussion

We found that the presence of Tp was related to the maturity status of *B. acuto-rostrata* and that most whales having a visible Tp were mature individuals, with a few exceptions. The presence of Tp in immature animals was previously reported in sei and Antarctic minke whales [13] [20] [21] [22]. Data on body length and age suggested the possibility that immature individuals exhibiting Tp had almost attained sexual maturity. We compared the presence of Tp in mature North Pacific common minke whales with that in other species (Table 4) [8] [13] [26]. Large proportions of fin and sei whale populations exhibited Tp. Dozens of males and females Antarctic minke whales, which are closely related to the common minke whale, also showed the presence of Tp. The reliability of identification of Tp in the common minke whale appears to be less than that in other whale species. We could not identify Tp in two cases of earplugs: those with growth layers with completely irregular or gradually diminished spacing. It is known that the general readability of common minke whale earplugs is low because of the unclear formation of growth layers [11] [27]. Regarding a earplug in which Tp could not be identified because of its gradually diminished spacing, Kato [21] reported a similar growth layer pattern in a earplug without Tp in an Antarctic minke whale. Furthermore, the core length of earplug is smaller in mature common minke whales than in other whales (Table 5); thus, it is considered that the interval of growth layers is originally narrow, which hampers the identification of Tp in this species. However, the present study revealed that Tp could be recognized in the North Pacific common minke whale.

**Table 4.** Percentage of North Pacific common minke whales in which the transition phase (Tp) was observed, among four species.

	Fin whale Southern hemisphere	Sei whale Southern hemisphere	Antarctic minke whale Southern hemisphere	Common minke whale North Pacific
Mature male	81 - 87	88	61.8	53.5
Mature female	80 - 83	86	60.0	58.6
	Lockyer, 1972	Lockyer, 1974	Zenitani, 2011	this study



**Table 5.** Mean core length (cm) in mature whales among four species. The core length was measured from the neonatal line to the germinal layer.

Species	Mean	<i>SD</i>	<i>n</i>	
Fin whale (Southern hemisphere)	6.01	2.02	48	(Maeda, in press)
Sei whale (North Pacific)	2.07	1.00	68	(Maeda, in press)
Antarctic minke whale	2.04	1.02	234	(Zenitani, 2011)
Minke whale (North Pacific)	1.18	0.56	114	(Maeda, in press)

It was found that the growth rate of skull width clearly changed in immature and mature whales in both sexes. Age at the inflection point of regression lines almost corresponds to the age at first ovulation. Mean age at Tp in both sexes was slightly lower than age at the crossing point of regression lines; however, in consideration of the range of age at which Tp appears, it is roughly appropriate. Therefore, formation of Tp in common minke whales was related to changes in the rate of skull growth, as was true for other species [13] [14]. Regarding the identification of Tp in mature whales, it was difficult to recognize Tp in younger whales that had recently attained sexual maturity. This difficulty may occur because too few layers were formed in the earplug to distinguish Tp clearly, resulting in a temporal lag in perception of Tp. Kato [21] and Ohsumi [22] reached similar conclusions, stating that Tp could be identified 4 - 5 years after the attainment of sexual maturity. To avoid potential bias from this temporal lag in perception, the use of Tp to monitor changes in age at sexual maturity should be restricted to whales that are more than 12 years old after birth, the age at which Tp becomes reliably observable at a rate of more than 50%.

## 5. Conclusion

In conclusion, we found that Tp could be identified in the North Pacific common minke whale. Most whales with an apparent Tp had attained sexual maturity. We also showed a significant correlation between the number of corpora and time after maturation in female minke whales. From these results, we concluded that Tp was a valid indicator for estimating age at sexual maturity in individual common minke whales. To date, earplugs of this species have been regarded as poor characteristics for evaluating age. However, we demonstrated that earplugs of common minke whales can reveal not only age, but also age at which sexual maturity was attained.

## Acknowledgements

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