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FETAL DEVELOPMENT IN TAIL FLUKES OF THE ANTARCTIC MINKE WHALE

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Abstract

Tail flukes of the Antarctic minke whale fetuses were studied to expand the available knowledge on fetal development, which is relatively understudied, as previous studies are qualitative in nature. Seven measurement points on the tail flukes of 122 fetuses (after conception 12.9 to 259.5 days) were recorded. We quantitatively defined the developmental stages based on the measurements. Tail flukes formed after an estimated fetal age of 57.8 days (9.4 cm) and retained almost the same shape as postnatal at 124.6 days (47.3 cm). The results also revealed that the growth rates differed at each measurement point and each developmental stage.

Key words: Antarctic minke whale, Balaenopteridae, embryology, fetus, fluke, ontogeny.

Several descriptive studies on the morphological changes in tail flukes (hereafter, flukes) during intrauterine development were conducted on mysticetes (Ogawa, 1953; Ohsumi, 1960; Roston *et al.*, 2013) and odontocetes (Ogawa, 1953; Nishiwaki *et al.*, 1963; Amano and Miyazaki, 1993; Štěrba *et al.*, 2000; Thewissen, 2018). However, quantitative studies—measurements and statistical analysis—on the developmental changes of flukes which is one of the most attractive and drastic changes are expected during the intrauterine period, are limited as obtaining a sufficient number of fetal samples for such an analysis is difficult.

This paper provides such quantitative information as above on the development of fetal flukes in cetaceans using the Antarctic minke whales (*Balaenoptera bonaerensis*) and measurements obtained from the New Scientific Whale Research Program in the Antarctic Ocean (NEWREP-A) operation in 2017/18 under the special permit issued by Government of Japan in accordance with the International Convention for Regulation of Whaling (ICRW) (Bando *et al.*, 2018). Samples were collected from the Pacific sector of the Antarctic area bounded by 170°W, 120°W, 60°S, and sea ice edge (or coastline), which corresponds to Area VI as defined by the International Whaling Commission (IWC).

NEWREP-A commenced in the austral summer of 2017/18 and the seasons after with the first author, Kim Y. as well as Bando T. and Nishimura F., on board the research base R/V *Nisshin Maru* in that year (8,030 GT). Through the sampling exercises, 122 fetuses from 122 pregnant females were examined by Kim alongside Bando and Nishimura.

Measurements, including the body length and fluke morphometrics with five measurement points, namely, *total span* (*TS*), *maximum span* (*MS*), *depth of flukes* (*DF*), *anterior length* (*AL*), and *posterior length* (*PL*) as defined in Fig. 1, derived from Ohsumi (1960) for fin whales (*B. physalus*) and Amano and Miyazaki (1993) for Dall's porpoises (*Phocoenoides dalli*), were made during the field survey. *TS* and *MS* were treated as the same for fetuses with less than 47.3 cm in body length because the fetal flukes have indistinguishable tips during the initial developmental stages as their skin lobes are rounded. The body length was measured parallel to the body axis, from the tip of the upper jaw to the notch of the flukes to the nearest 0.1 cm. Body lengths of small fetuses with bent heads and tails were mea-

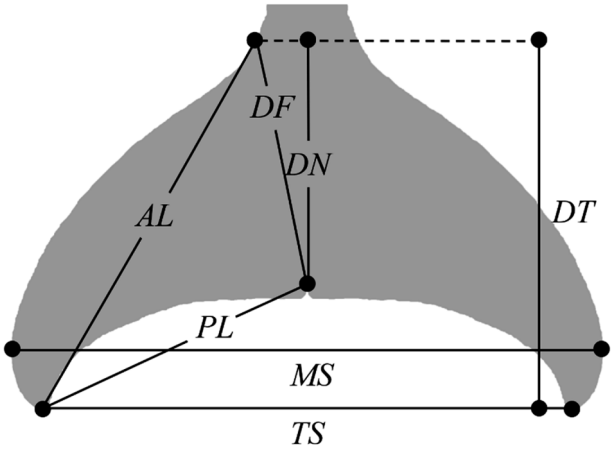

Fig. 1. Measurement points of flukes from the Antarctic minke whale fetuses: *total span* (*TS*), from left tip to right tip; *maximum span* (*MS*), from left ridge to right ridge; *depth of flukes* (*DF*), from anterior insertion, the most anterior point of skin lobes which was visually determined by the first author, to notch; *anterior length* (*AL*), from anterior insertion to tip; *posterior length* (*PL*), from notch to tip; *distance to notch* (*DN*), from the left anterior insertion and right anterior insertion lines to the notch; and *distance to total span* (*DT*), from left anterior insertion and right anterior insertion lines to *TS*.

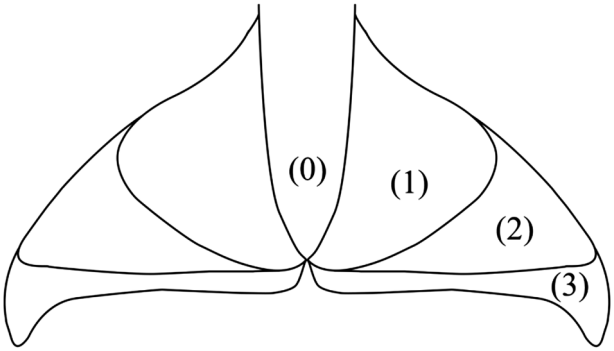

Fig. 2. Developmental stages of the flukes as the Antarctic minke whale fetuses develop: Stage (0), *tail shape*; Stage (1), *diamond shape*; Stage (2), *triangle shape*; Stage (3), *boomerang shape*.

sured along the curved body axis. These measurement points were measured to three decimal places along a straight line from point to point using 30 or 40 cm vernier calipers. Two additional measurement points, namely, *distance to notch* (*DN*) and *distance to total span* (*DT*), were measured. Because the flukes' tips are curved ventrally and both points could not be measured accurately in the field, both measurement points were measured to three decimal places using digital photographs of flukes in the laboratory using the ImageJ software developed by Schneider *et al.* (2012). Fetal age (number of days after conception) was estimated from the body length of the Antarctic minke whale using methods devised by Kato and Miyashita (1991) and seconded by Bando (2017) (see Appendix for the details).

The developmental stages of the fetal flukes were categorized based on Ohsumi (1960) and were conventionally defined as follows (Fig. 2): Stage (0), *tail shape*, with a completely tail-like shape with no skin lobes on both sides; Stage (1), *diamond shape*, when skin lobes form on both sides and flukes have a diamond-like shape (*DT* is less than or equal to half of *DN*); Stage (2), *triangle shape*, when flukes become triangular in shape (*DT* is more than half of *DN*); and Stage (3), *boomerang shape*, when both of the flukes' tips are pointed and the flukes take a boomerang-like shape (*DT* is more than *DN*). The typical shapes of the Antarctic minke whale flukes during their developmental stages [(0)–(3)] are exemplified as in Fig. 3. We also incorporated the allometric equation (Huxley, 1950). Growth pattern analyses were evaluated using *t*-test. Mean values of the ratios of each measurement point to

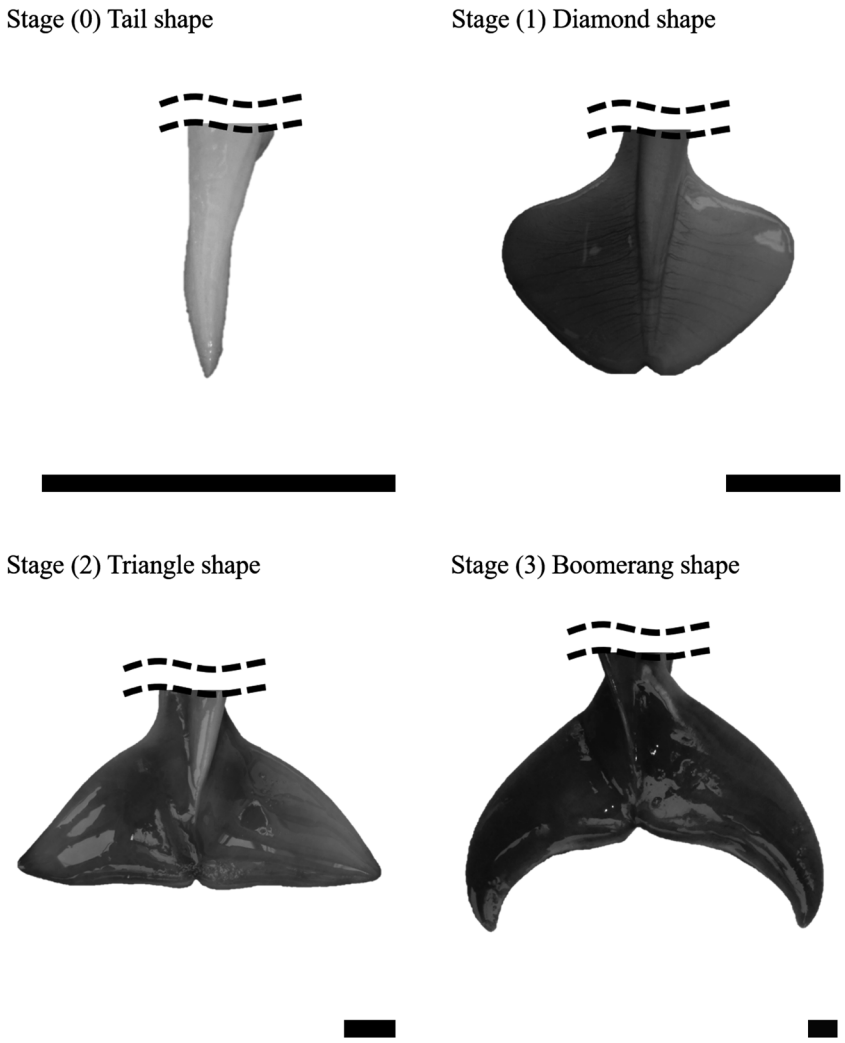

Fig. 3. Photographs of the flukes of the Antarctic minke whale fetuses at each developmental stage. Stage (0), *tail shape* (4.3 cm, 26.4 days, sex unknown); Stage (1), *diamond shape* (17.1 cm, 94.4 days, male); Stage (2), *triangle shape* (34.5 cm, 112.2 days, female); and Stage (3), *boomerang shape* (53.1 cm, 130.1 days, female). Black scale bar indicates 1.0 cm, respectively.

the body length at each developmental stage were evaluated using Mann–Whitney U test. Significant levels of both statistical tests were set at 0.05.

The *MS*/*DF* and *AL*/*PL* ratios indicate the flukes' aspect and diagonal ratios, respectively. These ratios increased as fetuses develop with the following results (Fig. 4): Stage (1), the *MS*/*DF* and *AL*/*PL* ratios increased from 1.0 to 1.4 and from 0.4 to 0.9, respectively; Stage (2), *MS*/*DF* increased from 1.7 to 2.9, and *AL*/*PL* increased from 0.8 to 1.3; and Stage (3), *MS*/*DF* leveled and increased from 2.2 to 3.2, and *AL*/*PL* increased from 1.2 to 1.7. At the beginning of Stage (1), *MS* and *DF* were almost the same. However, *MS* became larger than *DF* as the fetal flukes developed and elongated along the transverse axis direction. Meanwhile, at this stage, *AL* was less than half of *PL*. This changed late in Stage (2) when *AL* became larger than *PL*. This means that the anterior edge of flukes grows faster than their posterior part.

The result of this study showed that fetal age which corresponded to the developmental stages were 12.9–41.8 days in Stage (0), 57.8–96.0 days in Stage (1), 99.0–118.0 days in Stage (2), and 124.6–259.5 days in Stage (3) (Table 1). The flukes started developing skin lobes 57.8 days after conception.

The growth patterns of each measurement point to body length are shown in Table 2. In Stage (1), four measurement points showed positive growth patterns relative to the body length. These were *TS*,

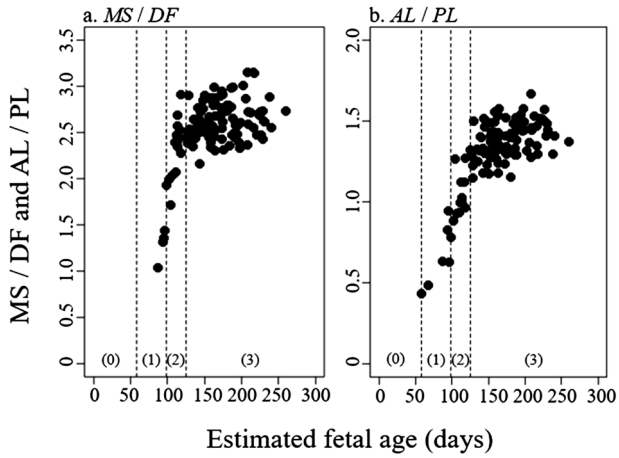

Fig. 4. The relationship between two ratios of fetal flukes (y axis) and estimated fetal age (x axis) at developmental stages of the Antarctic minke whale. The aspect ratio of the flukes, *MS*/*DF* (a), and the diagonal ratio of the outer edges, *AL*/*PL* (b). The dotted lines indicate the starting point of each developmental stage of fetal flukes, from the left to the right: (0), (1), (2), and (3). Stage (0), *tail shape*; Stage (1), *diamond shape*; Stage (2), *triangle shape*; and Stage (3), *boomerang shape*.

Table 1. The body length and estimated fetal age of the Antarctic minke whale at each developmental stage of fetal flukes.

Developmental stage	*N*	Body length (cm)		Estimated fetal age (days)	
		Range	Mean (SD)	Range	Mean (SD)
(0)	3	2.1–6.8	4.4 (2.4)	12.9–41.8	27.0 (14.5)
(1)	9	9.4–18.6	14.5 (3.0)	57.8–96.0	84.7 (13.3)
(2)	14	21.5–40.5	33.1 (5.8)	99.0–118.0	110.8 (5.8)
(3)	96	47.3–203.0	105.1 (35.3)	124.6–259.5	176.6 (30.8)

Stage (0), *tail shape*; Stage (1), *diamond shape*; Stage (2), *triangle shape*; Stage (3), *boomerang shape*.

MS, *AL*, and *PL* ($P<0.01$). Isometric growth patterns were found in the other three points—*DF*, *DN*, and *DT* ($P>0.05$). Positive growth patterns were observed in *TS*, *MS*, *AL*, *PL*, and *DT* in Stage (2) ($P<0.05$), while *DF* and *DN* showed isometric growth patterns to body length ($P>0.05$). In Stage (3), *AL*, *PL*, and *DT* showed positive growth patterns ($P<0.01$), and isometric growth patterns were produced at *MS*, *DF*, and *DN* ($P>0.05$). Negative growth pattern to body length were observed at *TS* ($P<0.05$). In all developmental stages, positive patterns were observed at *AL* and *PL*, while *DF* and *DN* showed isometric patterns. As the flukes developed from Stage (2) to (3), the growth patterns of *TS* and *MS* changed. In Stage (2), the growth patterns to body length of both measurement points were positive, but in Stage (3), those two measurement points were different. In Stage (3), a negative growth pattern was observed at *TS*, and an isometric growth pattern was observed at *MS*. The growth pattern of the *DT* changed from isometric in Stage (1) to positive in Stage (2).

The changes in ratios of each measurement point of the fetal flukes relative to their body length at each developmental stage are as follows (Table 3, Fig. 5). Each ratio was compared using the average values, and the standard deviations were shown with the average values. *TS* and *MS* ratios were 11.1±4.0% in Stage (1) and increased to 22.4±2.6% in Stage (2) ($P<0.01$). In Stage (3), while *TS* is constant at 22.0±2.5% ($P>0.05$), *MS* increased to 24.3±1.9% ($P<0.01$). The *DF* ratio ranged from 9.7±0.6% to 10.8±1.0% for Stage (1) and (2) ($P>0.05$). In Stage (3), DF decreased to 9.2±0.5% ($P<0.01$). The *AL* and *PL* ratios increased with the developmental stages. *AL* increased from 5.8±2.1% to 11.7±1.9% when the developmental stage became from Stage (1) to Stage (2) ($P<0.01$).

Table 2. The growth patterns of fetal flukes at developmental stages of the Antarctic minke whale.

Developmental stage	Measurement point	*N*	α[1]	ln β[1]	Growth pattern[2]	r^2	*P*-value
(1)	*TS*	7	2.605	0.001	Positive	0.980	**
	MS	7	2.605	0.001	Positive	0.980	**
	DF	4	0.883	0.149	Isometric	0.319	n.s.
	AL	6	2.341	0.002	Positive	0.934	**
	PL	6	1.423	0.028	Positive	0.995	**
	DN	7	0.787	0.178	Isometric	0.902	n.s.
	DT	7	0.937	0.049	Isometric	0.778	n.s.
(2)	*TS*	14	1.544	0.033	Positive	0.956	**
	MS	14	1.544	0.033	Positive	0.956	**
	DF	14	0.940	0.120	Isometric	0.889	n.s.
	AL	14	1.737	0.009	Positive	0.921	**
	PL	14	1.366	0.032	Positive	0.904	*
	DN	13	1.068	0.079	Isometric	0.944	n.s.
	DT	14	1.970	0.003	Positive	0.884	**
(3)	*TS*	79	0.921	0.313	Negative	0.882	*
	MS	92	1.034	0.208	Isometric	0.957	n.s.
	DF	96	0.982	0.100	Isometric	0.973	n.s.
	AL	92	1.176	0.076	Positive	0.974	**
	PL	91	1.095	0.080	Positive	0.949	**
	DN	65	0.990	0.102	Isometric	0.931	n.s.
	DT	65	1.316	0.036	Positive	0.944	**

[1] α and ln β are the allometric coefficient and constant, respectively. The asterisk indicates the significance levels of each allometric coefficient from a value of 1 (*t*-test: *, $P<0.05$; **, $P<0.01$, n.s., no significant difference). Stage (0), *tail shape*; Stage (1), *diamond shape*; Stage (2), *triangle shape*; and Stage (3), *boomerang shape*.

[2] The allometric equation $y=\beta x^{\alpha}$ was used to determine the growth curves and patterns in each developmental stage, where x is the body length (cm), y is the length of the measurement point (cm), α is the allometric coefficient, and β is the allometric constant. The growth patterns of each measurement point were classified into three based on the significance (*t*-test) of the allometric coefficients applied: positive when the allometric coefficient was significantly larger than 1, isometric when the allometric coefficient was not significantly different from 1, and negative when the allometric coefficient was significantly smaller than 1. All statistical analyses were made using R version 3.5.1 (R Core Team, 2018) running on RStudio version 1.1.456 (RStudio Team, 2016). Since no significant differences (ANCOVA, $P>0.05$) were observed in the growth patterns of all measurement points (results are not shown here) between males and females, they were not separated in our analysis.

In Stage (3), *AL* showed 17.2±1.5% ($P<0.01$). On the other hand, *PL* increased from 8.7±1.0% in Stage (1) to 11.5±1.2% in Stage (2) and to 12.4±1.2% in Stage (3) ($P<0.05$). The *DN* ratio ranged from 9.8±0.8% to 10.2±0.8% for all the developmental stages ($P>0.05$). The *DT* ratio increased through all the stages and ranged 4.2±0.5% in Stage (1), 7.7±1.6% in Stage (2), and 15.3±2.1% in Stage (3) ($P<0.01$). The ratios of each measurement point to body length varied depending on the developmental stage. Exceptionally, *DN* stayed within a certain range regardless of the developmental stages. As the flukes developed, the measurement points with increasing ratios were *MS*, *AL*, *PL*, and *DT*. The ratios of the other measurement points (*TS* and *DF*) changed with developmental stages.

Several authors have examined the fetal development of flukes, including Štěrba *et al.* (2000) for common dolphins (*Delphinus delphis*), pantropical spotted dolphins (*Stenella attenuata*), spinner dolphins (*S. longirostris*), and harbor porpoises (*Phocoena phocoena*), Nishiwaki *et al.* (1963) for sperm whales (*Physeter macrocephalus*), and Ohsumi (1960) for fin whales. This paper is the first attempt to incorporate a quantitative measure of fetal growth into drawing the morphological development of fetal flukes of the Antarctic minke whale.

Table 3. The ratios of fetal flukes to body length of the Antarctic minke whale at the developmental stages.

Developmental stage	Measurement point	*N*	The ratios of fetal flukes to body length (%)		*P*-value	
			Range	Mean (SD)	(2)	(3)
(1)	*TS*	7	5.3–15.5	11.1 (4.0)	**	
	MS	7	5.3–15.5	11.1 (4.0)	**	
	DF	4	9.5–11.8	10.8 (1.0)	n.s.	
	AL	6	3.1–8.6	5.8 (2.1)	**	
	PL	6	7.1–9.7	8.7 (1.0)	**	
	DN	7	9.2–11.7	10.2 (0.8)	n.s.	
	DT	7	3.5–4.7	4.2 (0.5)	**	
(2)	*TS*	14	17.9–26.6	22.4 (2.6)		n.s.
	MS	14	17.9–26.6	22.4 (2.6)		**
	DF	14	8.8–10.6	9.7 (0.6)		**
	AL	14	8.3–15.3	11.7 (1.9)		**
	PL	14	9.4–13.6	11.5 (1.2)		*
	DN	13	9.3–11.2	10.1 (0.5)		n.s.
	DT	14	5.1–10.3	7.7 (1.6)		**
(3)	*TS*	79	16.7–28.0	22.0 (2.5)		
	MS	92	20.4–30.1	24.3 (1.9)		
	DF	96	8.1–10.9	9.2 (0.5)		
	AL	92	13.3–20.7	17.2 (1.5)		
	PL	91	9.9–15.8	12.4 (1.2)		
	DN	65	7.5–12.0	9.8 (0.8)		
	DT	65	10.5–20.2	15.3 (2.1)		

The asterisk indicates the significance levels of difference to the average value of each ratio between the consecutive developmental stages (Mann–Whitney U test: *, $P<0.05$; **, $P<0.01$, n.s., no significant difference). Stage (0), *tail shape*; Stage (1), *diamond shape*; Stage (2), *triangle shape*; and Stage (3), *boomerang shape*.

Ohsumi (1960) showed the growth patterns of fin whale fetal flukes (*DF* and *PL* in our study) to body length. Their *DF* changed from a negative growth pattern to a positive one as the fetuses developed. This positive growth pattern was observed at the *PL*. Nishiwaki *et al.* (1963) observed the ratios to body length of three measurement points of sperm whale's fetal flukes, which correspond to the *TS*, *DF*, and *PL* in our study. They found that the *TS* and *PL*/body length ratios increased, while *DF* decreased. The growth patterns and ratios of the fetal flukes of the Antarctic minke whale indicated a rapid growth along the transverse axis direction during the fetal development. The results suggest that the growth rates of cetacean fetal flukes at these measurement points vary from species to species.

Štěrba *et al.* (2000) compared the fetal age development of flukes of four dolphin species: the common dolphin, the pantropical spotted dolphin, the spinner dolphin, and the harbor porpoise. They found that the flukes from each species started developing 32–42 days after conception, while we found that the Antarctic minke whale flukes began to develop at fetal age of 57.8 days. These results are evidence that the development of the Antarctic minke whale flukes begins about 15 days later than that of the dolphins, which would be key for comparing relative ratio to total gestation period and the methodological differences between previous studies and this study, which we plan to do in future investigations.

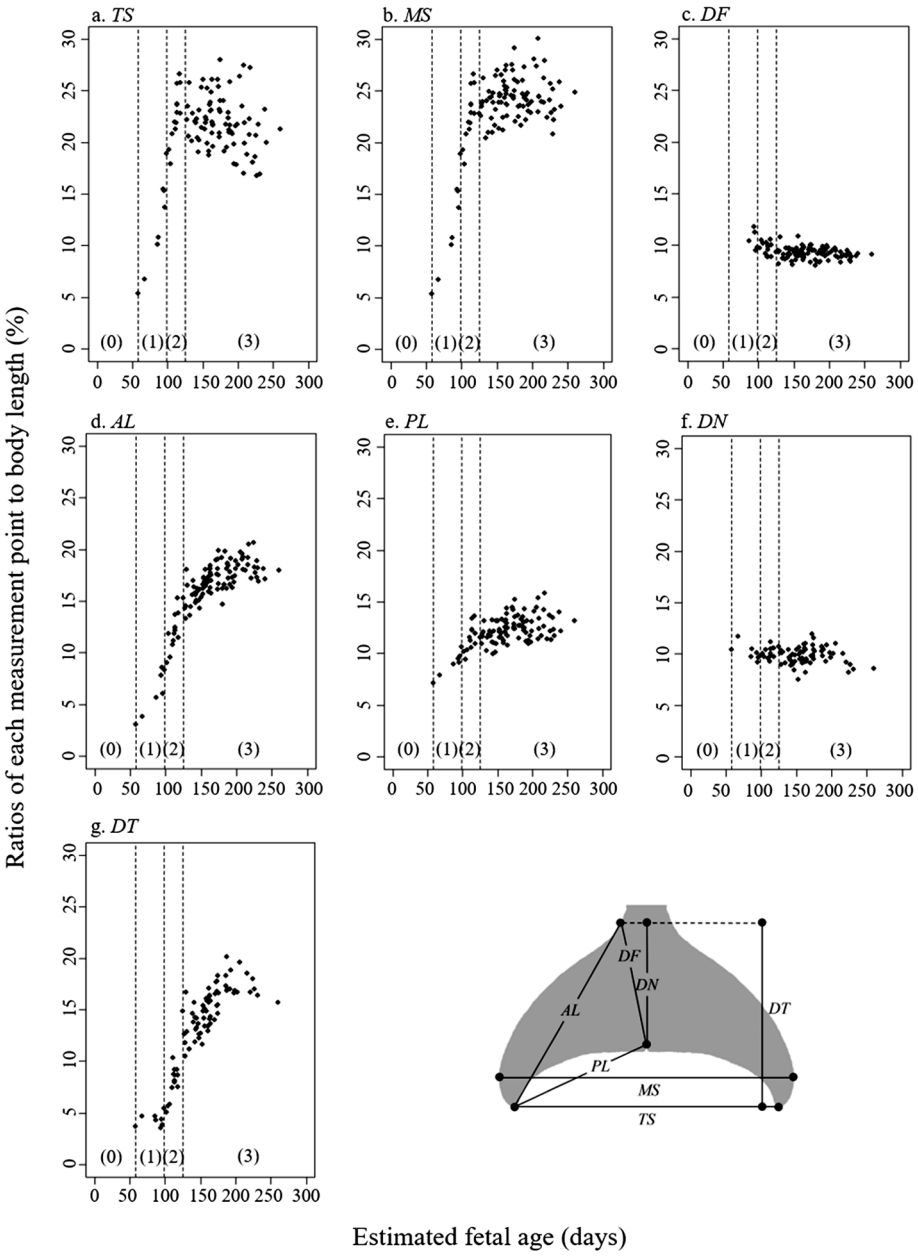

Fig. 5. The relationship between ratios of fetal flukes to body length (y axis) and estimated fetal age (x axis) of the Antarctic minke whale. *TS* (a), *MS* (b), *DF* (c), *AL* (d), *PL* (e), *DN* (f), *DT* (g). The dotted lines indicate the starting points of each developmental stage of fetal flukes, from the left to the right: (0), (1), (2), and (3). Stage (0), *tail shape*; Stage (1), *diamond shape*; Stage (2), *triangle shape*; Stage (3), *boomerang shape*.

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References

Amano, M. and Miyazaki, N. 1993. External morphology of Dall's porpoise (*Phocoenoides dalli*): Growth and sexual dimorphism. *Can. J. Zool.* 71 (6): 1124–1130.

Bando, T. 2017. Hokuseitaiheiyou niokeru nitarikujira *Balaenoptera edeni brydei* to iwashikujira *B. borealis* no keitai oyobi seibutsugakutekitokuseichi no hikakukentou [Comparison of the morphological and biological characters of Bryde's whale *Balaenoptera edeni brydei* and sei whale *B. borealis* in the western North Pacific.] (in Japanese). Ph. D. thesis, Tokyo University of Marine Science and Technology, Tokyo. 149 pp.

Bando, T., Nakai, K., Kanbayashi, J., Umeda, K., Kim, Y., Nishimura, F., Yoshida, T., Tsunekawa, M., Yoshimura, I., Mure, H., Kominami, T., Eguchi, H., Mogoe, T. and and Tamura, T. 2018. Results of the third biological field survey of NEWREP-A during the 2017/18 austral summer season. Paper SC/67B/SCSP/08 of the Scientific Committee of the International Whaling Commission. 17 pp. [Available from the secretariat of IWC]

Huxley, J. S. 1950. A discussion on the measurement of growth and form; relative growth and form transformation. *Proc. R. Soc. B.* 137 (889): 465–469.

Kato, H. and Miyashita, T. 1991. Migration strategy of southern minke whales in relation to reproductive cycle estimated from foetal lengths. *Rep. int Whal Commn*. 41: 363–369.

Laws, R. M. 1961. Reproduction, growth and age of southern fin whales. *Discovery Rep*. 3l: 327–486.

Nishiwaki, M., Ohsumi, S. and Maeda, Y. 1963. Change of form in the sperm whale accompanied with growth. *Sci. Rep. Whales Res. Inst.* 17: 1–14.

Ogawa, T. 1953. On the presence and disappearance of the hind limb in the cetacean embryos. *Sci. Rep. Whales Res. Inst.* 8: 127–132.

Ohsumi, S. 1960. Relative growth of the fin whale, *Balaenoptera physalus* (Linn.). *Sci. Rep. Whales Res. Inst.* 15: 17–84.

R Core Team. 2018. R: A language and environment for statistical computing. Version 3.5.1. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Roston, R. A., Lickorish, D. and Buchholtz, E. A. 2013. Anatomy and age estimation of an early blue whale (*Balaenoptera musculus*) fetus. *Anat. Rec*. 296 (4): 709–722.

RStudio Team. 2016. *RStudio: Integrated development environment for R*. Version 1.1.456. *RStudio, Inc.*, Boston, MA. http://www.rstudio.com

Schneider, C.A., Rasband, W.S. and Eliceiri, K.W. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods.* 9: 671–675.

Štěrba, O., Klima, M. and Schildger, B. 2000. Embryology of dolphins. Staging and ageing of embryos and fetuses of some cetaceans. *Adv. Anat. Embryol. Cell Biol.* 157: 1–133.

Thewissen, J. G. M. 2018. Highlights of cetacean embryology. *Aquat. Mamm.* 44: 591–602.

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Appendix

Formulas for estimating fetal age of the Antarctic minke whale

We used formula (1) based on Kato and Miyashita (1991) to estimate the fetal age (t, expressed in days) when the body length was 15 cm or more. The growth rate of the fetus changes with the formation of the placenta (Laws, 1961). The placenta of this species is formed when the body length is 15 cm (Kato and Miyashita, 1991). Bando (2017) studied Bryde's (*B. edeni brydei*) and sei whales (*B. borealis*) fetuses, and assumed that days after conception (i.e., fetal age) of fetuses with a body length of less than 15 cm were proportional to their body length. This study made a similar assumption. First, we calculated the fetal age at 15 cm in body length using formula (1) and estimated it at 92.2 days. Subsequently, we adapted $t=aL$ to estimate the growth coefficients (a), which we calculated as 6.147. Formula (2) was used when the body length was less than 15 cm.

$$t = 1.622\,L^{0.892} + 74 \quad (1)$$

$$t = 6.147\,L \quad (2)$$