



Fluctuations in serum steroid hormone concentrations and body mass during growth and sexual maturation in captive northern fur seals (*Callorhinus ursinus*)

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ABSTRACT. Northern fur seals (*Callorhinus ursinus*) have a distinct life history pattern comprising annual terrestrial breeding and oceanic migration, and the physiological changes associated with these patterns are of particular interest for understanding their environmental adaptations. However, owing to their oceanic distribution, limited information is available on the reproductive physiology of wild individuals during the immature stage and the winter migration period. This study aimed to determine the relationships among the seasonal hormone profiles, body growth, age, and pregnancy using monthly serum samples collected over 3–5 years from two male and two female captive individuals during pubescence and sexual maturation. Small increases in the serum testosterone signaled puberty in males aged 3 and 4 years. Thereafter, males showed considerable increases in testosterone during breeding seasons, indicating sexual maturity. Immature female serum progesterone was maintained at low levels, but after pubescence, females showed an increase in serum progesterone in August, the month next to the peak of delivery, followed by a decrease. In non-pregnant females, progesterone did not increase significantly until the next breeding season, but in pregnant females, they increased again from February to March and then gradually decreased. Immature males increased body mass constantly and reached puberty when their body mass exceeded 20 kg, and they showed seasonal weight fluctuations after puberty. These results provide fundamental information for determining sexual maturity and pregnancy in this species based on sex steroid hormones and body mass measurements.

KEY WORDS: *Callorhinus ursinus*, body mass, progesterone, puberty, testosterone

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The northern fur seal (*Callorhinus ursinus*) is an otariid marine mammal endemic to the northern Pacific Ocean [17, 22, 38]. From July to October, the seals form dense aggregations along the beaches of breeding islands and deliver offspring, copulate a few days after delivery, and nurse offspring [4, 17, 38]. Throughout the rest of the year, seals migrate in the open ocean to forage and overwinter without coming ashore. Their annual reproductive cycle is highly synchronized so that most delivery and copulation activities occur over a two-week period in July [17, 25, 38]. Total gestation period is about 360 days, which includes of 3–5 months of embryonic diapause [1, 11]. Females normally deliver a single pup in summer, nurse during the breeding season, and wean in early November. Then, the pups participate in migration for a few years until they become involved in reproductive activities on breeding islands [6, 15, 17, 39, 57]. Because northern fur seals have a distinct life history pattern consisting of terrestrial breeding and oceanic migration, the physiological changes associated with their annual reproductive cycle and ontogeny are of particular interest for understanding their environmental adaptations [27]. However, owing to their oceanic distribution during the migration period, limited information is available on the reproductive physiology of immature or overwintering adult northern fur seals.

The present study aimed to clarify the changes in the sex steroid hormone concentrations of four captive northern fur seals using

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serum samples collected monthly over 3–5 successive years during pubescence and sexual maturation and identify the timing of puberty in relation to body growth. Changes in the hormone profile before and after puberty and the feasibility of determining pregnancy using hormone levels are also discussed. As wild northern fur seal populations are largely declining [16] the present study provides information that is fundamental for understanding the reproductive physiology of this species, and to promote breeding in captivity.

MATERIALS AND METHODS

This study was conducted as a part of the research project by the former National Research Institute of Far Seas Fisheries, Fisheries Research and Education Agency, Japan (Yokohama, Japan). The animal experimental procedures were reviewed and approved by the Gifu University Animal Care and Use Committee (approval nos. 14094 and 17186) in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals [52].

Sample animals

Two male and two female northern fur seals housed at Izu Mito Sea Paradise, Shizuoka, Japan were used in this study (Table 1). One male seal (SEAL1) was born in late June 2007 in the “Fur Seals’ Cove” outdoor feedlot, which is a natural inlet partitioned by plastic netting and metal fences. SEAL1 showed signs of emaciation before the weaning period and was, therefore, moved to the indoor facility at 5 months post-partum and hand-reared with artificial milk until weaning using the same method as previously described [28]. Another male seal (SEAL2) was found stranded on a beach in Miyako, Iwate, northern Japan, on January 15, 2010, and was rescued with permission from the Ministry of Agriculture, Forestry and Fisheries, Japan. When rescued, SEAL2 was estimated to be less than 1 year old based on its body size (body length 73.0 cm, body mass 7.65 kg). After being raised in an aquarium near the rescue site for 2 years and 3 months, SEAL2 was brought to Izu Mito Sea Paradise. SEAL3, a female, was born in the outdoor feedlot in 2009 and was hand-reared with artificial milk from 1 week postpartum as she could not be successfully nursed by her mother [28]. SEAL3 was housed in the indoor breeding facility after reaching 2 months of age. SEAL4, another female, was born in the outdoor feedlot in 2010, and was nursed there successfully by her mother before being moved to the indoor facility at 2 years of age.

SEAL1 was housed with two other adult female fur seals (not assessed in this study) in an enclosure (approximately 46.8 m², with a pool measuring 40 m³) at the indoor facility throughout the experimental period from March 2009 to June 2012. SEAL2, SEAL3, and SEAL4 were placed together in another enclosure of the indoor facility (approximately 16.8 m², with a pool measuring 4.5 m³) from April 2013 to March 2018 (Table 1).

The fur seals were fed twice daily throughout the year, including the breeding season. Frozen mackerel were completely thawed and then fed to the seals. The daily rations were controlled according to the appetite of each animal. The daily food intake during the experimental period was approximately 3 kg day⁻¹ for SEAL1, 4–5 kg day⁻¹ for SEAL2, 2.5–3 kg day⁻¹ for SEAL3, and 2–2.5 kg day⁻¹ for SEAL4. The ratios of the daily food intakes to body mass were in the range of 5–15%. Regular observations from staff veterinarians revealed no health abnormalities in any of the animals during the study period. However, SEAL1 died suddenly in July 2012, probably owing to the diet switch from mackerel to Japanese sardine (*Engraulis japonicus*) for another experiment unrelated to this study. All the serum samples were collected before the diet switch.

Figure 1 shows the average monthly air and water temperatures from 2013 to 2017 and the average photoperiod in 2014 (Fig. 1) of the indoor facility used in this study. Other environmental conditions were similar to those described by Kohyama and Inoshima (2017).

Serum sampling

Blood samples were collected from each seal once a month. For SEAL1 this occurred over 3 years and 3 months until its death, and for the remaining seals over the full 5 years of the study period. Before sampling, each seal was introduced to the hallway adjacent to the enclosures before being caught in a cage to weigh the body mass in kg. The experimental animals were trained to enter the cage spontaneously without being forced. Blood samples were collected from the interdigital vessels of the hind flipper using disposable syringes and needles. During sampling, seals were physically restrained, with their hind flippers held partially out of the cage. Neither sedatives nor anesthetics were used. Collected blood samples were stored motionless in plain test tubes

Table 1. Northern fur seals (*Callorhinus ursinus*) used in this study

ID	Sex	Date of birth	Date of death	Sample collection period		Total number of serum samples obtained
				Date	Age [‡]	
SEAL1	Male	June 25, 2007	July 4, 2012	April 2009 to June 2012	1Y10M to 5Y0M	39
SEAL2	Male	Unknown, 2009 [†]	Alive	April 2013 to March 2018	3Y9M to 8Y8M	59
SEAL3	Female	July 26, 2009	Alive	April 2013 to March 2018	3Y10M to 8Y9M	60
SEAL4	Female	June 30, 2010	Alive	April 2013 to March 2018	2Y10M to 7Y9M	60

[†]: Stranded and rescued in Miyako, Iwate, Japan on January 15, 2010, at the age under 1 year judging from body size. Since northern fur seal deliveries on the breeding island are concentrated in July [17, 25, 38], SEAL2 is presumed to have been born in July. [‡]: Years (Y) and months (M) old.

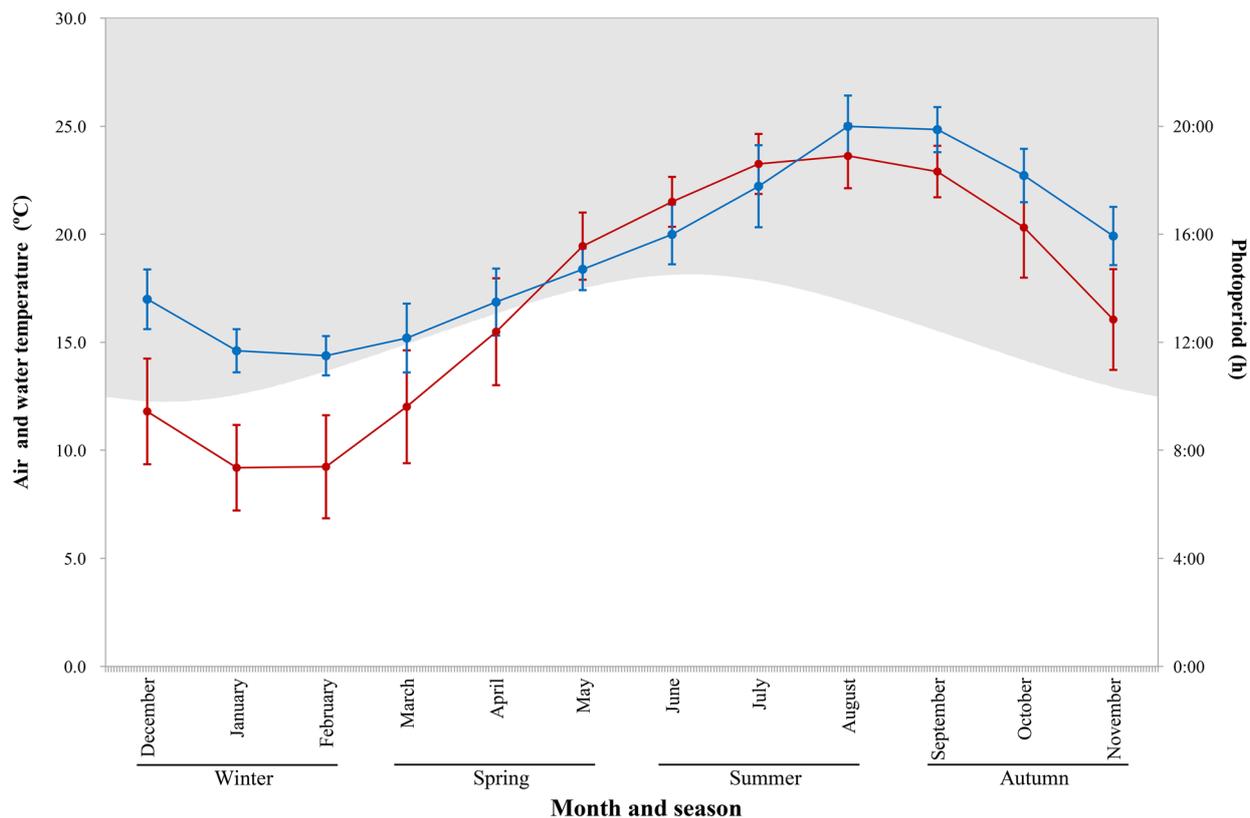


Fig. 1 Air temperature, water temperature and photoperiod in the breeding facility. Air and water temperatures are monthly averages \pm standard deviation from 2013 to 2017. Photoperiods have been listed considering 2014 as a typical year. Air temperature (AT, °C ●—), water temperature (WT, °C ●—), photoperiod (h □), and dark period (h □) of the indoor facility used in this study.

(VENOJECT II VP-AS109K, Terumo, Tokyo, Japan) placed in a cold box and were transported to the laboratory within a few hours. A total of 218 serum samples were obtained from the four seals.

Hormone assays

Male testosterone and female progesterone and estradiol were the sex steroid hormones measured in this study. All hormone assays were performed at a commercial clinical testing laboratory (SRL, Tokyo, Japan). The three hormones were assayed using electro-chemiluminescence immunoassay with the Cobas 8000 e801 modular analyzer (Hitachi High-Technologies Corp., Tokyo, Japan). According to the specifications of the manufacturer of the measuring instrument, the lower detection limits were 0.03 ng/ml for testosterone, 0.03 ng/ml for progesterone, and 5.0 pg/ml for estradiol. The cross-reaction rates of androstenedione, cortisol, and cortisone with testosterone were 2.66%, 0.016%, and 0.002%, respectively. The cross-reaction rates of androstenediol, androstenedione, allopregnanolone, corticosterone, and 11-deoxycorticosterone with progesterone were 0.01%, 0.107%, 0.347%, 0.921%, and 3.92%, respectively. The cross-reaction rates of estrone, estriol, and androstenedione with estradiol were 0.761%, 0.325%, and 0.005%, respectively.

Relationship between growth and puberty

The average body mass prior to the breeding season (BMPB) was calculated from body mass measurements of each individual from April to July before the breeding season. To clarify the relationship between growth and puberty, the relationship between the individual BMPB and the year in which puberty was achieved was analyzed. In this study, patterns of serum steroid hormone profiles were used to detect puberty as elevated levels of sexual steroids are linked to maturation of the reproductive system [46]. A previous study demonstrated the relationship between spermatogenic and steroidogenic activities in male northern fur seals during the breeding season, and thus, the first rise in serum testosterone concentrations was used as the sign of puberty in males [50]. The first large increase of serum progesterone concentrations similar to the typical pattern observed at estrus and ovulation in this species [24] was used as the basis for detecting puberty in females.

Analysis of old progesterone records

Past records of serum progesterone measured in Izu Mito Sea Paradise in the 1990s were used to examine their potential use to diagnose pregnancy. From November to May 1993–1995, serum progesterone was measured using the radioimmunoassay (RIA)

solid phase method from serum samples collected from seven captive adult female northern fur seals. All of these females were brought from the wild, and had experienced delivery in an aquarium. The lower detection limit of progesterone using the RIA solid phase method was 0.2 ng/ml. The monthly mean values of progesterone concentrations in pregnant and non-pregnant individuals were compared using the Welch's *t*-test (significance level 0.05, two-tailed). Pregnancy of the experimental females was confirmed by the presence or absence of delivery during the pupping season of the year. Statistical analyses were conducted using R 4.0.3 [40].

RESULTS

Males

Figures 2 and 3 show the monthly changes in serum testosterone concentrations and body mass measurements of SEAL1 and SEAL2, respectively. Serum sampling of SEAL1 was initiated in April 2009 at 1 year and 10 months of age. Testosterone was first detected at a level of 0.29 ng/ml in July 2010, when SEAL1 was 3 years of age and weighed 27 kg. SEAL1 is supposed to have entered puberty at 3 years of age in 2010. Slight increases in the serum testosterone concentrations were also detected in December 2010 and January 2011. In 2011, a sharp increase in the testosterone concentration (0.68 ng/ml) was detected in May, just before the breeding season, followed by another increase (0.27 ng/ml) in July. In 2012, the serum testosterone concentration began to increase in April and remained at elevated concentrations in the following months. The body mass of SEAL1 increased gradually without large fluctuations. During the experimental period, the females that lived in the same enclosure as SEAL1 did not become pregnant.

Serum sampling of SEAL2 was initiated in April 2013, when he was estimated to be 3 years and 9 months of age. A slight increase (0.17 ng/ml) in serum testosterone was detected after the peak breeding season in October 2013, when SEAL2 was 4 years old. At ≥ 5 years of age, SEAL2 consistently showed increased serum testosterone concentrations in the summer months during the breeding seasons. Judging from the serum testosterone profile, SEAL2 was likely to enter puberty at 4 years of age in 2013. The body mass of SEAL2 increased gradually from 2013 with cyclic fluctuations, being higher from spring to summer and lower from autumn to winter and exceeded 60 kg at 6 years of age in the 2015 breeding season. SEAL2 copulated with cohabiting SEAL3 in the 2015 breeding season, and SEAL3 delivered a pup in 2016.

Females

Figures 4 and 5 show the monthly fluctuations in the serum progesterone, serum estradiol, body mass of SEAL3 and SEAL4 and their delivery, respectively. At 3–5 years of age, the serum progesterone and estradiol concentrations in SEAL3 did not change

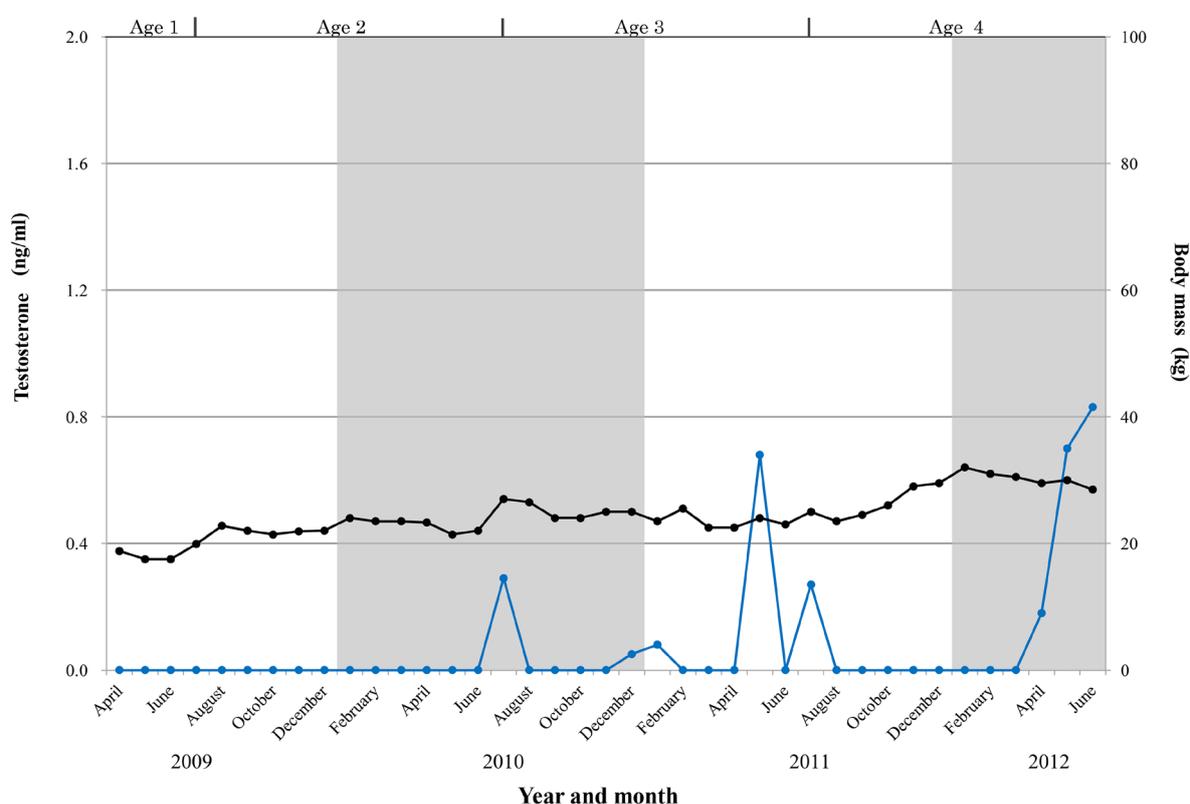


Fig. 2. Monthly changes in serum testosterone concentrations and body mass of SEAL1. Testosterone concentrations (ng/ml —●—) and body mass (kg —●—) of SEAL1 from April 2009 to June 2012. Ages are shown in years.

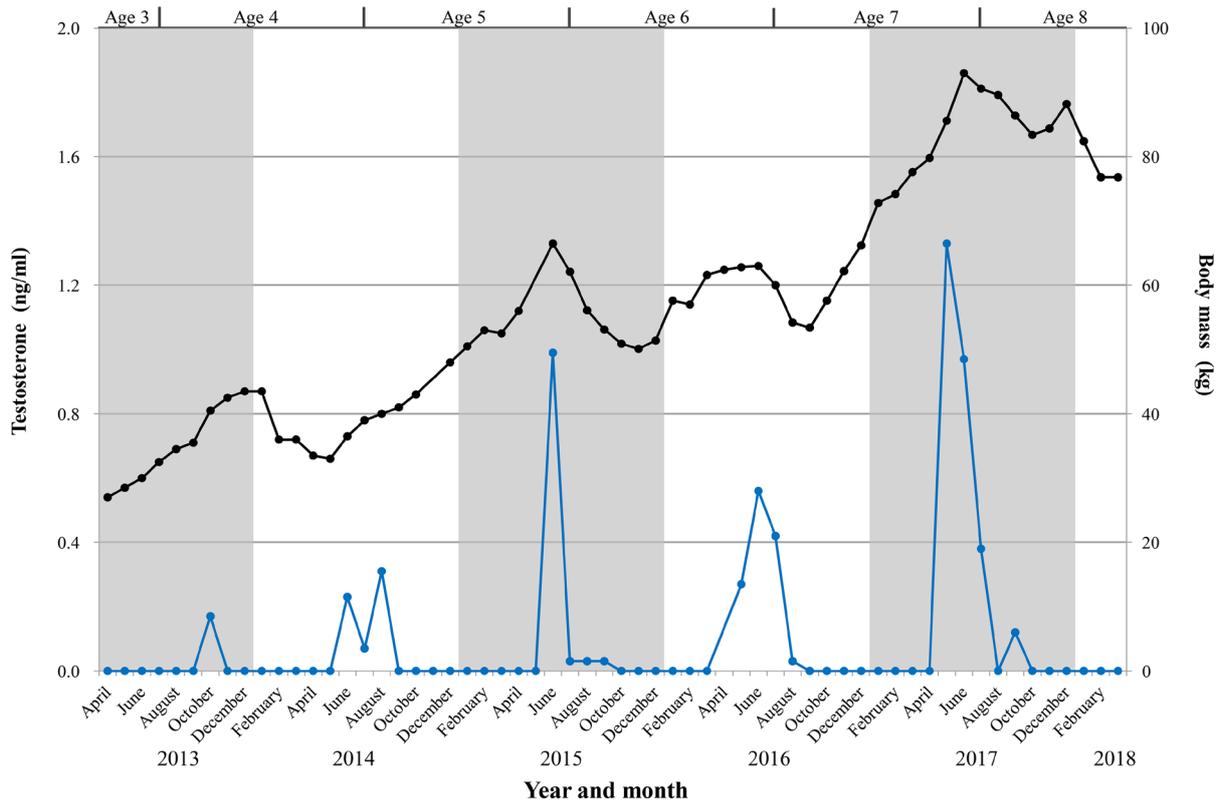


Fig. 3. Monthly changes in serum testosterone concentrations and body mass of SEAL2. Testosterone concentrations (ng/ml ●) and body mass (kg ●) of SEAL2 from April 2013 to March 2018. Ages are shown in years.

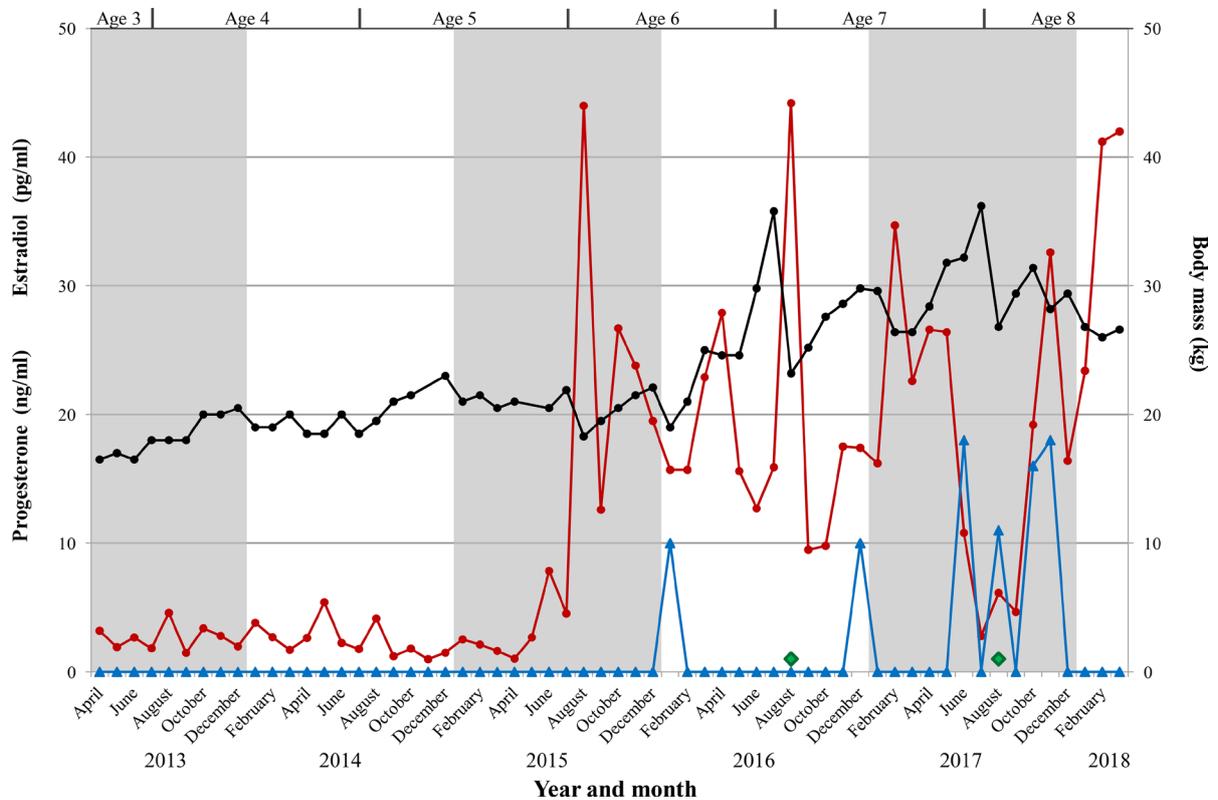


Fig. 4. Monthly changes in serum progesterone and estradiol concentrations, body mass, and delivery of SEAL3. Serum progesterone concentrations (ng/ml ●), serum estradiol concentrations (pg/ml ▲), body mass (kg ●), and delivery (◆) of SEAL3 from April 2013 to March 2018. Ages are shown in years.

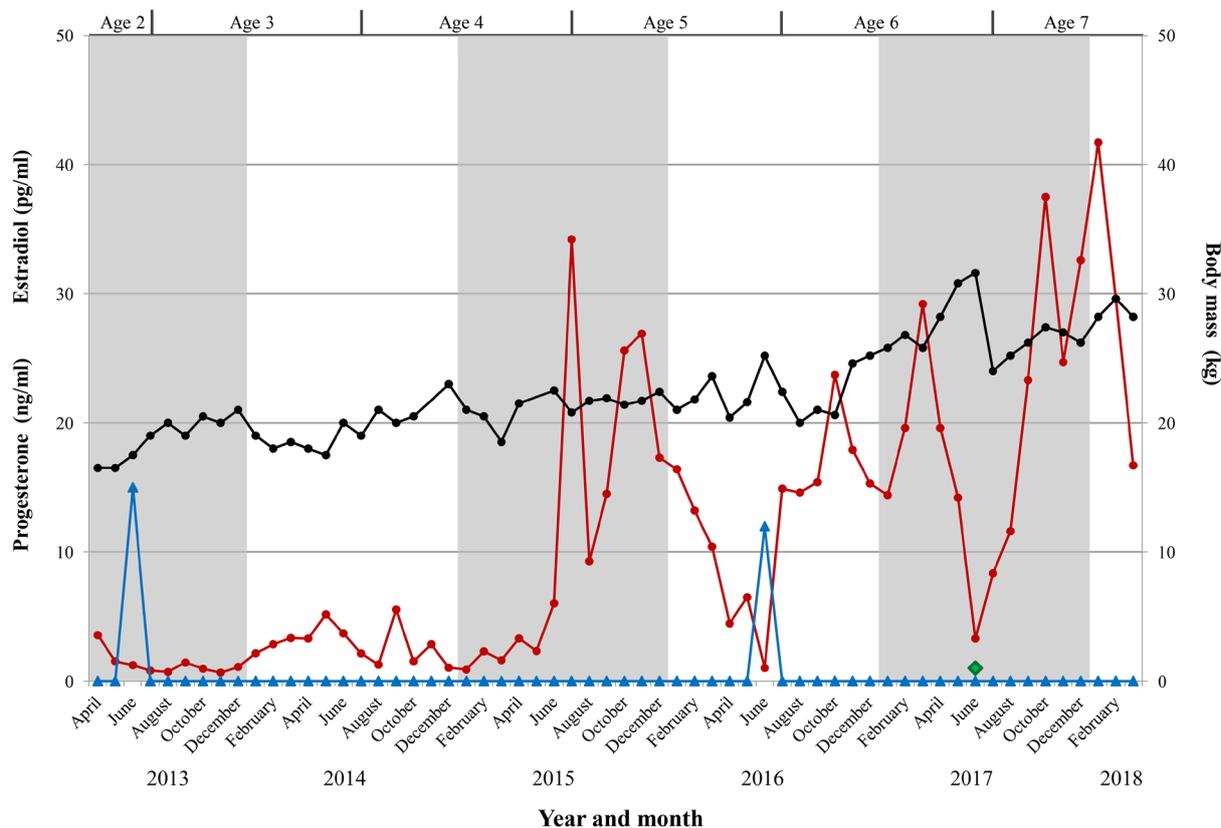


Fig. 5. Monthly changes in serum progesterone and estradiol concentrations, body mass, and delivery of SEAL4. Serum progesterone concentrations (ng/ml —●—), serum estradiol concentrations (pg/ml —▲—), body mass (kg —●—), and delivery (◆) of SEAL4 from April 2013 to March 2018. Ages are shown in years.

markedly; progesterone was maintained at the baseline level (average $2.71 \text{ ng/ml} \pm \text{SD } 1.49$ from April 2013 to July 2015), and the estradiol concentration was lower than the detection limit. A high level (44 ng/ml) of progesterone was measured for the first time at 6 years of age in August 2015. SEAL3 became pregnant in the 2015 breeding season and delivered a pup in the following August. From 2015 on, SEAL3 had higher serum progesterone concentrations in August, October to November, and March to April than in other months, and delivered a pup annually. The serum progesterone profile and parturition record indicate that SEAL3 entered puberty and came into estrus at 6 years of age in 2015. The body mass of SEAL3 increased gradually with small fluctuations from 2013 to 2015 but showed a marked rise from January to July 2016 just before the delivery of her first pup.

Similarly, the serum progesterone concentrations in SEAL4 did not change markedly at 3 and 4 years of age (average $2.34 \text{ ng/ml} \pm \text{SD } 1.47$ from April 2013 to June 2015) but increased dramatically up to 34.2 ng/ml at 5 years of age in July 2015. SEAL4 is supposed to have entered puberty and estrus at this time, but did not give birth the next year (2016). In the 2016 breeding season, when SEAL4 was considered to be pregnant, her serum progesterone concentrations increased in August, October, and the following February and March. Her body mass increased gradually from 2013 to 2015 but showed a large increase from November 2016 to June 2017 when she was pregnant.

Fluctuating increases in the serum estradiol concentrations of SEAL3 and SEAL4 were detected sporadically in June, August, November, December, and January. SEAL4 showed a spiky rise of estradiol in June 2016 followed by a gradual increase of progesterone, which represents a typical hormonal profile representing ovulation and corpus luteum formation in pinnipeds [7]. In 2015 and 2017, rises of estradiol in early reproductive seasons were inconspicuous in SEAL4. SEAL4 showed another rise of estradiol in June 2013, but this was not followed by progesterone increase. SEAL3 showed sporadic increases of estradiol in summer and winter.

Relationship between body mass and puberty

Table 2 shows the BMPB of each individual seal at each age and the age at which puberty was reached. Neither SEAL1 nor SEAL2 reached puberty when their BMPB was 20 kg or less. The BMPB of SEAL3 and SEAL4 was 21.1 kg and 21.6 kg, respectively, when they reached puberty in 2015 (Table 2). None of the seals reached puberty when the BMPB was 20 kg or less.

Table 2. The average body mass (kg) of seals prior to the breeding season* at each age

ID	Sex	Age (years)							
		2	3	4	5	6	7	8	
SEAL1	Male	18.4	23.4	23.6	29.3	-	-	-	
SEAL2	Male	-	-	29.5	35.5	61.5	62.1	87.3	
SEAL3	Female	-	-	17.0	18.9	21.1	28.7	32.2	
SEAL4	Female	-	17.4	18.6	21.6	22.4	28.7	-	

Bold: Age at which seals reached puberty during the breeding season that year. * From April to July.
-: Not weighed because the age is outside the experimental period.

Table 3. Comparison of monthly average serum progesterone concentrations (ng/ml) between pregnant and non-pregnant adult female northern fur seals (*Callorhinus ursinus*)

	November		December		January		February		March		April		May	
	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P
<i>n</i>	68	12	47	6	42	6	42	6	38	6	15	6	23	6
Average	12.5	16.9	8.5	17.0	6.2	17.0	5.2	12.6	4.4	9.5	4.9	6.8	3.0	4.8
SD	8.3	7.4	8.1	5.5	5.6	11.1	5.4	2.8	5.1	2.4	4.4	2.3	2.2	2.1
95% CI	10.5–14.4	12.7–21.1	6.1–10.8	12.6–21.4	4.5–7.9	8.2–25.9	3.5–6.8	10.4–14.9	2.8–6.1	7.6–11.5	2.7–7.2	4.9–8.6	2.1–3.9	3.1–6.5
<i>P</i> value	0.0794		0.0128*		0.0618		0.0002*		0.0015*		0.2320		0.1011	

P value: calculated using the two-sided Welch's *t*-test, * significant at the 0.05 level. NP, non-pregnant; P, pregnant; SD, standard deviation; CI, confidence interval.

Pregnancy diagnosis by progesterone concentration

Table 3 shows the monthly progesterone concentration comparisons between pregnant and non-pregnant females based on the past records in 1990s as mentioned above. The progesterone concentrations of pregnant females were significantly higher than non-pregnant females in December, February, and March (two-tailed Welch's test, $P < 0.05$).

DISCUSSION

The longitudinal monitoring of sex steroid hormones and body mass of captive northern fur seals revealed their physiological changes over the course of puberty. From the first increase in serum testosterone concentration, we determined that males in this study reached puberty at 3 (SEAL1) or 4 (SEAL2) years of age. Thereafter, serum testosterone concentrations increased regularly in the breeding season and showed the typical seasonal pattern reported previously [26, 36, 50]. Testosterone concentrations are generally known to increase around the time of sexual maturity in pinnipeds [41]. Our method of puberty assessment using the testosterone profile relies on the link between spermatogenetic and steroidogenetic activities [50], and is supported by a recent study demonstrating the relationship between spermatogenesis and testosterone levels in the hair of northern fur seals [37]. Results of the puberty assessment in the current study are comparable to previous anatomical and histological studies. A sharp increase in testicular weights and baculum lengths is known to occur in wild male northern fur seals at 3 to 4 years of age [43]. A recent study confirmed that testicular size and baculum length are effective indicators of sexual maturity and spermatogenesis [23]. The results of longitudinal observations of captive individuals in the present study were consistent with previous studies investigating transverse analyses of the male sexual maturation process, whereby male northern fur seals reached puberty and attained physiological maturity between the ages of 3 and 4.

The body mass of males before puberty gradually increased with small fluctuations. After puberty, male body mass increased with a clear seasonal cycle, ascending from spring to summer and descending from autumn to winter. These fluctuations in the body mass of mature male otariids are known as the fatted male phenomenon [12, 44]. Breeding males in the wild are thought to lose body mass because they fast during the breeding season [17, 49]. The males in this study were fed to fulfill their nutritional requirements even in the breeding season but showed large seasonal changes in body mass. The results suggest that body mass fluctuations of the adult males are related not only to their nutritional condition but also to their physiological conditions responsive to the anabolic effect of testosterone [12, 30, 35], in association with the annual reproductive cycle.

In this study, we defined puberty in females as the first large increase in serum progesterone concentrations. The females in this study showed seasonal fluctuations in progesterone concentrations and occasionally detectable levels of estradiol after the detected puberty. These seasonal patterns concur with the hormone profiles reported in captive adult female northern fur seals [24, 31]. Herein, pup delivery records indicated that the reproductive cycles of these females in this study were normal after puberty. SEAL3 became pregnant in her first ovulation and underwent repeated normal pup delivery in each of the following years. Although SEAL4 did not deliver a pup in 2016 after her first ovulation, she successfully delivered pups in 2017 and 2018.

Detecting the sexual maturation of females from monthly serum estradiol measurements is difficult because of its pulsatile surges at ovulation [18, 51]. Results of this study demonstrate that serum progesterone concentrations can be used for assessing pregnancy

in female northern fur seals. The large increase in serum progesterone concentration in the early breeding season reflects the growth of the corpus luteum after ovulation. The rise in serum progesterone in August was considered as the signal that puberty occurred at 6 years of age in SEAL3 and 5 years of age in SEAL4. Thereafter, the progesterone concentration declined in the following months and was re-elevated in late autumn. This increase in serum progesterone concentrations reflects the re-activation of the corpus luteum related to delayed implantation [7, 8, 55]. Pregnant females (SEAL3 2015–2016, SEAL3 2016–2017, and SEAL4 2016–2017) showed increased progesterone concentrations in February, March and April, whereas the non-pregnant female (SEAL4 2015–2016) did not show this increase in spring. It is considered that regression of the corpus luteum occurred from winter to spring in non-pregnant females. Such differences in the serum progesterone profile between pregnant and non-pregnant female seals can be used to diagnose pregnancy. The analysis of old records showed significant differences in the monthly progesterone concentrations between pregnant and non-pregnant seals in December, February, and March. These results indicate that pregnancy diagnosis based on serum progesterone concentrations is possible from winter to early spring. The serum relaxin concentration is reported to be an effective marker to distinguish pregnant and nonpregnant northern fur seals [5], however its effectiveness is limited to a late gestation period [45]. The use of serum progesterone concentrations for pregnancy diagnosis of captive animals has a practical advantage as it can be easily measured in a clinical laboratory for human medicine. The applicability of pregnancy diagnosis based on serum progesterone measurement is also indicated in other pinnipeds, such as the California sea lion (*Zalophus californianus*) [20], Steller sea lion (*Eumetopias jubatus*) [42], Australian sea lion (*Neophoca cinerea*) [14] and harbor seal (*Phoca vitulina*) [21]. Combining serum progesterone measurements with either the use of serum relaxin or ultrasonic image of the uterus is expected to result in a more accurate diagnosis of pregnancy [41]. According to the records in the Izu Mito Sea Paradise, most female northern fur seals kept in the outdoor feedlot generally reach puberty at 3 years of age and deliver their first pups at 4 years of age. The timing of puberty in SEAL3 was 3 years later than the typical pattern in captive females in Izu Mito Sea Paradise. Body condition is another factor that can affect the timing of puberty. Body growth of SEAL3 was slower than normal animals because she could not be nursed successfully by her mother and was hand-reared with artificial milk for a prolonged period [28, 32, 56]. Despite the difference in their ages, the BMPB of SEAL3 and SEAL4 did not exceed 20 kg before puberty, but attained 21.1 kg and 21.6 kg, respectively, when they reached puberty in 2015 (Table 2).

In wild female northern fur seals, age is often considered to be the key factor that determines puberty [15, 17, 54, 57]. However, the results of this study suggest that a BMPB of 20 kg appears to be the threshold for puberty in captive female northern fur seals. Male individuals also exceeded a BMPB of 20 kg when they reached puberty at approximately 3 years of age (Table 2). In wild northern fur seals, both males and females reach a body mass of 20 kg at an average of 3 or 4 years of age [3, 49], although large variations in body growth have been reported [49]. Trites and Bigg (1992) suggested that annual growth fluctuations could reflect the food conditions at sea. Relationships between sexual maturity and age or body mass have previously been suggested based on a cross-sectional analysis of different age classes and body sizes [34, 43, 49]. Based on the results of this study and the published literature [34, 43, 49], it can be assumed that body mass and nutritional conditions affect the timing of puberty and sexual maturation in northern fur seals, and that a BMPB above 20 kg may be a requirement for puberty. One possible reason for the delay of sexual maturation in SEAL3 is her slower initial growth and lower body mass at age compared with normal individuals. It is reported that body weight has a more important role than age in driving the maturation of testes in wild bores (*Sus scrofa*) [33]. Nutritional condition is known to work as a permissive factor for the timing and progression of pubertal development in humans (*Homo sapiens*) [2].

The relationship between body length and puberty initiation was also reported for other otariids such as Steller sea lion [53], New Zealand sea lion (*Phocarctos hookeri*) [9], and South American sea lion (*Otaria flavescens*) [19]. Herein, the body mass was used as an index of body size and condition because it reflects body condition and can be more easily measured in captive animals than body length. Body mass measurements can be performed without physical or chemical restraint by training animals to enter a cage or to ride on a scale. In humans, Baker (1985) reported that the relationship between body mass and puberty is stronger than the relationship between body length and puberty, which further supports the methods used here.

Additionally, observations of body mass variation are useful for monitoring the sexual maturation of captive northern fur seals. Rapid increases in body mass with cyclic seasonal fluctuations can indicate the onset of male puberty, whereas concentrations of serum progesterone greater than 10 ng/ml in December and February followed by a rapid increase in body mass can be used for pregnancy diagnosis in females.

We revealed the long-term serum hormone profiles of captive northern fur seals over the course of ontogeny, puberty, and sexual maturation that could not be examined in wild individuals. The longitudinal observations of physiology, body growth, and sexual maturation processes presented here provide useful information for the conservation of northern fur seals, a vulnerable species that is facing prolonged population decline owing to unidentified causes [10, 13, 16, 47, 48]. However, it is necessary to verify whether the results of this study are directly comparable to wild animals, because it is known that some blood characteristics of captive northern fur seals are slightly different from those of wild seals [29]. In addition, this study was conducted using a minimal number of northern fur seals due to restricted experimental conditions. In the future, it will be necessary to verify the results of this study by increasing the number of test individuals and accumulating more information.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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