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Skull morphology and genetic variation of the Kuril harbor seal (*Phoca vitulina stejnegeri*) and the spotted seal (*Phoca largha*) around Hokkaido, Japan

（北海道沿岸に生息するゼニガタアザラシ *Phoca vitulina stejnegeri* とゴマフアザラシ *Phoca largha* の頭蓋骨形態および遺伝学的比較）

Emiko Nakagawa

Contents

Chapter 4

Determination of the hybridization between the Kuril harbor seal and the spotted seal around Hokkaido, using mitochondrial DNA cytochrome *b* and SRY (sex determining region on the Y chromosome) gene

Preface

Five species of seals inhabit and/or migrate to coastal regions around Hokkaido, Japan: the Kuril harbor seal (*Phoca vitulina stejnegeri* Allen, 1902); the spotted seal (*P. largha* Pallas, 1811); the ringed seal (*P. hispida* Schreber, 1775); the ribbon seal (*P. fasciata* Zimmerman, 1783), and the bearded seal (*Erignathus barbatus* Erxleben, 1777). The major species are the Kuril harbor seal and the spotted seal.

The harbor seal (*P. vitulina*) is widely distributed in the Northern Hemisphere, five subspecies of which are recognized based on their geographic distribution and skeletal morphology (Bigg, 1981). The Kuril harbor seal is one of five subspecies of harbor seals. They do not migrate seasonally and settle in coastal areas all year around (Burns, 2009). Therefore, the Kuril harbor seal is the only pinniped observed throughout the year around Hokkaido.

Until recently, the spotted seal had been considered to be a subspecies of harbor seals. Now there are some differences between two seals not only in behavior and distribution, but also reproduction and morphology (See Chapter 1). Consequently, the spotted seal is recognized as a distinct species from the harbor seal.

The spotted seal occurs in the north and west of the North Pacific Ocean, mainly in the Yellow Sea, Okhotsk, Bering, Chukchi, and Beaufort seas (Rugh et al., 1997). It is a pagophilic (ice-breeding) seal, and is generally associates with ice from November through May-June (Lowry et al., 2000).

In recent years, the numbers of both seals have increased in Hokkaido. The increase in the number of spotted seals migrating to the

Sea of Japan is especially remarkable. Although approximately 320 spotted seals were observed in the Sea of Japan in March 2003, this number increased threefold by February 2006 (Hokkaido Government, 2006). Kuril harbor seals numbered approximately 650 around Hokkaido in 2000, and increased to approximately 800 by 2005 (Hokkaido Government, 2006).

The increase in the number of seal individuals has resulted in occasional damage to coastal fisheries. Damage to salmon fixed nets on the Pacific side by Kuril harbor seals has been reported; fishery damage by spotted seals was also reported (Hokkaido Government, 2006). Moreover, the invasion of fisheries by seals inevitably leads to the occurrence of bycatches. In fact, more than 150 seals die as a part of bycatch every year (Hokkaido Government, 2006).

Detailed information about these two seal species is necessary in order to reduce these interactions and to appropriately conserve and manage these animals. Conservation and management of both fisheries and seals should be conducted in each area because of the seals' widespread distributions and the extent of damage to fisheries in Hokkaido. Information about the existence of local groups of Kuril harbor seal and spotted seals is currently insufficient for areal conservation and management.

The Kuril harbor seal has been as an endangered species by the Ministry of Environment, Japan, and the spotted seal is under the control of the Hokkaido Government, Japan. These differences will affect the management infrastructure. However, studies about comparing with the two seal species around Hokkaido are inadequate.

In the present thesis, the skull morphology and genetic variation of the Kuril harbor seal and the spotted seal around Hokkaido were reported. In Chapter 1, ecological features of the two species from previous reports were described. In Chapter 2, growth variation in skull morphology of the two species was reported. In Chapter 3, genetic variation in the two species based on mitochondrial DNA cytochrome *b* sequences was reported. In Chapter 4, determination of the hybridization between the two species using mitochondrial DNA and SRY (sex determining region on the Y chromosome) gene was reported.

Chapter 1

Species characteristics of the Kuril harbor seal and the spotted seal around Hokkaido, Japan

In this chapter, ecological and morphological features of the Kuril harbor seal and the spotted seal around Hokkaido are described.

The Kuril harbor seal

Taxonomy

The harbor seal *P. vitulina* widely distributes in the Northern Hemisphere and five subspecies are recognized based on geographic distribution: the Eastern Atlantic harbor seal *P. v. vitulina* Linnaeus, 1758, in the eastern Atlantic Ocean; the Western Atlantic harbor seal *P. v. concolor* De Kay, 1842, in the western Atlantic Ocean; the Ungava seal *P. v. mellonae* Doutt, 1942, in fresh water lakes in the Ungava Peninsula, Canada; the Pacific harbor seal (a synonym is the eastern Pacific harbor seal) *P. v. richardsi* (Gray, 1864), in the eastern Pacific Ocean; and the Kuril harbor seal (synonyms are the western Pacific harbor seal and/or the insular seal) *P. v. stejnegeri* , in the western Pacific Ocean (Bigg 1981).

Distribution

The Kuril harbor seal distributes from the Cape Erimo where the southern limit of its distribution, northward along the Nemuro Peninsula, the Kuril Islands, and eastern Kamchatka as far as either the end of the Alaska Peninsula or the eastern Aleutians (King, 1983; Burns, 2009). The harbor seal does not migrate seasonally, and settles in coastal area throughout the year (Burns, 2009). The haul-out site of Kuril harbor seals around Hokkaido scattered along Pacific coast. Though the number of Kuril harbor seals has increased, the number of haul-out sites has decreased since 1970s (Hokkaido Government, 2006). Over 60% of Kuril harbor seals around Hokkaido are concentrated at two haul-out sites: Daikoku Island and Erimo (Hokkaido Government, 2006).

Appearance

The Kuril harbor seal is the largest harbor seal. Length and weight of adult males ranges from 174 to 186 cm and 87 to 170 kg and that of adult females from 160 to 169 cm and 60 to 142 kg (Burns, 2009). Newborn pups were up to 98 cm and 19 kg (Naito and Nishiwaki, 1972; Burns and Gol'tsev, 1984).

The pelage pattern and coloration of harbor seals are valuable, background colors range from yellowish or yellowishgray (light-phase) to blackish (dark-phase). The Kuril harbor seal is generally blackish and shows obvious light rings, especially on the dorsum (Burns, 2009). Because newborn harbor seals shed the lanugo before birth (*in utero*), their pelage is like adults (Burns, 2009).

Reproduction

The Kuril harbor seal is generally pagophobic (land-breeding), with breeding occurring on rocky islets at any suitable place within range (King, 1983). Its reproductive season (breeding, nursing, and mating) extends from April to early July (Hokkaido Government, 2006; Burns, 2009). Their mating system is promiscuous or weakly polygynous (Jefferson et al., 2008) and mating occurs at about the time that pups are weaned (Bigg, 1969; Boulva and McLaren, 1979; Burns, 1986). In Kuril harbor seals, the molt generally occurs during mid-summer to early autumn, within two or three months of the pupping season (Bigg, 1981).

The spotted seal

Taxonomy and distribution

The spotted seal (or largha seal) is a sibling species with the harbor seal and considered to be a monotypic species (Burns, 2009).

The spotted seal occurs in the seas north and west of the North Pacific Ocean: the Yellow Sea, Okhotsk, Bering, Chukchi, and Beaufort seas mainly (Rugh et al., 1997). In some areas of the North Pacific their distributions overlap with harbor seals'.

Appearance

In general, length and weight of adult males ranges from 161 to 176 cm and 85 to 110 kg and that of adult females from 151 to 169 cm and 65 to 115 kg (Burns, 1986). Newborn pups were 78 to 92 cm and 7 to 12 kg (Trukhin, 2005).

The pelage of spotted seal is generally pale with grey saddle, dark spots overall (Shaughnessy and Fay, 1977). They uniform in color and pattern and tend to resemble light-phase harbor seals, so that the differentiation of these two species in the field is sometimes very difficult (Rugh et al., 1997).

Reproduction

It is a pagophilic (ice-breeding) seal and generally associates with ice from November through May-June (Lowry et al., 2000). There are great seasonal expansions and contractions of range, and then eight known breeding concentrations of spotted seals: Liaodong Gulf; Peter the Great Bay; the western coast of Sakhalin Island in the Tatar Strait; the eastern coast of Sakhalin Island extending to northern Hokkaido; northern Shelikova Gulf; northeast from Kronotsky Cape on the eastern side of the Kamchatka Peninsula to Olyutorski Gulf; the Gulf of Anadyr in the Northwest Bering Sea; and from Bristol Bay, Alaska, to west of the Pribilof Islands (King, 1983; Rugh et al., 1997). Their mating also occurs at about the time that pups are weaned (Bigg, 1969; Boulva and McLaren, 1979; Burns, 1986).

Migration

They migrate on the Sea of Japan and Okhotsk Sea sides of Hokkaido from November to following May, and their reproductive season is from March to April (Naito and Nishiwaki, 1975; Mizuno et al., 2001). Spotted seal pups retain their whitish woolly lanugo for about 2-4 weeks (Burns, 2002). Individual spotted seals appear to migrate from the coast of Hokkaido to adjacent waters, and most likely spend summers on the coast of Sakhalin. However, it remains unclear where they stay outside of the summer months. In addition, some spotted seals are observed on the Pacific side throughout the year.

Chapter 2

Growth variation in skull morphology of the Kuril harbor seal and the spotted seal around Hokkaido

Introduction

Some morphological studies on the skulls of harbor and spotted seals have been performed. In general, the harbor seal’s skull is more massive, the premolar teeth of adults are mostly obliquely set, the caudal margin of the jugal bone is mostly angular, and the hyoid bones are incomplete (Naito and Nishiwaki, 1975; Shaughnessy and Fay, 1977; Burns et al., 1984). In adult spotted seals, the line of the premolar teeth is straight, the caudal margin of the jugal bone is rounded, and the hyoid bones are complete (Naito and Nishiwaki, 1975; Shaughnessy and Fay, 1977; Burns et al., 1984).

However, some morphological features of each species around Hokkaido are in the process of changing. The cranial measurements of the Kuril harbor seal in Nosappu have downsized in comparison to specimens from 20 years ago (Hata, 2007). Spotted seals had no sexual dimorphism in the 1980s (Uno, 1986); however, it was apparent in specimens from 1997–98 (Mizuno and Ohtaishi, 2001). Although intraspecies variations in the cranial features of each species have been described, the recent interspecies variations between Kuril harbor and spotted seals have not been adequately characterized.

In general, adult skull specimens are used in morphological studies because of their physiological maturity. Both harbor and spotted seal pups have a high mortality rate during the nursing period (Burns et al., 1984); in Hokkaido, the majority of seals that are washed ashore or that die as bycatch are pups (Hokkaido Government, 2006). In the wild, the dead bodies of seals are often putrid or skeletonized, making it problematic to identify these sympatric species by the pelage. Therefore, it is extremely useful to identify these sibling species by their skull characteristics. However, there is a paucity of information regarding the morphological characteristics of the skulls of seal pups and their associated developmental morphological changes.

The aim of this study was to provide detailed information on cranial development in the Kuril harbor seal and the spotted seal around Hokkaido. We examined metric and non-metric characteristics to reveal growth variations and to assess the application of skull characteristics for the identification of the two sibling species in the field.

Materials and methods

Samples

We collected the skulls from 80 Kuril harbor seals and 41 spotted seals. Kuril harbor seals were taken from Nosappu (41 males [M] and 39 females [F]) in 2005. Spotted seals were taken from Nosappu (6M, 2F), Erimo (1M, 1F), Hamamasu (3M, 4F), Yagishiri Island (5M, 2F), and Rausu (9M, 8F) in 2005 and 2006. The locations of the sampling sites are shown in Fig. 2-1. We previously demonstrated that Kuril harbor seals in Hokkaido can be divided into two populations, the Erimo and the eastern Hokkaido populations (Akkeshi and Nosappu), on the basis of mitochondrial DNA (mtDNA) cytochrome *b* region sequences (See Chapter 3). To reduce the biases of genetic populations, we only used specimens of Kuril harbor seals from Nosappu. There are no local populations of spotted seals in Hokkaido (Mizuno et al., 2003), so we collected individuals from the five sampling sites described above. The seals from Nosappu, Erimo, and Hamamasu were bycatch from fishery nets. The seals from Rausu were killed for fishery damage control and those from Yagishiri Is. were dead seals washed ashore. Seal capture was conducted under a license from the Japanese Ministry of the Environment.

Skulls were boiled for 15–20 min and skeletonized, then placed in water containing 10% proteinase (Tasinase N-11-100: Kyowa hakkou kogyo, Tokyo, Japan) at 50°C for 7–8 h and cleaned. The skulls were then bleached white by soaking them in 4% hydrogen peroxide for a few days and dried under natural conditions for two or more days (Hachiya and Ohtaishi, 1994).

Measurements for metric and non-metric cranial characteristics

Metric and non-metric cranial characteristics are shown in Table 2-1 and Figs. 2-2 and 2-3. Twenty-nine metric characteristics were measured for each specimen using calipers to the nearest 0.1 mm, following previous studies (Burns et al., 1984; Mizuno and Ohtaishi, 2001). Six non-metric characteristics were ranked and assigned a numerical score, based on our judgment of its conformity to one of the diagrams in Fig. 2-3. Low scores for these characteristics were features of spotted seals; while, high scores were features of harbor seals (Naito and Nishiwaki, 1975; Burns et al., 1984).

Characteristic I described the shape of the temporozygomatic suture, score 1 indicated a rounder shape, and score 3 indicated more right-angled shape. Characteristic II described the angle of the upper second premolar, score 1 indicated a straight angle along the line of teeth, and score 4 indicated a more slanted shape. Characteristic III described the extent of the nasal-incisive suture, score 1 indicated wide contact, and score 4 indicated no contact. Characteristic IV described the shape of the pterygoid hamulus, score 1 indicated an inward shape, and score 4 indicated an outward shape. Characteristic V described the shape of the bulla, score 1 indicated a less ungular shape, and score 3 indicated a more ungular shape. Characteristic VI described the shape of the nares, score indicated an upper distended shape, and score 3 indicated a lower distended shape. All specimens were measured by one person (E. Nakagawa) to avoid interobserver errors.

Growth class determination

Specimens were grouped by species, sex, and growth class. Three growth classes were defined according to the postnatal developmental stage as described by Uno (1986): pups (0 year), subadults (1–4 years old), and adults (more than 5 years old). These classes were assigned depending on the condylobasal length (CBL) as described in Mizuno and Ohtaishi (2001) and Hata (2007) basically. Furthermore, the data in Uno (1986) were referenced complementarily.

For male Kuril harbor seals, the CBL of pups were less than 187 mm, those of subadults ranged from 187 to 212 mm, and those of adults were more than 212 mm; for female Kuril harbor seals, the CBL of pups were less than 182 mm, those of subadults ranged from 182 to 205 mm, and those of adults were more than 205 mm.

For male spotted seals, the CBL of pups were less than 182 mm, those of subadults ranged from 182 to 210 mm, and those of adults were more than 210 mm; for female spotted seals, the CBL of pups were less than 181 mm, those of subadults ranged from 181 to 200 mm, and those of adults were more than 200 mm.

Statistical analysis

Statistical analyses were carried out using R for Windows version 2.9.1 (R Development Core Team, 2007). Standard statistical values obtained using metric data included the mean, standard deviation (SD), coefficient of variation (CV), and the range of each growth class. The *t*-test was performed to compare sexual differences, and analysis of variance (ANOVA) was used to compare the interspecies differences for each growth class. Principle component analysis (PCA) and canonical discriminant analysis were used in order to investigate interspecies variation for each growth class. Canonical discriminant analysis was performed, with the exception of the CBL because of its artificial bias, and the values of the original variables determined by both analyses were standardized so that each variable had an equal weight. Pearson's chi-square test revealed no sexual differences with regard to the non-metric data; therefore, we combined the male and female data. The frequency of each score was calculated and interspecies differences were investigated using Pearson's chi-square test.

As many of the skulls were partly broken, the full suite of 35 characteristics could not be determined for all specimens. For that reason, sample sizes varied among analyses, depending on which of the characteristics were being compared and the type of statistical treatment employed. The sample sizes are stated in the Tables and Figures.

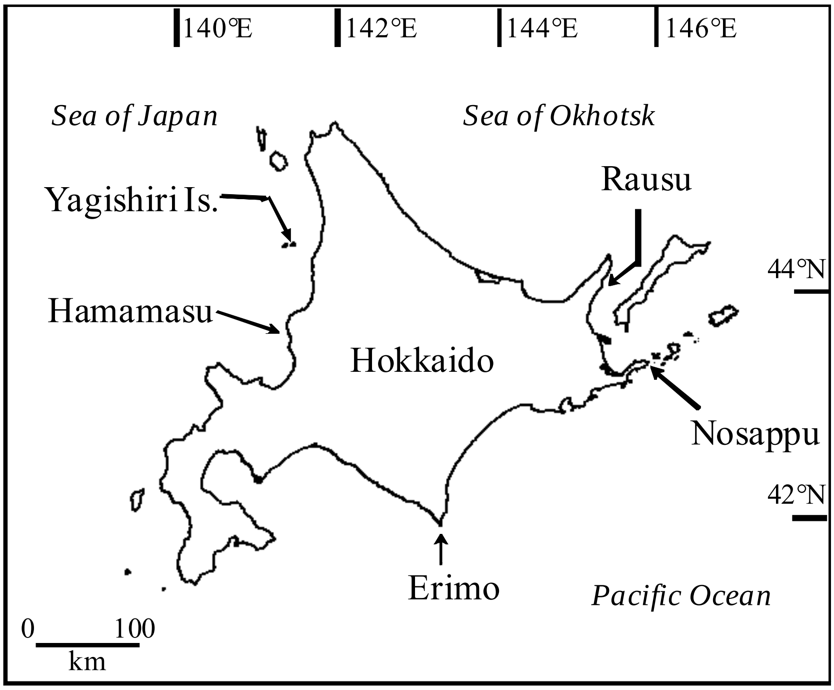

Fig. 2-1 Geographical locations of the sampling sites in Hokkaido, Japan

Table 2-1 Cranial characteristics of Kuril harbor seals and spotted seals used in this study (as shown in Fig.2-2 and 2-3).

No.	Characteristics
Metric characteristics	
1	Condylobasal length
2	Palatal length
3	Upper tooth row length
4	Mastoid width
5	Greatest width of cranium
6	Zygomatic width
7	Cranium height
8	Mandible length
9	Height of mandible at coronoid process
10	Lower tooth row length
11	Height of mandible behind the molar
12	Overall length of nasals
13	Length of maxillo-frontal suture to caudal end of nasals
14	Width of nasals at maxillo-frontal suture
15	Maximal width of external nares
16	Width of snout at canine
17	Least interorbital width
18	Greatest mesial-distal length of second upper premolar
19	Width of palate behind first molars
20	Least width of palate at pterygoid hamulus
21	Width of bulla from notch rostral to auditory process to middle of carotid foramen
22	Length of bulla
23	Width at condyles
24	Foremen magnum width
25	Foremen magnum height
26	Length of snout from rostral edge of nasals
27	Jugal length
28	Width of bulla from tip of auditory process to rostral edge of carotid foramen
29	Width of corocoid process at rostral margin
Non-metric characteristics [1]	
Ⅰ	Shape of temporozygomatic suture (1-3)
Ⅱ	Angle of second upper premolar (1-4)
Ⅲ	Extent of nasal-incisive suture (1-4)
Ⅳ	Shape of pterygoid hamulus (1-4)
Ⅴ	Shape of bulla (1-3)
Ⅵ	Shape of nares (1-3)

[1] The numeral scores of the non-metric characteristics are shown in parentheses.

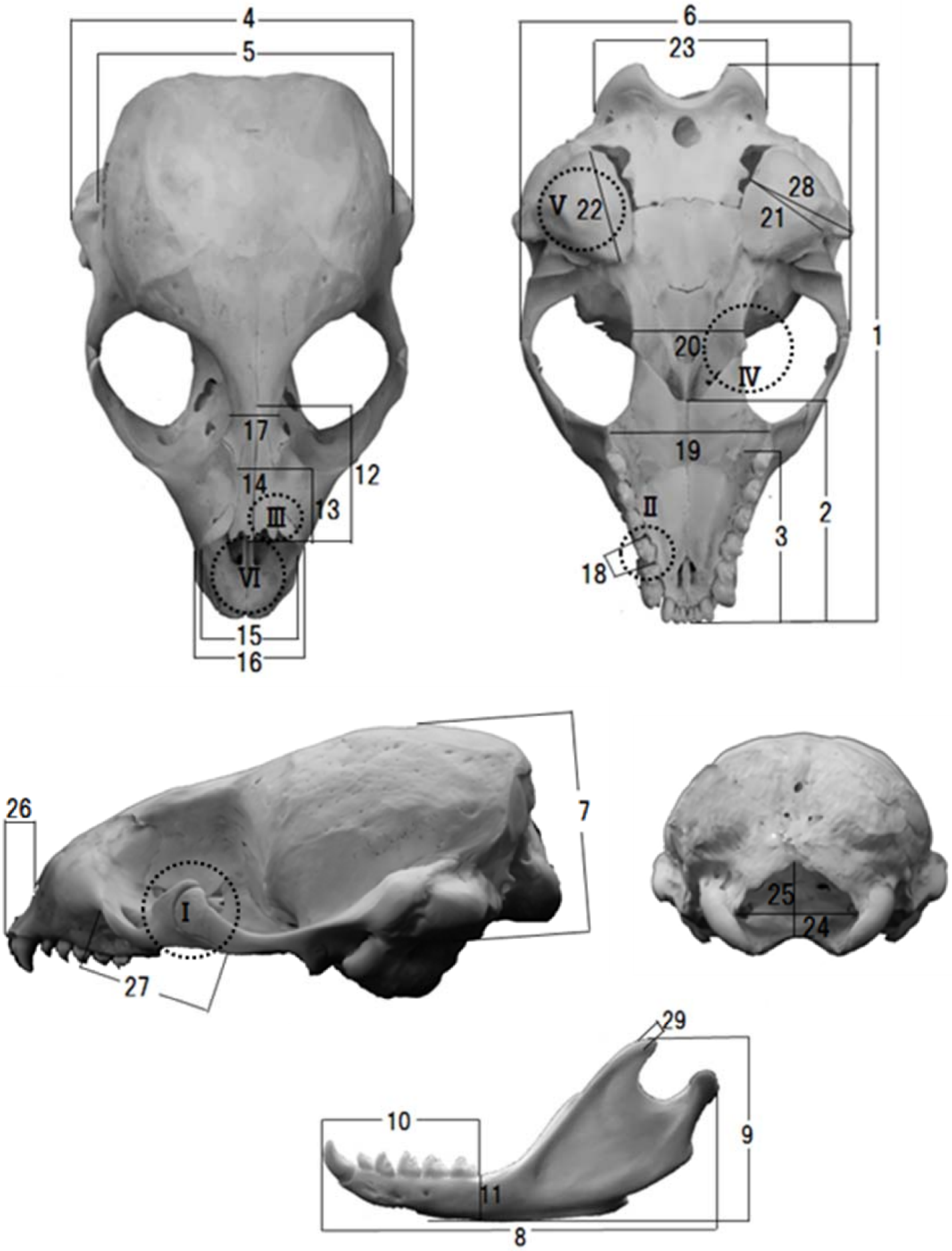

Fig.2-2 Cranial characteristics used in this study (photographs are from the spotted seal). Numbers refer to the characteristics listed in Table 2-1 and circles show the points of non-metric characteristics in Fig. 2-3.

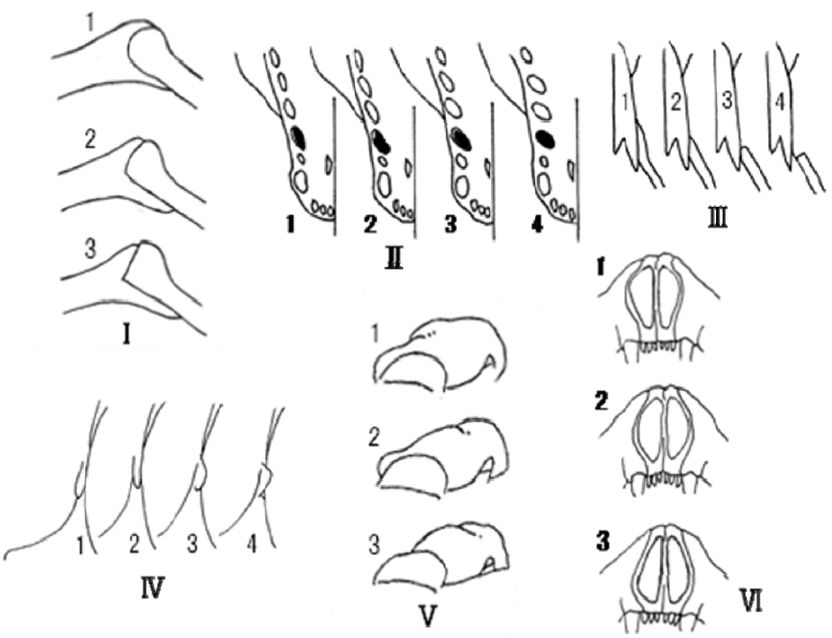

Fig.2-3 Non-metric characteristics of seal skulls used in this study. Roman numerals refer to the characteristics listed in Table 2-1. Illustrations were reformed from a previous study (Burns et al., 1984).

Results

Kuril harbor seals

Twenty-one metric cranial characteristics of Kuril harbor seal pups, 11 characteristics of subadults, and 15 characteristics of adults showed significant sexual differences by the *t*-test (Table 2-2). Characteristics nos. 2, 3, 4, 5, 6, 16, and 20 demonstrated sexual differences in all growth classes, and, among these, nos. 4, 5, 16, and 20 were also accepted as interspecies differences in all growth classes by ANOVA (Table 2-2). In addition, 20 metric cranial characteristics of pups, 25 characteristics of subadults, and 8 characteristics of adults demonstrated interspecies differences (Table 2-2).

PCA for the combined sample from both species was performed because of no prior grouping of material. The first principle component accounted for 91.0% of the total variation, and the second principle component accounted for 2.1%. The scatter plot of the first and second principle components revealed a clear separation among the growth classes between the two species (Fig. 2-4). The first principle component was mainly influenced by metric cranial characteristics nos. 1, 4, 5, 7, 10, 15, 20, 21, 22, 23, 24, and 28 (Table 2-3).

Canonical discriminant analysis was performed using the same samples as the PCA. This analysis correctly classified all Kuril harbor seals, but three female Kuril harbor seal subadults were incorrectly classified as one male pup and two male subadults (Table 2-4). The percentage contribution of the first canonical variate accounted for

69.6% and the second canonical variate accounted for 14.3%. The standardized discriminant coefficients revealed that the variables that contributed to the discrimination between species, were nos. 4, 5, 7, 10, 15, 20, 21, 22, 23, 24, and 28 (Table 2-5). These were the same as the influential characteristics determined by PCA, with the exception of the CBL (no. 1).

Pearson's chi-square test on six non-metric cranial characteristics revealed significant interspecies differences for characteristics I and III (P < 0.05). For characteristics I and III, many of the Kuril harbor seals were ascribed to score 3 (Fig. 2-5). In addition, characteristics I and VI showed the variation in the proportion of growth class associated with the scores (Fig.2-5). For characteristic IV, Kuril harbor seals were equally represented in each score category (Fig. 2-5).

Spotted seals

The spotted seal samples were particularly biased towards pups, and the *t*-test was not used to compare the sexual differences among adult spotted seals because of the small sample size (Table 2-2). Four characteristics of spotted seal pups and 10 characteristics of subadults showed significant sexual differences by the *t*-test ($P<0.05$; Table 2-2).

Canonical discriminant analysis correctly classified each growth class of all spotted seals (Table 2-4). However, one male spotted seal pup was incorrectly classified as female (Table 2-4).

For the non-metric cranial characteristics, I, II, III, and VI, many spotted seals were scored as 1 (Fig. 2-5). On the contrary, the majority of spotted seals scored 4 for characteristic IV (Fig. 2-5).

Table 2-2 Standard statistics for the skull characteristics of Kuril harbor seals and spotted seals.

Characteristic number[1]	Species	Sex	Pup						Subadult						Adult					
			N	Mean ± S.D. (mm)	C.V. (%)	Range (mm)	*t*-test[2]	ANOVA[3]	N	Mean ± S.D. (mm)	C.V. (%)	Range (mm)	*t*-test[2]	ANOVA[3]	N	Mean ± S.D. (mm)	C.V. (%)	Range (mm)	*t*-test[2]	ANOVA[3]
1	Kuril harbor seal	M	8	181.18 ± 4.09	2.26	174.00 – 186.4			20	198.88 ± 5.97	3.00	187.9 – 209.8			13	219.03 ± 7.06	3.22	212.0 – 235.5		
		F	5	172.22 ± 4.26	2.47	165.50 – 178.4			19	195.23 ± 5.16	2.64	186.1 – 203.9			15	213.97 ± 7.22	3.37	206.0 – 229.3		
	Spotted seal	M	19	174.00 ± 5.34	3.07	156.00 – 181.1			4	191.65 ± 4.83	2.52	187.3 – 199.6			1	214.80	–			
		F	13	173.06 ± 4.54	2.62	165.80 – 180.5			2	184.95 ± 0.55	0.30	184.4 – 185.5			2	217.35 ± 2.25	1.04	215.1 – 219.6		
2	Kuril harbor seal	M	8	75.60 ± 1.44	1.90	73.50 – 77.8	*	††	20	85.25 ± 3.02	3.54	79.4 – 90.3	*	††	13	99.03 ± 9.60	9.69	90.0 – 130.0	*	
		F	5	72.44 ± 2.38	3.28	68.80 – 76.0			19	83.05 ± 3.40	4.09	76.0 – 89.0			15	92.79 ± 4.64	5.00	87.6 – 103.1		
	Spotted seal	M	19	71.86 ± 3.17	4.41	66.30 – 80.0			4	81.28 ± 1.35	1.66	79.7 – 83.3			1	92.40	–			
		F	13	70.44 ± 2.69	3.82	63.10 – 75.2			2	75.65 ± 2.25	2.97	73.4 – 77.9			2	94.55 ± 1.05	1.11	93.5 – 95.6		
3	Kuril harbor seal	M	8	61.61 ± 1.33	2.16	59.8 – 63.1	*	†††	20	67.23 ± 2.52	3.74	63.2 – 72.3	*	†††	13	74.93 ± 2.86	3.81	71.9 – 80.6	**	
		F	5	58.78 ± 1.99	3.38	55.8 – 60.9			19	65.72 ± 2.25	3.43	61.4 – 70.7			15	71.53 ± 2.34	3.27	68.2 – 75.8		
	Spotted seal	M	19	55.85 ± 4.54	8.13	51.8 – 73.5			4	60.58 ± 1.08	1.78	59.3 – 62.0	*		1	70.00	–			
		F	13	54.36 ± 2.47	4.54	49.7 – 59.7			2	58.30 ± 0.30	0.51	58.0 – 58.6			2	72.30 ± 0.50	0.69	71.8 – 72.8		
4	Kuril harbor seal	M	8	116.80 ± 2.43	2.08	112.3 – 120.3	**	†††	20	124.24 ± 3.60	2.90	117.4 – 132.3	**	†††	13	133.84 ± 3.48	2.60	127.6 – 140.4	**	††
		F	5	110.90 ± 1.91	1.72	108.6 – 113.9			19	121.56 ± 3.06	2.52	114.9 – 127.4			15	129.55 ± 4.01	3.10	124.5 – 137.1		
	Spotted seal	M	19	108.29 ± 3.40	3.14	98.3 – 115.3			4	116.00 ± 1.83	1.58	113.2 – 118.2	*		1	120.50	–			
		F	13	106.98 ± 2.62	2.44	103.7 – 111.6			2	109.60 ± 1.70	1.55	107.9 – 111.3			2	122.90 ± 0.50	0.41	122.4 – 123.4		
5	Kuril harbor seal	M	8	94.78 ± 1.58	1.67	92.2 – 97.4	**	†††	20	98.05 ± 2.13	2.18	95.1 – 102.4	*	†††	13	100.45 ± 2.15	2.14	97.3 – 104.3	**	†††
		F	5	92.28 ± 1.10	1.19	90.9 – 93.5			19	96.85 ± 1.57	1.62	92.9 – 99.5			15	97.61 ± 2.35	2.41	94.3 – 103.8		
	Spotted seal	M	18	89.16 ± 2.58	2.89	83.5 – 94.5			4	91.10 ± 1.49	1.64	89.9 – 93.6			1	94.00	–			
		F	13	88.61 ± 1.76	1.99	85.5 – 90.9			2	88.45 ± 1.25	1.41	87.2 – 89.7			2	92.00 ± 1.60	1.74	90.4 – 93.6		
6	Kuril harbor seal	M	8	110.79 ± 1.81	1.64	107.4 – 113.3	***	†††	20	120.60 ± 4.52	3.75	111.9 – 132.3	*	†††	13	134.47 ± 3.39	2.52	128.2 – 142.4	*	
		F	5	104.20 ± 1.81	1.73	102.8 – 107.7			19	117.51 ± 3.50	2.98	108.4 – 124.1			15	128.73 ± 7.93	6.16	119.2 – 149.8		
	Spotted seal	M	19	101.36 ± 3.16	3.12	91.0 – 105.0			4	111.28 ± 2.96	2.66	108.4 – 115.9	*		1	126.40	–			
		F	13	100.62 ± 2.46	2.45	95.8 – 106.3			2	104.10 ± 0.10	0.10	104.0 – 104.2			2	134.25 ± 2.55	1.90	131.7 – 136.8		
7	Kuril harbor seal	M	8	68.24 ± 2.25	3.30	64.9 – 72.0	*	†††	20	68.83 ± 2.85	4.14	64.2 – 74.7		†††	13	71.71 ± 2.02	2.82	68.5 – 75.6	*	
		F	5	64.66 ± 2.64	4.08	59.5 – 66.7			19	67.57 ± 2.44	3.61	64.4 – 73.7			15	69.95 ± 2.74	3.91	64.2 – 74.0		
	Spotted seal	M	19	63.63 ± 2.69	4.23	59.1 – 68.0			4	63.45 ± 1.50	2.37	61.4 – 65.6			1	70.80	–			
		F	13	62.18 ± 2.84	4.57	54.7 – 66.3			2	62.05 ± 0.85	1.37	61.2 – 62.9			2	69.90 ± 3.10	4.43	66.8 – 73.0		
8	Kuril harbor seal	M	8	117.43 ± 2.07	1.77	113.3 – 119.5	***	†††	20	129.81 ± 4.82	3.71	119.3 – 137.8		††	11	146.50 ± 5.01	3.42	140.6 – 158.7	*	
		F	5	110.96 ± 2.02	1.82	108.8 – 114.4			19	128.23 ± 4.32	3.37	116.7 – 134.0			14	142.46 ± 5.59	3.92	135.8 – 155.4		
	Spotted seal	M	19	110.41 ± 3.30	2.99	100.7 – 115.7			4	122.08 ± 2.78	2.28	118.0 – 125.8	*		1	141.70	–			
		F	13	108.96 ± 3.19	2.92	102.7 – 114.0			2	118.20 ± 0.20	0.17	118.0 – 118.4			2	143.60 ± 2.00	1.39	141.6 – 145.6		
9	Kuril harbor seal	M	8	48.38 ± 2.26	4.66	45.2 – 52.2		†††	20	54.38 ± 3.55	6.52	48.4 – 65.2		††	13	64.35 ± 3.86	6.00	58.6 – 70.8		
		F	5	46.38 ± 1.89	4.08	44.8 – 49.6			19	52.81 ± 2.38	4.52	49.6 – 57.4			15	62.00 ± 6.02	9.71	55.2 – 72.2		
	Spotted seal	M	19	44.78 ± 2.01	4.48	39.0 – 47.7			3	50.37 ± 0.26	0.52	50.0 – 50.6			1	65.00	–			
		F	13	44.10 ± 1.85	4.20	41.6 – 47.4			2	46.90 ± 2.20	4.69	44.7 – 49.1			2	65.60 ± 1.90	2.90	63.7 – 67.5		
10	Kuril harbor seal	M	8	56.38 ± 0.77	1.36	55.5 – 57.7	***	†††	19	59.40 ± 1.81	3.05	56.3 – 63.5		†††	11	64.75 ± 1.65	2.55	62.7 – 68.0		
		F	5	52.92 ± 1.18	2.24	51.3 – 54.9			17	58.09 ± 3.24	5.58	50.5 – 66.9			14	61.29 ± 1.60	2.62	59.8 – 64.7		
	Spotted seal	M	19	51.23 ± 1.50	2.93	48.0 – 54.5	*		4	54.00 ± 0.57	1.06	53.1 – 54.7			1	60.80	–			
		F	13	50.05 ± 1.93	3.86	47.4 – 54.1			2	52.65 ± 0.55	1.04	52.1 – 53.2			1	61.10	–			

[1] Characteristic numbers are referred to Table 1.

[2] Significant differences between the sexes by the *t*-test (***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$).

[3] Significant differences between the species by ANOVA (†††, $P < 0.001$; ††, $P < 0.01$; †, $P < 0.05$).

Table 2-2 Continued.

Characteristic number[1]	Species	Sex	Pup						Subadult						Adult					
			N	Mean ± S.D. (mm)	C.V. (%)	Range (mm)	*t*-test[2]	ANOVA[3]	N	Mean ± S.D. (mm)	C.V. (%)	Range (mm)	*t*-test[2]	ANOVA[3]	N	Mean ± S.D. (mm)	C.V. (%)	Range (mm)	*t*-test[2]	ANOVA[3]
11	Kuril harbor seal	M	8	18.04 ± 1.14	6.31	16.2 – 19.8	*	†††	20	19.97 ± 1.18	5.89	18.0 – 22.5	**	†††	13	23.62 ± 1.23	5.20	21.3 – 26.2		
		F	5	16.40 ± 1.16	7.05	15.2 – 18.5			18	18.82 ± 1.15	6.12	17.1 – 20.6			15	21.22 ± 1.57	7.42	19.3 – 25.3		
	Spotted seal	M	19	15.06 ± 1.09	7.22	13.6 – 17.7	*		4	16.50 ± 0.87	5.30	15.5 – 17.9			1	19.90	–			
		F	13	14.17 ± 0.92	6.52	13.1 – 16.7			2	16.25 ± 0.05	0.31	16.2 – 16.3			2	20.65 ± 0.45	2.18	20.2 – 21.1		
12	Kuril harbor seal	M	8	42.63 ± 1.68	3.95	40.3 – 45.2	***		20	46.36 ± 2.96	6.39	40.7 – 51.0		†	13	53.38 ± 2.88	5.40	48.1 – 59.8		
		F	5	38.12 ± 1.16	3.04	36.5 – 39.7			19	45.87 ± 3.30	7.20	39.8 – 51.0			15	51.53 ± 5.07	9.83	47.0 – 66.6		
	Spotted seal	M	19	40.17 ± 2.02	5.04	36.1 – 43.5			4	43.10 ± 2.77	6.44	39.8 – 47.1			1	48.60	–			
		F	13	39.69 ± 3.22	8.10	30.7 – 43.1			2	39.95 ± 1.85	4.63	38.1 – 41.8			2	48.75 ± 3.65	7.49	45.1 – 52.4		
13	Kuril harbor seal	M	8	22.10 ± 1.21	5.49	20.3 – 24.0	**		20	23.09 ± 1.52	6.60	19.8 – 25.3			13	27.25 ± 1.55	5.70	25.4 – 31.1		
		F	5	19.96 ± 0.94	4.68	18.5 – 21.2			19	23.03 ± 2.16	9.39	19.0 – 28.0			15	26.01 ± 3.28	12.63	21.9 – 34.5		
	Spotted seal	M	19	21.97 ± 1.14	5.21	19.6 – 24.2			4	24.68 ± 2.52	10.21	21.2 – 27.9			1	30.20	–			
		F	13	21.35 ± 1.98	9.29	16.0 – 24.3			2	21.05 ± 2.15	10.21	18.9 – 23.2			2	25.45 ± 2.45	9.63	23.0 – 27.9		
14	Kuril harbor seal	M	8	10.45 ± 1.09	10.42	8.9 – 12.1			20	12.17 ± 1.06	8.75	10.2 – 14.2	*		13	14.33 ± 1.05	7.33	11.8 – 15.8		
		F	5	9.88 ± 0.84	8.55	9.0 – 11.4			19	11.36 ± 1.10	9.67	9.4 – 13.7			15	13.49 ± 2.32	17.22	8.1 – 19.0		
	Spotted seal	M	19	10.41 ± 0.78	7.47	9.3 – 12.0			4	11.25 ± 1.06	9.46	10.0 – 12.7			1	14.60	–			
		F	13	10.34 ± 1.00	9.68	8.8 – 12.5			2	10.60 ± 0.80	7.55	9.8 – 11.4			2	13.85 ± 0.75	5.42	13.1 – 14.6		
15	Kuril harbor seal	M	8	25.04 ± 1.09	4.36	23.5 – 26.7			20	27.18 ± 1.58	5.81	24.6 – 30.2		†	13	30.56 ± 0.98	3.22	28.1 – 32.3	*	
		F	5	24.22 ± 0.28	1.15	23.9 – 24.7			19	27.00 ± 1.24	4.60	24.6 – 29.0			15	29.69 ± 1.51	5.09	26.3 – 31.7		
	Spotted seal	M	19	24.17 ± 1.15	4.75	22.0 – 26.0			4	26.73 ± 1.20	4.49	24.8 – 28.1	**		1	29.70	–			
		F	13	24.56 ± 1.48	6.01	21.4 – 26.1			2	23.95 ± 0.25	1.04	23.7 – 24.2			2	28.10 ± 0.10	0.36	28.0 – 28.2		
16	Kuril harbor seal	M	8	37.09 ± 1.40	3.78	35.4 – 40.0	**	†††	20	40.53 ± 1.92	4.73	35.5 – 45.0	**	†††	13	45.52 ± 2.12	4.66	41.8 – 48.9	**	†
		F	5	34.36 ± 1.03	2.99	32.8 – 35.6			19	38.90 ± 1.40	3.59	37.0 – 42.2			15	42.37 ± 2.97	7.02	39.4 – 49.8		
	Spotted seal	M	19	30.56 ± 0.99	3.22	29.0 – 32.2			4	32.05 ± 0.39	1.22	31.5 – 32.6	*		1	39.80	–			
		F	13	30.00 ± 3.05	10.16	22.0 – 36.6			2	29.65 ± 0.45	1.52	29.2 – 30.1			2	38.50 ± 2.70	7.01	35.8 – 41.2		
17	Kuril harbor seal	M	8	14.53 ± 1.22	8.43	11.6 – 15.7	*	†	20	15.14 ± 1.00	6.62	13.1 – 16.8		†††	13	17.16 ± 0.91	5.33	15.2 – 18.7	**	
		F	5	12.90 ± 0.94	7.26	11.6 – 14.3			19	13.57 ± 0.90	6.66	11.8 – 16.0			15	15.39 ± 1.94	12.61	13.1 – 20.0		
	Spotted seal	M	19	13.15 ± 0.97	7.35	10.9 – 14.8			4	12.63 ± 1.85	14.65	10.0 – 15.0			1	16.90	–			
		F	13	12.92 ± 1.35	10.46	10.8 – 15.1			2	11.10 ± 0.60	5.41	10.5 – 11.7			2	15.30 ± 0.20	1.31	15.1 – 15.5		
18	Kuril harbor seal	M	8	9.68 ± 0.24	2.52	9.3 – 10.0	**	†††	20	10.06 ± 0.64	6.32	9.0 – 11.5	**	†††	13	10.17 ± 0.41	4.03	9.4 – 10.8		
		F	5	8.94 ± 0.31	3.51	8.4 – 9.3			19	9.45 ± 0.85	9.00	6.6 – 10.6			15	9.82 ± 0.72	7.29	8.8 – 11.8		
	Spotted seal	M	19	8.37 ± 0.59	7.06	7.5 – 9.6	**		4	8.43 ± 0.45	5.33	7.8 – 8.9	**		1	9.20	–			
		F	13	7.60 ± 0.75	9.89	6.0 – 8.9			2	6.90 ± 0.10	1.45	6.8 – 7.0			2	10.15 ± 0.45	4.43	9.7 – 10.6		
19	Kuril harbor seal	M	8	51.74 ± 1.65	3.18	48.6 – 53.8	**	†††	20	55.75 ± 1.84	3.30	51.5 – 58.0		†††	13	61.52 ± 1.52	2.46	59.0 – 64.0	**	†
		F	5	48.76 ± 1.52	3.12	47.2 – 50.7			19	55.47 ± 1.73	3.12	51.9 – 58.6			15	58.65 ± 3.73	6.36	53.4 – 66.5		
	Spotted seal	M	19	45.23 ± 2.51	5.55	39.5 – 50.2			4	49.68 ± 2.23	4.50	47.2 – 52.5	*		1	51.40	–			
		F	13	44.86 ± 2.37	5.28	42.0 – 51.4			2	45.70 ± 0.60	1.31	45.1 – 46.3			2	56.20 ± 0.20	0.36	56.0 – 56.4		
20	Kuril harbor seal	M	8	36.70 ± 1.57	4.27	34.1 – 38.7	**	†††	20	38.67 ± 1.48	3.84	34.9 – 41.5	*	†††	13	41.56 ± 1.54	3.71	38.1 – 43.8	**	††
		F	5	34.20 ± 1.29	3.77	32.0 – 35.9			19	37.77 ± 1.31	3.47	35.0 – 41.5			15	38.99 ± 2.65	6.79	32.0 – 42.5		
	Spotted seal	M	19	33.07 ± 1.16	3.51	30.0 – 35.5			4	34.10 ± 1.02	3.00	33.2 – 35.8			1	35.00	–			
		F	13	32.38 ± 1.66	5.11	29.9 – 36.5			2	33.25 ± 0.25	0.75	33.0 – 33.5			2	35.00 ± 1.00	2.86	34.0 – 36.0		

[1] Characteristic numbers are referred to Table 1.

[2] Significant differences between the sexes by the *t*-test (***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$).

[3] Significant differences between the species by ANOVA (†††, $P < 0.001$; ††, $P < 0.01$; †, $P < 0.05$).

Table 2-2 Continued.

No.[1]	Species	Sex	Pup						Subadult						Adult					
			N	Mean ± S.D. (mm)	C.V. (%)	Range (mm)	*t*-test[2]	ANOVA[3]	N	Mean ± S.D. (mm)	C.V. (%)	Range (mm)	*t*-test[2]	ANOVA[3]	N	Mean ± S.D. (mm)	C.V. (%)	Range (mm)	*t*-test[2]	ANOVA[3]
21	Kuril harbor seal	M	8	29.26 ± 3.39	11.57	24.0 – 33.8			20	31.20 ± 1.19	3.81	29.6 – 34.0	*	†††	13	32.05 ± 1.51	4.72	29.6 – 34.8	*	††
		F	5	29.34 ± 0.91	3.11	28.2 – 30.3			19	30.92 ± 1.11	3.60	28.8 – 33.4			15	30.77 ± 1.72	5.60	27.7 – 34.6		
	Spotted seal	M	19	28.27 ± 1.40	4.96	25.6 – 30.4			4	28.58 ± 1.79	6.25	26.7 – 31.1			1	25.00	–			
		F	13	27.87 ± 1.44	5.16	25.4 – 30.0			2	27.65 ± 0.15	0.54	27.5 – 27.8			2	29.50 ± 0.10	0.34	29.4 – 29.6		
22	Kuril harbor seal	M	8	39.65 ± 0.81	2.04	38.1 – 40.8	*		20	41.14 ± 1.40	3.40	38.1 – 43.5		†	13	42.77 ± 1.24	2.90	40.8 – 44.5	*	
		F	5	37.24 ± 1.61	4.33	35.0 – 39.8			19	40.11 ± 1.46	3.65	36.2 – 42.2			15	41.48 ± 1.60	3.86	38.6 – 44.6		
	Spotted seal	M	19	37.97 ± 1.68	4.44	35.7 – 42.1			4	39.55 ± 0.93	2.35	38.1 – 40.5			1	41.00	–			
		F	13	37.77 ± 1.31	3.48	35.7 – 39.9			2	37.60 ± 1.10	2.93	36.5 – 38.7			2	40.55 ± 1.25	3.08	39.3 – 41.8		
23	Kuril harbor seal	M	8	59.60 ± 2.92	4.90	53.3 – 63.4		††	20	59.60 ± 1.65	2.76	56.9 – 63.2			13	61.03 ± 1.99	3.26	58.0 – 64.9		
		F	5	57.24 ± 1.72	3.00	55.1 – 59.6			19	57.86 ± 5.04	8.72	40.0 – 64.2			15	60.29 ± 2.34	3.89	56.3 – 64.0		
	Spotted seal	M	19	56.69 ± 2.03	3.58	52.3 – 60.5			4	58.18 ± 1.18	2.04	57.2 – 60.2	*		1	61.00	–			
		F	13	55.85 ± 2.19	3.92	53.1 – 61.4			2	55.65 ± 0.65	1.17	55.0 – 56.3			2	60.85 ± 0.55	0.90	60.3 – 61.4		
24	Kuril harbor seal	M	8	35.33 ± 1.34	3.79	32.6 – 36.9	*	†††	20	33.79 ± 1.67	4.95	31.7 – 37.6		†††	13	33.38 ± 2.20	6.58	30.7 – 38.4		
		F	5	34.22 ± 0.62	1.81	33.2 – 35.0			19	34.07 ± 1.69	4.96	31.3 – 36.8			15	32.93 ± 1.46	4.43	29.6 – 34.9		
	Spotted seal	M	19	31.49 ± 1.70	5.40	28.6 – 35.0	*		4	30.70 ± 1.19	3.87	29.0 – 32.2			1	33.10	–			
		F	13	29.82 ± 2.34	7.84	24.9 – 34.4			2	31.50 ± 0.90	2.86	30.6 – 32.4			2	30.05 ± 1.55	5.16	28.5 – 31.6		
25	Kuril harbor seal	M	8	28.94 ± 1.63	5.64	26.9 – 31.7		†††	20	27.96 ± 2.35	8.40	24.5 – 32.3		†††	13	27.70 ± 2.07	7.48	23.4 – 31.9		††
		F	5	27.22 ± 1.56	5.74	25.0 – 29.4			19	27.72 ± 1.84	6.64	24.8 – 31.3			15	26.49 ± 1.36	5.15	23.4 – 29.0		
	Spotted seal	M	19	24.03 ± 2.01	8.37	20.3 – 27.9			4	22.95 ± 1.49	6.48	21.5 – 25.4			1	25.70	–			
		F	13	24.57 ± 3.40	13.85	20.3 – 34.5			2	22.25 ± 1.15	5.17	21.1 – 23.4			2	22.35 ± 0.05	0.22	22.3 – 22.4		
26	Kuril harbor seal	M	8	25.63 ± 1.12	4.35	23.7 – 27.3			20	30.10 ± 1.34	4.46	28.0 – 32.8		†	13	34.85 ± 2.58	7.40	31.9 – 40.2		
		F	5	24.66 ± 1.10	4.48	22.5 – 25.6			19	29.82 ± 1.64	5.50	27.0 – 33.5			15	33.72 ± 1.56	4.63	31.6 – 37.6		
	Spotted seal	M	19	25.09 ± 1.83	7.30	21.3 – 27.3			4	28.83 ± 0.71	2.46	27.8 – 29.8			1	36.00	–			
		F	13	24.54 ± 1.79	7.30	21.6 – 27.5			2	26.80 ± 1.30	4.85	25.5 – 28.1			2	37.25 ± 0.05	0.13	37.2 – 37.3		
27	Kuril harbor seal	M	8	48.05 ± 1.34	2.78	46.0 – 50.2	*	†††	20	53.36 ± 2.61	4.89	46.8 – 58.5		†††	13	60.91 ± 2.48	4.07	58.2 – 66.7		
		F	5	46.74 ± 0.84	1.80	45.8 – 47.9			19	52.61 ± 2.58	4.90	46.4 – 56.8			15	59.07 ± 3.83	6.49	53.8 – 68.4		
	Spotted seal	M	19	44.53 ± 2.11	4.74	40.3 – 48.1			4	48.68 ± 0.49	1.01	48.1 – 49.3			1	57.30	–			
		F	13	42.99 ± 2.73	6.35	38.0 – 48.7			2	45.95 ± 1.85	4.03	44.1 – 47.8			2	60.90 ± 0.00	0.00	60.9 – 60.9		
28	Kuril harbor seal	M	8	39.83 ± 1.89	4.75	35.6 – 42.6	**		20	42.20 ± 1.06	2.52	39.8 – 44.1		†	13	46.05 ± 1.55	3.36	43.9 – 49.6	**	†††
		F	5	37.28 ± 0.75	2.02	36.2 – 38.4			19	42.69 ± 4.24	9.92	39.6 – 59.7			15	44.08 ± 1.81	4.11	41.3 – 48.6		
	Spotted seal	M	19	38.35 ± 1.30	3.38	35.0 – 40.1			4	39.73 ± 1.37	3.45	38.2 – 41.5			1	38.50	–			
		F	13	37.75 ± 1.79	4.74	32.2 – 39.8			2	38.95 ± 1.05	2.70	37.9 – 40.0			2	41.15 ± 0.15	0.36	41.0 – 41.3		
29	Kuril harbor seal	M	8	3.26 ± 0.47	14.29	2.5 – 3.9	*	†	20	3.97 ± 0.73	18.49	2.7 – 6.3		†††	13	5.90 ± 1.05	17.83	4.4 – 8.1		
		F	5	2.68 ± 0.50	18.79	2.2 – 3.5			19	4.06 ± 0.75	18.35	2.5 – 5.6			15	5.59 ± 1.19	21.23	3.8 – 8.2		
	Spotted seal	M	19	2.55 ± 0.45	17.48	1.7 – 3.2			3	2.90 ± 0.16	5.63	2.7 – 3.1	*		1	5.90	–			
		F	13	2.68 ± 0.61	22.81	2.3 – 4.7			2	2.15 ± 0.15	6.98	2.0 – 2.3			2	4.65 ± 0.45	9.68	4.2 – 5.1		

[1] Characteristic numbers are referred to Table 1.

[2] Significant differences between the sexes by the *t*-test (***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$).

[3] Significant differences between the species by ANOVA (†††, $P < 0.001$; ††, $P < 0.01$; †, $P < 0.05$).

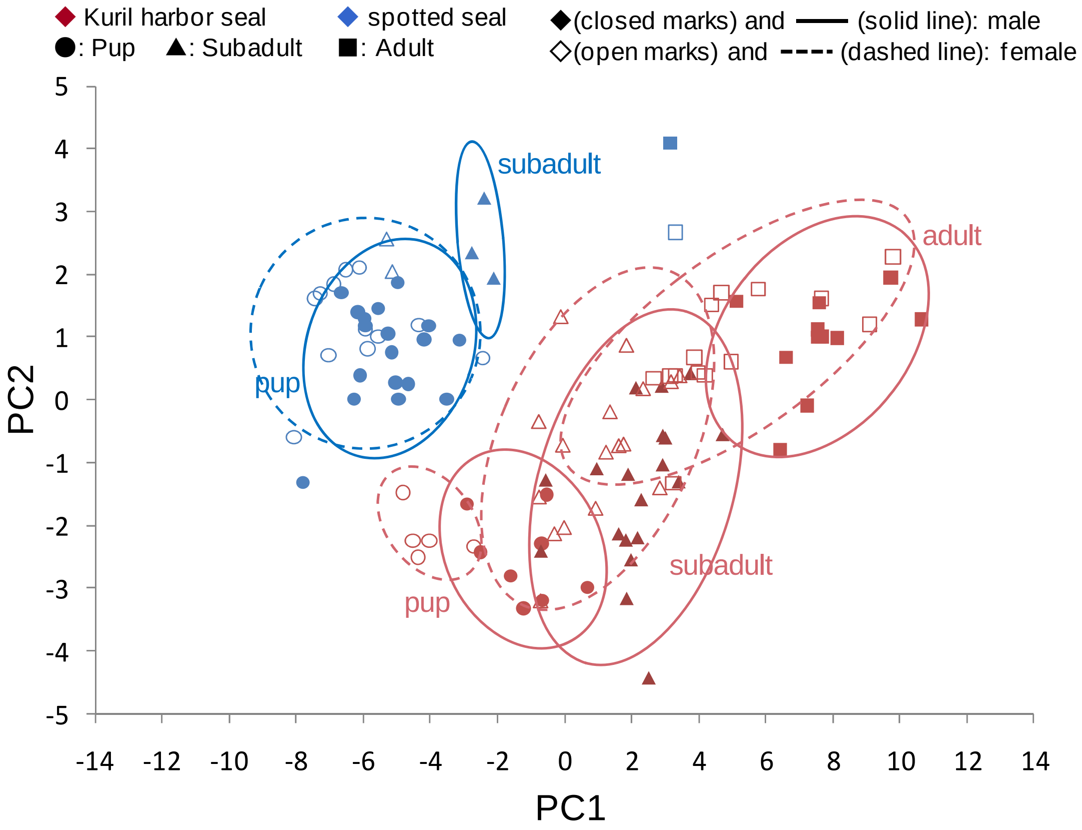

Fig.2-4 Scatter plots of the first principle component (PC1) and the second principle component (PC2). Circles show the probability ellipsoid 95% of plotted values for each species, sex, and growth class.

Table 2-3 Factor matrix from the 29 value principle component analyses of both the Kuril harbor seal and the spotted seal specimens.

Characteristic number	First Principle component	Standardized first principle component
1	0.212	11.060
2	0.206	7.868
3	0.209	8.671
4	0.215	12.299
5	0.190	20.317
6	0.214	9.273
7	0.175	16.486
8	0.213	9.106
9	0.209	6.863
10	0.209	11.211
11	0.209	5.727
12	0.197	8.341
13	0.153	8.614
14	0.165	6.172
15	0.197	10.352
16	0.209	6.518
17	0.164	7.754
18	0.163	8.252
19	0.210	8.383
20	0.198	10.998
21	0.137	13.887
22	0.181	18.226
23	0.116	17.788
24	0.075	13.975
25	0.091	9.205
26	0.197	6.887
27	0.208	7.775
28	0.172	11.572
29	0.185	2.592

[1] Characteristic numbers are referred to Table 2-1.

Table 2-4 Numbers of specimens that predicted the species, sex, and growth class[1] by discriminant analysis.

Original			Predicted											
			Kuril harbor seal						Spotted seal					
Species	Sex	Growth	Male			Female			Male			Female		
			P	S	A	P	S	A	P	S	A	P	S	A
Kuril harbor seal	Male	P	8	0	0	0	0	0	0	0	0	0	0	0
		S	0	19	0	0	0	0	0	0	0	0	0	0
		A	0	0	11	0	0	0	0	0	0	0	0	0
	Female	P	0	0	0	5	0	0	0	0	0	0	0	0
		S	1	2	0	0	14	0	0	0	0	0	0	0
		A	0	0	0	0	0	14	0	0	0	0	0	0
Spotted seal	Male	P	0	0	0	0	0	0	17	0	0	1	0	0
		S	0	0	0	0	0	0	0	3	0	0	0	0
		A	0	0	0	0	0	0	0	0	1	0	0	0
	Female	P	0	0	0	0	0	0	0	0	0	12	0	0
		S	0	0	0	0	0	0	0	0	0	0	2	0
		A	0	0	0	0	0	0	0	0	0	0	0	1

[1] Pup(P), Subadult(S), and Adult(A)

Table 2-5 Discriminant coefficients and standardized discriminant coefficients from discriminant analysis.

Characteristic[1] number	Discriminant coefficient	Standardized discriminant coefficient	Characteristic[1] number	Discriminant coefficient	Standardized discriminant coefficient
2	0.056	7.882	**16**	-0.128	6.577
3	-0.138	8.718	**17**	0.032	7.827
4	-0.097	12.331	**18**	0.039	8.365
5	-0.035	20.365	**19**	0.098	8.400
6	-0.074	9.297	**20**	-0.051	11.073
7	-0.026	16.536	**21**	-0.020	13.960
8	-0.209	9.137	**22**	0.284	18.179
9	0.174	6.868	**23**	0.077	17.800
10	-0.107	11.273	**24**	-0.334	14.149
11	-0.451	5.934	**25**	-0.031	9.248
12	0.000	8.378	**26**	0.050	6.922
13	0.220	8.589	**27**	-0.003	7.807
14	0.100	6.207	**28**	0.020	11.615
15	0.305	10.310	**29**	-0.061	2.767

[1] Characteristic numbers are referred to Table 2-1.

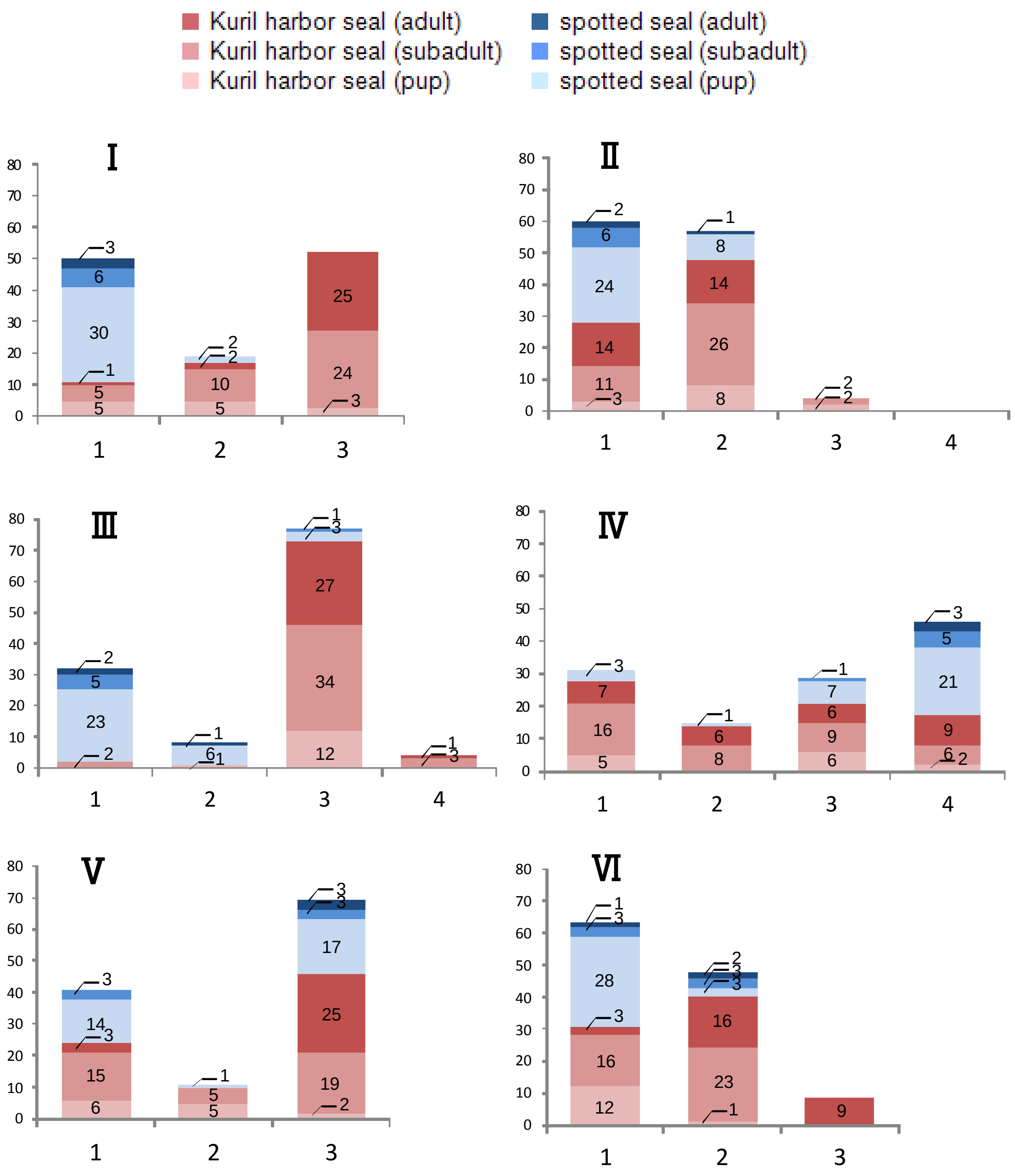

Fig. 2-5 Frequencies of each numeric score for non-metric characteristics. The numbers of specimens show on each bar.

Discussion

Kuril harbor seals

Sexual differences in the metric cranial characteristics identified by the *t*-test were more likely to be detected in each growth class of Kuril harbor seals than of spotted seals. This feature has been observed by other studies (Naito and Nishiwaki, 1975; Shaughnessy and Fay, 1977; Burns et al., 1984; Uno, 1986) and is probably a result of this species' polygamy by competing for females at limited haul-out sites (Clutton-Brock et al., 1982). This sexual dimorphism of the Kuril harbor seal was observed not only in adults but also in pups and subadults. The numbers of Kuril harbor seals have increased, but their haul-out sites have decreased over the last two decades (Hokkaido Government, 2006); therefore, their density at haul-out sites has grown. This density effect may be related to sexual dimorphism. The cerebral cranium and supramaxilla sizes of male Kuril harbor seals were larger than those of females; additionally, they were also larger in Kuril harbor seals than in spotted seals as determined with ANOVA. The body length of the Kuril harbor seal is larger than the spotted seal (Naito and Nishiwaki, 1975), and the skull sizes of the two seals are reflected by their body sizes.

PCA was performed using pooled data from both species, and the first principle component accounted for a very high proportion (91.0%). The measurements, which could be used to determine the whole size of the skull (characteristic nos. 1, 4, 5, 7, 20, 23, and 24), the rostrum (no. 15), mandible (no. 10), and auditory bulla (nos. 21, 22, and 28) influenced the growth classes of both seals. The growth of certain metric data was associated with that of the other data, and we could not identify the best characteristic with which to effectively identify either species.

In canonical discriminant analysis, only two specimens were discriminated incorrectly with regard to the sex and growth class; furthermore, the species of all specimens were discriminated correctly. Consequently, it is possible to almost exactly place any unknown specimen in their species, sex, and growth class with the cranial data set generated in this study.

When the shape of the temporozygomatic suture (characteristic I) and the extent of the nasal-incisive suture (Characteristic III) were significantly different between Kuril harbor and spotted seals in Pearson's chi-square test, the shape of the temporozygomatic suture and the shape of nares (characteristic VI) were indicators of the growth class of Kuril harbor seals. The differences in non-metric characteristics between the two seals became clear with the growth variation of the Kuril harbor seal.

Spotted seals

Although ANOVA revealed interspecies differences between the two seals for many metric characteristics of pups and subadults, we observed fewer differences for adults. Adult spotted seals were remarkably large in this study; besides, the number of subadult and adult spotted seals was biased and inadequate for a definitive conclusion about the growth variation in their skull morphology.

Though the spotted seal had few sexual differences in skull morphology (Burns et al., 1984; Uno, 1986), differences were recognized for some metric characteristics by the *t*-test. The measurements, which could determine the width of the skull (characteristics nos. 4 and 6), foremen magnum (nos. 23 and 24), the size of the supramaxilla (nos. 3, 15, and 16), mandible (nos. 8, 10, 11, and 29), and second upper premolar (no. 18) showed sexual differences among spotted seal pups and subadults.

The non-metric characteristics of the spotted seal did not have as much growth variation as was observed in the Kuril harbor seal. Alternatively, we obtained different results for the shapes of the pterygoid hamulus (characteristic IV) of both seals as compared to those obtained in a previous study (Burns et al., 1984), in particular, this was a confirmed characteristic (score 4) in the majority of spotted seals. The pterygoid hamulus is related to the motion of the mandible. The sexual differentiation of masticatory function was also observed in the metric cranial characteristics. The increase in the number of spotted seals migrating to the Sea of Japan (Hokkaido Government, 2006) could be responsible for this morphological variation in masticatory function, which differed from that observed in previous studies (Burns et al., 1984; Uno, 1986), and may be related to the variation of prey species. To clarify the variation in masticatory function and the growth variation in their skull morphology, we need to collect a larger number of specimens from a range of sites.

Although non-metric cranial characteristics have a lower discriminating power than metric characteristics (Burns et al., 1986; Perrin et al., 1994), they are easy to use in the field and can be used by inexperienced researchers. It is possible to identify the Kuril harbor seal or the spotted seal with considerable accuracy on the basis of the two non-metric characteristics (characteristics I and III). These findings will be very useful to identify the species and growth class of unknown seal skulls in the field, where the two species are sympatric.

The characteristics of Kuril harbor seals were confirmed with those of other harbor seal subspecies, and the morphological differences between the Kuril harbor seal and the spotted seal corresponded with those observed in a previous study (Burns et al., 1984; Uno, 1986). Future studies with a larger number of samples would clarify the degree of morphological variation both within and between the two species.

Summary

Morphological growth variations in skull features between the Kuril harbor seal and the spotted seal were examined. Skulls from 80 Kuril harbor seals and 41 spotted seals were collected, and we measured 29 metric and 6 non-metric cranial characteristics. Three growth classes were defined according to the postnatal developmental stage: pups (0 year), subadults (1–4 years old) and adults (more than 5 years old). Sexual dimorphism in Kuril harbor seal pups, subadults, and adults were detected. Although interspecies differences were detected in each growth class, Kuril harbor seals were larger and more massive than spotted seals; this feature was already detectable in pups. Certain cranial characteristics with which to identify the two species were not detected, but it was possible to identify any unknown specimens to their species, sex, and growth class using the cranial data generated in this study. Using 6 non-metric cranial characteristics, we identified significant interspecies differences with regard to the shape of the temporozygomatic suture and the extent of the nasal-incisive suture; the shape of the temporozygomatic suture and the shape of the nares were indicators of growth class in Kuril harbor seals. Although non-metric cranial characteristics have a lower discriminating power than metric characteristics, they are easy to use in the field even by inexperienced researchers.

Chapter 3

Genetic variation in the Kuril harbor seal and the spotted seal around Hokkaido, based on mitochondrial DNA cytochrome *b* sequences

Introduction

The increase in the number of seal individuals has resulted in occasional damage to coastal fisheries. Damage to salmon fixed nets on the Pacific side by Kuril harbor seals has been reported; fishery damage by spotted seals in Rausu was also reported (Hokkaido Government, 2006). Moreover, the invasion of fisheries by seals inevitably leads to the occurrence of bycatches. In fact, more than 150 seals die as a part of bycatch every year (Hokkaido Government, 2006). Detailed information about these two seal species is necessary in order to reduce these interactions and to appropriately conserve and manage these animals. Conservation and management of both fisheries and seals should be conducted in each area because of the seals' widespread distributions and the extent of damage to fisheries in Hokkaido. Information about the existence of local groups of Kuril harbor and spotted seals is currently insufficient for areal conservation and management.

There is no report on the genetic population structures of Kuril harbor seals around Hokkaido. Further, the study on local populations of spotted seals did not recognize the genetic population structures in the Sea of Japan and Okhotsk areas based on the mitochondrial DNA (mtDNA) control region because the haplotypes were too varied to indicate clear geographic differences (Mizuno et al., 2003). Therefore, other genetic markers are necessary to investigate the genetic populations of spotted seals. MtDNA has many advantages as a molecular marker because it evolves faster than nuclear DNA (Brown et al., 1982), probably due to inefficient replication repair (Clayton, 1984). The cytochrome *b* region has a smaller mutation rate in the non-coding region than the control region (Desjardins and Morais, 1990). Cytochrome *b* is suitable for studying the local populations of the two seal species because the haplotypes of the control region were too varied to allow for recognition of the genetic populations of spotted seals (Mizuno et al., 2003).

In the present study, the variations in mtDNA cytochrome *b* sequences and genetic population structures of Kuril harbor and spotted seals throughout the Hokkaido area were investigated. Availability of genetic information about the local populations of the two seal species will contribute to effective areal conservation and management of seals and fisheries.

Materials and methods

Sample collection

Blood or tissue (muscle) samples were collected from 39 Kuril harbor seals and 31 spotted seals (Fig. 3-1 and Table 3-1). Blood samples were obtained from Erimo seals captured with permission of the Japanese Ministry of the Environment for scientific investigation. Blood and muscle samples from Nosappu, Akkeshi, and Hamamasu were obtained from seals as a part of bycatch in fixed nets. Muscle samples from Rausu were obtained from seals killed for fishery damage control, while those from Yagishiri Island were obtained from seals that washed ashore. Sampling seasons of both species are shown in Table 3-1.

DNA extraction, amplification, and sequencing

Total DNA was extracted from whole blood or tissue samples using the DNeasy Tissue Kit (Qiagen, Hilden, Germany). The entire region (1,140 bp) of the mtDNA cytochrome *b* gene was amplified by polymerase chain reaction (PCR). The PCR primer pair was as follows: 5′-AGG CGT CGA AGC TTG ACA TGA AAA GCC ATC GTTG-3′ and 5′-CGA ATT CCA TTT TTG GTT TAC AAG AC-3′ (Árnason et al., 1995). PCR was performed using Ex Taq (Takara Bio Inc., Shiga, Japan), and amplifications were carried out with the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). Amplification consisted of an initial 5 minutes of denaturation at 94°C, followed by 25 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 90 s and 72°C for 7 minutes. The PCR products were recognized by electrophoresis in 2% agarose gels stained with ethidium bromide, and then purified with the QIAquick PCR Purification Kit (Qiagen). The purified products were cycle-sequenced with the BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems), and then the final products were sequenced with the BigDye® XTerminator Purification Kit (Applied Biosystems) using the ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems). The nucleotide sequences have been deposited in the DDBJ, EMBL, and GenBank nucleotide sequence databases under accession numbers AB510408–AB510445.

DNA data analysis

The 1,140 bp cytochrome *b* sequences were aligned using the MEGA 4.0 program (Tamura et al., 2007). Phylogenetic trees for all the haplotypes were constructed using the neighbor-joining (NJ) method (Saitou and Nei, 1987) based on Kimura's two-parameter distances (Kimura, 1980) and the maximum parsimony (MP) method by using MEGA 4.0. Bootstrap analyses for the NJ and MP methods were carried out with 1,000 replications. The Baikal seal (*Phoca sibirica*; DDBJ/EMBL/GenBank accession no. AY140977) was used as the outgroup in the phylogenetic trees. An analysis of molecular variance (AMOVA: Excoffier et al., 1992) as implement in ARLEQUIN 3.0 (Excoffier et al., 2005) compared the components of genetic diversity for the variance among populations to that observed within each population. Genetic variations within each population were quantified by haplotype diversity (*h*) and nucleotide diversity (π) (Nei, 1987) using DnaSP ver.4.50.3 (Rozas et al., 2003). Genetic differentiations among populations were assessed by the fixation index (*F*st) as defined by Wright (1969) and Nei's genetic distance (Nei, 1987) among populations using ARLEQUIN 3.0. The significance of *F*st values was calculated by Fisher's exact test ($P < 0.05$).

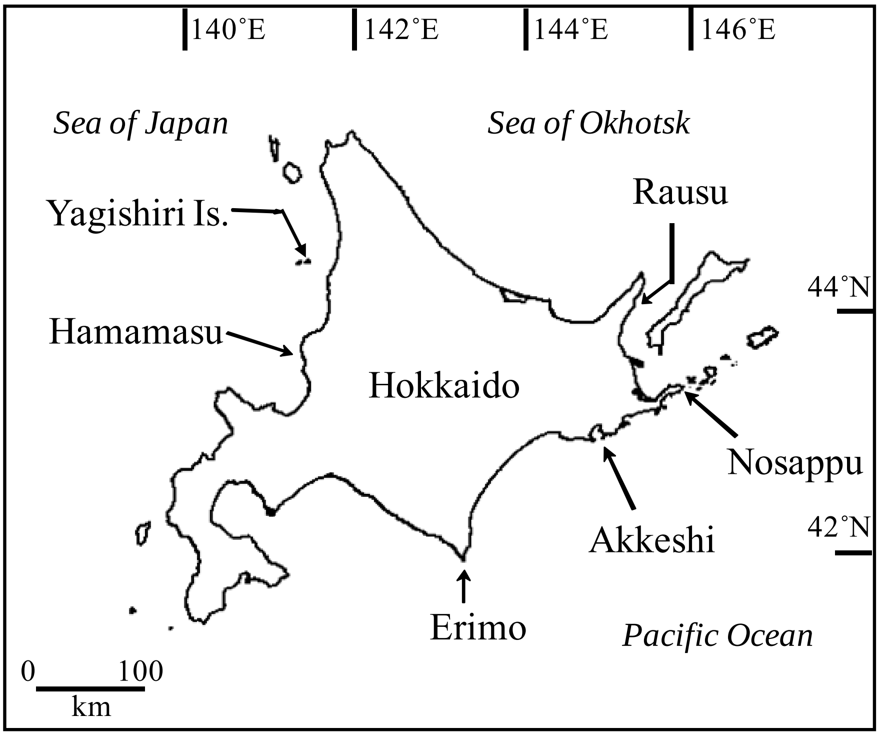

Fig. 3-1 Geographical locations of sampling sites in Hokkaido, Japan

Table 3-1 Sampling sites and dates of Kuril harbor seals and spotted seals.

Species	Sampling site	n	Sampling date
Harbor seal	Erimo	23	June and July 2006/2007/2008
	Akkeshi	4	September 2004
	Nosappu	12	September-November 2005
Spotted seal	Erimo	3	June and July 2005
	Akkeshi	4	May and June 2005
	Nosappu	7	September-November 2005
	Rausu	5	March and April 2005
	Yagishiri	5	February, March, April, and December 2006 and January 2007
	Hamamasu	7	January, March, and April 2006 and March 2008

Results

The Kuril harbor seal

In total, 19 variable sites defining 15 haplotypes were observed in all of the cytochrome *b* sequences from 39 Kuril harbor seal samples. Seven haplotypes (Pvs 4-10) were detected in 23 Erimo individuals, 6 haplotypes (Pvs 10-15) were detected in 12 Nosappu individuals, and 4 haplotypes (Pvs 1-3,12) were detected in 4 individuals (Table 3-2). There were 2 haplotypes observed in multiple sites: haplotype Pvs 10 was observed in 16 (16/23; 69.6%) Erimo individuals and 3 (3/12; 25.0%) Nosappu individuals, and haplotype Pvs 12 was observed in 1 (1/4; 25.0%) Akkeshi individual and 2 (2/12; 16.7%) Nosappu individuals. In addition, Pvs 11 was observed in 4 Nosappu individuals, and the other haplotypes were each detected in one individual.

There were no common haplotypes between Erimo and Akkeshi individuals. Phylogenetic trees using the NJ and MP methods showed that the Kuril harbor seal haplotypes formed a single cluster that was clearly phylogenetically divided from those of the spotted seal (Figs. 3-2 and 3-3). The Kuril harbor seal haplotypes were divided into two major lineages: Group I was primarily comprised of Erimo haplotypes and Group II contained Akkeshi and Nosappu haplotypes.

AMOVA of the Kuril harbor seals showed that genetic variance among populations (53.5%) were slightly higher than that within populations (46.5%) (Table 3-4). Except for the Kuril harbor seals in Erimo, haplotype diversity (*h*) of Kuril harbor seals was more than 0.8

and nucleotide diversity (π) of the Kuril harbor seals in Erimo was also lower than that in other populations (Table 3-5). Nucleotide diversity was also calculated 0.0034 in Group I (SD = 0.0006), 0.0038 in Group II (SD = 0.0008), and 0.0047 between Groups I and II (SD = 0.0011).

The *F*st estimates of Erimo–Akkeshi and Erimo–Nosappu in the Kuril harbor seal were significantly high, and the genetic distances between the Akkeshi and Nosappu populations were the lowest among Kuril harbor seals (Table 3-6). Genetic distances among local populations of Kuril harbor seal indicated that the Akkeshi–Nosappu value was higher than others (Table 3-6). However, there was no significant difference in genetic distance for Kuril harbor seals.

The spotted seal

Thirty-four variable sites defining 23 haplotypes were observed in the cytochrome *b* sequences of 31 spotted seal samples. Only 4 haplotypes were observed in multiple sites: haplotype Pl 5 was observed in 3 individuals (Erimo, Rausu, and Yagishiri Is.); Pl 18 in 2 individuals (Rausu and Hamamasu); Pl 20 in 2 individuals (Rausu and Yagishiri Is.); and Pl 22 in 5 individuals (two Akkeshi, Rausu, Yagishiri Is., and Hamamasu; Table 3-3). The other haplotypes were detected in one individual each. However, Nosappu individuals had no haplotypes in common with other sites.

Spotted seal haplotypes seemed to fall into two lineages: one comprised Pl 8 (observed in Hamamasu only) and Pl 11 (observed in Nosappu only) and the other comprised the remaining haplotypes (Figs.

3-2 and 3-3). Therefore, no clear geographic lineages of spotted seals were observed.

AMOVA of the spotted seals showed that the genetic variance within populations (97.3%) was far higher than that among populations (2.7%) (Table 3-4). All haplotype diversity (*h*) of spotted seals was more than 0.8 and nucleotide diversity (π) was higher in Nosappu (Table 3-5).

The *F*st estimates of spotted seals were far lower than those of Kuril harbor seals, with no significant differences found between sampling sites (Table 3-6). Genetic distances among spotted seal populations indicated that Nosappu population had a higher value than others; however, there was also no significant difference in genetic distance observed for spotted seals (Table 3-6).

Table 3-2 Mitochondrial sequence variations from 1,140 bp of the cytochrome b region of 39 Kuril harbor seal individuals and haplotype frequencies at each sampling site.

Haplotype	Nucleotide position																								Sampling site			Total
	43	206	336	411	466	501	529	555	589	592	602	640	646	669	703	719	777	788	792	858	870	895	1057	1066	Erimo	Akkeshi	Nosappu	
Pvs 1	A	A	A	A	G	A	C	A	C	C	A	G	G	T	C	C	C	A	T	C	A	G	C	A		1		1
Pvs 2	·	T	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	G	·	·	·	C	·	·		1		1
Pvs 3	·	T	·	·	A	·	·	·	·	·	·	·	·	·	·	·	T	G	C	·	·	C	·	·		1		1
Pvs 4	·	T	·	·	A	·	·	·	·	·	·	·	·	C	·	·	T	G	C	·	·	C	A	G	1			1
Pvs 5	·	T	·	·	A	·	·	·	·	·	C	·	·	C	·	·	T	G	C	·	·	C	·	·	2			2
Pvs 6	·	T	·	·	A	·	·	·	A	G	·	·	·	C	·	·	T	G	C	·	·	C	·	·	1			1
Pvs 7	·	T	·	·	A	·	·	·	·	·	·	·	A	C	·	A	T	G	C	·	·	C	·	·	1			1
Pvs 8	·	T	·	·	A	·	·	·	·	·	·	·	A	C	·	·	T	·	C	·	·	C	·	·	1			1
Pvs 9	·	T	·	·	A	·	·	·	·	·	·	·	·	C	T	·	T	G	C	·	·	C	·	·	1			1
Pvs 10	·	T	·	·	A	·	·	·	·	·	·	·	·	C	·	·	T	G	C	·	·	C	·	·	16		3	19
Pvs 11	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	T	·	C	·	·			4	4
Pvs 12	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	C	·	·		1	2	3
Pvs 13	·	T	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	G	·	·	·	C	·	·			1	1
Pvs 14	·	T	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	C	·	·			1	1
Pvs 15	·	T	·	G	·	·	·	·	·	·	·	·	·	C	·	·	·	G	C	·	·	C	·	·			1	1
Total																									23	4	12	39

Table 3-3 Mitochondrial sequence variations from 1,140 bp of the cytochrome b region of 31 spotted seal individuals and haplotype frequencies at each sampling site.

Haplotype	Nucleotide position																																					Sampling site						Total
	99	132	147	153	201	240	273	324	367	375	496	501	562	573	595	672	673	675	696	773	834	862	864	904	927	988	989	993	1043	1066	1071	1098	1103	1107	1113	1114	1129	Erimo	Akkeshi	Nosappu	Rausu	Yagishiri	Hamamasu	
Pl1	T	G	G	A	C	T	C	A	G	T	G	G	G	T	T	C	A	A	C	C	C	C	A	G	G	G	C	C	T	A	A	A	T	C	C	G	C		1					1
Pl2	·	·	·	·	·	C	·	G	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	T	·	·	·	G	·	·	·	·	·	·		1					1
Pl3	·	·	A	·	·	·	·	·	·	C	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	T	T	·	·	1						1
Pl4	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	A	·	1						1
Pl5	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	1			1	1		3
Pl6	·	·	A	·	·	·	·	·	·	C	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·						1	1
Pl7	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	T	·	·						1	1
Pl8	·	·	A	·	A	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·						1	1
Pl9	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	T	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·						1	1
Pl10	C	·	·	·	·	·	·	·	·	·	C	·	·	·	·	T	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·						1	1
Pl11	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	T	C	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·			1				1
Pl12	·	·	A	G	·	·	·	·	A	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·			1				1
Pl13	·	·	·	·	·	C	·	G	·	·	·	·	·	·	·	T	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·			1				1
Pl14	·	·	A	·	·	·	·	·	·	·	·	A	·	·	A	T	·	C	·	·	·	T	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·			1				1
Pl15	·	·	·	·	·	C	·	G	·	·	·	·	·	·	·	T	·	·	·	G	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·			1				1
Pl16	·	·	A	·	·	·	·	·	·	C	·	·	·	·	·	T	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	T	·	G			1				1
Pl17	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	G	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·			1				1
Pl18	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·				1		1	2
Pl19	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	G	A	·	·	·	·	·	·	·	·	·	·	·	·	·				1			1
Pl20	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·				1	1		2
Pl21	·	·	A	·	·	·	T	·	·	·	·	·	·	·	·	T	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·					1		1
Pl22	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·		2		1	1	1	5
Pl23	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·					1		1
	Total																																					3	4	7	5	5	7	31

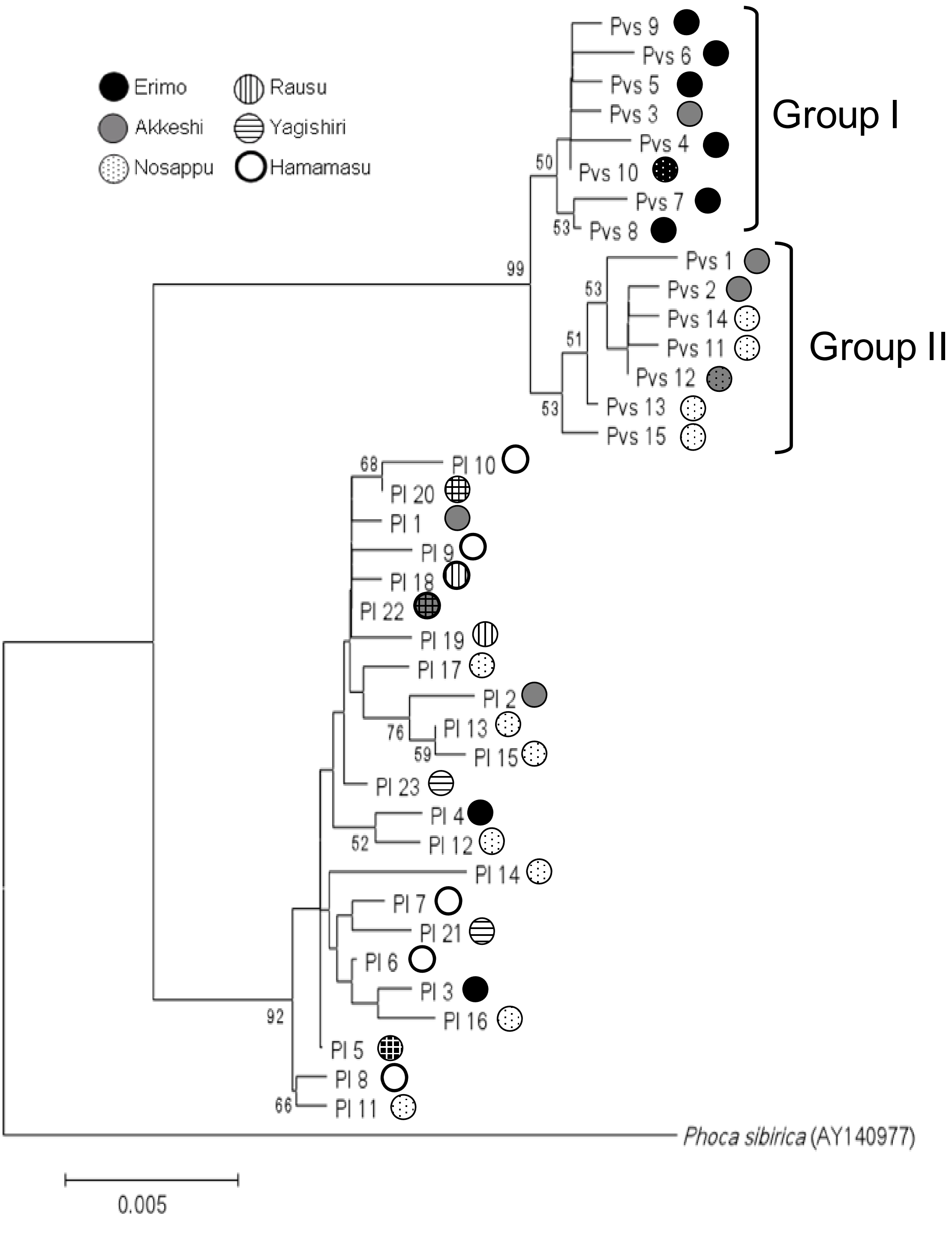

Fig. 3-2 Neighbor-joining (NJ) tree constructed for the cytochrome *b* region of mtDNA haplotypes of the Kuril harbor seal (Pvs) and the spotted seal (Pl). Alphanumeric characters in parentheses after *Phoca sibirica* are DDBJ/EMBL/GenBank accession numbers. Numbers at nodes indicate bootstrap values, with only values >50% being shown. Shaded circles indicate sites where each haplotype was confirmed.

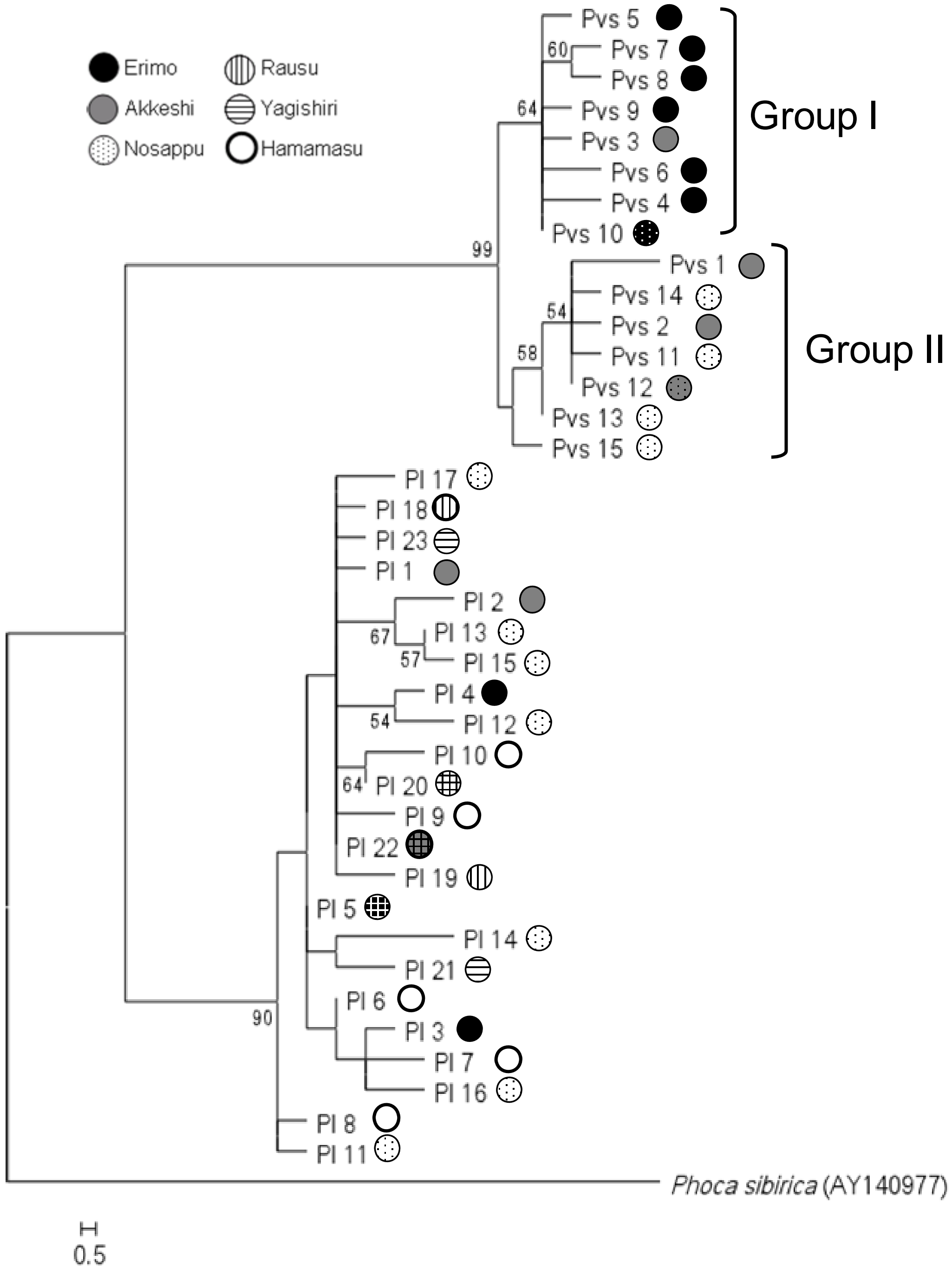

Fig. 3-3 Maximum parsimony (MP) tree constructed for the cytochrome *b* region of mtDNA haplotypes of the Kuril harbor seal (Pvs) and spotted seal (Pl). Alphanumeric characters in parentheses after *Phoca sibirica* are DDBJ/EMBL/GenBank accession numbers. Numbers at nodes indicate bootstrap values, with only values >50% being shown. Shaded circles indicate sites where each haplotype was confirmed.

Table 3-4 Analysis of molecular variance (AMOVA) for Kuril harbor seals (above) and spotted seals (below) among and within local populations.

Kuril harbor seal

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	2	22.23	0.963	53.5
Within populations	36	30.18	0.838	46.5
Total	38	52.41	1.802	

Spotted seal

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	5	10.66	0.051	2.7
Within populations	25	46.76	1.870	97.3
Total	30	57.42	1.922	

Table 3-5 Haplotype and nucleotide diversities of Kuril harbor seals and spotted seals

Species	Sampling site	n	Number of haplotype	Haplotype diversity *h*±SD	Nucleotide diversity π±SD
Kuril harbor seal	Erimo	23	7	0.522±0.124	0.00083±0.00025
	Akkeshi	4	4	1.000±0.177	0.00307±0.00085
	Nosappu	12	6	0.848±0.074	0.00233±0.00035
Spotted seal	Erimo	3	3	1.000±0.272	0.00409±0.00138
	Akkeshi	4	3	0.833±0.222	0.00219±0.00092
	Nosappu	7	7	1.000±0.076	0.00526±0.00065
	Rausu	5	5	1.000±0.126	0.00175±0.00039
	Yagishiri	5	5	1.000±0.126	0.00228±0.00067
	Hamamasu	7	7	1.000±0.076	0.00326±0.00052

Table 3-6 Fixation index (*F*st; below diagonal) and genetic distance (above diagonal) of Kuril harbor seals and spotted seals (*, $P<0.05$).

Kuril harbor seal

	Erimo	Akkeshi	Nosappu
Erimo		3.7283	4.7065
Akkeshi	0.6887*		3.0833
Nosappu	0.5656*	0.0286	

Spotted seal

	Erimo	Akkeshi	Nosappu	Rausu	Yagishiri	Hamamasu
Erimo		3.9167	5.3810	3.4000	3.4000	3.9048
Akkeshi	0.1160		4.8214	2.2500	2.6500	3.2500
Nosappu	0.0171	0.0699		4.6286	4.6286	5.2449
Rausu	0.0867	0.0064	0.1026		2.1600	2.7143
Yagishiri	0.0226	0.0366	0.0456	0.0648		2.9429
Hamamasu	0.0487	0.0209	0.0739	0.0695	0.0826	

Discussion

The Kuril harbor seal

On the basis of the haplotypes of mtDNA cytochrome *b* regions, two phylogenetic trees showed that Hokkaido Kuril harbor seals have two lineages—Group I (Erimo) and Group II (Akkeshi and Nosappu). Nucleotide diversity was slightly higher between Groups I and II than within Group I and Group II. Despite larger samples, Erimo population showed fewer haplotypes and lesser nucleotide diversity than the other populations. Moreover, *F*st which indicates genetic divergence between populations (Kikko et al., 2008) showed high values (>0.5) in Erimo population. The results of all the analyses performed in this study indicated marked differentiation of Erimo population. Erimo is the southernmost region of Kuril harbor seal distribution in Hokkaido, with the nearest haul-out site of this species being Akkeshi, approximately 200 km from Erimo (Fig. 3-1). Erimo is isolated from other haul-out sites, because there are no haul-out sites between Erimo and Akkeshi. In Erimo, population bottlenecks were observed in the 1970s and 1980s where the observed number of Kuril harbor seals was reduced to less than 100 (Itoo and Syukunobe, 1986). Recently, because hunting of seals has been prohibited, their numbers have rapidly increased to over 400 (Hokkaido Government, 2006). Thus, strong settlement of Erimo population on rock reefs and rapid increase in their numbers after bottleneck were attributed to their marked differentiation. Further, unbroken distribution of harbor seals over a 100 km stretch between Akkeshi and

Nosappu (Fig. 3-1) was observed, where some small haul-out sites are located (Itoo and Shukunobe, 1986). Low genetic divergence between Akkeshi and Nosappu populations was indicated from *F*st value; therefore, Kuril harbor seals in these two sites were considered to be of the same lineage. Thus, it is concluded that there are two lineages of Kuril harbor seals around Hokkaido, namely, the Erimo population and the eastern Hokkaido population (Akkeshi and Nosappu). No geographical differences are present in ecology or morphology of Kuril harbor seals around Hokkaido. These two lineages need to be studied from the viewpoint of population conservation and management. However, two haplotypes (Pvs 3 and Pvs 10) detected in Akkeshi and Nosappu populations were clustered into Group I of the phylogenetic trees (Figs. 3-2 and 3-3). Kuril harbor seals around Hokkaido were thought to have extended their distribution southward from Kamchatskaya to Erimo (Burg et al., 1999); further, the two haplotypes might be of the ancestral type. This assumption needs to be validated by future studies in evolutionary genetics.

The spotted seal

Despite the geographic structuring of the distribution of breeding concentrations of spotted seals, no genetic differentiation has been found (Rugh et al., 1997). In this study, haplotype diversity of all the spotted seal populations showed high values (>0.8), and some haplotypes (Pl 5, Pl 18, Pl 20, and Pl 22) were observed at several sites. The phylogenetic trees of this seal did not show any geographic lineages; further, no significant differences were observed in the *F*st values among the populations. The percentage of variation within populations was markedly high and that among populations was low (Table 3-4). In contrast to Kuril harbor seals, the genetic populations of the spotted seal are not found in the Pacific, Sea of Japan, and Okhotsk areas of Hokkaido. Because spotted seals exhibit seasonal migrations and travel over hundreds of kilometers a day (Lowry et al., 1998), the chances of genetic exchange occurring among populations are high. No geographic lineages of spotted seals were recognized in studies on both the control region (Mizuno et al., 2003) and the cytochrome *b* region with different mutation rates from each other. It has been observed that spotted seals have exceptional migratory abilities and exhibit a various population differentiation in their manner of adaptive evolution. In addition, the genetic patterns of these populations could have been the result of a bottleneck effect. However, information about the past population dynamics of the spotted seals was not available.

Our study has revealed that around Hokkaido, there are two geographic lineages of Kuril harbor seals but no geographic lineage of spotted seals; the reason for this could be the ecological differences between the two species. mtDNA is a maternally inherited genetic marker that would be predicted to show segregated patterns among breeding grounds (Bowen, 1992). Other genetic markers may be useful for recognition of the geographic lineages of spotted seals, because these seals do not have any geographic population structures, *e.g.*

microsatellite DNA is inherited biparentally and has been found to reveal substantial variation in species that exhibit low variability in other classes of nuclear markers (Hughes and Queller, 1993).

This study provided basic and meaningful information, but the task of conservation and management of both fishery and seals remains a challenge. The Kuril harbor seal has been as an endangered species by the Ministry of Environment, and the spotted seal population is under the control of the Hokkaido Government. These differences will affect the management infrastructure. Furthermore, some fishery damage by seals was observed in all the sites in this study, except for Hamamasu (Hokkaido Government, 2006). The extent of fishery damage by the seals and the degree of fishermen's victimization varied in these areas. Conservation and management strategies should be implemented despite these challenges. It is especially necessary to emphasize the isolation of the Erimo Kuril harbor seal group. Future studies on Kuril harbor and spotted seals based on the present study will contribute toward the conservation and management of fishery and seals throughout the Hokkaido area.

Summary

The Kuril harbor seal and the spotted seal are major seal species around Hokkaido. While some investigations have been conducted on ecology and morphology of the two seal species, there is a lack of genetic information. Therefore, the variations of mitochondrial DNA (mtDNA) cytochrome *b* sequences in the two species were studied. Fifteen haplotypes were observed in 39 Kuril harbor seals from Erimo, Akkeshi, and Nosappu, and 23 were observed in 31 spotted seals from Erimo, Akkeshi, Nosappu, Rausu, Yagishiri Island, and Hamamasu. The phylogenetic trees showed two Kuril harbor seal lineages: Group I primarily contained haplotypes from Erimo, and Group II contained haplotypes from Akkeshi and Nosappu. Because Erimo population had fewer haplotypes and less nucleotide diversity than Akkeshi and Nosappu populations, it was considered to be isolated from the others. In contrast, the genetic variance within populations of spotted seals (97.3%) showed far higher than the variance among populations (2.7%) by analysis of molecular variance. There was no significant difference between spotted seal populations, indicating that the absence of lineage throughout Hokkaido. The differences in the genetic population structures between the two species could have been generated by their ecological differences. This study provided the basic genetic information about these seal species and will contribute to the conservation and management of fishery and seals throughout Hokkaido.

Chapter 4

Determination of the hybridization between the Kuril harbor seal and the spotted seal around Hokkaido, using mitochondrial DNA cytochrome *b* and SRY (sex determining region on the Y chromosome) gene

Introduction

When closely related species overlap in distribution, interspecific hybridizations occur sometimes. In the Pinnipedia, for example, hybridizations between the Subantarctic (*Arctocephalus tropicalis*) and the Antarctic fur seals (*A. gazelle*) have been identified at the Prince Edward Islands, Macquarie Island, and Iles Crozet (Kingston and Gwilliam, 2007). In addition, the New Zealand fur seal (*A. forsteri*) is also present on the Macquarie Island, high level of hybridizations were detected among three species of fur seal (Lancaster et al., 2006). In the Phocidae, a hybrid between harp (*Phoca groenlandica*) and hooded seal (*Cystophora cristata*) was identified in the Gulf of St. Lawrence (Kovacs et al., 1997), where each two species breed sympatrically.

The hybridization between harbor and spotted seals is suggested because of the occurrence of several craniologically intermediate specimens from the areas of probable sympatric (Shaughnessy and Fay, 1977). In fact, it is confirmed that harbor and spotted seals hybridize in captivity and their hybrids have the ability of reproduction (Katsumata et al., 2003), but that is not confirmed in the wild.

Spotted seals tend to resemble light-phase harbor seals in color and pattern, which has contributed to the confusion about these two species (Burns, 2009). Since some spotted seals have been observed to haul-out with Kuril harbor seals on the Pacific side, both of those species are sympatric throughout the year at Erimo and Akkeshi. Since the observed numbers of spotted seals at Kuril harbor seals' haul out sites on the

Pacific coast of Hokkaido increase recently, it is possible to occur the hybridization between two seals.

Hybrid identification based on phenotype can be problematic since individuals within species can vary widely in pelage pattern and coloration and hybrids may not necessarily be morphologically intermediate to their parent species (Campton, 1987). Considering that phenotypic hybrid identification can be difficult and unreliable, it is essential to establish techniques that facilitate this process. The use of genetic markers which show differences between species is an alternative method to phenotypic differentiation and has been employed in several studies for hybrid identification (Nijman et al., 2003; Vila et al., 2005; Lancaster et al., 2006; Kingston and Gwilliam, 2007).

In this study, mtDNA and Sex determining region on the Y chromosome (SRY) gene are utilized to determine the hybridization between Kuril harbor seal and spotted seal in the wild.

Materials and methods

Sample collection

Blood or tissue (muscle) samples were collected from 46 Kuril harbor seals from two sites, Erimo (n=28) and Nosappu (n=18), and 27 spotted seals from three sites, Erimo (n=5), Nosappu (n=10), and Hamamasu (n=12) (see Fig. 3-1). Blood samples were obtained from Erimo seals captured with permission of the Japanese Ministry of the Environment for scientific investigation. Blood and muscle samples from Nosappu and Hamamasu were obtained from seals as a part of bycatch in fixed nets. All seals were identified the species from their pelage pattern and coloration by the researchers. Most samples were also used in Chapter 3, but any samples were excluded in the study of Chapter 3 because of having difficulties of species identification or suspicions of hybridization.

DNA extraction, amplification, and sequencing

Total DNA was extracted from whole blood or tissue samples using the DNeasy Tissue Kit (Qiagen, Hilden, Germany). The amplification and sequenceing of mtDNA cytochrome *b* gene were performed in the same way of Chapter 3.

Some male samples were also sequenced with SRY gene. The PCR primer pair was designed by reference to sequence of SRY available in DDBJ/GenBank/EMBL Accession nos. AY424662 (*P. vitulina*) and AY424664 (*P. largha*) using Primer3 (http://frodo.wi.mit.edu/cgi-bin/

primer3/primer3.cgi). The region (272 bp) of the SRY gene was amplified by polymerase chain reaction (PCR). The PCR primer pair was as follows: 5′-ACA ATC TTT CCT ACA CAT TCC TCC T-3′ and 5′-ACT CTG GTG CTG TAA CTT TTG TTT C-3′. PCR was performed using Ex Taq (Takara Bio Inc., Shiga, Japan), and amplifications were carried out by the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). Amplification consisted of an initial 5 minutes of denaturation at 94°C, followed by 25 cycles of 94°C for 30 s, 57°C for 30 s, 72°C for 90 s, 72°C for 7 minutes. The PCR products were recognized by electrophoresis in 2% agarose gels stained with ethidium bromide, and then purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). The purified products were cycle-sequenced with the BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA), and then the final products were sequenced with the BigDye® XTerminator Purification Kit (Applied Biosystems, Foster City, CA) using the ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems, Foster City, CA).

The Fisher's exact test ($P<0.05$) was used to compare results among sampling sites using software R (R Development Core Team, 2007).

Results

mtDNA

Out of 15 male and 13 female Kuril harbor seals from Erimo, the spotted seal haplotype of mtDNA was detected in one female (Table 4-1). There were no spotted seal haplotypes detected in 13 male and 5 female Kuril harbor seals from Nosappu.

Out of 3 male and 2 female spotted seals from Erimo, the Kuril harbor seal haplotypes of mtDNA were detected in one male and one female (Table 4-1). All spotted seals from Nosappu and Hamamasu showed the spotted seal haplotypes of mtDNA.

SRY

In order to identify the hybridization, the SRY sequences of the two species could be aligned. Eight male Kuril harbor seals (4 Erimo and 4 Nosappu individuals) were detected to have the same haplotype of harbor seal from DDBJ/EMBL/GenBank database (Table 4-2). However the SRY sequences of six spotted seals (4 Nosappu, 1 Erimo, and 1 Hamamasu) could be also aligned, they were detected to have the haplotype of harbor seal (Table 4-2). Therefore, Kuril harbor seals and spotted seals had the same SRY haplotype. The male spotted seal from Erimo which was detected Kuril harbor seal haplotype of mtDNA also had the SRY haplotype of harbor seal. Since two seal species had the same SRY haplotype, it failed to identify their paternal species and the hybrid between Kuril harbor seals and spotted seals.

Table 4-1 Pelage patterns and mtDNA haplotype were shown on Kuril harbor seals and spotted seals from each sampling site.

Sampling Site	Pelage patterns	mtDNA	
		Kuril harbor seal	Spotted seal
Erimo	Kuril harbor seal	96.4 (27 / 28)	3.6 (1 / 28)
	Spotted seal	40.0 (2 / 5)	60.0 (3 / 5)
Nosappu	Kuril harbor seal	100.0 (17 / 17)	0.0 (0 / 17)
	Spotted seal	0.0 (0 / 10)	100.0 (10 / 10)
Hamamasu	Spotted seal	0.0 (0 / 12)	100.0 (12 / 12)

Table 4-2 SRY sequence of the Kuril harbor seal (*P. v. stejnegeri*) and the spotted seal (*P. largha*).

Species	nucleotide positions							
	101		111	120	161		171	180
Phoca vitulina (AY424662)	A A T A A	C A G T A	A A G T T	T G T T T	T A G A T	G G T A G	A G C A C	A T A G G
P. largha (AY424664)	. . . . .	. . . . .	. . A . .	. . . . .	. . . . .	. . . . .	. . – . .	. . . . .
P. vitulina stejnegeri, Hokkaido	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
P. largha, Hokkaido	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .

Discussion

In general, pelage patterns are used to identify whether the Kuril harbor seal or the spotted seal. However, pelages of Kuril harbor seals show the various patterns and there are some individual looks like spotted seals around Hokkaido. Sometimes it is very difficult to identify the two seal species, even by experienced observers. There is no information about the pelage of hybrids between Kuril harbor seal and spotted seal, and then it is impossible to identify the hybrids from their pelage.

It was from only Erimo that three seals showed that their mtDNA haplotype differed from species by their pelage. There were no such individuals from other sites. Consequently, the probability of hybridization was higher in the Erimo individuals than others.

One of the potential barriers to hybridization is temporally discrete receptive periods. Such displacement of the timing of breeding at sympatric sites may reduce the levels of inter-specific mating. Kuril harbor seals in Nosappu seemed to make their habitat in the Kuril Islands, where the reproductive seasons of the two species clearly separated. The number of spotted seals in Erimo has increased recently (Hokkaido Government, 2006), therefore it seems that the displacement of the timing of breeding does not occur yet.

Spotted seals spend the breeding and nursing season on seasonal sea ice, and then mating occurs in the water at about the time that pups are weaned (Burns, 2009). However, the seasonal sea ice seldom appeared on the coast of Erimo, and then it is not thought that spotted seal breed around Erimo without the ice. If the hybridizations occur, it is possible that male spotted seals mate with female Kuril harbor seals after puping in the late April to the early May.

Although, there was an individual showing Kuril harbor seal pelage and spotted seal mtDNA haplotype, it is also thought to that the hybridization between male Kuril harbor seal and female spotted seal occurs. It is also possible that they are just Kuril harbor seals that resemble spotted seals or spotted seals that resemble Kuril harbor seals, because only maternal DNA haplotypes were investigated.

There are two genetic differences, one substitution and one deletion in the sequence of SRY gene, between harbor seals and spotted seals in the DDBJ, EMBL, and GenBank nucleotide sequence databases. However, the SRY gene sequence of the two species in Hokkaido were same, those differences were not found in spotted seals in Hokkaido. Therefore, it is thought that spotted seals which showed the sequence in DDBJ/EMBL/GenBank belong to different population from spotted seals around Hokkaido. It is no wonder that the two species showed the same SRY sequence, because the two species are the sibling species.

However, if these suspicious individuals which look like spotted seals are hybrids indeed, it will be very high rate of hybridization. This study was preliminary but it is necessary to survey the hybridization in Erimo. Because SRY gene is unuseful for the determination of hybridization between the Kuril harbor seal and the spotted seal around Hokkaido, it is necessary to apply other paternal genetic markers or nuclear DNA.

Summary

To determine the hybridization between Kuril harbor seals and spotted seals in the wild, mtDNA and SRY gene were studied. Blood or tissue (muscle) samples were collected from 46 Kuril harbor seals and 27 spotted seals. One female Kuril harbor seal from Erimo showed the spotted seal haplotype of mtDNA. One male and one female spotted seal from Erimo showed the Kuril harbor seal haplotype of mtDNA. It was from only Erimo that three seals showed that their mtDNA haplotype differed from species by their pelage. The probability of hybridization was higher in the Erimo individuals than others. However, Kuril harbor seals and spotted seals had the same SRY haplotype, and it failed to identify their paternal species and the hybrid between Kuril harbor seals and spotted seals. The SRY gene sequence of the two species in Hokkaido was same, the differences were not found in spotted seals in Hokkaido. Therefore, it is thought that spotted seals which showed the sequence in DDBJ/EMBL/GenBank belong to different population from spotted seals around Hokkaido. It is no wonder that the two species showed the same SRY sequence, because the two species are the sibling species. Because SRY gene is unuseful for the determination of hybridization between the Kuril harbor seal and the spotted seal around Hokkaido, it is necessary to apply other paternal genes and/or nuclear DNA.

Conclusion

Kuril harbor seals and spotted seals are the top predators of coastal ecosystems around Hokkaido. Because the two species distribute in limited sites around Hokkaido, the dynamics of them have an effect on lower predators and prey species on the ecosystems.

Detailed information about these two seal species is necessary in order to reduce these interactions and to appropriately conserve and manage these animals. The Kuril harbor seal has been as an endangered species by the Ministry of Environment, and the spotted seal population is under the control of the Hokkaido Government. These differences will affect the management infrastructure. Furthermore, some fishery damage by seals was observed in all the sites in this study (Hokkaido Government, 2006). The extent of fishery damage by the seals and the degree of fishermen's victimization varied in these areas. Conservation and management of both fisheries and seals should be conducted in each area because of the seals' widespread distributions and the extent of damage to fisheries in Hokkaido. Information about the existence of local groups of Kuril harbor seal and spotted seals is currently insufficient for areal conservation and management.

In the present thesis, the skull morphology and genetic variation of the Kuril harbor seal and the spotted seal around Hokkaido were reported. Morphological growth variations in skull features between the Kuril harbor seal and the spotted seal were examined. Sexual dimorphism in Kuril harbor seal pups, subadults, and adults were detected by skull features. Although interspecies differences were detected in each growth class, Kuril harbor seals were larger and more massive than spotted seals; this feature was already detectable in pups. Certain cranial characteristics with which to identify the two species were not detected, but it was possible to identify any unknown specimens to their species, sex, and growth class using the cranial data generated in this study. The shape of the temporozygomatic suture and the shape of the nares were indicators of growth class in Kuril harbor seals.

The variations of mtDNA cytochrome *b* sequences in the two species were shown and 15 haplotypes were observed in 39 Kuril harbor seals from Erimo, Akkeshi, and Nosappu, and 23 were observed in 31 spotted seals from Erimo, Akkeshi, Nosappu, Rausu, Yagishiri Island, and Hamamasu. The phylogenetic trees showed two Kuril harbor seal lineages: Group I primarily contained haplotypes from Erimo, and Group II contained haplotypes from Akkeshi and Nosappu. Because Erimo population had fewer haplotypes and less nucleotide diversity than Akkeshi and Nosappu populations, it was considered to be isolated from the others. In contrast, the genetic variance within populations of spotted seals (97.3%) showed far higher than the variance among populations (2.7%) by analysis of molecular variance. There was no significant difference between spotted seal populations, indicating that the absence of lineage throughout Hokkaido. The differences in the genetic population structures between the two species could have been generated by their ecological differences.

Blood or tissue (muscle) samples were collected from 46 Kuril harbor seals and 27 spotted seals. One female Kuril harbor seal from

Erimo showed the spotted seal haplotype of mtDNA. One male and one female spotted seal from Erimo showed the Kuril harbor seal haplotype of mtDNA. It was from only Erimo that three seals showed that their mtDNA haplotype differed from species by their pelage. The probability of hybridization was higher in the Erimo individuals than others. However, Kuril harbor seals and spotted seals had the same SRY haplotype, and it failed to identify their paternal species and the hybrid between Kuril harbor seals and spotted seals. The SRY gene sequences of the two species in Hokkaido were same, the differences were not found in spotted seals in Hokkaido. Therefore, it is thought that spotted seals which showed the sequence in DDBJ/EMBL/GenBank belong to different population from spotted seals around Hokkaido. Because SRY gene is unuseful for the determination of hybridization between the Kuril harbor seal and the spotted seal around Hokkaido, it is need to apply other genetic markers.

This study provided basic and meaningful information, but the task of conservation and management of both fishery and seals remains a challenge. It is especially necessary to emphasize the isolation of the Erimo Kuril harbor seal group. Future studies on Kuril harbor and spotted seals based on the present study will contribute toward the conservation and management of fishery and seals throughout the Hokkaido area.

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References

Árnason Ú, Bodin K, Gullberg A, Ledje C, Mouchaty S (1995) A molecular view of pinniped relationships with particular emphasis on the true seals. J Mol Evol 40: 78–85

Bigg MA (1981) Harbour seal *Phoca vitulina* Linnaeus, 1758 and *Phoca largha* Pallas, 1811. In "Handbook of marine mammals, vol. 2: Seals" Ed by SH Ridgway and RJ Harrison, Academic Press, London, pp 1-28

Bowen BW, Meylan AB, Ross MJ, Limpus CJ, Balazs GH, Avise JC (1992) Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. Evolution 46: 865-881

Brown WM, Prager EM, Wang A, Wilson AC (1982) Mitochondrial DNA sequences of primates; tempo and mode of evolution. J Mol Evol 18: 225–239

Burg TM, Trites AW, Smith MJ (1999) Mitochondrial and microsatellite DNA analyses of harbor seal population structure in the northeast Pacific Ocean. Can J Zool 77: 930-943

Burns JJ (2009) Harbor seal and spotted seal *Phoca vitulina* and *P. largha*. In "Encyclopedia of Marine Mammals" Ed by WF Perrin, B Würsig, JGM Thewissen, Academic Press, pp 533–542

Burns JJ, Fay FH, Fedoseev GA (1984) Craniological analysis of harbor and spotted seals of north Pacific region. U. S. Dep. Commer., NOAA Tech Rep NMFS 12: 5-16

Campton DE (1987) Natural hybridization and introgression in fishes: methods of detection and genetic interpretations. In: Ryman N, Utter F Eds, Population genetics and fishery management. University of Washington Press, pp 161-192

Clayton DA (1984) Transcription of the mammalian mitochondrial genome. Ann Rev Bioch 53: 573–594

Clutton-Brock TH, Guinness FE, Albon SD (1982) Red Deer. Behavior and Ecology of Two Sexes. University of Chicago Press, Chicago

Desjardins P, Morais R (1990) Sequence and gene organization of the chicken mitochondrial genome. A novel gene order in higher vertebrates. J Mol Evol 212: 599–634

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evol Bioinform Online 1: 47–50

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479–491

Hachiya N, Ohtaishi N (1994) Methods of preparing osteal specimens. Hokkaido Univ. press, Sapporo (in Japanese)

Hata S (2007) Skull growth and morphology of the Kuril harbor seal *Phoca vitulina stejnegeri* in Hokkaido. Master's thesis. Graduate School of Fishery Science, Hokkaido University, Hakodate (in Japanese)

Hokkaido Government (2006) Report on conservation and management of seals. Hokkaido Government (in Japanese)

Hughes CR, Queller DC (1993) Detection of highly polymorphic microsatellite loci in a species with little allozyme polymorphism. Mol Ecol 2: 131-137

Itoo T, Syukunobe T (1986) Number and present status of the Kuril seal. In "Proceedings of the Symposium on Ecology and Protection of the Kuril Seal" Ed by K Wada, T Ito, A Niizuma, S Hayama, M Suzuki, Tokai Univ Press, Tokyo, pp 18–58 (in Japanese with English summary)

Jefferson TA, Webber MA, Pitman RL (2008) Marine mammals of the world. 2nd ed. Academic Press, New York

Kikko T, Kuwahara M, Iguchi K, Kurumi S, Yamamoto S, Kai Y, Nakayama K (2008) Mitochondrial DNA population structure of the white-spotted charr (*Salvelinus leucomaenis*) in the Lake Biwa water system. Zool Sci 25: 146-153

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16: 111–120

King JE (1983) Seals of the world. 2nd ed. British Museum (Natural History), London

Kingston JJ and Gwilliam J (2007) Hybridization between two sympatrically breeding species of fur seal at Iles Crozet revealed by genetic analysis. Conservation Genetics 8: 1133-1145

Kovacs KM, Lydersen C, Hammill MO, White BN, Wilson RJ and Malik S (1997) A harp seal × hooded seal hybrid. Marine mammal science

13(3): 460-468

Lancaster ML, Gemmell NJ, Negro S, Goldsworthy SD, Sunnucks P (2006) Ménage à trois on Macquarie Island: hybridization among three species of fur seal (*Arctocephalus* spp.) following historical population extinction. Mol Ecol 15: 3681-3692

Lowry LF, Burkanov VN, Frost KJ, Simpkins MA, Davis R, DeMaster DP, Suydam R, Springer A (2000) Habitat use and habitat selection by spotted seals (*Phoca largha*) in the Bering Sea. Can J Zool 78: 1959-1971

Lowry LF, Frost KJ, Davis R, DeMaster DP, Suydam RS (1998) Movements and behavior of satellite-tagging spotted seals (*Phoca largha*) in the Bering and Chukchi seas. Polar Biol 19: 221–230

Ministry of the Environment (2002) Red Data Book 1 (Mammals). Japan Wildlife Research Center, Tokyo (in Japanese)

Mizuno AW, Ohtaishi N (2001) Cranial features of the spotted seal, *Phoca largha*, in the Nemuro Strait, considering age effects. J Vet Med Sci 64: 137-144

Mizuno AW, Onuma M, Takahashi M, OhtaishiN (2003) Population genetic structure of the spotted seal *Phoca largha* along the coast of Hokkaido, based on mitochondrial DNA sequences. Zool Sci 20: 783-788

Mizuno AW, Suzuki M, Ohtaishi N (2001) Distribution of the spotted seal *Phoca largha* along the coast of Hokkaido, Japan. Mamm Study 26: 109-118

Naito Y, Nishiwaki M (1972) The growth of two species of the harbor seal in the adjacent water of Hokkaido. Sci Rep Whales Res Inst 24: 127-144

Naito Y, Nishiwaki M (1975) Ecology and morphology of *Phoca vitulina largha* and *Phoca kurilensis* in the southern sea of Okhotsk and northeast of Hokkaido. Rapp P-v Réun Cons int Explor Mer 169: 379-386

Nei M (1987) Molecular Evolutionary Genetics. Colombia University Press, New York

Nijman IJ, Otsen M, Verkaar ECL, de Ruijter C, Hanekamp E, Ochieng JW, Shamshad S, Rege JEO, Hanotte O, Barwegen MW, Sulawati T, Lenstra JA (2003) Hybridization of banteng (*Bos javanicus*) and zebu (*Bos indicus*) revealed by mitochondrial DNA, satellite DNA, AFLP and microsatellites. Heredity 90: 10-16

O'Corry-Crowe GM, Westlake RL (1997) Molecular investigations of spotted seals (*Phoca largha*) and harbor seals (*P. vitulina*), and their relationships in areas of sympatry. In "Molecular Genetics of Marine Mammals" Ed by AE Dizon, SJ Chivers, WF Perrin, the Society for Marine Mammalogy, Lawrence, KS, pp 291-304

Perrin WF, Yablokov AV, Barlow J, Mina MV (1994) Comparison of the resolving power of metric and non-metric cranial characters in defining geographical populations of dolphins. Contrib Sci (Los Angel) 447: 1-15

Primer 3 (URL: http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi)

R Development Core Team (2007) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL: http://www.R-project.org.

Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19: 2496–2497

Rugh DJ, Shelden KEW, Withrow DE (1997) Spotted Seals, *Phoca largha*, in Alaska. Mar Fish Rev 59: 1-18

Saito S, Watanabe Y (2004) Outline and difficulty on kuril harbor seal. In "Management of marine mammals along the coast of Hokkaido, Japan" Ed by M Kobayashi, T Isono, K Hattori, Marine Wildlife Center of Japan, Sapporo, pp 23–28 (in Japanese)

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406–425

Shaughnessy PD, Fay FH (1977) A review of the taxonomy and nomenclature of North Pacific harbour seals. J Zool 182: 385-419

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24: 1596–1599

Uno H (1986) Comparison of cranial growth and development between Kuril and Largha seals. In "Proceedings of the Symposium on Ecology and Protection of Kuril seal" Ed by K Wada, T Ito, A Niizuma, S Hayama, M Suzuki, Tokai University Press, Tokyo, pp 158-178 (in Japanese with English abstract)

Vilà C, Walker C, Sundqvist AK, Flagstad O, Andersone Z, Casulli A, Kojola I, Valdmann H, Halverson J, Ellegren H (2003) Combined use of maternal, paternal and bi-parental genetic markers for the identification of wolf–dog hybrids. Heredity 90: 17-24

Wright S (1969) Evolution and Genetics of Populations Vol.2, The Theory of Gene Frequencies. University of Chicago Press, Chicago

Summary in Japanese

北海道沿岸には5種のアザラシ類が生息あるいは回遊しており，中でも個体数が多いのがゼニガタアザラシおよびゴマフアザラシである．近年，両種ともに個体数の増加が報告されており，それに伴って沿岸漁業への被害や混獲によるアザラシの死亡個体数も増加している．沿岸生態系において高次捕食者に位置するアザラシ類の個体数変動は，下位の生物相にも大きな影響を与える．日本で唯一通年生息しているゼニガタアザラシは絶滅危惧ⅠB 類として環境省が管理しており，北海道内では個体数の60%以上がえりもと厚岸に集中している．それに対して，冬から春に北海道の日本海およびオホーツク海沿岸に来遊するゴマフアザラシは，環境省ではなく北海道の管理下にある．アザラシの漁業被害量や混獲死亡個体数は地域差が大きく，さらに両種が同所的に生息している地域もある．しかし，それらの多くはどちらか一方を対象としたものであり，分類学的に近縁な2種の相違をより明確にするためには，2種を対象とした比較研究が必要である．本研究では，北海道沿岸に生息するゼニガタアザラシとゴマフアザラシについて，形態学的および遺伝学的比較を行った．

ゼニガタアザラシとゴマフアザラシの成長に伴う頭蓋骨形態の変化を調べたところ，ゼニガタアザラシでは幼獣の段階から雌雄差が認められた．また，ゴマフアザラシよりもゼニガタアザラシの方が大型であることもすべての成長段階で認められた．ゴマフアザラシは1980年代には雌雄差がほとんど認められないと報告されていたが，本研究では特に咀嚼機能に関与する計測部位で雌雄差が認められた．これは近年の個体数増加に伴って，繁殖競争や餌生物を巡る競争が激化したためではないかと考えられた．成長段階による頭蓋骨の非計測的特徴の変化を調べたところ，側頭頬骨縫合と鼻骨切歯縫合の形状には種差が認められた．また，両種は幼獣の段階では特徴が類似しているが，成長に伴ってゼニガタアザラシの形状が変化して種差が明確になっていくことが明らかとなった．

mtDNA・チトクローム*b*領域配列の解析の結果，ゼニガタアザラシではえりもの個体のハプロタイプ多様度が他に比べて低く，系統樹でも厚岸や納沙布の個体とは異なる集団に分かれることが認められた．このことから，北海道沿岸のゼニガタアザラシはえりも個体群と道東個体群が存在することが明らかとなった．それに対して，

ゴマフアザラシのハプロタイプは多様であり，系統樹でも明確な地域個体群を認めることは出来なかった．この個体群の有無は両種の生態学的相違を反映しており，上陸場への定着性の強いゼニガタアザラシが明確な地域個体群を示したのに対して，回遊性があり特定の上陸場への定着性があまりみられないゴマフアザラシには地域個体群が認められないということが確認された．

mtDNA と SRY による両種の種間交雑判定を試みたところ，えりものゼニガタアザラシ28個体中1個体とゴマフアザラシ5個体中2個体で，斑紋と異なる種の mtDNA ハプロタイプを持つことが確認された．その他の地域では，すべての個体は斑紋と同じ種の mtDNA ハプロタイプを示した．しかし，SRY 配列を調べたところ，ゼニガタアザラシとゴマフアザラシは同じ配列であり，SRY ハプロタイプを利用して交雑判定を行うことは不可能であった．しかし斑紋と mtDNA ハプロタイプが異なる個体が確認されたことから，えりもで種間交雑が生じている可能性が考えられた．

本研究の結果から，北海道沿岸に生息するゼニガタアザラシとゴマフアザラシの頭蓋骨形態の成長に伴う特徴，mtDNA による地域個体群の存在有無,種間交雑個体の存在の可能性が示された.特に,えりも地域では遺伝的特異性の高いゼニガタアザラシの地域個体群が認められるとともに，ゴマフアザラシとの交雑が生じている可能性も考えられた．従って，北海道におけるアザラシ類の保護管理を行う上で，えりも地域のモニタリングを重点的に進める必要があると考えられた．アザラシ類や沿岸漁業を含めた沿岸生態系の保全管理を実行していくにあたり，本研究の結果を基盤として，更に多様な調査研究へと発展させていくことが望まれる．