RESEARCH ARTICLE

Morphological identification in skull between spotted seal and harbor seal using geometric morphometrics

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Abstract

The morphology of the skull contains considerable ecological information about a species, because the skull contains sensory organs that are used to look for food, compete for mates, or to migrate. Spotted seals (*Phoca largha*) and harbor seals (*Phoca vitulina*) are similar in body size and pelage color but differ in habitat use and reproductive biology. The current study aims to clarify differences in the shapes of skulls in the spotted and harbor seals using geometric morphometrics and to discuss whether ecological differences can explain morphological differences in skulls. First, we discovered that the age at which the shape of skulls stopped changing was 7 years in both species, using the linear-threshold model. Using a total of 75 landmarks, 54 individuals (25 spotted seals, 29 harbor seals) that were older than the age at which skulls stopped changing were correctly identified at a rate of 100%. The total of 75 landmarks was narrowed down to eight key landmarks that resulted in an identification accuracy rate of 100% using random forests. Of the eight landmarks, seven were related to feeding apparatus, indicated that the harbor seal had a broader mouth and mandible than the spotted seal. Because of both species were dietary generalists and classified as pierce feeders, we suggested that the different features in the shapes of their skulls were caused not only by differences in their feeding behavior but also other differences related to reproductive behavior.

KEYWORDS
landmark, linear-threshold model, morphology, random forests, shape

1 | INTRODUCTION

The shapes of the skull evolved as a consequence of diet, how prey is captured, and intraspecific competition. Previous comparative studies demonstrated that differences in the shape of the skull were associated with different types of food and different ways of feeding (Adam & Berta, 2002; Kienle & Berta, 2016; Kienle et al., 2018; Kienle & Berta, 2016; Kienle & Berta, 2019). However, the skull also contains sensory organs such as eyes, ears, and nose, which are used to look

for food, mates, and to migrate. The position of the nose in whales, for example, changed during evolution from an anterior location to a dorsal position so that they could take a breath at the water surface (Reidenberg & Laitman, 2008).

The subject species of the current study are spotted seals (*Phoca largha*) and harbor seals (*Phoca vitulina*) whose ranges around Hokkaido, Japan, overlap and are occasionally observed hauled out together. Until the 1980s, the spotted seal was considered to be a subspecies of the harbor seal because the body size and pelage color of those species are

similar. However, ecological differences in habitat and reproductive biology (McLaren, 1966; Burns, 1970) and detailed morphological and craniometric analyses (Shaughnessy & Fay, 1977; Burns et al., 1984) confirmed that substantial differences exist between these two seals (Lowry et al., 1998). Spotted seals give birth on sea ice and are monogamous. They migrate during ice-free months, and their breeding season occurs during the months of sea ice cover. Harbor seals, on the other hand, give birth on land and use haul-out sites throughout the year, during the pupping, nursing, breeding, and molting seasons (Naito & Nishiwaki, 1972), and they are polygynous (Sullivan, 1981; Boness et al., 2002). In addition, spotted seal pups possess a white lanugo. This pelage provides insulation for the neonates of ice-breeding seals until they develop a blubber layer, and it may also serve as camouflage for protection from predators (Yochem & Stewart, 2018). Harbor seal pups, however, are normally born with a pelage similar to adults; they lose their white coat in the uterus before birth. A previous study argued that the spotted seal was, therefore, more plesiomorphic than the harbor seal (McLaren, 1966, 1975). A more recent study reported that the two species were closely related genetically, but their mitochondrial DNA is distinct (O'Corry-Crowe & Westlake, 1997).

An allometric comparison of the skulls showed that the harbor seal had a longer face and rostrum than the spotted seal (Shaughnessy & Fay, 1977; Burns et al., 1984; Uno, 1986). However, those features were thought to be associated with specialized types of food or the method of feeding on euphausiids in newly weaned pups (Kato, 1982; Uno, 1986). Another study reported that the same types of food were frequently observed in the stomachs of both species located in the same area (Nakaoka et al., 1986). They are generalist pierce feeders that grip and swallow their prey whole (Adam & Berta, 2002; Kienle & Berta, 2016; Kienle et al., 2018; Kienle & Berta, 2019). We, therefore, believe that the shapes differences of these species' skulls are not necessarily a result of their diet or the way they feed. It may be possible that the differences in skull features are due to other ecological differences. Although allometric methods are convenient, it is difficult to use such methods to describe complex closed contours (Tatsuta et al., 2018). Previous studies also attempted to categorize and classify the non-metrical characteristics of skulls in these species, however they were not able to quantify them (Burns et al., 1984; Nakagawa et al., 2009).

The current study aims to analyze differences in the shapes of skulls of spotted and harbor seals using geometric morphometrics and discuss morphological differences of the skulls in the context of ecological differences.

2 | MATERIALS AND METHODS

2.1 | Collection of samples

2.1.1 | Collection and treatment of skulls

The skulls of spotted seals (Phoca largha Pallas, 1811) and harbor seals (Phoca vitulina Linneus 1758) were collected along the coast of Hokkaido in northern Japan from 2002 to 2015 (Figure 1). A total of 270

dead seals were bycatch in fishing net which samples were provided from Fisheries Cooperatives. Forty-one of spotted seal samples were provided by hunters that exterminated seals for assuagement of fisheries damage by seals. The exterminations were carried out under the permission of Hokkaido Government, and the hunters belongs to Fisheries Cooperatives. Seven samples of spotted seal were captured for the scientific purpose to investigate the fishery damage by seals, complying the Wildlife Protection and Hunting Management Law and under permission of Hokkaido Government with the permission numbers of 351(2008), 353(2010), and 35(2011). A stranded and dead spotted seal was additionally included in samples. After muscles were removed from the skulls, whole skulls without damaged crania or mandibles were selected and used. Finally, we used 112 spotted seal skulls and 207 harbor seal skulls (Table 1). Spotted seals comprised 50 males and 62 females from 0 to 35 years old, and harbor seals comprised 104 males and 103 females from 0 to 32 years old (Table 1). All samples are deposited at Tokyo University of Agriculture (supplementary online material).

2.1.2 | Age determination

The upper right canine teeth were taken from each skull, sectioned at 10 μm with a cryostat (Leica Biosystems), and stained with Delafield's hematoxylin, following fundamental methods (Hachiya & Ohtaishi, 1994; Hohn, 2002) to determine the age. Ages were estimated based on the counts of cementum annuli (Mansfield & Fisher, 1960).

2.2 | Preprocessing

2.2.1 | Photography of skulls

Skulls were placed on a cutting mat (57 × 640 cm, Clover), and a digital camera (D3000, Nikon) mounted on a tripod (FHD-41Q, Velbon) was used to photograph the dorsal and ventral sides, and the lateral crania and mandibles. Distance was approximately 40 cm from skull to camera. All skulls were photographed in the same place at Tokyo University of Agriculture Abashiri, Hokkaido, Japan. Adobe Photoshop CS2 was used to align the scales of the photographs.

2.2.2 | Landmarks on skulls

Geometric morphometrics based on two-dimensional landmarks were used to quantify the shapes of skulls. A total of 75 homologous landmarks (shown in Figure 2 and described in Table 2) were digitized from each of four images using tpsDig2 (Rohlf, 2013). Landmarks were based on Bookstein (1991). They were located at the juncture points of bones, ridge curves, and edge points, all chosen from previous studies to capture the overall shape of the skull (Shaughnessy & Fay, 1977; Burns et al., 1984; Abe, 2000; Adam & Berta, 2002;
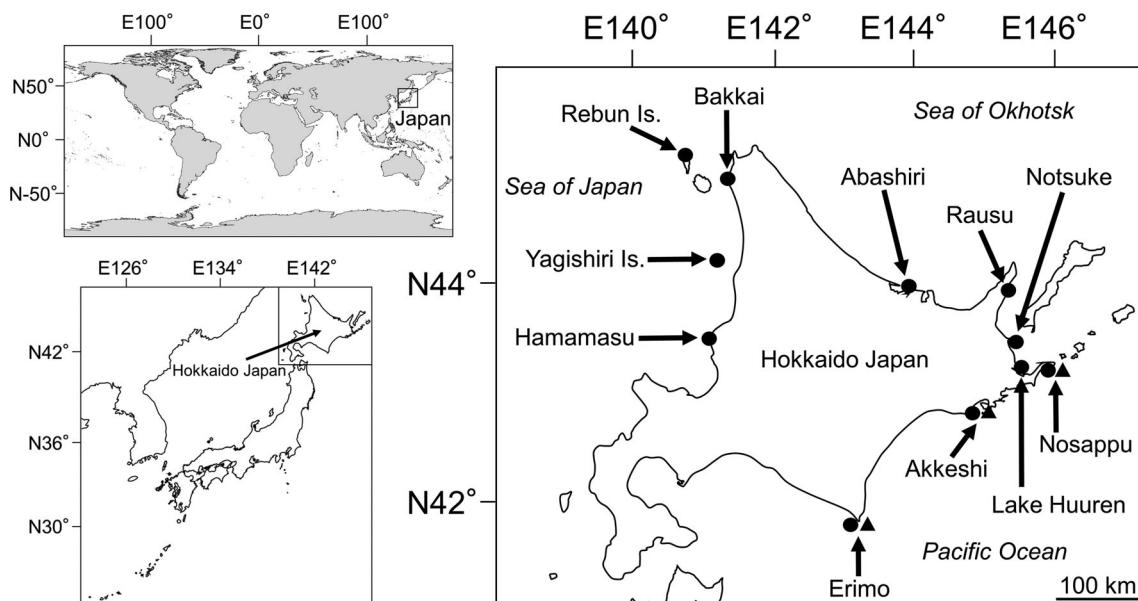
FIGURE 1 Geographical locations of the sampling sites in Hokkaido, Japan. Circles represent spotted seal (*Phoca largha*) and triangles represent harbor sea (*Phoca vitulina*)

Daniela & Thales 2008; Nakagawa et al., 2009; Kienle & Berta, 2016; Kienle & Berta, 2019).

With each landmark as (x_i, y_i), $\left(\frac{\sum x_i}{n}, \frac{\sum y_i}{n}\right)$ was taken as the centroid point (x_t, y_t) and as the original point. With its relative coordinates as $(x_i' = x_i - x_t, y_i' = y_i - y_t)$ from the original point, the distance from the original point was calculated as $d_i = \sqrt{x_i'^2 + y_i'^2}$ and the angle was calculated as $\theta = \arccos\left(\frac{d_i}{x_i'}\right)$, where x_i' was positive if $\theta < \frac{\pi}{2}$ or negative if $\theta > \frac{\pi}{2}$. These distance and angle (CDA) were used in the analysis. In addition, the direction of the reference landmark (dorsal cranium: No. 1, ventral cranium: No. 21, lateral cranium: No. 58, lateral mandible: No. 63) was set on the y- or x-axis, respectively. It was rotated to allow for comparison between individuals.

2.3 | Analysis

2.3.1 | Determining impact of age on shape change

In general, the growth of the body in mammals changes from rapid to gradual. Morphology of the skull also changes with growth after birth (Moore, 1981). We estimated the age at which shape changes stopped in the skull, because to capture the shape and character after the age. We considered that, when comparing the characteristics of skulls between species, it was simpler to use the age at which shape changes stopped rather than using major shape changes in the skulls that occurred during the growth of the species. We used the linear-threshold model (Clark et al., 1985), which is presents a normal linear regression up to a boundary and a linear regression with zero angle above a boundary, to estimate the age at which the shapes of skulls in each species stopped changing. The linear-threshold model consists of one regression and threshold value. These equations are described below:

$$obs_{age_i,j} = \begin{cases} \beta_{1,i,j} + \beta_{2,i,j} age_i + \varepsilon_{i,j} & age_i \leq age_\theta \\ \beta_{0,i,j} + \varepsilon_{i,j} & age_i > age_\theta \end{cases}$$

where age_i denotes the age of an individual, $obs_{age_i,j}$ is the measurement value of the CDA ($j = 1 \sim 150$) for each individual, age_θ denotes the age at which the shape of the skull stopped changing, $\beta_{0,i,j}$ represents a constant, $\beta_{1,i,j}$ denotes an intercept, and $\beta_{2,i,j}$ denotes the slope of the line. The error $\varepsilon_{i,j}$ was assumed to be normally distributed with mean 0 and variance σ. To estimate the age_θ, the AIC (Akaike, 1973) is calculated by varying value of age_θ from 0 to 35 and we defined the candidate value of age_θ with lowest AIC as the optimal value.
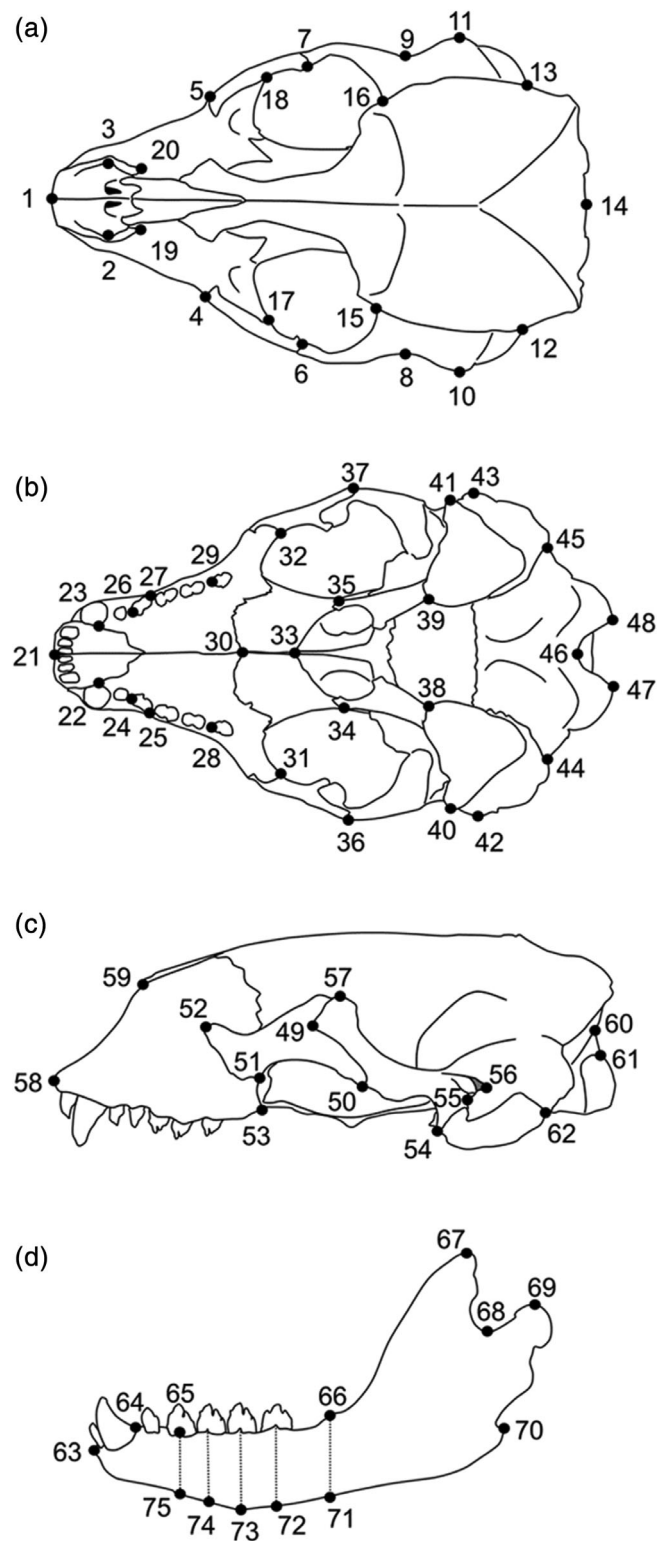
2.3.2 | Discrimination rate and important landmarks

We obtained the discrimination rate using random forests (Breiman, 2001) from samples of the two species that were of an age when the shape of the skull had stopped changing. Recently, some studies have used random forests for speciation by morphology (e.g., Machida et al., 2020). Random forests is an algorithm for classification developed by Breiman (2001) that uses an ensemble of classification trees (Breiman & Friedman, 1984; Ripley, 1996; Hastie et al., 2001). The algorithm for random forests in the classification is shown below. (1) Extract a bootstrap sample from the dataset (B₁, B₂, ..., B$_{ntree}$). The number of data in each bootstrap sample (B$_i$) was the same as the number of data in the original data set. About

TABLE 1 List of skull specimens used in this study

Age (year)	Spotted seal (*Phoca largha*) ♂	♀	Total	Harbor seal (*Phoca vitulina*) ♂	♀	Total
0	15	15	30	19	19	38
1	5	6	11	13	9	22
2	7	8	15	24	20	44
3	2	4	6	16	17	33
4	2	7	9	8	7	15
5	5	7	12	9	9	18
6	2	2	4	4	4	8
7	2	1	3	1	2	3
8	0	1	1	3	1	4
9	0	0	0	0	1	1
10	2	3	5	2	1	3
11	3	1	4	2	1	3
12	0	0	0	1	0	1
13	1	1	2	0	0	0
14	0	0	0	0	0	0
15	2	2	4	0	2	2
16	0	1	1	1	0	1
17	1	1	2	0	0	0
18	0	0	0	0	2	2
19	1	0	1	0	2	2
20	0	1	1	0	1	1
21	0	0	0	0	0	0
22	0	0	0	1	1	2
23	0	0	0	0	1	1
24	0	0	0	0	0	0
25	0	0	0	0	0	0
26	0	0	0	0	0	0
27	0	0	0	0	0	0
28	0	0	0	0	2	2
29	0	0	0	0	0	0
30	0	0	0	0	0	0
31	0	0	0	0	0	0
32	0	0	0	0	1	1
33	0	0	0	0	0	0
34	0	0	0	0	0	0
35	0	1	1	0	0	0
Total	50	62	112	104	103	207

FIGURE 2 Landmarks shown on the cranium (a) Dorsal; (b) Ventral; (c) Lateral; and Mandible (d). Descriptions of each landmark are provided in Table 2

two-thirds of the original data were extracted as training data, while the remaining one-third is called out of bag (OOB) data and used for verification. (2) In each bootstrap sample (B_i), these processes of randomly selecting the number of *mtry* in explanatory variables from among all variables and generating a decision tree (T_i) were repeated the number of *ntree*. (3) Determine the misclassification rate by a

majority vote by fitting the model constructed for OOB in each trial where the accuracy was verified using OOB data. The response variable of the model was species (i.e. spotted or harbor seal), and

TABLE 2 Description of landmarks

Dorsal landmarks	
1	Juncture between incisors on the premaxilla
2, 3	Maximal width of external nares point
4, 5	Anterior edge of the maxilla-jugal suture
6, 7	Edge of the jugal-squamosal suture
8, 9	Medial point of the squamosal
10, 11	Anterior edge of the mastoid process
12, 13	Posterior point of squamosal
14	Medial point of the occipital
15, 16	Posterior point of the orbit
17, 18	Medial point of the maxilla-jugal suture
19, 20	Posterior edge of the incisor
Ventral landmarks	
21	Juncture between incisors on the premaxilla
22, 23	Premaxilla-maxilla suture where it intersects the medial edge of the canine
24, 26	Anterior edge of the P2
25, 27	Posterior edge of the P3
28, 29	Anterior edge of the M1
30	Posterior-most premaxilla-maxilla suture on the palate
31, 32	Medial edge of the maxilla-jugal suture
33	Maxilla-palatine midline suture
34, 35	Anterior edge of the pterygoid hamular
36, 37	Lateral edge of the jugal-squamosal suture
38, 39	Anterior edge of the auditory
40, 41	Anterior edge of the external auditory meatus
42, 43	Anterior edge of the mastoid process
44, 45	Posterior edge of the squamosal-basioccipital
46	Anterior-most point on the foramen magnum
47, 48	Posterior edge of the occipital condyle
Lateral landmarks	
49	Anterior edge of the jugal-squamosal suture
50	Posterior edge of the jugal-squamosal suture
51	Medial edge of the premaxilla-jugal suture
52	Anterior edge of the premaxilla-jugal suture
53	Posterior edge of the maxilla
54	Posterior edge of the glenoid fossa
55	Anterior edge of the external auditory meatus
56	Posterior edge of the external auditory meatus
57	Dorsal point of the squamosal
58	Anterior edge of the premaxilla
59	Anterior-most point on the nasal-premaxilla suture
60	Medial point of the occipital
61	Dorsal point of the occipital condyle
62	Posterior edge of the auditory-squamosal
Mandible landmarks	
63	Anterior point of the mandibular ramus

(Continues)

TABLE 2 (Continued)

Mandible landmarks	
64	Posterior edge of the canine
65	Medial point of the neck of the p2
66	Posterior point of the tooth row
67	Dorsal apex of the curve on the coronoid process
68	Anterior edge of the mandibular condyle
69	Posterior edge of the mandibular condyle
70	Tip of the angular process
71	The ventral of the posterior point of the body of the mandible
72	The ventral point of the medial neck of the m1
73	The ventral point of the medial neck of the p4
74	The ventral point of the medial neck of the p3
75	The ventral point of the medial neck of the p2

explanatory variables were the CDA from each landmark. The discrimination rate was calculated by subtracting OOB error rate (Díaz-Uriarte & Alvarez de Andrés, 2006) (%) from 100. The OOB error rate was obtained by setting each parameter of *ntree* and *mtry* (Immitzer et al., 2012). The *ntree* and *mtry* indicate the number of trees in the forest and the number of input variables randomly chosen at each split, respectively (Genuer et al., 2010).

We conducted the model selection by adopting appropriate explanatory variables modified by Baba and Matsuishi (2015). The model selection was based on variable importance (Breiman, 2001). The variables were set for each landmark. We conducted a model with all the CDAs, and for each CDA we calculated the Mean Decrease of Gini (Navega et al., 2014). Then for each landmark, the Mean Decrease of Gini for distance and angle were summed together and we used this summed value as an index of importance for each landmark. The algorithm for calculating Mean Decrease of Gini is shown below. (1) Estimate the overall misclassification rate (err^{oob}) of the kth ($k = 1, ..., ntree$) decision tree. (2) Randomly reorder all the values of the explanatory variables of the mth ($m = 1, ..., mtry$) in the OOB data corresponding to the kth decision tree to estimate the overall misclassification rate ($\widetilde{err_m^{oob}}$) at that time. (3) The above 1)-2) was performed on all decision trees and the average value calculated by the following equation was taken as the Mean Decrease of Gini of the mth explanatory variable (Imp_m).

$$Imp_m = \widetilde{err_m^{oob}} - err^{oob}$$

The model was reconstructed by removing the landmark one by one in order of lowest importance for each landmark estimated by full model and continued to remove variables at same or lower misclassification rate (err^{oob}).

Important landmarks were defined by the least the number of landmarks for discrimination which were evaluated by Mean Decrease of Gini and included in the smallest explanatory variable that did not change the percentage of accuracy rate. Finally, we clarified the rank
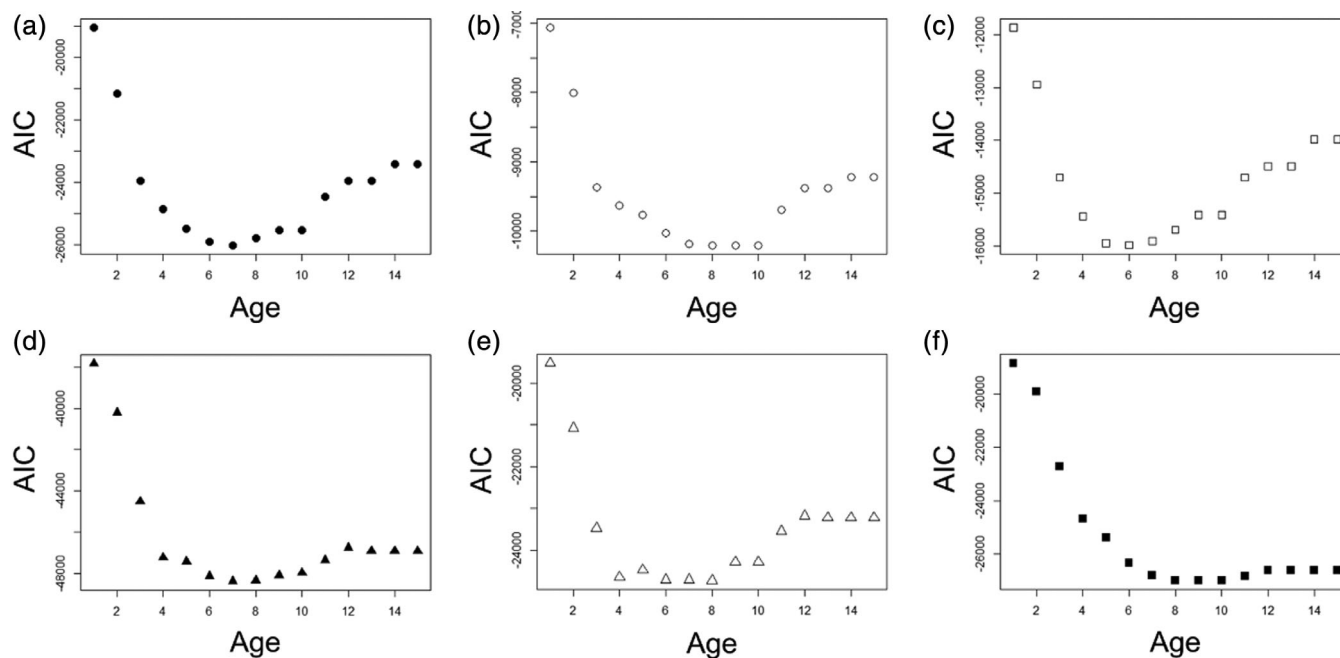
FIGURE 3 The linear-threshold model fitted to coordinate values of landmarks and represented AIC in each age group (a) All spotted seal, (b) Male of the spotted seal, (c) Female of the spotted seal, (d) All harbor seal, (e) Male of the harbor seal, (f) Female of the harbor seal)

of important landmarks and the necessity to use that the number of landmarks for discrimination.

We conducted a principal components analysis (PCA) using CDA to determine the major axes of variation between variables. All analyses were conducted in R version 3.5.0 (R Development Core Team, 2018). Random forest analyses were conducted using the randomForest version 4.6–14 package (Liaw & Wiener, 2002) under R.

3 | RESULTS

3.1 | Estimation of the age at which the shape of the skull stopped changing

Using the linear-threshold model, the age at which the skull stopped changing (age_0) was found to be 7 years old in both species (Figure 3). AIC was lower value as the divided into species than sex in the model (Figure 3).

3.2 | Identification rates of species and calculation of important landmarks

The 25 spotted seals and 29 harbor seals were at least 7 years old, or older. A comparison of plots at each average coordinate showed that the skull of the harbor seal was larger than that of the spotted seal (Figure 4).

For the analysis of random forests, the overall misclassification rate for OOB data, (err^{oob}), showed the lowest value when the number

of features (*mtry*) was set the number of the square roof of input variables (Shi & Horvath, 2006), and it was confirmed that the estimation was sufficiently stable when the number of decision trees (*ntree*) was 2000, from the change in each misclassification rate. Therefore, we tuned the model using the number of decision trees (*ntree*) with 2000 of features (*mtry*) for each parameter. With 75 landmarks, the identification accuracy rate was 100%. For each landmark, we could narrow down the 75 landmarks to eight landmarks that were most important for identification purposes while maintaining the same identification rate of 100% (Figure 5, supplementary online material). Figure 4 and Figure 5 shows the eight important identification landmarks: the posterior orbit (No. 15), molar (No. 28), edge of the pterygoid hamular (Nos. 34–35), under the posterior external auditory meatus (No. 56), the tip of the angular process (No. 70), and the tooth row of the body of the mandible (Nos. 72–73).

Landmarks 15, 34, and 35 (on the orbit and pterygoid hamular) in harbor seals were at the more posterior location than those of the spotted seals (Figure 4a, b). Landmark 28 (molar) in harbor seals was located more on the buccal compared with the spotted seals (Figure 4b).

Landmark 56 (posterior of the external auditory meatus) in the spotted seal was located toward the posterior of the centroid point in the lateral view. In contrast, in the harbor seal, it was located more posteroventral (Figure 4c). Furthermore, the external acoustic meatus of the spotted seal was cover by a bony process from the lateral view, while that of the harbor seal was relatively open.
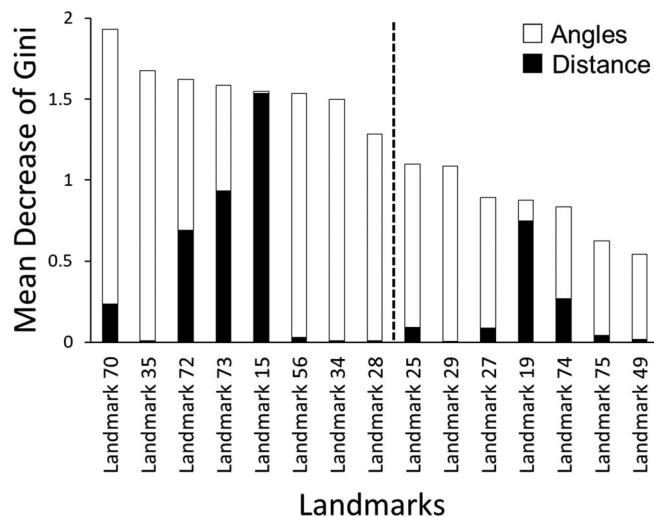
Landmark 70 (angular process) in harbor seals was located more posteroventral compared with the spotted seals (Figure 4d). Landmarks 72 and 73 (ventral of the body of the mandible landmarks) in the harbor seal were located at an inferior position than in the spotted
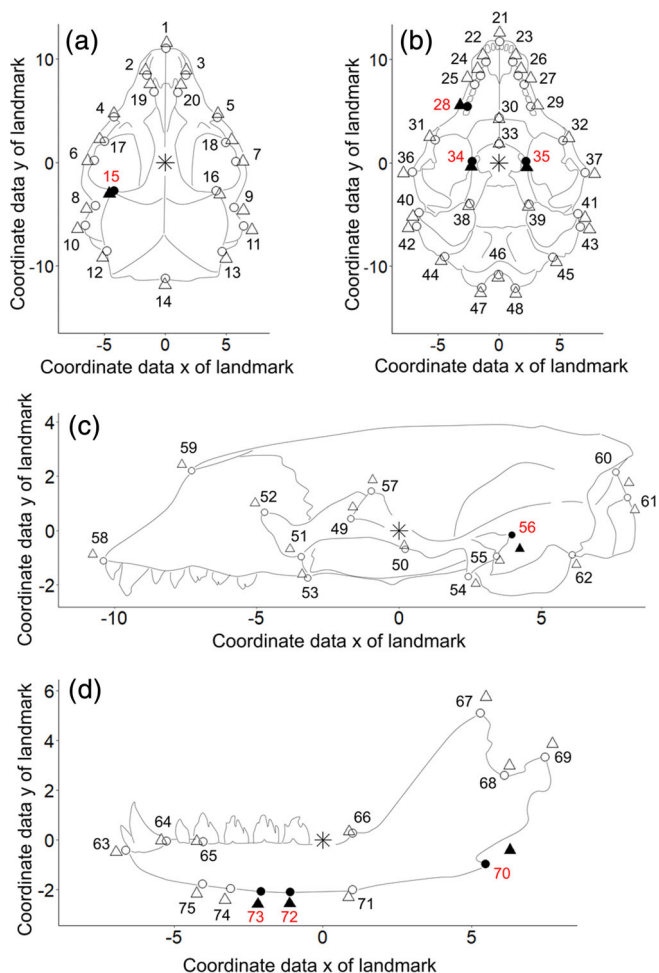
FIGURE 4 Comparisons of the mean for each landmark between spotted seal (circle) and harbor seal (triangle) (a) Dorsal view of the cranium; (b) Ventral view of the cranium; (c) Lateral view of the cranium; (d) Lateral view of the mandible. Asterisk (*) indicates the centroid point in each view. Outline of the skull are fitted to landmarks of the spotted seal (circle) to compare with the harbor seal (triangle). Each number indicates the landmark. Solid circles, triangles, and red number show important landmarks for discrimination
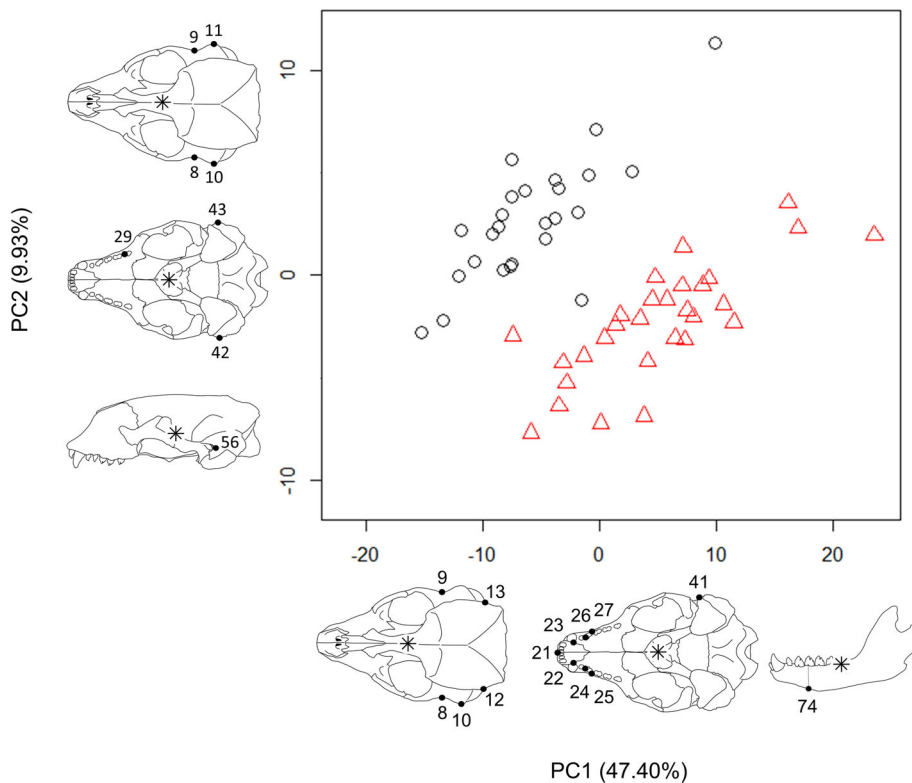

FIGURE 5 The top fifteen important identification landmarks as revealed by the mean decrease of Gini value in each landmark, which represents the total value of the distance and angle from the centroid point. The distance represents the difference in size, and the angle represents the difference in the position relative to the distance to compare landmarks as coordinate data. The left side of the dotted line represents eight important identification landmarks for spotted seals and harbor seals aged 7 years or older

Landmark 74 (ventral of the body of the mandible), which was reflected in PC1, and important identification landmarks 72 and 73 calculated by random forests were close to each other and showed tendencies similar to the selected landmark (Figures 4, 6). Landmark 29 (molar), which was reflected in PC2, and important identification landmark 28 calculated by random forests also showed a similar position at the posterior rostrum (Figures 4, 6). Landmark 56 (posterior of the external auditory meatus) of PC2 was also a common important identification landmark selected by random forests (Figures 4, 6). Landmarks 21–27 (anterior of the rostrum) and landmarks 8–10 and 12–13 (around the squamosal) were reflected in PCs in PCA, but these were not selected by the random forests (Figures 4, 6).

4 | DISCUSSION

We showed that the age at which the shape of the skull stopped changing was 7 years in the spotted seal and 7 years in the harbor seal. Using all 75 landmarks, 54 individuals (25 spotted seals, 29 harbor seals) that were older than the age at which the skull stopped changing were correctly identified at an accuracy of 100%. Since each average position of landmarks on harbor seals was more on the lateral than on spotted seals (Figure 4), the skull of the harbor seal is broader and larger than that of the spotted seal. Burns (2002) and Nakagawa et al. (2009) reported that the skull of the harbor seal was more massive and robust than that of the spotted seal, a finding that was also supported by the present study.

A total of eight identification landmarks (15, 28, 34, 35, 56, 70, 72, 73) were narrowed down while maintaining the same

seal (Figure 4d). Landmark 73 was located at the most ventral position among the anterior on the mandible of the harbor seal (Figure 4d).

The PCA revealed that 47.40% and 9.93% of the variances in CDA for spotted seal and harbor seal aged 7 years or older were explained by the PC1 and the PC2, respectively (Figure 6). PC1 reflected the distances from the centroid point to the landmarks around the squamosal (8–10, 12–13), the anterior rostrum landmarks (21–27), the anterior edge of the external auditory meatus (41), and the ventral landmark of the body of the mandible (74) at the dorsal and ventral view of the cranium and the lateral view of the mandible (Figure 6, supplementary online material). PC2 reflected the angle from the centroid point to the landmarks around the squamosal (8–11, 42–43), the posterior of the rostrum (29), and the posterior of the external auditory meatus (56) at the dorsal, ventral and lateral view of the cranium (Figure 6, supplementary online material).

FIGURE 6 Scatterplots of the first two axes of principal component analysis based on variables of the landmarks of spotted seals and harbor seals aged 7 or older. Circles represent spotted seals and triangles represent harbor seals. Variables that were strongly reflected in each axis are represented by the illustrations of the cranium and landmarks. Filled circles and numbers represent the landmarks. The asterisk (*) in the illustrations of cranium indicates the centroid point

discrimination ratio (100%) among the total number of 75 landmarks. Of the eight landmarks, seven were related to the feeding apparatus (15, 28, 34, 35, 70, 72, 73). Differences in landmarks 15, 28, 34 and 35 indicated that the harbor seal has a longer rostrum and broader palate than the spotted seal. Slater et al. (2009) reported that a broader rostrum would enable to have a larger and stronger bite, and the longer, broader rostrum of the harbor seal would therefore enable it to open its mouth wider and have a stronger bite than the spotted seal. Differences in position of landmark 70 (angular process) was indicated that the harbor seal has a more developed angular process relative to the spotted seal (Figure 4). The angular process serves for the attachment of the pterygoids medially and the masseter laterally (Evans & de Lahunta, 2013). Expansion of the angular process reflect increased size of the masseter musculature (Radinsky, 1985). Therefore, this suggests that the harbor seal may have larger masseter musculature and stronger bite force. Differences in the position of landmarks 72 and 73 indicated that the mandible of the harbor seal was broader than that of the spotted seal. Differences in position of landmark 73 indicated that the body of the mandible around landmark 73 was more angular than the spotted seal. Those positions (72, 73) relate to area of attachment for the digastric muscle, which helps with opening the mouth. The broader of the body of the mandible under the row of teeth and the more ventral position of the 73 seems to support the attachment of much digastric muscle that could be developed for strong biting movement. Although bite performance is generally related to the type of prey, both species are dietary generalists and are classified as pierce feeders (Adam & Berta, 2002) and thus do not need to crush hard prey. Euphausiids are the prey of newly weaned spotted seal pups (Kato, 1982) and schooling fishes are the main prey of older seals (Kobayashi, 2015). On the other hand, harbor seals are opportunistic feeders on locally abundant and easily available prey items (Telmann & Galatius, 2018), and they have been found to prefer foraging demersal fishes in shallow waters and also cephalopod species (Andersen et al., 2004; Bromaghin et al., 2013; Nakaoka et al., 1986). Additionally, a comparative study of the feeding habitats of spotted seals and harbor seals in the same coastal area in the Nemuro district of eastern Hokkaido, Japan, for example, demonstrated that the harbor seal depends on benthic animals in shallow waters, while the spotted seal foraged for prey from the surface to middle layers (Nakaoka et al., 1986). However, a variety of common prey was also found in spotted seals and harbor seals stomachs (Nakaoka et al., 1986), suggesting that the harbor seal could not necessarily need a longer mouth and stronger bite than the spotted seal. Also, information on the stomach contents may vary temporary, therefore it is difficult to evaluate the feeding habitat on a long-term. However, the information of the feeding habits in the spotted seal and harbor seals along the coast of Hokkaido is insufficient (e.g., Itoo et al., 1983; Kaimoto et al., 2018; Kato, 1982; Masubuchi et al., 2017; Masubuchi et al., 2019; Nakaoka et al., 1986; Wada et al., 1992). It was also suggested that dietary constraints in the past could reflect the differences in the skull shape of the spotted seals and harbor seals.

Alternatively, differences in the skull shape related to bite force may be related to differences in reproductive behavior. In pinnipeds, polygynous species produce larger theoretical bite forces than monogamous species, they have large muscle attachment areas, and

both males and females of combative species have more robust jaw morphology (Jones et al., 2013). In addition, highly - aggressive species have wider zygomatic arches likely to allow the emplacement of large jaw–closing muscles in the skull (Dewar et al., 2015). Male–male combat is influenced by bite force evolution in pinnipeds (Jones et al., 2013; Jones & Ruff, 2011). Relationship between combat style and skeletal morphology also reported between the intraspecific combat behaviors and morphology of the cervical vertebrae in the ruminant mammals, the morphology differed in relation to the combat style and the role of cervical vertebrae was supported to resist the forces generated in male – male combat (Vander Linden & Dumont, 2019). Scars were frequently observed around the neck of adult male harbor seals (Naito, 1973; Niizuma, 1986; Sullivan, 1981), and biting at the surface of the water between adult males for access to estrous females was observed during the breeding season (Sullivan, 1981), because the mating system of the harbor seal is polygamous and they use haul-out sites throughout the year (Burns, 2002). The spotted seal, however, gives birth and nurses pups on sea ice, and triads consisting of a female, her pup, and one attending male can be seen on the ice, or with the attending male close by, in an annually monogamous system where there is little competition between males (Burns, 2002). We conclude that a broader mouth and mandible in harbor seal had compared to spotted seal might be due to their different mating systems. Additionally, it has been reported that some spotted seals attained sexual maturity at the age of 3 years, though most reached sexual maturity at 5–6 years of age (Naito & Nishiwaki, 1972), and that most harbor seals reached sexual maturity at the age of 5 years (Suzuki & Yamashita, 1986). Mizuno and Ohtaishi (2002) also reported that skull growth ceased mostly around 5 years old and the completion ages for character development were after 5 years old in the spotted seal. Because the age at which the shape of the skull stopped changing was 7 years in both species, we found that the shape of the skull also changed after the age of sexual maturity and showed similar tendency of the previous study. Furthermore, Nakagawa et al. (2009) reported that the occipital width contributed to the discrimination between the skulls in the spotted seals and harbor seals and the feature also showed in this study as the landmarks around the squamosal contributed to the major axes (Figure 6). On the other hand, the landmarks around the squamosal were selected by PCA but also these landmarks were not selected in the random forests as the important identification landmarks. We estimated that the shape changes around squamosal would be great variation for individuals and occur the interaction close to landmarks, because some muscles are attachment around the area. For example, the splenius capitis, longissimus capitis, and sternocleidomastoid attach to mastoid process, furthermore, previous study reported that these muscles and the morphology of the mastoid process were associated with male – male competitions through the neck movements in some pinniped species (Bohórquez-Herrera et al., 2017). However, in the current study, landmarks around the mastoid process were not selected as the important identification landmarks. It was indicated that the important identification landmarks would represent the original features and reflect the difference of ecological behaviors in the spotted seal and harbor seal.

In conclusion, we used geometric morphometrics to show detailed differences in the shapes of skulls in the spotted and harbor seals, which are closely related species. We could discriminate between the two species with a high degree of accuracy and narrow down important landmarks for identification. We suggest that the different features in the shapes of their skulls are caused not only by differences in their feeding but also to differences in reproductive behavior.

AUTHOR CONTRIBUTIONS

Arai Yuichi: Formal analysis; investigation; writing - original draft. **Minoru Kanaiwa:** Methodology. **Mio Kato:** Data curation. **Mari Kobayashi:** Conceptualization; funding acquisition; writing - original draft.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/jmor.21397.

DATA AVAILABILITY STATEMENT

Data available on request from the authors

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