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Foreword

Remarkable evolution in cetacean studies in recent decades owes much to major journals that have made significant contribution to the development of modern cetology: Discovery Reports, published by the National Institute of Oceanography in the United Kingdom, and Norwegian Whaling Gazette in Norway, as well as The Scientific Reports of the Whales Research Institute in Japan.

The Scientific Reports of the Whales Research Institute was first published in 1948, a year after the Whales Research Institute was established. Aiming to share valuable research findings and scientific knowledge worldwide, the publication was formatted in English since its beginning, quite an ambitious attempt in Japan still recovering from the devastation of World War II.

Since its first publication, a total of 246 scientists contributed 419 scientific papers to The Scientific Reports of the Whales Research Institute. It is widely acknowledged and appreciated that these scientific papers were the foundation for the development of cetacean studies worldwide, and in today's terms, it was a research journal that had a significant impact factor, or high number of citations. Regrettably, however, The Scientific Reports of the Whales Research Institute was discontinued in 1988 with the 39th volume after the institute was reorganized into the Institute of Cetacean Research.

In the 30 years since then, various types of journals on cetacean studies have been published globally, each offering different perspectives on scientific research outcomes. As for Japan, no research journal matching The Scientific Reports of the Whales Research Institute in its quality has been published. It is probably because many domestic cetologists have sought to publish their papers in international research journals based outside Japan.

As the global environment surrounding the issue of whaling became increasingly complex, we have observed a shift in publishing policies among these journals, rejecting papers whose findings are based on specific research methods such as lethal sampling. Because of this, no small numbers of papers submitted by biological scientists using samples collected through lethal surveys, even just for some parts, have been denied proper reviews. While we agree that animal ethics should be given high priority when writing a research paper, if a paper, the research method of which is allowed under domestic and international rules, is rejected, it is a decision made beyond scientific judgment.

Our new journal for cetacean population studies intends to follow the scientific policy of The Scientific Reports of the Whales Research Institute, that is, to contribute to global development of cetacean studies. As long as submitted papers conform to scientifically-accepted animal ethics, we do not make distinctions based on research methods. At the same time, to maintain the journal's neutrality in the complex global environment surrounding whaling issues, the journal will be published from a newly organized committee, rather than as a bulletin type scientific report from a specific research institute. The title of the new journal will be Cetacean Population Studies to be abbreviated CPOPS, and we aim to keep our door wide-open for researchers worldwide, contribute to the scientific development of resource studies for marine mammals especially focusing on cetaceans, and nurture many aspiring scientists.

Seiji Ohsumi

Seiji Ohsumi, Ph. D.
Chairman
Publication Committee for the Cetacean Population Studies

Full paper

SATELLITE TRACKING OF COASTAL BRYDE’S WHALES *BALAENOPTERA EDENI* ALONG THE SOUTHWEST COAST OF JAPAN

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Abstract

To examine the movements of Bryde’s whales, *Balaenoptera edeni*, in coastal waters off Japan, satellite tagging was conducted off Kochi, the southwest coast of Tosa Bay from 2004 to 2008, and off Nomaike, on the southwest coast of the Satsuma Peninsula in 2005. Using an air gun, an Argos satellite tag was attached on the animal from a whale watching boat. A total of 20 shots were taken at 17 individuals, and tags were successfully attached to 11. Geographical locations from the Argos Satellite were obtained from seven animals. The maximum tracking period was 30 days in Tosa Bay, and 40 days off Nomaike. Most individuals stayed in the same waters for at least several weeks in the summer season (July to August). However, one individual moved from Tosa Bay to the Kii Peninsula, and one from Nomaike to the Goto Islands in the East China Sea. No animals moved to the offshore waters across the Kuroshio Current. This result supports the current classification of the stock of coastal Bryde’s whales (the East China Sea Stock). Further technical improvements are necessary to increase the tagging success rates and extend the tracking period to investigate movements and distributional ranges of Bryde’s whales in the coastal waters off Japan.

Key words Coastal Bryde’s Whale, Satellite Tracking, Movement.

Introduction

Bryde’s whales *Balaenoptera edeni* are distributed worldwide in warm temperate oceans between around 40° N to 40° S (Omura, 1959). Currently, at least two genetically distinct forms are recognized (Kato and Perrin, 2009, 2017): coastal Bryde’s whales (*B.e.edeni*) and offshore Bryde’s whales (*B.e.brydei*). Coastal Bryde’s whales are distributed in coastal waters around southwest Japan and have been utilized in commercial whale watching activities by the local fishermen in southwestern Tosa Bay, Kochi prefecture since 1989, and in Nomaike, on the southwest coast of the Satsuma Peninsula, Kagoshima prefecture since around 1996 (Morioka, 2000). Offshore Bryde’s whales are distributed broadly in offshore waters from the equator to around 40° N in the western North Pacific.

To elucidate the status and stock structure of the coastal Bryde’s whales around Kochi and Kagoshima, several studies have been carried out using line transect shipboard sighting surveys, as well as photo-identification studies, and genetic analyses. The results of these surveys and studies indicated that Bryde’s whales off Kochi and Kagoshima are an isolated coastal population isolated from the offshore Bryde’s whales, separated by the Kuroshio Current (Kato *et al*., 1996; Kishiro *et al*., 1997; Yoshida and Kato, 1999; Kato and Kishiro, 1999). Bryde’s whales off Kochi are sighted within 15 nautical miles from the southwest coast of Tosa Bay year round and have apparent seasonal changes in density with a peak in the summer season (Kishiro *et al*., 1997). Bryde's whales near Kagoshima are sighted in the regional waters between Koshiki Island and off Nomaike, and are also frequently sighted during the summer season (Kato and Kishiro, 1999). However questions concerning the seasonal movements and distributional range of Bryde's whales remain.

The satellite telemetry technique is one efficient way to investigate the migration of marine mammals, and recent development in tagging techniques have increased the opportunity to investigate animal movements (Mate *et al*., 2007; Heide-Jørgensen *et al*., 2001). To examine individual movements and distributional ranges of Bryde's whales in the coastal waters off Japan, this study applied this technique to track tagged Bryde's whales off Kochi and Kagoshima.

An earlier version of the present paper was consisting of the author's doctoral thesis submitted to Tokyo University of Marine Science and Technology.

Materials and Methods

Study area and shipboard surveys

The two study areas off Nomaike, Kagoshima (a) and off Kochi (b) are shown in Figure 1. Both

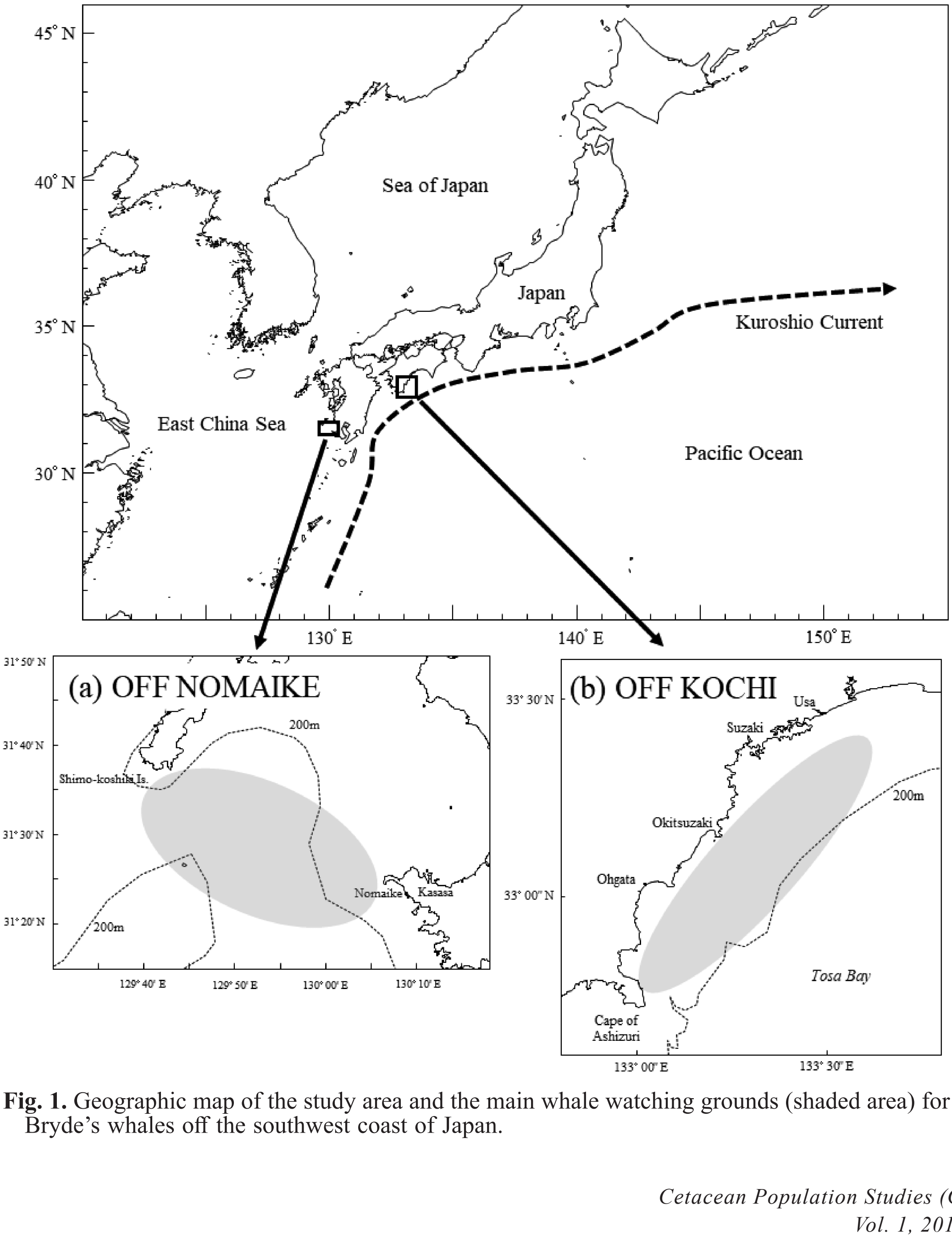

Fig. 1. Geographic map of the study area and the main whale watching grounds (shaded area) for Bryde's whales off the southwest coast of Japan.

areas are known as commercial whale watching areas for Bryde's whales.

Shipboard surveys were carried out using local whale watching boats (5 to 12 GT) off Kochi from 2000 to 2015 and off Nomaike from 2001 to 2005. Each survey lasted 3 to 14 days in the summer season (July to August), which was thought to be the peak migration of the whales in both areas (Kato and Kishiro, 1999). During a survey, 2 to 5 boats were used simultaneously, with 1 to 3 researchers on board each vessel. The vessel GPS locations, sea surface temperature, and sea weather conditions were recorded every 15 minutes during the cruises. Using 7 × 50 binoculars and the naked eye, searching was carried out non-randomly. Search area and route of individual vessels was determined daily based on the weather conditions and sighting information from commercial vessels. When a whale was sighted, the vessels approached to confirm species identification, record the GPS position, determine group size and behaviors, and take photographs for individual identification.

Tagging device and tracking methods

As a part of the shipboard surveys, satellite tagging was attempted in the waters off Kochi from 2004 to 2008, and off Nomaike in 2005. As per agreement with local whale watching operators, the number of whales to be tagged was pre-determined to be 2–3 animals per year in the respective waters.

A Spot-5 implantable tag with a two-month battery life (Wildlife Computers, USA) was used as the Argos transmitter. A 40 mm air gun (Miroku Machinery Co. Ltd, Japan) was used for tagging (Fig. 2), with the filling pressure set to 110 kgf/cm^2. The forecastle deck was used as the tagging platform. The tagging dart used in this study consisted of a dart with a 3-bladed tip, an Argos transmitter, and retrievable float (Fig. 3). The float was connected to the dart by a water-soluble string. When the dart hit the

Fig. 2. Air gun used for attachment of the satellite tags from 2004 to 2008.

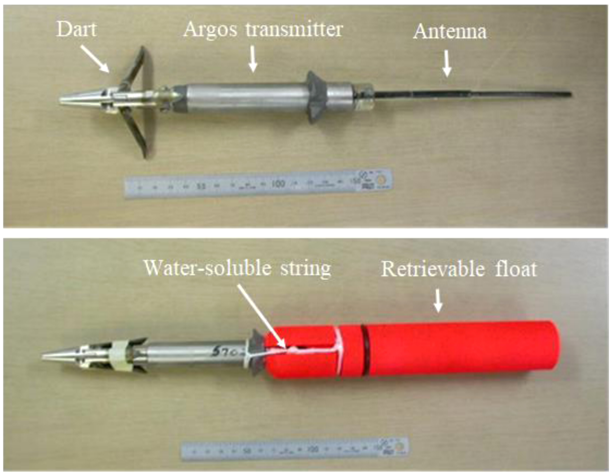

Fig. 3. The satellite tag used for Bryde's whales from 2004 to 2008. Upper photograph indicates Argos transmitter with a dart. Lower photograph indicates the tag with a retrievable float.

whale, it was embedded through the blubber into the muscle, the string dissolved, the float detached, and the antenna of the transmitter was exposed on the body surface. When the target was missed, the dart floated on the sea surface and was then retrieved by a hand net. The dart was coated with povidone iodine to reduce physical damage and potential infection caused by tagging. The tracking data from tagged animals were obtained from the Argos satellite.

Animal tagging was carried out following the basic guidelines of the 3R principle for animal experiments in Japan issued by the Ministry of Agriculture, Forestry and Fisheries.

Results

Tagging results

A total of 20 tagging attempts were carried out, and 11 tags were successfully attached on 11 whales. In Tosa Bay (off Kochi), a total of 12 shots were made at 12 individuals from 2004 to 2008. Among them, 10 darts hit their target, and nine transmitters were successfully attached. For successful tagging, the chase time ranged from 5 to 23 minutes (mean: 14.7 minutes), and shooting distance ranged from 5 to 6 m (mean: 5.3 m). When the darts missed the target, the chase time ranged from 6 to 62 minutes (mean: 33.0 minutes), and shooting distance ranged from 5 to 10 m (mean: 7.6 m).

Off Nomaike, a total of eight shots were taken at five individuals in 2005, and two individuals were successfully tagged. For successful tagging, chase time ranged from 6 to 11 minutes (mean: 8.5 minutes), and shooting distance was 7 m. When the darts missed, chase time ranged from 3 to 79 minutes (mean: 33.5 minutes), and shooting distance ranged from 7 to 10 m (mean: 8.2 m) (Table 1).

Table 1. Results of the tagging for Bryde's whales in Tosa Bay and off Nomaike from 2004 to 2008.

Area	Whale ID	Shooting Date	Shooting Time	Argos ID	School size	Sea state*	Time for chasing (minutes)	Shooting distance (m)	Verdicts	Transmitter attached
Tosa Bay	–	18 Jul. 2004	12:31	49483	1	3	31	10	Hit	Lost
	#1	20 Jul. 2004	14:38	49482	1	3	17	6	Hit	Fix
	#2	16 Jul. 2005	14:40	57022	2	2	5	5	Hit	Fix
	#3	16 Jul. 2005	14:56	57023	2	2	16	5	Hit	Fix
	–	20 Jul. 2005	16:02	–	2	2	6	8	Ricochet	Retrive
	#4	20 Jul. 2005	16:20	57021	2	2	10	6	Hit	Fix
	#5	24 Jul. 2006	13:54	64634	1	3	18	5	Hit	Fix
	#6	24 Jul. 2006	15:57	64635	1	2	23	5	Hit	Fix
	#7	25 Aug. 2006	12:05	64636	2	2	14	5	Hit	Fix
	–	11 Aug. 2007	12:40	–	1	3	62	5	Miss	Retrive
	#8	11 Aug. 2007	16:21	64637	1	2	21	5	Hit	Fix
	#9	31 Aug. 2008	15:18	64639	1	3	8	6	Hit	Fix
Off Nomaike	–	10 Aug. 2005	9:26	–	1	2	5	7	Miss	Retrive
	–	10 Aug. 2005	11:17	–	same ind.	2	56	7	Miss	Retrive
	–	10 Aug. 2005	13:56	–	1	2	5	10	Miss	Retrive
	#10	11 Aug. 2005	12:29	57026	1	1	11	7	Hit	Fix
	–	13 Aug. 2005	10:28	–	2	2	3	9	Miss	Retrive
	–	13 Aug. 2005	11:18	–	same ind.	2	53	9	Miss	Retrive
	–	13 Aug. 2005	11:44	–	same ind.	3	79	7	Miss	Retrive
	#11	13 Aug. 2005	12:53	57025	1	3	6	7	Hit	Fix

* Beaufort scale

Fig. 4. Example of the tag attachment on a Bryde's whale. The tag was attached on 20 July 2004 in Tosa Bay (whale #1), and photographed on 23 July 2004.

Tags were attached on either side of the body anterior to the dorsal fin (Fig. 4). All targeted whales swam quickly during chasing, and no behavioral change was observed during or after the tagging operation.

Transmission received by the Argos Satellite

Table 2 summarizes the results of tag transmissions received by the Argos Satellite. Although 11 tags were successfully deployed, only ten tags transmitted signals that were received by the satellite, and no signal was obtained from one tag (whale #3). For the ten active tags, the time period between tag attachment and final reception of the signals lasted from 1 to 40 days, and the number of transmission signals ranged from 2 to 191 (Fig. 5). From this signal data, geographic locations were obtained 17 times from five animals in Tosa Bay, and 19 times from two animals off Nomaike. The accuracy for these locations was classified and recorded as class 0 (one time), class A (14 times), and class B (21 times) by the Argos data collection and location system. According to the accuracy from a past calibration study (class 0: ≥ 1 km; class A: ≤ 46 km; class B: ≤ 73 km (Baba et al., 1997)), the accuracy of this study ranged from 1 km to 73 km.

Table 2. The number of transmission signals received, and locations estimated by the Argos satellite from Bryde's whales tagged in Tosa Bay and off Nomaike from 2004 to 2008.

Area	Whale ID	Tagging date	Argos ID	Reception period (days)	No. of signals received	Tracking period (days)	No. of locations estimated	Accuracy class		
								0	A	B
Tosa Bay	#1	20 Jul. 2004	49482	4	10	4	4	0	2	2
	#2	16 Jul. 2005	57022	34	81	30	1	0	1	0
	#3	16 Jul. 2005	57023	0	0	–	–	–	–	–
	#4	20 Jul. 2005	57021	5	37	0	0	–	–	–
	#5	24 Jul. 2006	64634	4	17	4	3	0	3	0
	#6	24 Jul. 2006	64635	7	2	0	0	–	–	–
	#7	25 Aug. 2006	64636	1	6	0	0	–	–	–
	#8	11 Aug. 2007	64637	15	41	13	5	1	2	2
	#9	31 Aug. 2008	64639	2	20	2	4	0	2	2
Off Nomaike	#10	11 Aug. 2005	57026	4	10	2	1	0	0	1
	#11	13 Aug. 2005	57025	40	191	40	18	0	4	14

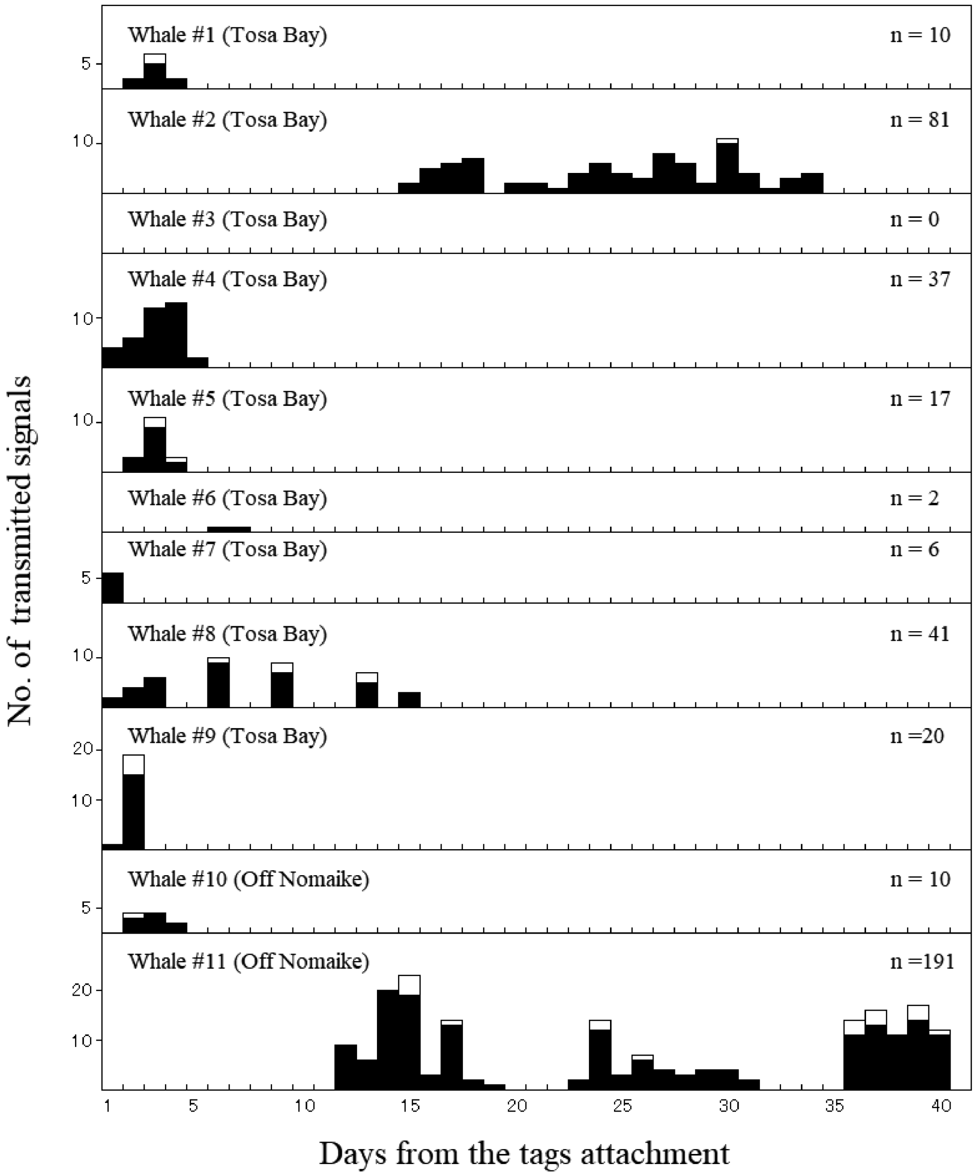

Fig. 5. Frequency distribution of the transmitted signals received by the Argos satellite, with days from tag attachment on Bryde's whales. Open bar: Location was determined; closed bar: Only reception.

Geographical movements of tagged animals

Four out of five animals tagged in Tosa Bay (whale #1, #2, #5, and #9: Fig. 6 a-d) stayed in the Bay throughout the transmission period (2 to 30 days). One animal tagged in the Bay on 11 August 2007 (whale #8) moved out of the Bay, moving eastward and reaching the east coast of the Kii Peninsula (off Owase) on 19 August, nine days after tagging. It then turned westward and returned to the Bay by 23 August (Fig. 6 e).

Off Nomaike, two individuals were tracked (Fig. 7 a-b). One individual (whale #10) was located near the tagging site the next day; however, no location could be determined after that, even though transmitted signals were sporadically received until two days later. Another individual (whale #11) was tracked for 40 days after tagging. After staying near the tagging site for 15 days (13 to 27 August), #11 moved to the Goto Islands then returned to near the tagging site (29 August), and stayed in this vicinity until transmitting signals were lost on 21 September.

Re-sightings of tagged animals

One tagged individual (whale #1) was re-sighted during the transmitting period (Fig. 6 a). The loca-

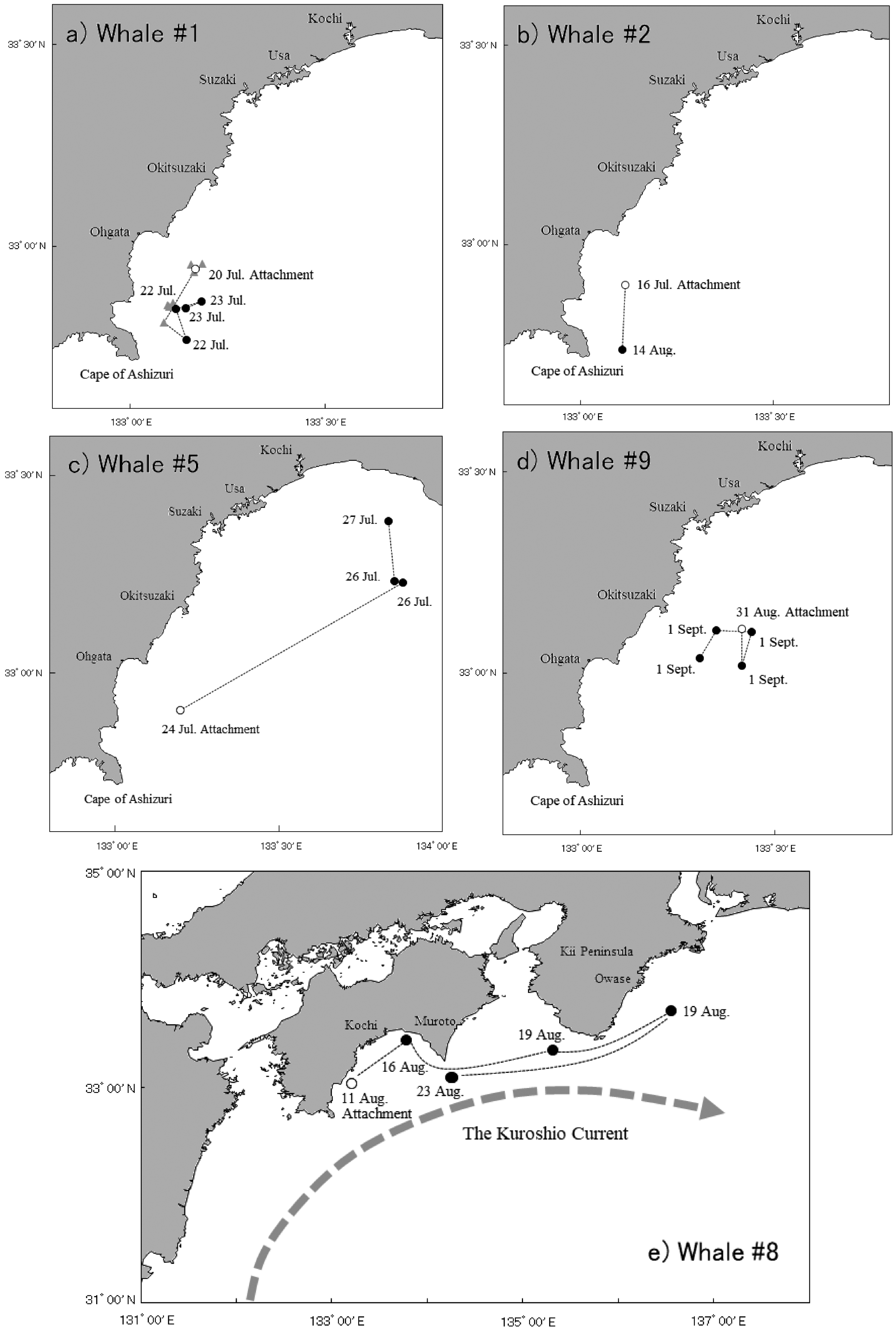

Fig. 6. Satellite-based movements of five Bryde's whales tagged in Tosa Bay in 2004 (whale #1), 2005 (whale #2), 2006 (whale #5), 2007 (whale #8), and 2008 (whale #9). Open circles: Positions at tag attachment; closed circle: Positions determined by the Argos satellite; grey triangle: re-sighting positions during the transmitting period.

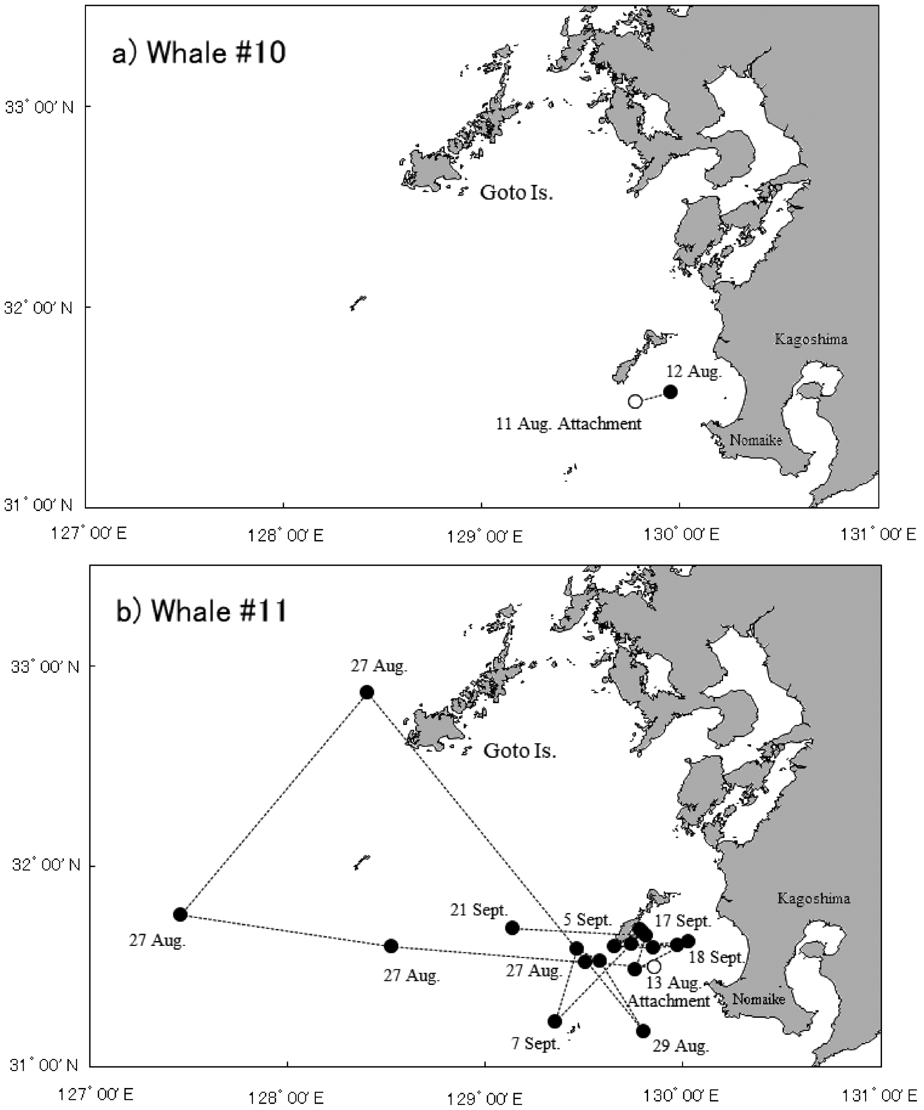

Fig. 7. Satellite-based movements of two Bryde's whales tagged in the waters off Nomaike in 2005 (whale #10 and #11). Open circles: Positions at tag attachment; closed circle: Positions estimated by the Argos satellite.

tions determined by transmitted signals (accuracy class A and B) coincided well with actual sighted positions of the whale.

Two tagged individuals in Tosa Bay were re-sighted years later. Whale #2 was tagged on 16 July 2005, and was re-sighted on 6 August 2007 and again on 23 and 27 July 2008 in the Bay. Whale #4 was tagged on 20 July 2005, and re-sighted on 22 August 2006 and 6 August 2007 in the Bay. These re-sightings were determined by Photo-ID (Fig. 8), and it was noted that the tags had fallen off. Tagging scars on the body had healed similarly to old cookie-cutter shark bites (Fig. 8). Animals did not show any behavioral changes. No problems were observed by approaching the whales in the whale watching boat, and one (whale #4) remained calm during a 70-minute observation. Whale #2 associated with another individual, and both animals remained calm during observations. These instances suggested that tagging impacts were not severe or lasting in these animals.

Discussion

The trials and results reported in the present study are the first attempt at the satellite tracking of Bryde's whales in the coastal waters off Japan. Although the number of transmitted signals was small

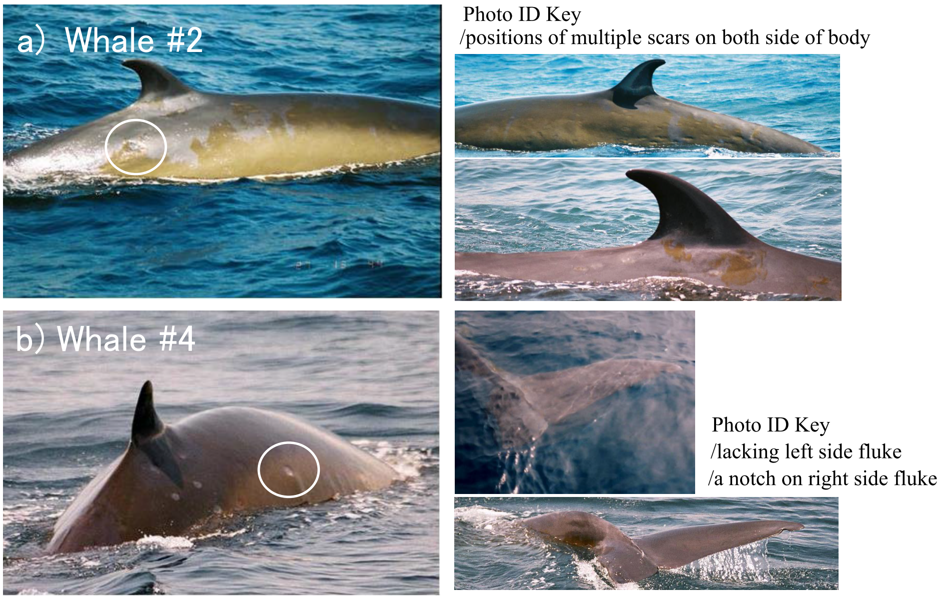

Fig. 8. Re-sightings of two tagged Bryde's whales in Tosa Bay, with photo ID keys to identify the animals. White circles indicate the tagging site of the whales. a) whale #2: Tagged on 16 July 2005, and re-sighted on 27 July 2008; b) whale #4: Tagged on 20 July 2005, and re-sighted on 22 August 2006.

and the tracking period was short, the results suggested that the Bryde's whales in the coastal waters did not move long distances, and possibly stayed in Tosa Bay and off Nomaike for at least several weeks during the summer season (Figs. 6 and 7). East-west movements of one animal from Tosa Bay to the Kii Peninsula indicated that whales could move beyond the Bay, and their distributional range possibly expands around the Kii Peninsula along the Pacific coastline. Movements of one animal off Nomaike to the Goto Islands indicated that the distributional range is possibly wider than the regional waters off Nomaike in the East China Sea. Both results revealed that the whales stayed in the same general location, with occasional movements to a wider area than the regional waters of Tosa Bay and Nomaike.

In the western part of the North Pacific, the Scientific Committee of the International Whaling Commission (IWC) sets two management stocks for the Bryde's whales: the western North Pacific stock and the East China Sea Stock (International Whaling Commission, 1997). The western North Pacific stock corresponds to the offshore Bryde's whales distributed in a broad area from the equator to around 40° N and west of 160° W in the North Pacific. The East China Sea Stock corresponds to whales distributed in the East China Sea. Based on previous studies, the Bryde's whales off Kochi (in Tosa Bay) have been treated as an extension of the East China Sea stock, and their range is thought to expand to at least off Kochi in Tosa Bay (Kato *et al*., 1996; Kishiro *et al*., 1997; Yoshida and Kato, 1999; Kato and Kishiro, 1999). However, the tracking results from this study (whale #8) imply that the northern limit of their range might expand beyond Tosa Bay, with possible migrations along the coast to the Kii Peninsula. If so, it would be necessary to re-consider their range to improve management of the East China Sea stock.

Based on the sighting distributions, Kato and Kishiro (1999) suggest that the warm Kuroshio Current acts as a physical barrier between the two stocks. No whales tagged in this study crossed the Kuroshio Current towards the offshore waters in the western North Pacific region. Although the sample size was small, this result may support the IWC stock classification and suggestion for the Kuroshio as the barrier between the two stocks.

In this study, transmitted signals from the tags and resultant estimated locations were sporadic, and poor reception by the satellite decreased the accuracy of the estimations. One possible reason might be mechanical trouble with the tag caused by the physical shock of deployment or striking the whale body. Another reason might be the location of tag attachment on the body. In this study, the tags were mainly attached near the base of the dorsal fin. However, the base of the dorsal fin emerges just before long dives, and is not as frequently exposed compared to anterior parts of the body (around the blowhole). To estimate accurate locations, the satellite needs at least three successive transmitted signals while passing over the animal. The low frequency of tag exposure caused inadequate signal reception. If the tag could be attached more anteriorly, the transmission frequency might increase enough to improve uplink to the satellite.

To elucidate the movements of the whales after the summer season, a longer tracking period is desired; unfortunately, the maximum tracking period was 30 days in Tosa bay, and 40 days off Nomaike. It is presumed that tags fell off of individuals after the last signal transmission. To extend the longevity of the tags, further technical improvements to the dart, such as a modification of the shape and number of blades, introduction of a flexible mechanism in the anchor blades, and using adapted materials to prevent biological reactions will be needed. To control the pressure and depth of dart penetration, use of other shooting gear such as the Norwegian LK-Arts system (e.g. Heide-Jorgensen et al., 2001; Olsen et al., 2009) that can easily control the filling pressure may also be useful.

Increasing the tagging success rate and extension of the tracking period are desirable for future studies, and further improvements to the darts and shooting gear as mentioned above will be needed. It is important to continue to monitor whale movements and to build a larger dataset including a greater number of individuals. The accumulated data obtained could bring further insight into the movements and distributional ranges of Bryde's whales around Japan. In addition, if possible, simultaneous biopsy sampling and genetic analyses would be valuable for further elucidation and confirmation of stock structure.

Acknowledgment

The author wishes to express sincere thanks for the assistance of many anonymous researchers, skippers, and staff of the whale watching organizations in Tosa Bay and Nomaike. The field surveys were conducted by the National Research Institute of Far Seas Fisheries in cooperation with local prefectural governments and the whale watching organizations as a contract with the Fisheries Agency of Japan (FAJ). Sincere thanks are due to the FAJ, Kochi Prefectural Government, and Kagoshima Prefectural Government for providing the opportunity to conduct the surveys.

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Full paper

COMPARISON OF SHAPES OF THE WHITE FLIPPER PATCH BETWEEN TWO SUB-SPECIES OF COMMON MINKE WHALES (*BALAENOPTERA ACUTOROSTRATA*)

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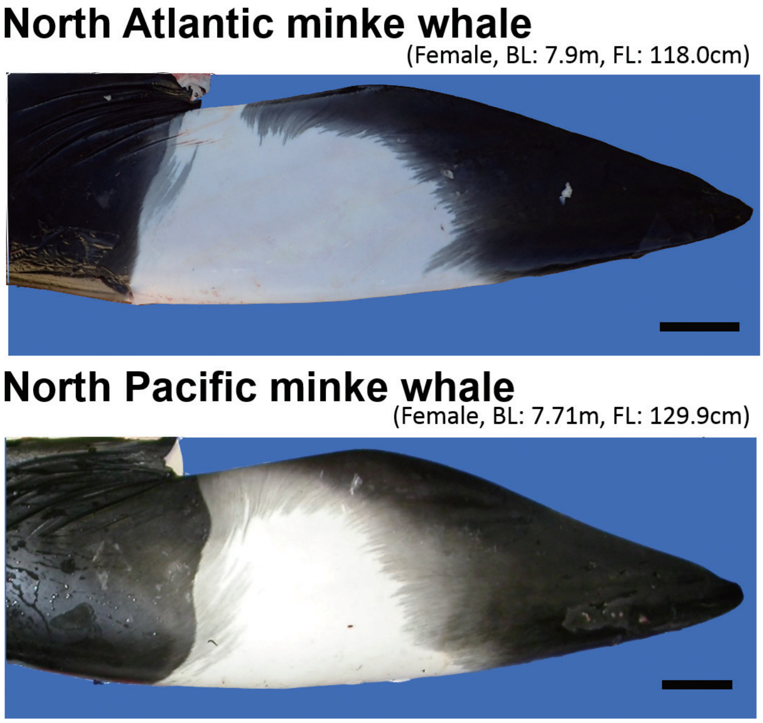
Abstract

The North Atlantic and North Pacific minke whales are regarded as two different subspecies. In this study we aimed to clarify the morphological differences of the white patch on the flipper between these subspecies. Morphological measurements were taken from the left flipper of sexually mature animals, collected from the North Atlantic (N=15) and the North Pacific (N=16) oceans. The length between the tip of flipper to the distal border of the white patch relative to the total flipper length showed no statistical differences between the two subspecies. However, the length between the tip of flipper to the proximal border of white patch relative to the total flipper length was significantly larger in the North Atlantic (74.31%) as compared to the North Pacific (63.62%) minke whales. Also, the mean angle between the proximal boundary line of the white patch and the longitudinal axis of the flipper was significantly different between the North Atlantic (70.05 degrees) and the North Pacific (92.29 degrees) minke whales. These results show that there are clear morphological differences in the white patch of the flipper between the two minke whale subspecies.

Key words common minke whale, *Balaenoptera acutorostrata*, flipper, white patch morphology, subspecies, taxonomy.

Introduction

The common minke whale (*Balaenoptera acutorostrata*) is distributed worldwide and is regarded as an important marine resource, particularly in the North Atlantic and North Pacific. Taxonomically, common minke whales are classified into two subspecies: *B. a. acutorostrata* in the North Atlantic and *B. a. scammoni* in the North Pacific. Although a distinctive population exists in the Southern hemisphere, generally known as the "dwarf" minke whale (*B. a.* subsp.), the taxonomical status of this subspecies is still under discussion (Rice, 1998). Since external characteristics are important classification criteria for taxonomy, various studies have focused on the differences in these characteristics to distinguish minke whale populations within and between oceans (Omura and Sakiura, 1956; Christensen *et al.*, 1990; Kato *et al.*, 1992; Nakamura *et al.,* 2014). A unique characteristic of the common minke whale is the white patch on their flippers. Compared to the North Pacific minke whale, the dwarf minke



North Atlantic minke whale
(Female, BL: 7.9m, FL: 118.0cm)

North Pacific minke whale
(Female, BL: 7.71m, FL: 129.9cm)

Fig. 1. Examples of typical appearances of minke whale flippers for the North Atlantic minke whale (top) and the North Pacific minke whale (bottom). See also photographs in Appendix 1. Scale bars indicate 10 cm.

whale has much larger white patches which extend to their shoulders. This feature has been used to distinguish these two subspecies (Arnold *et al.*, 1987; Kato and Fujise, 2000; Arnold *et al.*, 2005). Nakamura *et al.,* (2015) reported that the shape of the white patch would be a powerful taxonomical character to morphologically distinguish the stocks in the North Pacific. Horwood (1989) reviewed and described the differences in the shape of the white flipper patch between the North Atlantic and North Pacific common minke whales. Notably, the latter has a small central projection of the white area into the black area of the flipper, whereas in the former, the white area is more angled with no such projection.

Fig. 1 shows the color patterns of flippers from North Atlantic and North Pacific common minke whales. These different characteristics of the white patches have not yet been thoroughly described and compared. Using a photogrammetric method, Nakamura *et al.* (2014) showed that the white patch is significantly larger in the North Atlantic common minke whale as compared to the North Pacific one. However, to clarify the morphological differences of flipper between the two common minke whale subspecies, a more detailed analysis based on unified methodologies and controlled conditions is needed. The aim of this study was to specify the relative size and differences of the white flipper patch between the North Atlantic and North Pacific common minke whales, now based on measurements made directly on flippers obtained from animals taken in commercial (North Atlantic) and research (North Pacific) catches.

Materials and Methods

The data from North Atlantic common minke whales were collected during Norwegian commercial whaling operations conducted south of the Svalbard islands during the summer season in 2016 (Fig. 2). The North Pacific common minke whale data were collected from the survey of the second phase of the Japanese Whale Research Program under Special Permit in the Western North Pacific (commonly known as JARPN II), conducted in the coastal and offshore waters of Japan in 2012 and 2013

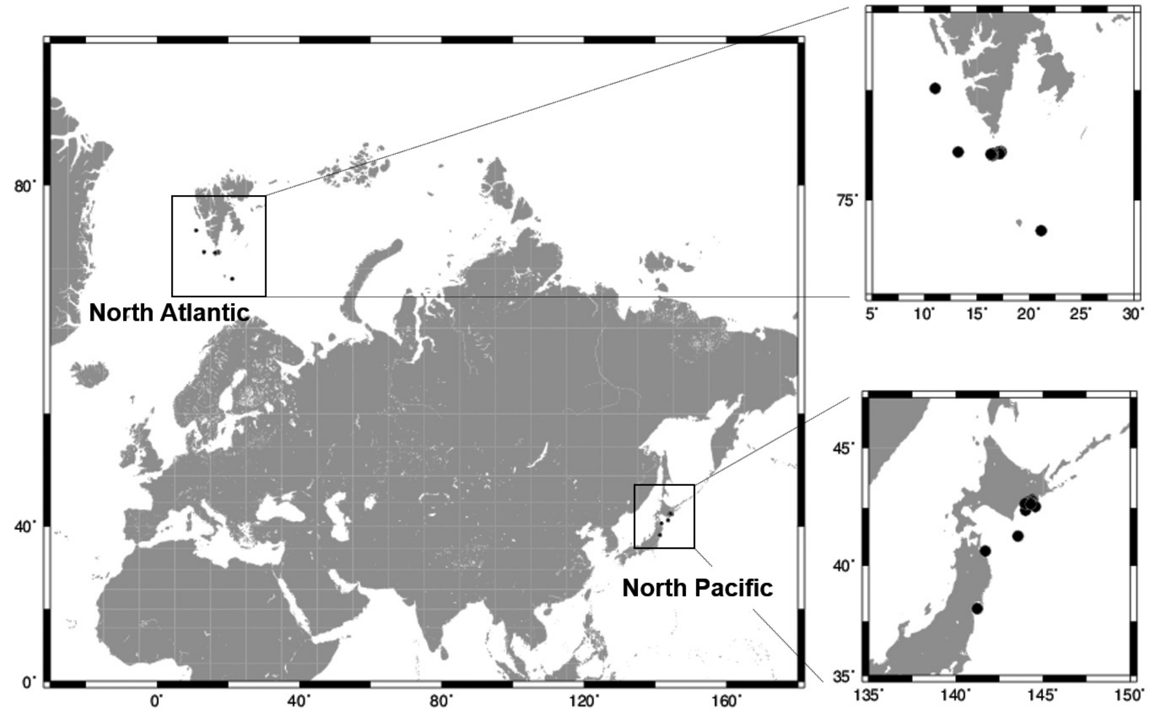


Fig. 2. Location where materials of the North Atlantic and North Pacific minke whales were collected.

(Fig. 2). This research was conducted in accordance with Article VIII of the International Convention for the Regulation of Whaling, and Japanese law.

To minimize the effects of sex-specific and growth-dependent differences on the white patch, we analyzed sexually mature female whales only. A total of 15 animals, mean body length 8.07 m (range: 7.2−8.7 m) from the North Atlantic population, and 16 animals, mean body length 7.96 m (range: 7.10−8.68 m) from the North Pacific population were used in this study. Body length was measured from the tip of the snout to the notch of flukes, rounded to the nearest 10 cm for the North Atlantic minke whales and the nearest 1 cm for the North Pacific minke whales. Sexual status was determined by examining ovaries; whales possessing ≥ 1 corpus albicans or corpus luteum were regarded as sexually mature. The left flipper was removed and photographed. For each photograph, we carefully ensured that the camera was placed in the upper direction confronting the flipper. Then, the following lengths were measured in a straight line using a measuring tape to the nearest 0.5 cm: *Total flipper length*: The tip of flipper (a) to the end of the articular process of the humerus (c); *Measurement point A*: The tip of flipper (a) to the distal border of white patch (A); and *Measurement point B*: The tip of flipper (a) to the proximal border of white patch (B). *Measurement point C* (*Angle* θ) is the angle between the proximal boundary line of white patch and the longitudinal axis of the flipper. The proximal boundary line of white patch was defined as the line passing from the ventral (B) to dorsal (b) sides of the proximal border of white patch, whereas the longitudinal axis of the flipper is the straight line from the tip of flipper to the end of the articular process of the humerus. This angle was calculated by a photogrammetric method using the graphic software Canvas X (Fig. 3). T -testing was applied to test the differences between the two subspecies. It was considered that t was significant at $P < 0.05$. Standard deviation (S.D.) was adapted to measure the dispersion of the data. All statistical analyses were carried out using packaged tools in the statistics software R (R Development Core Team 2015).

Results and Discussion

To clarify inter-subspecies differences in the relative size of the white patch, the proportion of *Measurement points A* and *B* relative to the *Total flipper length* in percent, and *Measurement point C* in

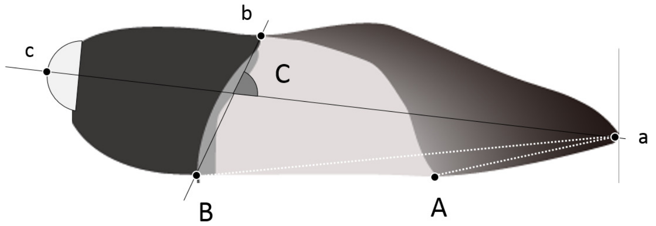
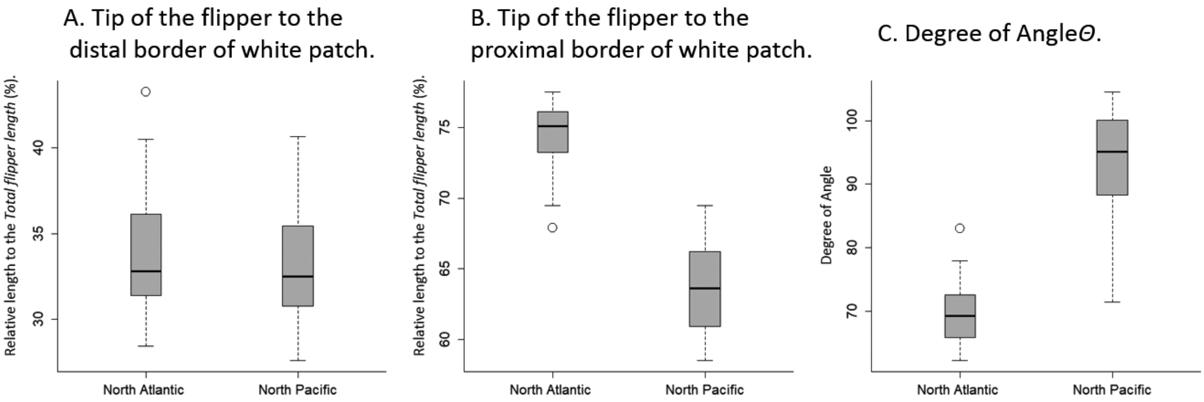


Fig. 3. Measurement points of the white patch of the left flipper from the common minke whale. *Total flipper length*: The tip of flipper (a) to the end of the articular process of the humerus (c), *Measurement point A*: The tip of flipper to the distal border of white patch (A), *Measurement point B*: The tip of flipper to the proximal border of white patch (B) and *Measurement point C* (Angle θ): The angle between the proximal boundary line of white patch (B-b) and the longitudinal axis of the flipper (a-c) were measured.


A. Tip of the flipper to the distal border of white patch.

B. Tip of the flipper to the proximal border of white patch.

C. Degree of AngleΘ.

Fig. 4. Comparison of the mean values of three measurement points of the white patch on the flipper from the North Atlantic and North Pacific minke whales. A and B show relative size of the *Measurement point A* and *B* (see Fig. 3) to the *Total flipper length*.

Table 1. Mean values of each measurement points of white patch on the flipper of the North Atlantic and North Pacific minke whales.

Subspecies	Number of samples	A. Relative length between the tip of flipper to the distal border of white patch to the total flipper length (%)	B. Relative length between the tip of flipper to the proximal border of white patch to the total flipper length (%)	C. AngleΘ(°)
North Atlantic	15	33.97 ± 3.96	74.31 ± 2.58	70.05 ± 5.74
North Pacific	16	33.08 ± 3.64	63.62 ± 2.91	92.29 ± 10.15

degrees were compared between the two subspecies.

The mean proportion of *Measurement point A* relative to *Total flipper length* was 33.97% (S.D. = 3.96) and 33.08% (S.D. = 3.64) in the North Atlantic and North Pacific common minke whales, respectively. No significant differences were detected (Fig 4-A, Table 1). The mean proportion of *Measurement point B* relative to the *Total flipper length* was significantly larger in the North Atlantic (74.31%, S.D. = 2.58) as compared to the North Pacific (63.62%, S.D. = 2.91) common minke whales ($P < 0.01$; Fig 4-B, Table 1). The mean of *Measurement point C* was significantly distinct between the North Atlantic (70.05 degrees, S.D. = 5.74) and the North Pacific (92.29 degrees, S.D. = 10.15) common minke whales ($P < 0.01$; Fig 4-C, Table 1).

The results of our analysis indicate that clear morphological differences exist in the relative size of the white flipper patch between the two subspecies. Although the mean relative length of *Measurement point A* showed almost the same values between the two subspecies, the relative length of *Measurement point B* was significantly greater in the North Atlantic common minke whales than in the North Pacific ones. This indicates that overall, the North Atlantic common minke whales have a relatively larger white patch area than the North Pacific common minke whales. As mentioned above, the white patch of the dwarf minke whales covers its shoulder region and is much larger than that of North Pacific and North Atlantic common minke whales (Best, 1985; Kato and Fujise, 2000; Arnold *et al*., 2005). Pastene *et al*. (2007) reported that dwarf minke whales are genetically closer to the North Atlantic common minke whales than to the North Pacific ones. Therefore, the shape of the white patch may well serve as a suitable taxonomic feature to distinguish between subspecies of the common minke whale.

Acknowledgements

We thank the captain, Mr. Dag Myklebust, and the crew of KATO and Mr. Truls Soløy, the Chairman of the Norwegian Whalers Union for their contribution during sampling in the North Atlantic Ocean. We also express our gratitude toward the former leaders of the JARPN II Survey; Drs. Genta Yasunaga and Takeharu Bando of the Institute of Cetacean Research; Chief in biological surveys, Mr. Shigeo Tabata and Mr. Takahiro Hara of the National Research Institute of Far Seas Fisheries; Mr. Atsushi Wada and Mr. Tatsuya Isoda of the Institute of Cetacean Research; and the staff of the Kyodo Senpaku Co., who were largely involved in the offshore surveys. Finally, we sincerely indebted to Dr. Hidehiro Kato, Professor Emeritus of Tokyo University of Marine Science and Technology for his supervision throught the present study.

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Appendices: Appendix 1-1. Pictures of the left flipper of the North Atlantic minke whale.

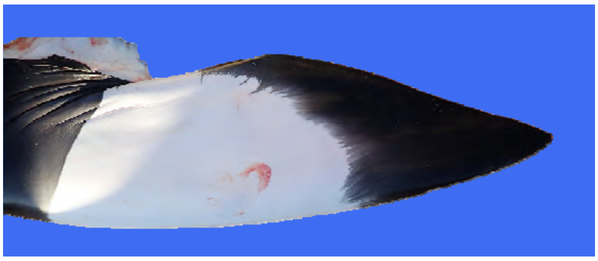
50cm

2016Norway_47 (7.9m)

2016Norway_49 (7.7m)

2016Norway_50 (8.4m)

2016Norway_51 (8.5m)



2016Norway_52 (7.2m)

2016Norway_53 (8.4m)

2016Norway_54 (7.8m)

2016Norway_57 (7.3m)

Appendix 1-2, (continued).

2016Norway_58 (7.7m)

2016Norway_59 (8.2m)

2016Norway_60 (8.7m)

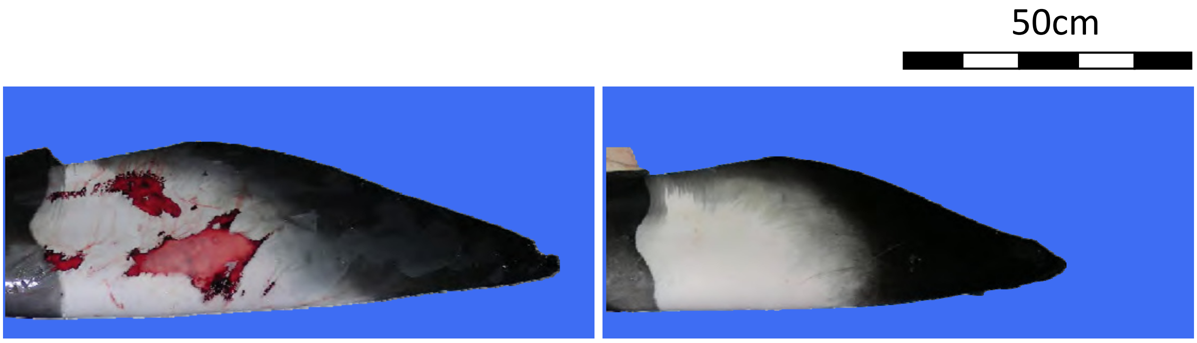
2016Norway_61 (8.0m)

2016Norway_62 (8.5m)

2016Norway_63 (8.5m)

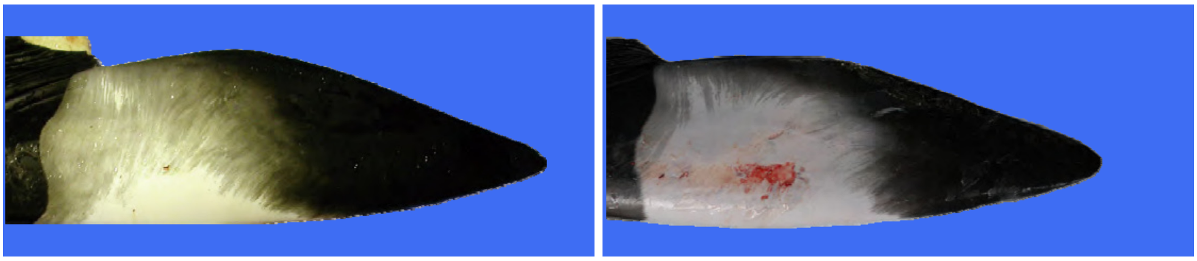
2016Norway_65 (8.3m)

Appendix 1-3. Pictures of the left flipper of the North Pacific minke whale.



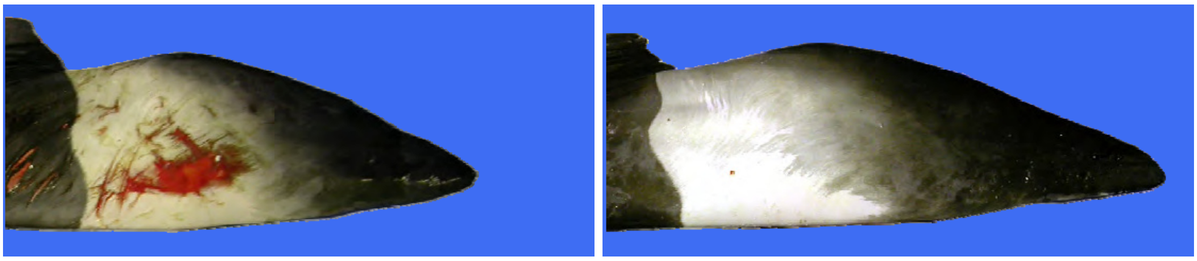
12NPCS-M029 (8.1m)

12NPCS-M049 (7.1m)



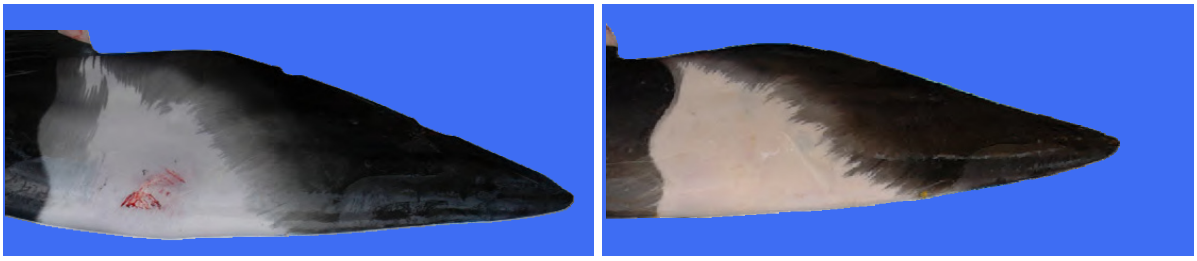
12NPCK-M011 (8.1m)

12NPCK-M028 (8.1m)



12NPCK-M031 (8.2m)

12NPCK-M037 (8.2m)

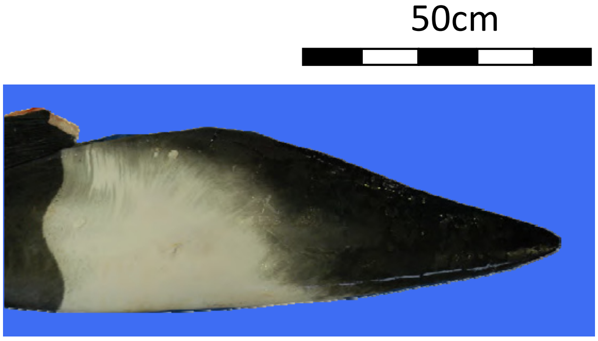


12NP-M014 (8.3m)

12NP-M046 (7.7m)

Appendix 1-4, (continued).

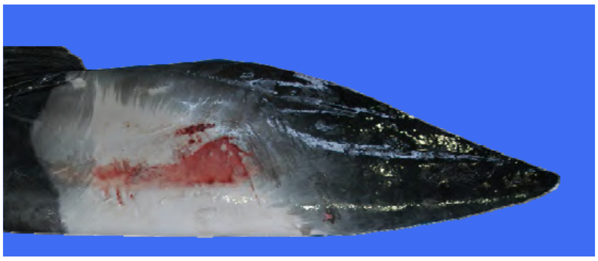

12NP-M064 (7.9m)



13NPCK-M023 (7.9m)


13NPCK-M028 (7.5m)


13NPCK-M040 (7.7m)


13NPCK-M049 (8.3m)


13NPCK-M052 (8.7m)


13NPCK-M053 (7.6m)


13NPCK-M057 (8.1m)

Short note

NASAL MUCOSA RESEMBLING AN OLFACTORY SYSTEM IN THE COMMON MINKE WHALE (*BALAENOPTERA ACUTOROSTRATA*)

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Abstract

Although it has long been assumed that modern cetaceans lack nervous system structures that mediate olfaction, recent studies suggest that mysticetes still maintain olfactory nerves. We collected samples of the mucous membrane covering the cribriform plate at the bottom of the dorsal nasal meatus from a mature female common minke whale. The samples were then thin-sectioned and stained with hematoxylin and eosin. Microscopic observations revealed that the mucosa was covered with a pseudostratified columnar epithelium with vessels, glands, and nerve plexuses in its lamina propria. These histological characteristics resembled those of the olfactory epithelium in terrestrial mammals, suggesting that mysticetes do indeed possess a sense of smell.

Key words Baleen whale, Olfaction, Histology, Nasal cavity, Olfactory epithelium.

In terrestrial mammals, the external nares and nasal passages function not only as a respiratory organ but also as an olfactory organ. Meanwhile, cetaceans use their blowhole as a respiratory tract, but their olfactory system seems to be highly degenerated. Extremely, odontocetes are reported to possess no nervous systems that mediate olfaction (Glezer, 2002; Oelschläger and Oelschläger, 2009).

In contrast, recent studies suggest that mysticetes can smell in air partly due to the presence of olfactory bulbs in their brain (Thewissen, George, Rosa and Kishida, 2011; Kishida, Thewissen, Usip, Suydam and George, 2015). The bony nasal passages of mysticetes branch into two sections: the ventral nasal meatus, which is broad and connects the blowhole and larynx, and the dorsal nasal meatus, a narrower passage the posterior part of which leads to the brain case (Godfrey, Geisler and Fitzgerald, 2013; Fig. 1). Because these structures resemble those of terrestrial mammals, previous studies support the presence of an olfactory system in mysticetes (Cozzi, Huggenberger and Oelschläger, 2016). Analyses of the olfactory marker protein gene further supports this, suggesting the existence of a functional olfactory system in mysticetes, even albeit much reduced compared with terrestrial mammals (Kishida and Thewissen, 2012; Springer and Gatesy, 2017).

Of the various functions and morphologies of the nasal epithelium, the portion known as the olfactory region affects an animal's olfactory ability (Kato and Yamauchi, 2003). The olfactory epithelium is a pseudostratified columnar epithelium composed of three types of cells: basal cells, sustentacular cells and olfactory receptor cells. Olfactory receptor cells have olfactory cilia projecting into the nasal cavity which react to odoriferous substances, stimulating the part of the brain known as olfactory bulbs (Wheater, Burkitt and Daniels, 1979) and subsequently the sense of smell will arise. The olfactory region is located near the cribriform plate of the ethmoid bone with great inter-species variations

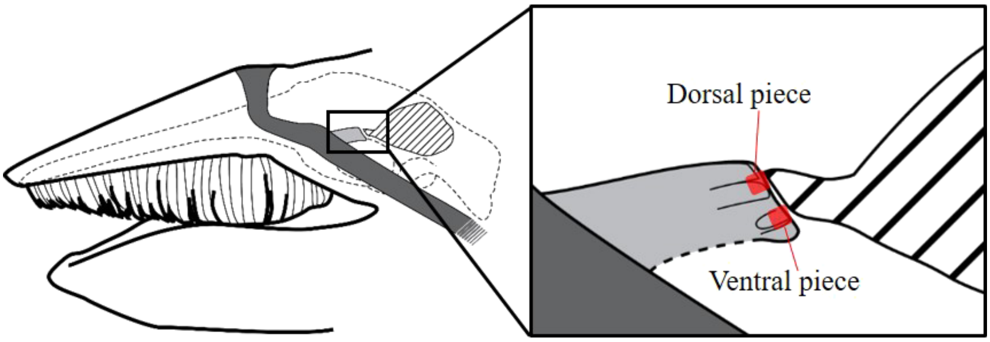

Fig. 1. The positions of mucosa sampling on the cribriform plate. Lateral view of the head showing structure of the ventral nasal meatus (dark gray), the dorsal nasal meatus (light gray), and brain case (stripes). There were a series of deep folds at the bottom of dorsal nasal meatus.

among mammals (Kato and Yamauchi, 2003). There are small serous glands below the olfactory epithelium known as Bowman's glands, the ducts of which penetrate into the epithelium and opening of the nasal cavity. Olfactory nerve fascicles are also found in the lamina propria.

Thus, since the presence of an olfactory epithelium indicates the existence of olfaction, it is a good starting point in discussing the feasibility of olfaction in mysticetes. In this study, we carried out histological observations (light microscopy) of nasal mucosa samples from the common minke whale to describe the olfactory anatomy in this species.

We analyzed a female common minke whale (*Balaenoptera acutorostrata*) with a body length of 7.68 m. This individual was captured in 2016 during the second phase of the Japanese Whale Research Program under the special permit in the Western North Pacific (JARPNII), and was appeared to be sexually matured based on analysis of its ovaries.

Samples were prepared immediately after the animal's death (approx. within 4.5 h). Since the location of the olfactory epithelium was thought to be inside the bony nasal passages, the head was separated into two halves along the mid-sagittal plane using a chainsaw. After carefully locating the right dorsal nasal meatus, two transverse sections were cut off to remove anterior part of the dorsal nasal meatus and posterior part of the ethmoid bone. Lateral bony parts were also removed, leaving the entire dorsal nasal meatus inside the specimen. Finally, the specimen was trimmed into a cube of approximately 10×5×20 cm using a hand saw, and the tissues were fixed in 10% formalin and preserved in 70% ethanol.

Three epithelium samples were collected from the mucosa on the cribriform plate. The location of the cribriform plate was assumed to be the posterior wall of the recess at the bottom of the dorsal nasal meatus (Fig. 1). Three square pieces of mucosa with 2–3 mm thick including the tissue beneath the epithelium were collected from the preserved specimen using a surgical knife. Two samples were collected from the dorsal side of the wall and one from the ventral side.

The epithelial samples were then processed according to standard histological techniques and stained with hematoxylin and eosin (H/E). The epithelium was observed under an optical microscope with magnification of 10× and 40×, paying particular attention to whether the epithelial cells consisted of a pseudostratified columnar epithelium typical of olfactory organs. The thickness of the epithelium and structure of the lamina propria were also determined.

Dorsal epithelial samples of mucosa, which constituted the posterior wall of the recess, showed features of a pseudostratified columnar epithelium 70–80 μm thick (Fig. 2). A similar epithelium was also observed on the ventral side; however, the thickness varied. On the dorsal portion of this ventral sample, the thickness was 80 μm or more, while the ventral portion was approximately 50 μm thick. Nerve plexuses and glandular cells were observed in the lamina propria in all three samples, and in one dorsal sample, some of these serum ducts appeared to open into the nasal cavity.

Thickness of the epithelium exceeds 100 μm in some areas, but less than 100 μm in other areas. In

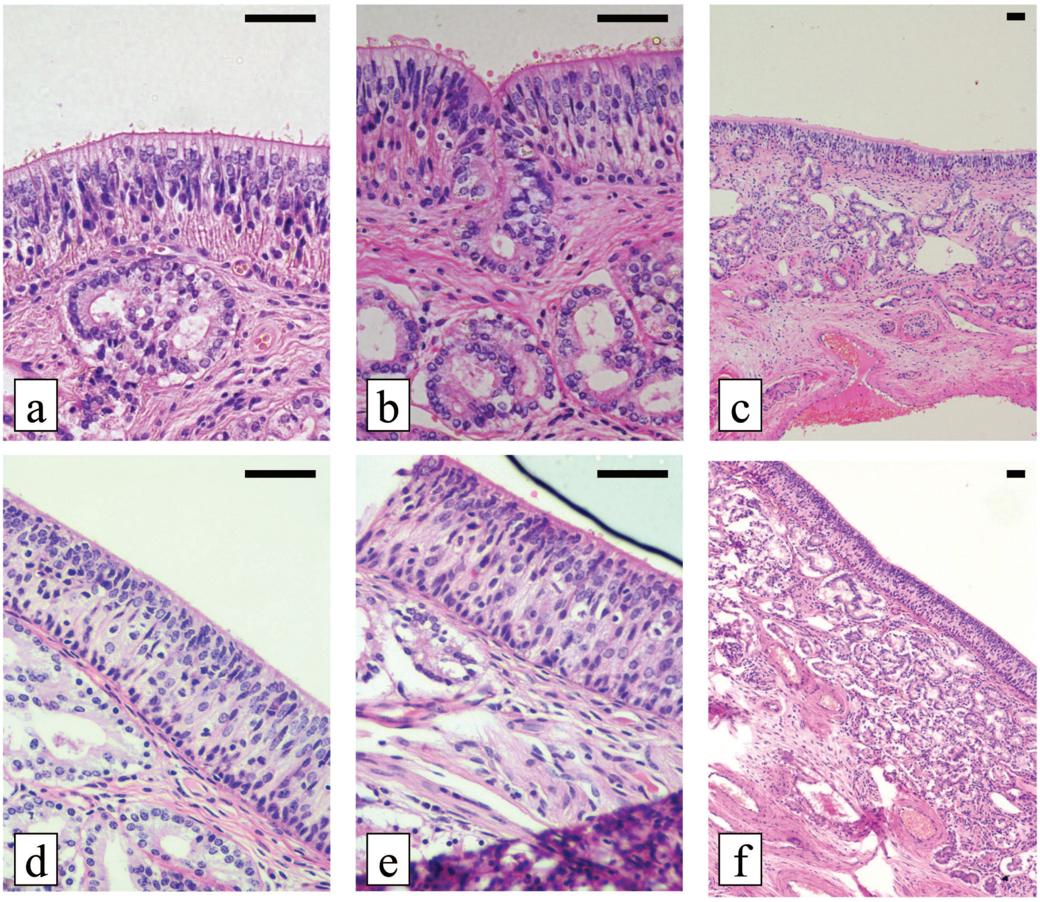

Fig. 2. Sections showing the epithelium samples from the cribriform plate. Sections were 6 μm thick and stained with H/E. Scale bars = 50 μm. The lumen (nasal cavity) is located at the top of the pictures. Close-up of epithelial cells (a, b; 40×) and epithelial tissue showing the lamina propria (c; 10×) from the dorsal side of the cribriform plate. Similar images from the ventral side (d, e, f).

terrestrial mammals, the olfactory epithelium is typically composed of a pseudostratified columnar epithelium approximately 100–120 μm thick (Kato and Yamauchi, 2003). The epithelial samples of nasal mucosa observed here show the morphological characteristics similar to those in terrestrial mammals.

The lamina propria of the thick pseudostratified columnar epithelium contained numerous glands, and these glands opened into the lumen in the dorsal sample. Although more detailed examination is required to conclude, we assume that these are Bowman's glands. Numerous blood vessels were also observed, suggesting a role in helping warm inhaled air. Near these vessels, peripheral nerve fascicles were also observed. Overall, the distribution of these tissues in the lamina propria resembled the olfactory region of the nasal mucosa in other mammals (Kato and Yamauchi, 2003; Harkema, Carey and Wagner, 2006; Chamanza and Wright, 2015).

Areas of pseudostratified columnar epithelium were located near the olfactory bulbs. Meanwhile, in megascopic observations of parasagittal sections of the animal's head, the bony canal representing the olfactory tract, or anterior elongation of the brain case, faced the olfactory recess. This elongated area is occupied by the olfactory bulb, and the bone dividing the brain case and bony nasal passage is the cribriform plate of the ethmoidal bone (Thewissen *et al*., 2011; Godfrey *et al*., 2013; Ichishima, 2016). Epithelium samples of nasal mucosa from the cribriform plate therefore resembled the olfactory epithelium in terrestrial mammals, supporting the feasibility of olfaction in mysticetes.

In general, mysticetes have left and right nasal passages completely isolated from each other (Berta, Ekdale and Cranford, 2014), suggesting that left and right nasal passages could serve as independent olfactory organs. That is, since the left and right auditory organs are able to locate a sound source, these two olfactory organs may be capable of locating the origin of an odoriferous substance via different stimuli of chemical substances between the left and right nasal cavities (Kikuta, Sato, Kashiwadani, Tsunoda, Yamasoba and Mori, 2010). We therefore hypothesize that mysticetes have two nasal openings with symmetrical nasal passages, which they use in olfaction (Kishida, 2016).

The microscopic structure of the epithelium samples observed in this study strongly supports the possibility of an olfactory epithelium. However, it should be noted that similar morphologies do not always suggest an identical function. Further analyses of the role of this epithelium are required to confirm the existence of olfaction in the common minke whales.

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