SPECIAL FEATURE The ornithological application of stable isotope analysis

Relationship between non-breeding migratory movements and stable isotopes of nitrogen and carbon from primary feathers of Black-tailed Gull Larus crassirostris

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Abstract Various seabird foraging strategies during the non-breeding season have recently been revealed by combining the use of bio-logging devices and the study of stable isotopic signatures (δ15N and δ13C) from various tissues. In this study, we used these combined methods to determine the relationships between stable isotopic signatures in Black-tailed Gulls Larus crassirostris primary feathers and the areas in which the feathers are presumed to have been grown. The fifth primary (P5) feathers are replaced during late August, and although the migratory movements for seven of the eight gulls studied during this replacement period differed, the isotopic δ15N and δ13C values were similar. These values indicated that the seven gulls fed on a wide range of prey from krill to demersal fish species. The isotopic values from P5 for the individual gull that moved southward after breeding, were much higher than for the seven other birds. In contrast, all eight gulls showed a relatively narrow distribution during the replacement of their outermost primaries (P10), which were replaced during mid October and November. However, the isotopic values from P10 of the individual that moved southward during replacement of P5 were also much higher. The unique isotopic values of this gull might indicate specialization in anthropogenic food resources or high trophic level resources through the migration period, regardless of location. Contrary to previous studies, our research did not detect links between migratory movements and stable isotopic signatures from feathers in Larus gulls migrating through a relatively narrow range and having considerable individual variation in diet.

Key words Black-tailed Gull, δ13C and δ15N, Diet, Migratory movement, Non-breeding period

After breeding, seabirds often migrate away from their breeding areas to areas with productive waters where they may recover their body condition, undertake molt, or build up reserves in preparation for future breeding (Gill 2007; Newton 2007). Foraging strategies during migration strongly associated with body condition are assumed to affect survival and/or breeding success in seabirds (Sorensen et al. 2009; Bogdanova et al. 2011). However, because of the lack of methods for simultaneously tracing movements (e.g., route and timing) and diet during seabird migration, many aspects of foraging strategies during migration have remained unexplored.

Recently, it has become possible to study simultaneously migratory movements and diet by combining the use of miniature bio-logging devices and the analysis of stable isotopic signatures of nitrogen (δ15N) and carbon (δ13C) in various tissues (Phillips et al. 2009; Catry et al. 2011). Global location sensors (GLS, geolocator) have enabled the long-distance and long-term (i.e. over a year) tracking of individual seabird movements, thereby demonstrating migration strategies (Phillips et al. 2007b; Egevang et al. 2010;
Stable isotopic signatures of δ15N and δ13C from various tissues can provide dietary information in various time series (Hobson & Clark 1992a, b; Hobson 1993; Hobson et al. 1994). In the marine environment, δ15N increases in a stepwise manner by 3-5‰ at each trophic level, which can provide information about trophic levels of consumers, while δ13C can provide spatial information typically about foraging area, such as inshore versus offshore, or benthic versus pelagic, and latitude of the area (Hobson et al. 1994; Bearhop et al. 2000; Cherel et al. 2006; Michener & Kaufman 2007).

Stable isotopic values from feathers, which become metabolically inert when fully formed, can reflect and record the dietary composition and foraging locations visited during migratory movements (Wassenaar 2008; Ramos et al. 2009a, b). Recent studies of seabirds with known molting patterns using combined methods (GLS and stable isotopic values from feathers), have revealed various foraging strategies within single species or breeding population (Furness et al. 2006; Phillips et al. 2007a; González-Solis et al. 2011). Black-legged Kittiwake Rissa tridactyla breeding on Hornoya, Norway showed two distinct migration strategies and the isotopic signatures of their primary feathers corresponded well with the areas to which they had migrated and in which their primary feathers were grown (González-Solis et al. 2011). Thus these methods when combined are also effective for demonstrating variations in key foraging areas and prey items within a species or population during the non-breeding period (Fort et al. 2012).

However, these studies have only shown a link between the migratory movements and stable isotopic signatures of feathers in seabird species that migrate through a relatively wide range (e.g., trans-equatorial migration) and that show little individual variation in trophic level (i.e., specialist predators) during migration (Furness et al. 2006; Phillips et al. 2007a; González-Solis et al. 2011). In such species the isotopic signatures from feathers can easily reflect the spatial information of limited dietary items. In contrast, different, probably more complicated, links can be predicted in species that migrate through relatively narrow ranges and that exhibit considerable individual variation in trophic level (i.e., generalist predators), such as Larus gulls (Watanuki 1992; Kazama et al. 2013). In such species, spatial variation in isotopic signatures can be small, and, in addition, large variations in trophic levels within an individual (e.g., seasonal variation) and/or among individuals can mask those spatial variations.

There is a large breeding colony of Black-tailed Gull Larus crassirostris on Kabushima (Kabu Island), northeastern Japan (Narita & Narita 2004). Although nothing was known of the migratory movements of the gulls from Kabushima, previous GLS studies on the migratory movements of gulls from another island showed that they migrated only through a narrow range along the coast of Japan (Kazama et al. 2013). Furthermore, the Black-tailed Gull is a generalist predator that feeds widely on krill, pelagic and demersal fishes, carcasses, and garbage during the breeding season (Kazama et al. 2008; Tomita et al. 2009; Yoda et al. 2012), although the species’ diet during the non-breeding season remain poorly known.

In the present study, we describe the migratory movements of the Kabushima Black-tailed Gull population using GLS; then, we combine GLS and stable isotope analyses to determine links between the stable isotopic signatures in primary feathers and the areas in which the feathers are presumed to have been grown.

**MATERIALS AND METHODS**

1) **Field work**

The study was conducted on Kabushima (40°32′N, 141°33′E), Hachinohe City, northeastern Honshu, Japan (Fig. 1), where 30,000 to 35,000 Black-tailed Gulls breed annually (Narita & Narita 2004). We captured 10 breeding gulls during the incubation period in early June 2010. Global location sensors (GLS-Mk15, 16×14×6 mm, 2.5 g, manufactured by British Antarctic Survey; Afanasyev 2004) were attached to the study birds using plastic leg rings. The total mass of the GLS including the ring was 5.0 g, representing < 1% of the mean body mass of the 10 captured gulls (581.2±55.3 SD g).

We recaptured the study birds near their nests during the incubation period of the following breeding season, in early May 2011, and all GLSs were retrieved. However, light data for two gulls were unavailable. When we retrieved the GLSs, feather samples were also collected from the study birds to analyze stable isotopic values of carbon and nitrogen during the non-breeding period. In adult Black-tailed Gulls, the primary feathers are replaced completely between late June (the end of the breeding period) and the middle of November (during the migration period) (Olsen & Larsson 2003). We collected the
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top 2–3 cm of two primary feathers (the fifth primary (P5), and the outermost primary (P10)). Replacement of primary feathers begins with the innermost primary (P1) during the brooding period in late June and progresses distally to P5 in late August and ends with P10 between mid October and mid November (Olsen & Larsson 2003). Thus, we considered that the isotopic values of $\delta^{15}$N and $\delta^{13}$C from these feath-
Fig. 1. Migration routes during the non-breeding season (from August 2010 to March 2011) and nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotopic signatures of primary feathers for eight Black-tailed Gulls breeding on Kabushima. Movement patterns are indicated with half-monthly (14, 15 or 16 d) spatial medians of validated daily data. Months are indicated by numbers within circles (January=1). White letters on a black background indicate the first half of the month while the opposite combination indicates the second half. Lines join each individual gull’s successive positions, but do not indicate the path travelled. P5 (red circles and line) and P10 (blue circles and line) feathers in adults are replaced in late August and between mid October and mid November (Olsen & Larsson 2003). The location of Kabushima is indicated with a star.
ers reflected their diets during the period of their replacement.

2) Positioning

GLS devices record time, light intensity, immersion in seawater and water temperature (for details see Takahashi et al. 2008). Light data were analyzed with ‘TransEdit’ and ‘BirdTracker’ software developed by the British Antarctic Survey. Sunset and sunrise times were estimated from thresholds in the light curves; latitude was derived from day length, and longitude from the time of local midday with respect to Greenwich Mean Time and Julian day, providing two locations at noon and midnight per day (Phillips et al. 2004). We used location data only at noon for the analyses.

We analyzed light data following the procedures in Yamamoto et al. (2010) and Kazama et al. (2013). Light records with obvious interruptions around sunset and sunrise, or during the night were identified and removed. Furthermore, location errors were minimized by comparing the water temperature and the light-based longitudes experienced by each bird with remotely sensed 8-d composite sea surface temperature (SST) data (resolution 9 km, measured by Aqua-MODIS, http://oceancolor.gsfc.nasa.gov/cgi/l3?per=DAY) using an algorithm described by Teo et al. (2004). Locations that required unrealistic flight speeds, i.e. more than 35 km h⁻¹ (mode of flight speed for Black-tailed Gull; Yoda et al. 2012), were excluded. Location data during breeding (April to July) were excluded from creating migration maps (Fig. 1) since light-based geolocation can be unreliable for incubating or brooding birds. This is because when birds incubate or brood, light levels decrease while the birds are on the nest (Kazama et al. 2013).

The spatial errors inherent in GLS tracking are approximately 186 km on average (Phillips et al. 2004). Therefore, we visualized the overall movement patterns using half-monthly spatial medians of the daily latitude and longitude positions at noon following Guilford et al. (2011) and Kazama et al. (2013). Migration maps for the gulls were created with Quantum GIS 1.7.1 (Quantum GIS Development Team 2011, http://qgis.osgeo.org) and ChizuTaro 5.03 (Tokyo Cartographic Co., Ltd).

3) Feather sample preparation and stable isotope analysis

Feather samples were washed in 0.25 M sodium hydroxide solution to remove surface contamination, rinsed in distilled water, dried in an oven at 50°C for 24 hours to constant weight, and then ground to a fine powder using an electric crusher (TK-AM5, Taiotec) operating at liquid nitrogen temperature prior to stable isotope analysis (Bearhop et al. 1999). Ground feathers were sealed in tin capsules for combustion; samples weighed between 0.51 and 0.65 mg.

The carbon and nitrogen stable isotopic values of feathers were measured using a gas-source isotopic value mass spectrometer (ANCA-GSL and Hydra 20–20, Sercon Ltd., UK). All feather samples were run in duplicate. Stable isotopic values expressed in δ notation as parts per thousand (‰) deviation from the PeeDee Belamnite for 13C and atmospheric N₂ for 15N according to the following equation (Bearhop et al. 1999):

\[ \delta X = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000 \]

where X is 13C or 15N and R is the isotopic value ([13C/12C or 15N/14N]). Measurement precision of both δ15N and δ13C was estimated to be 0.2‰. Data are presented as means ± standard deviation (SD).

RESULTS

1) Overall migration routes during the non-breeding period

The eight gulls studied followed various migration routes (Fig. 1). Four gulls (ID: B22, C21, B75, and B41) migrated after breeding along the coast of east Hokkaido and the Sea of Okhotsk from August to October, they then moved southward off the coast of east Hokkaido, to the Sanriku and Joban (Honshu) coasts during October and December (Fig. 1a-d). Three gulls (ID: B62, E61, and B36) stayed mainly off the coast of west Hokkaido, in the Tsugaru Strait between Hokkaido and Honshu, and along the eastern part of the Shimokita Peninsula from August to December (Fig. 1e-g). B36 moved southward to Sendai Bay between late August and early September (Fig. 1g). In contrast, the migration route of one gull (ID: C19) differed considerably from the others (Fig. 1h), as it moved southward to Torishima (Bird Island) and to the western Ogasawara Islands during August. It then moved northward and remained in the Sea of Japan and the Tsugaru Strait until December. From January to February, all eight gulls, including C19, wintered off the Kanto and Joban coasts of Honshu, then moved northward to Kabushima.
2) Movements during replacement of P5 and P10 feathers and isotopic signature

The P5 feathers (n=8), which were replaced in late August, had isotopic $\delta^{15}N$ values of 13.41±1.73‰ and $\delta^{13}C$ values of −18.98±0.74‰. Migration routes during replacement of P5 varied. Some birds visited northeast Hokkaido (ID: B22, C21, B75, B41; Fig. 1a-d), some south Hokkaido and northern Honshu (ID: B62, E61; Fig. 1e-f), Sendai Bay (ID: B36; Fig. 1g), and even the western Ogasawara Islands (ID: C19; Fig. 1h). Isotopic $\delta^{15}N$ and $\delta^{13}C$ values of P5 feathers did not differ among the gulls following these three routes ($\delta^{15}N$: 12.81±0.45‰, $\delta^{13}C$: −19.20±0.45‰, Fig. 1a-g); however, those values for bird C19 ($\delta^{15}N$: 17.62‰, $\delta^{13}C$: −17.46‰) were much higher (Fig. 1h).

The P10 feathers of all eight individual gulls, which were replaced between mid October and mid November, had isotopic $\delta^{15}N$ values of 14.98±2.59‰ and $\delta^{13}C$ values of −18.64±1.08‰. During replacement of their P10 feathers all eight gulls ranged around southeast Hokkaido and northern Honshu (Fig. 1a-h). With the exception of C19, the isotopic $\delta^{15}N$ and $\delta^{13}C$ values obtained from P10 feathers did not differ significantly among the seven gulls ($\delta^{15}N$: 14.10±0.83‰, $\delta^{13}C$: −18.92±0.80‰, Fig. 1a-g). Although C19 remained near the seven other individuals during replacement of P10, its isotopic $\delta^{15}N$ and $\delta^{13}C$ values ($\delta^{15}N$: 21.10‰; $\delta^{13}C$: −16.69‰, Fig. 1h) were much higher than those of the other gulls (Fig. 1a-g).

DISCUSSION

The results of this study suggest that neither the isotopic $\delta^{15}N$ and $\delta^{13}C$ values of P5 nor P10 were related to migratory movements during replacement of those feathers in the Black-tailed Gulls breeding on Kabushima. Although the migratory movements of seven of the eight gulls (the exception being C19) during replacement of P5 were varied (Fig. 1), the isotopic $\delta^{15}N$ and $\delta^{13}C$ values of the seven gulls were similar. The $\delta^{15}N$ and $\delta^{13}C$ enrichment factors for primary feathers of seabirds are 3.6‰ and 1.4‰ respectively (Becker et al. 2007). The isotopic $\delta^{15}N$ and $\delta^{13}C$ values of P5 overlapped widely with the values for krill Euphausia pacifica ($\delta^{15}N$: 8.0±0.2‰, $\delta^{13}C$: −19.7±0.2‰; Mitani et al. 2006), Japanese Common Squid Todarodes pacificus ($\delta^{15}N$: 9.1±0.7‰, $\delta^{13}C$: −19.1±0.9‰; Mizutani & Wada 1988), pelagic fishes such as Japanese Anchovy Engraulis japonicus ($\delta^{15}N$: 8.8±0.9‰, $\delta^{13}C$: −18.9±0.5‰); Pacific Saury Cololabis saira ($\delta^{15}N$: 9.3±1.4‰, $\delta^{13}C$: −19.6±0.6‰; Mitani et al. 2006); Sandlance Ammodytes personatus ($\delta^{15}N$: 9.6±0.5‰, $\delta^{13}C$: −19.2±0.3‰; Ito et al. 2009), and demersal fishes such as Greenling Pleurogrammus azonus ($\delta^{15}N$: 9.6±0.5‰, $\delta^{13}C$: −18.2±0.4‰; Ito et al. 2009), which are available as prey for the Black-tailed Gull during the breeding season (Kazama et al. 2008; Tomita et al. 2009; Kentaro Kazama personal observation). For C19, which moved southward after breeding, isotopic $\delta^{15}N$ and $\delta^{13}C$ values derived from P5 were much higher than for the other individuals. It is possible that C19 might have scavenged anthropogenic food resources (e.g., waste from fisheries) or dead marine mammals. However, we considered that the unique isotopic values of this gull reflected individual-specific dietary composition, but not location-specific composition (see below). We were unable to explain why the migration routes of individual gulls soon after breeding differed so much and in comparison with other periods of this study. In the Black-legged Kittiwake it has been shown that individuals that experienced breeding failure in the previous breeding season dispersed more widely soon after breeding than successful breeders (Bogdanove et al. 2011). Individual variation in foraging activities soon after breeding is relatively large in the Black-tailed Gull (Kazama et al. 2013). Thus, migratory movements soon after breeding may be influenced by ecological factors, although the breeding success of the study individuals was unknown in our study.

All gulls (including C19) occurred across a relatively narrow distribution during replacement of P10 (Fig. 1). Excluding C19, isotopic $\delta^{15}N$ and $\delta^{13}C$ values from P10, and during replacement of P5, were similar among the seven gulls and indicated that they had fed on a wide range of prey from krill to demersal fishes. Marine productivity is high in late autumn off Kushiro (east Hokkaido) and Tohoku (northern Honshu) where the gulls stayed during replacement of P10 (Shinada et al. 2000; Taki 2007). Also indicative of the marine productivity in those regions is the fact that other breeding or non-breeding seabirds migrate there to feed during the same period (Ito & Watanuki 2008; Matsumoto et al. 2012; Kazama et al. 2013). In contrast, the isotopic $\delta^{15}N$ and $\delta^{13}C$ values from P10 of C19 were much higher than those from the other seven gulls, although C19 remained in similar areas to the other gulls during replacement of P10 (Fig. 1h). It is presumed that C19 may have
scavenged on anthropogenic food resources or dead marine mammals during the period of replacement of P10.

C19 may have specialized in anthropogenic or high trophic level food resources during the migration period regardless of the location. *Larus* gulls are typically generalist predators at the species level, but sometimes exhibit individual diet specialization during the breeding season (McCleery & Sibly 1986; Watanuki 1992). This study has indicated that *Larus* gulls may also be specialist predators at the individual level during the non-breeding period. Thus, unique isotopic values of P5 and P10 from C19 might reflect a record of individually specific dietary composition rather than a location-specific one. In this respect, further studies with larger sample sizes should be undertaken.

Contrary to previous studies on wide-range-migratory seabirds that have shown them to exhibit little individual variation in diet (Furness et al. 2006; Phillips et al. 2007a; González-Solís et al. 2011), our studies did not detect links between migratory movements and stable isotopic signatures from the feathers of the Black-tailed Gull. It might be difficult to demonstrate the foraging strategies or key foraging areas of migratory seabirds with narrow ranges during the non-breeding period, given their potential for such large individual variation in diet when using only one method of GLS and stable isotope analysis. Thus, combining methods can be a powerful way of investigating avian foraging strategies during the migration period in such species.

**ACKNOWLEDGMENTS**

We would like to thank T. Yamamoto and A. Takahashi for their help with analysis of tracking data of GLSs, N. Yamamoto for her help with preparation of feather samples for stable isotope analysis, and A. Narita for his help with data collection on Kabushima. We greatly appreciate K. Kazama, the editor, and two anonymous reviewers for their critical and helpful comments on the manuscript and Mark Brazil for correcting the English used in the manuscript. The Japanese Environmental Ministry and Agency for Cultural Affairs gave permission to work on Kabushima. This work was supported by the Strategic Research Foundation Grant-aided Project for Private Universities (S0801056) from the Ministry of Education, Culture, Sport, Science, and Technology, Japan (MEXT).

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