RELATIONSHIPS BETWEEN STABLE ISOTOPES AND MERCURY CONTAMINANTS IN FEATHERS OF BLACK-TAILED GULL *LARUS CRASSIROSTRIS*

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ABSTRACT

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The feathers of seabirds are widely used as appropriate indicators for various aspects of avian ecology and toxicology. To reveal total mercury (Hg) concentrations and the connection to diet using stable isotope analysis in Black-tailed Gulls *Larus crassirostris*, feathers were collected from 10 carcasses from the breeding colony on Kabushima Island during the incubation period in 2020. Total Hg concentrations in primary remiges were found to decrease during the molt sequence. The innermost primaries (P1), the first to be replaced after breeding, had the highest Hg concentrations of all feathers (7.73 ± 2.37 μg/g dry weight). The concentration of Hg in P1 feathers may reflect the Hg load in birds' body tissues. The outermost primaries (P10), the last to be replaced during primary molt, had not only the lowest Hg concentrations but also the smallest variation among individuals among all primary feathers (1.11 ± 0.29 μg/g dry weight). This property of P10 concerning Hg contamination may be useful when comparing Hg contamination between populations. There were no significant relationships between total Hg concentration and nitrogen isotope ratios (δ^{15}N) or carbon isotopes ratios (δ^{13}C) in most feathers. The lack of a relationship between total Hg concentration and δ^{15}N could be due to low variation in diet during the molt period or to differences between the mobilization of Hg and the incorporation of δ^{15}N into feathers during the period of feather growth. Having less variation in δ^{13}C values among individuals could be due to similarity of locations while the gulls migrate, molting along the way, as well as the irrelevance of total Hg concentration and δ^{13}C values in feathers.

Key words: diet, feathers, mercury, molt, monitoring, stable isotope analysis

INTRODUCTION

Seabirds that forage in the upper levels of marine food webs are exposed to a wide range of threats, including marine pollution, making them ideal sentinel organisms for assessing the status of marine ecosystems (Lescroël et al., 2016). Seabird feathers are used widely in avian ecology and toxicology studies as they are good indicators of diet and therefore also of exposure to contaminants (Tomita at al., 2015; Watanuki et al., 2015). Feathers preserve chemical compositions acquired during their growth, remain metabolically inert after synthesis, and can be collected easily and noninvasively (Furness, 1993; Lodenius & Solonen, 2013). Many studies have used feathers as indicators for mercury (Hg) pollution in the marine environment (Furness, 1993; Furness et al., 1986; Lodenius & Solonen, 2013). Other studies have used stable isotope analysis to investigate diet. Nitrogen stable isotope (δ^{15}N) and carbon stable isotope (δ^{13}C) ratios of feathers allow us to understand differences in individual diets and foraging location, because isotope values vary with trophic levels of prey and/or location of foraging (Bearhop et al., 2002; Hobson & Clark 1992; Wada et al., 1987).

It is known that Hg concentrations in feathers vary widely within and between individuals. In many adult seabirds, the primary flight feathers are molted from the innermost (P1) to the outermost (P10) after breeding (Monteiro & Furness, 1995; Pyle et al., 2018). In accordance with the molting sequence, Hg concentrations in primary feathers typically decline from P1 to P10 (Braune, 1987; Furness et al., 1986). This is likely to be the result of depletion of the internal Hg pool stored in tissues as feathers grow in the molt (Furness et al., 1986; Thompson et al., 1998). In addition to leading to variations in internal Hg, changes in the diet at the time of feather growth are reflected in variations in feather δ^{15}N values (Grecian et al., 2015; Thompson & Furness, 1995). As Hg and δ^{15}N accumulating in feathers are derived from the diet (Carravieri et al., 2014; Furness et al., 1986; Gómez-Ramírez et al., 2023; Monteiro & Furness, 1995), a significant correlation between Hg contaminant levels and δ^{15}N in feathers would be expected if Hg and δ^{15}N are assimilated into feathers via the same process. Therefore, when comparing individuals, those with higher δ^{15}N values should have higher Hg due to bioaccumulation, assuming that they have similar exposure and are foraging in the same area. Indeed, several studies have investigated the relationships between Hg contaminant levels and δ^{15}N values across different types of feathers (Bearhop et al., 2000; Bond & Diamond, 2009; Carravieri et al., 2014; Nisbet et al., 2002). In addition, δ^{13}C in feathers, which indicates regional variation in foraging areas (neritic or pelagic offshore waters) at the time of feather growth (Cherel et al., 2000), may also affect Hg values.

This study investigates the accumulation patterns of Hg and stable isotopes in primary feathers in relation to molt in the Black-tailed Gull *Larus crassirostris*. I collected 10 carcasses from the Kabushima breeding colony in 2020 to analyze total Hg concentrations and stable isotope values in various feathers. I predicted that total Hg concentrations in primaries would decrease in line with the molt sequence from P1 to P10, as has been previously reported, while δ^{15}N in primary feathers would be independent of the sequences, as δ^{15}N is derived from the diet during feather development.

Consequently, I expected no relationship between them. Since the Black-tailed Gull is a short-distance migrant, dispersing along the coast of Japan, no link between the timing of feather growth and $\delta^{13}C$ values in feathers has been found so far (Tomita et al., 2015). I also predicted that $\delta^{13}C$ in primaries would be independent of the sequences.

METHODS

Ten adult Black-tailed Gull carcasses were collected from the breeding colony on Kabushima during April and May 2020. All carcasses sampled were fresh, likely killed within two days by either red foxes *Vulpes vulpes* or domestic cats *Felis catus* (Tomita et al., 2010). Although no obvious external injuries were apparent, puncture marks consistent with canine teeth were found on the pectoral muscles upon dissection. Specimens were placed in plastic bags and then frozen at −20 °C before feather removal.

The frozen specimens were thawed and weighed (±0.1 g), and then sexes were identified by examining the gonads. The selected feathers were removed from all 10 specimens: primaries P1–P10, rectrices R1–R6, breast feathers (*ca*. 10 feathers), and back feathers (*ca*. 10 feathers). One bird (ID: ID10) was missing R4. Prior to total Hg and stable isotope analysis, feather samples were washed in 0.25 M sodium hydroxide solution and rinsed in Milli-Q water to remove surface contamination, then dried in an oven at 50 °C for 24 h to constant weight. Feathers were ground into fine powder using an electric crusher (TK-AM5, Taitec) operating at liquid nitrogen temperature. Approximately 5 cm of the tips of primary remiges and rectrices were analyzed, in contrast to the entirety of breast and back contour feathers. Powdered feather samples were used for total Hg and stable isotope analysis after being divided into three parts as previously described by Tani et al. (2023). The average body mass of six males was 586.1 ± 233.3 g (standard deviation) and of four females was 593.1 ± 86.9 g.

Total Hg concentrations in feathers were measured using a direct thermal decomposition mercury analyzer (MA-3000; Nippon Instruments). A standard solution was prepared to dilute a Hg atomic absorption standard solution (1,005 mg/L, Kanto Kagaku Co., Ltd.) 1,000 times and 100,000 times with L-cysteine solution. After preparation of the calibration standards using the standard solution, total Hg concentration was measured by thermal decomposition. I conducted two measurements per sample. If the coefficient of variation between the two values was within 5%, I used their mean value as the representative value. The recovery rate of Hg in the reference material (NIMD-01, 0.794 ± 0.05 μg g^{-1} dry weight, human hair, National Institute for Minamata Disease, Japan) was 99.0% ± 2.9% (n = 11).

Powdered feathers were sealed in tin capsules for combustion. The $\delta^{13}C$ and $\delta^{15}N$ values of feathers were measured using a gas-source isotopic values mass spectrometer (ANCA GSL and Hydra 20-20, Sercon Ltd., UK). All samples were run in duplicate. Stable isotopic values expressed in δ notation as parts per thousand (‰) deviation from the PeeDee Belemnite for ^{13}C and atmospheric nitrogen for ^{15}N according to the following equation (Bearhop et al. 1999):

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 1{,}000$$

where X is ^{13}C or ^{15}N, and R is the isotopic value ($^{13}C/^{12}C$ or $^{15}N/^{14}N$). To ensure analytical accuracy and precision, an internal standard (alanine) was used to calibrate the ^{13}C:^{12}C and ^{15}N:^{14}N ratios. Intra- and inter-run variations were 0.22‰ and 0.28‰ for $\delta^{15}N$, and 0.07‰ and 0.11‰ for $\delta^{13}C$, respectively.

A linear mixed model, with an identity link function and normally distributed errors, was used to test for differences among feathers in total Hg concentrations, $\delta^{15}N$ values, and $\delta^{13}C$ values, where feathers were explanatory variables and individual identity was a random effect in the model. The likelihood-ratio test was used to assess the effect of feathers on total Hg concentrations and on $\delta^{15}N$ and $\delta^{13}C$ ratios. When estimating parameters from the linear mixed model, P1 was used as the intercept. A linear model was used to test whether there was a significant relationship between total Hg concentrations and $\delta^{15}N$ or $\delta^{13}C$ values in feathers, where total Hg concentrations were independent variables and $\delta^{15}N$ or $\delta^{13}C$ values were explanatory variables. All statistics were performed using R (version 4.1.2) (R Core Team, 2018). We used the "car" and "lme4" packages for these models. The results were considered significant when $p < .05$. All graphs were created using the "ggplot2" package.

RESULTS

Total Hg concentrations differed significantly among feathers (Fig. 1a, χ^2 = 804.8, df = 17, $p < .001$); $\delta^{15}N$ and $\delta^{13}C$ values also differed significantly among feathers ($\delta^{15}N$ values: Fig. 1b, χ^2 = 28.30, df = 17, $p < .05$; $\delta^{13}C$ values: Fig. 1c, χ^2 = 95.70, df = 17, $p < .001$). Total Hg concentrations in primary feathers declined, as expected, from P1 to P10 (Table 1). P1 had the highest total Hg concentration and exhibited the most extreme variation among individuals, whereas P10 had the lowest value and the smallest variation among individuals (Fig. 1a). $\delta^{15}N$ and $\delta^{13}C$ values in primary feathers did not decline from P1 to P10 (Fig. 1b, c; Tables 2, 3).

Total Hg concentrations and $\delta^{15}N$ values in feathers did not show significant relationships, except for P3 (Fig. 2a–r). In P3 there was a significant positive relationship between total Hg concentrations and $\delta^{15}N$ values ($F_{1,8}$ = 7.64, p = .025, R^2 = 0.42, total Hg concentration = −9.30 × 1.12 × $\delta^{15}N$, Fig. 2c).

Total Hg concentrations and $\delta^{13}C$ values in feathers did not show significant relationships, with the exception of P7 (Fig. 3a–r). In P7, there was a significant negative relationship between total Hg concentrations and $\delta^{13}C$ values ($F_{1,8}$ = 7.37, p = .026, R^2 = 0.41, total Hg concentration = −11.44 × −0.72 × $\delta^{13}C$, Fig. 3g).

DISCUSSION

Total Hg concentrations in the primary remiges of the Black-tailed Gull decreased in line with the molt sequence from P1 to P10, whereas $\delta^{15}N$ and $\delta^{13}C$ values did not. There were no significant relationships between total Hg concentrations and $\delta^{15}N$ or $\delta^{13}C$ values in feathers, except for $\delta^{15}N$ in P3 and $\delta^{13}C$ in P7.

Total Hg concentrations in Black-tailed Gull primaries decreased in sequence with molt as has been reported for many avian species (Braune,1987; Furness et al., 1986; Gatt et al., 2021; Martínez et al., 2012; Peterson et al., 2019). Hg that is ingested in the diet between the end of one period of molting and the beginning of the next accumulates in body tissues because it cannot be transferred to existing feathers, although small amounts may be eliminated in eggs and excreta (Monteiro & Furness, 1995). Once molt begins,

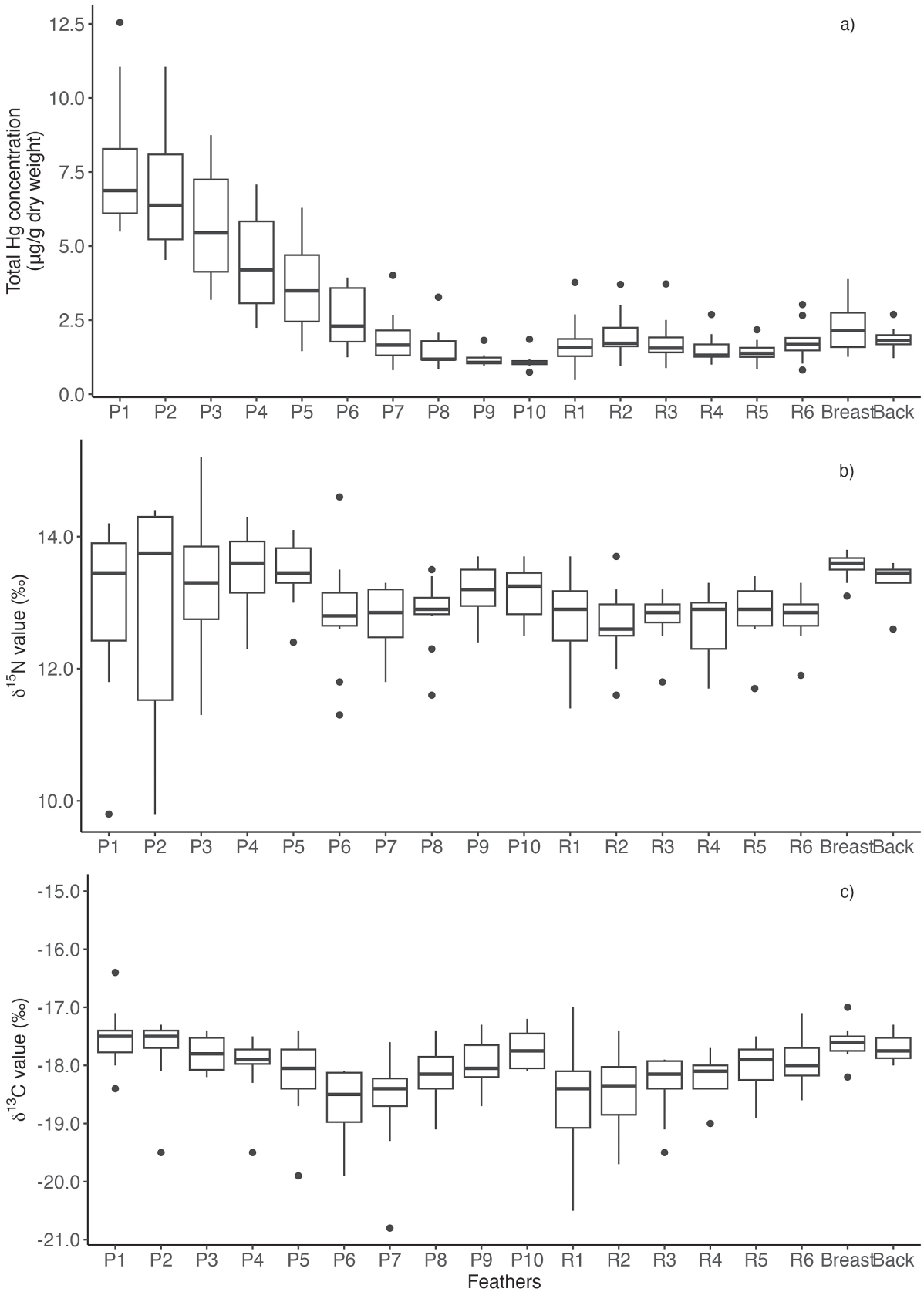

Fig. 1. (a) Relationship between total Hg concentrations and each feather type in Black-tailed Gulls *Larus crassirostris*; (b) relationship between feather type and δ^{15}N values; and (c) relationship between feather type and δ^{13}C values. "P1–P10" represent primaries P1–P10, "R1–R6" represent rectrices R1–R6, "Breast" represents breast feathers, and "Back" represents back feathers. In the boxplots, the box represents the interquartile range (IQR; Q1–Q3), the line inside indicates the median, the whiskers extend to values within 1.5 × IQR, and dots denote outliers beyond this range.

TABLE 1
Parameter estimates from linear mixed models for the analysis of the effects of feathers on total Hg concentrations in the Black-tailed Gulls *Larus crassirostris* investigated in this study

Type of feather	Estimate	Standard error	*df*	*t*-value	*p*
P1 (Intercept)	7.73	0.38	42.21	20.16	< .001
P2	–0.69	0.42	153.00	–1.65	.1
P3	–2.09	0.42	153.00	–5.02	< .001
P4	–3.22	0.42	153.00	–7.71	< .001
P5	–4.14	0.42	153.00	–9.92	< .001
P6	–5.18	0.42	153.00	–12.42	< .001
P7	–5.84	0.42	153.00	–14.01	< .001
P8	–6.20	0.42	153.00	–14.85	< .001
P9	–6.56	0.42	153.00	–15.73	< .001
P10	–6.62	0.42	153.00	–15.87	< .001
R1	–5.97	0.42	153.00	–14.30	< .001
R2	–5.74	0.42	153.00	–13.76	< .001
R3	–5.93	0.42	153.00	–14.22	< .001
R4	–6.09	0.42	153.00	–14.59	< .001
R5	–6.29	0.42	153.00	–15.06	< .001
R6	–5.97	0.42	153.00	–14.31	< .001
Breast feather	–5.50	0.42	153.00	–13.17	< .001
Back feather	–5.87	0.42	153.00	–14.07	< .001

Hg is mobilized into the newly growing feathers and, therefore, decreases as fresh feather growth progresses (Furness et al., 1986; Monteiro & Furness, 1995).

P1 feathers contain the highest concentrations of Hg of all feathers, because they are the first to be replaced during the post-breeding molt. Therefore, Hg concentration in P1 feathers may reflect the Hg load in the body tissues before molt, including during the breeding season. When assessing the effect of Hg loading on avian reproduction and life history, the Hg concentration in P1 feathers may be an appropriate indicator. In contrast, among all primary feathers, P10 exhibited the lowest

TABLE 2
Parameter estimates from linear mixed models for the analysis of the effects of feathers on δ^{15}N values in the Black-tailed Gulls *Larus crassirostris* investigated in this study

Type of feather	Estimate	Standard error	*df*	*t*-value	*p*
P1 (Intercept)	12.97	0.25	122.84	52.95	< .001
P2	–0.08	0.32	152.02	–0.25	.80
P3	0.32	0.32	152.02	0.99	.32
P4	0.52	0.32	152.02	1.62	.11
P5	0.48	0.32	152.02	1.49	.14
P6	–0.13	0.32	152.02	–0.40	.69
P7	–0.19	0.32	152.02	–0.59	.56
P8	–0.13	0.32	152.02	–0.40	.69
P9	0.17	0.32	152.02	0.53	.60
P10	0.17	0.32	152.02	0.53	.60
R1	–0.16	0.32	152.02	–0.50	.62
R2	–0.31	0.32	152.02	–0.96	.34
R3	–0.22	0.32	152.02	–0.68	.50
R4	–0.29	0.33	152.27	–0.86	.39
R5	–0.12	0.32	152.02	–0.37	.71
R6	–0.20	0.32	152.02	–0.62	.54
Breast feather	0.57	0.32	152.02	1.77	.08
Back feather	0.38	0.32	152.02	1.18	.24

TABLE 3
Parameter estimates from linear mixed models for the analysis of the effects of feathers on $\delta^{13}C$ values in the Black-tailed Gulls *Larus crassirostris* investigated in this study

Type of feather	Estimate	Standard error	*df*	*t*-value	*p*
P1 (Intercept)	−17.52	0.18	64.43	−97.65	< .001
P2	−0.23	0.21	152.01	−1.08	.28
P3	−0.29	0.21	152.01	−1.36	.17
P4	−0.50	0.21	152.01	−2.35	.02
P5	−0.67	0.21	152.01	−3.15	.002
P6	−1.15	0.21	152.01	−5.41	< .001
P7	−1.12	0.21	152.01	−5.27	< .001
P8	−0.70	0.21	152.01	−3.29	.001
P9	−0.46	0.21	152.01	−2.16	.03
P10	−0.21	0.21	152.01	−0.99	.32
R1	−1.05	0.21	152.01	−4.94	< .001
R2	−0.90	0.21	152.01	−4.23	< .001
R3	−0.82	0.21	152.01	−3.86	< .001
R4	−0.66	0.22	152.13	−3.04	.003
R5	−0.48	0.21	152.01	−2.26	.03
R6	−0.43	0.21	152.01	−2.02	.04
Breast feather	−0.08	0.21	152.01	−0.38	.71
Back feather	−0.17	0.21	152.01	−0.80	.43

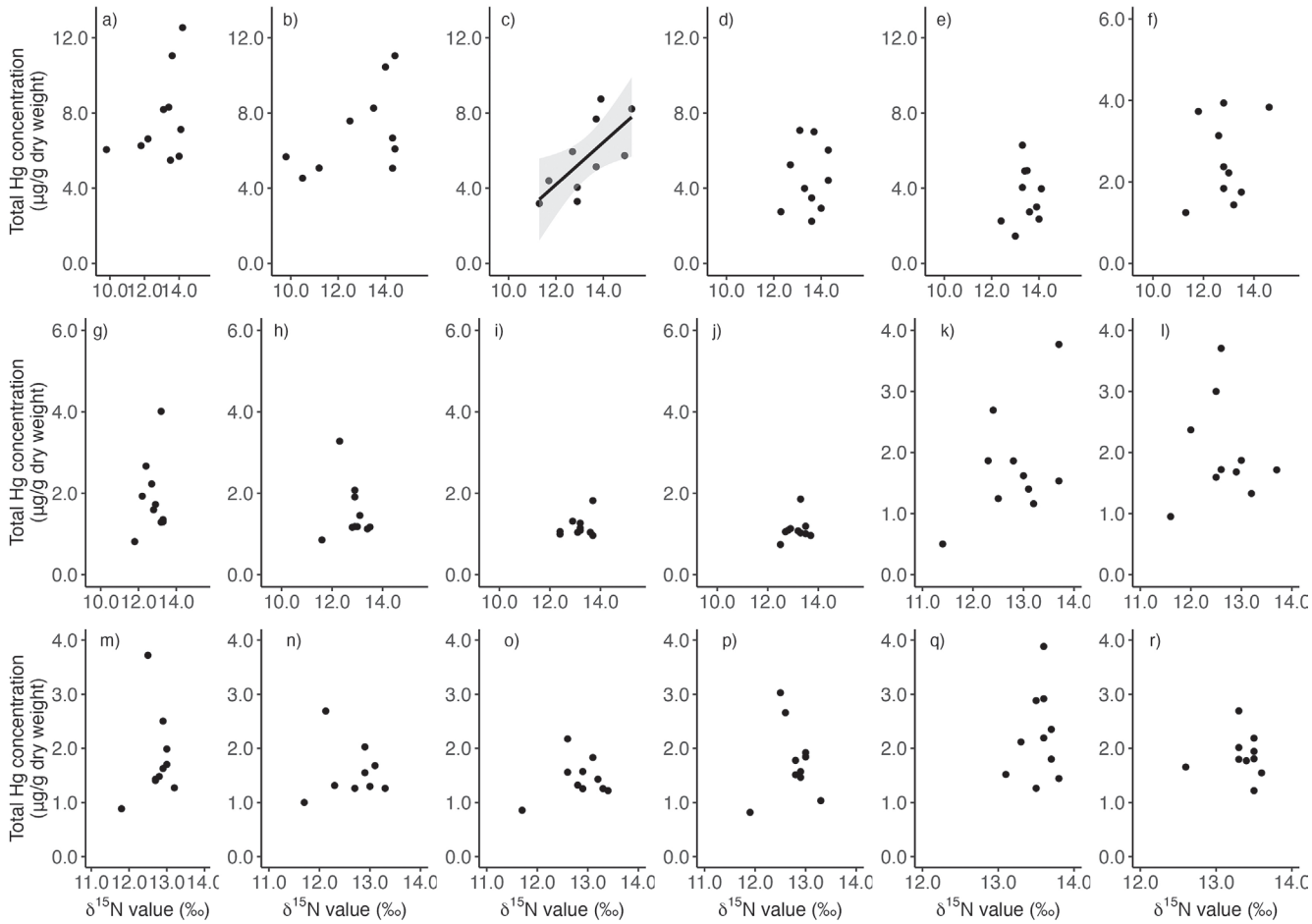

Fig. 2. The relationship between total Hg concentrations and $\delta^{15}N$ values in Black-tailed Gull *Larus crassirostris* primary feathers (P1 [a], P2 [b], P3 [c], P4 [d], P5 [e], P6 [f], P7 [g], P8 [h], P9 [i], and P10 [j]), rectrix feathers (R1 [k], R2 [l], R3 [m], R4 [n], R5 [o], and R6 [p]), breast feathers (q), and back feathers (r).

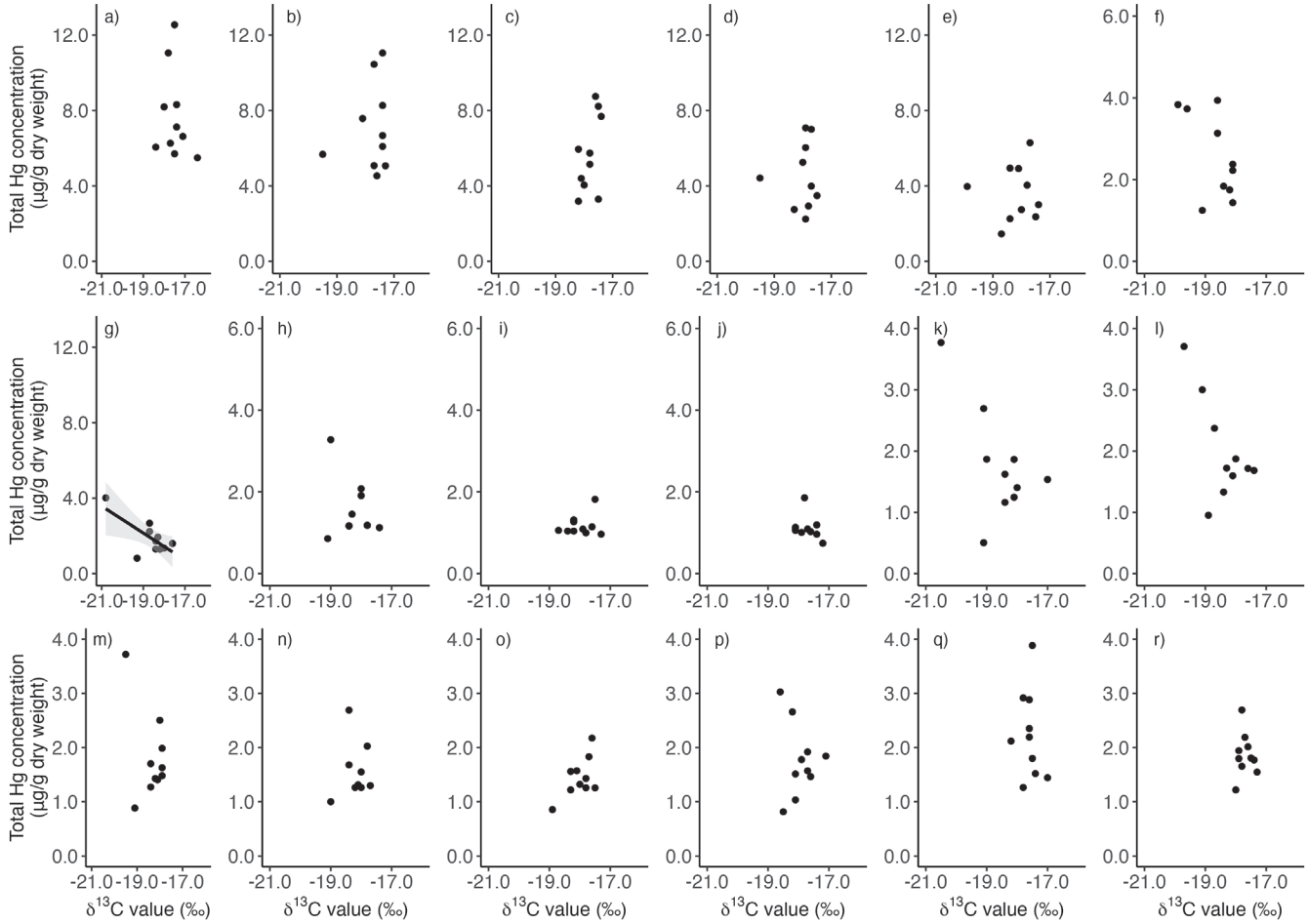

Fig. 3. The relationship between total Hg concentrations and δ^{13}C values in Black-tailed Gull *Larus crassirostris* primary feathers (P1 [a], P2 [b], P3 [c], P4 [d], P5 [e], P6 [f], P7 [g], P8 [h], P9 [i], and P10 [j]), rectrix feathers (R1 [k], R2 [l], R3 [m], R4 [n], R5 [o], and R6 [p]), breast feathers (q), and back feathers (r).

Hg concentrations and the smallest variation among individuals. This limited variation between individuals in Hg concentration in P10 may be useful when comparing Hg contamination between populations. Although no information is available, to my knowledge, concerning the molt of Black-tailed Gull rectrices, breast feathers, and back feathers, many gulls commence replacing those feathers after completing primary molt (Olsen, 2004). Since Hg concentrations in rectrices were lower and less variable than those of primary remiges, the Hg loads in their body tissues during the period of rectrix molt might be less than during primary molt. Black-tailed Gulls may mobilize Hg into growing rectrices from their body tissues and from freshly ingested food. Breast and back feathers had slightly higher Hg concentrations than did rectrices, indicating that their replacement may coincide with the replacement of the primaries, and Hg may be mobilized mainly from that accumulated in their body tissues.

Unlike total Hg concentrations, the δ^{15}N and δ^{13}C values measured in primaries did not relate to the molt sequence. The δ^{15}N values of feathers reflect the diet during the feather-growing period (Mizutani et al., 1990; Rubenstein & Hobson, 2004). Ingolfsson (1970) estimated that primary feathers of Glaucous Gulls *L. hyperboreus* grow 7.8 mm/day and those of Great Black-backed Gulls *L. marinus* grow 8.7 mm/day. If Black-tailed Gull primary feathers grow at a comparable rate, the amount necessary for stable isotope analysis would represent 5.7–6.4 days of growth. In this study, the birds integrate stable isotopes into their tissues from their diet, and what appears in their feathers accumulates in a week at most. As Bond (2010) pointed out, the lack of a significant relationship between total Hg concentrations and δ^{15}N values in feathers could be a consequence of their being different processes for the mobilization of Hg and δ^{15}N as feathers grow. The δ^{13}C values in primaries (ranging from −20.8‰ to −16.4‰ during this study) were similar to those found previously in the same colony by Tomita et al. (2015) and did not decrease with molt sequence, as I had initially predicted. Although the migratory areas used by the gulls in this study were not identified, environmental δ^{13}C values might not vary very much in the relatively small region in which this population occurs during the period when they molt their primaries (Kazama et al., 2013; Tomita et al., 2015).

In conclusion, the results of this study suggest that total Hg concentrations in the P1 feathers of Black-tailed Gulls are an appropriate indicator of the Hg load in their body tissues, which can be useful in the assessment of the effects of Hg loading on their reproduction and life history. The results also suggest that P10 feathers can be useful in comparing Hg contamination between populations.

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