Seabirds are important components of marine ecosystems, and many studies have attempted to define their trophic status (Ainly & Sanger 1979; Sanger 1987; Schneider et al. 1987; Croxall & Lishman 1987). Previous studies have focused on the prey types brought back to chicks by parents (Guinet et al. 1998; Kitaysky & Golubova 2000; Drant et al. 2003; Boyd et al. 2006; Wanless et al. 2007), and those in the stomachs of parents during the chick-rearing period (Bost et al. 1994; Chelel et al. 1996; Wilson et al. 2004). Seabirds need to invest nutrients in their eggs during the egg formation period, thus their trophic status might differ during that phase from that during chick-rearing. However, because of methodological difficulties, few studies have attempted to determine food sources used for egg formation.

Stable-carbon and nitrogen isotope analyses of avian tissues can provide valuable information on diet (Hobson et al. 1994). This approach is based on the fact that stable-isotope ratios in a consumer's tissues are related to those in its diet (Hobson 1993). Because eggs are composed of nutrients that are ultimately derived from the diet of adult females, the relative abundance of naturally-occurring stable-isotopes of carbon and nitrogen in egg yolks should be related to those in female diet, and this may form the basis of a method for tracing diet during egg formation (Hobson et al. 1995).

On Teuri (44°25′N, 142°19′E) and Rishiri (45°14′N, 141°09′E) islands in the northern Sea of Japan (Fig. 1), there are some of the largest seabird colonies in Japan. Large numbers of Rhinoceros Auklets Cerorhinca monocerata, Black-tailed Gulls Larus crassirostris, Japanese Cormorants Phalacrocorax capillatus and Slaty-backed Gulls Larus schistisagus breed in these colonies (Osa & Watanuki 2002), and are important components of the marine ecosystem of the northern Sea of Japan. In this region, the main diet of Rhinoceros Auklets and Black-tailed Gulls during early spring is thought to be krill and small pelagic fishes, and the availability of these prey may influence seabird breeding performance (Kazama et al. 2008; Tomita et al. 2009; Ito et al. 2009). However, information on the food sources used by these two species, and by Japanese Cormorants and Slaty-backed Gulls, when producing eggs is still lacking.
In this study, carbon and nitrogen stable-isotopes were measured in egg yolks of the Rhinoceros Auklet, Japanese Cormorant and Slaty-backed Gull on Teuri Island, and the Black-tailed Gull on Rishiri Island. We also measured stable-isotopes of potential prey. We estimated the food sources invested in producing egg yolks using measured stable-isotopes of their potential prey and reported the enrichment factors of yolks to provide basic dietary information for seabirds during egg formation.

MATERIALS AND METHODS

1) Field work

Eggs of Rhinoceros Auklets (2003: N=5, 2005: N=10), Slaty-backed Gulls (2005: N=6) and Japanese Cormorants (2005: N=7) from Teuri Island, and the Black-tailed Gull on Rishiri Island were collected under permit from the Japanese Environmental Agency (number 04315003, 050318001), the Agency for Cultural Affairs (permit number 16-4-1810), and the Hokkaido Souya sub-prefectural office (permit numbers 7-1 and 7-2). Nest contents were checked every day during the egg-laying period and only newly laid eggs (<1 day old) were collected.

Potential prey were collected around the colonies during early spring. Krill *Thysanoessa inermis*, a potential food source for Rhinoceros Auklets and Black-tailed Gulls (Tomita et al. 2009; Ito et al. 2009; Kentaro Kazama personal observation), were sampled by bongo net (0.33-mm mesh aperture, 60-cm mouth diameter) in the surrounding water on May 2003 by the research vessel *Tankai Maru* (National Fisheries Research Institute, Fisheries Research Agency). Over 1 age class (1+) Japanese Sand lance *Ammodytes personatus* (Average FL=210 mm), 0 age class (0+) Japanese Sand lance (Average FL=65 mm) and 0+ Japan Sea Greenling *Pleurogrammus azonus* (Average FL=110 mm), also prey items of Rhinoceros Auklets and Black-tailed Gulls (Deguchi et al. 2004; Kazama et al. 2008; Ito et al. 2009), were collected from bill-loads of Rhinoceros Auklets on Teuri at night in May 2004 and 2005. Japanese Sand lance >110 mm in fork-length were classified as >1+ age class (1+) and those smaller than 110 mm were 0+ age class (1+). Japan Sea Greenling smaller than 180 mm were classified as 0+ age classes (Isikawa & Watanuki 2002; Takahashi et al. 2001). Black-edged Sculpin *Gymnocanthus herzensteini* (Average FL=281 mm), Littlemouth Flounder *Pleuronectes herzensteini* (Average FL=184 mm), Kurosoi Rockfish *Sebastes schlegeli* (Average FL=172 mm) and large Japan Sea Greenling (Average FL=265 mm), which are prey items of Slaty-backed Gulls and Japanese Cormorants (Watanuki 1987; Ishikawa & Watanuki 2004; Watanuki et al. 2004), were sampled from commercial fishing catches in the surrounding water on May 2005. Those samples were kept at −30°C for later analysis.

2) Stable-isotope analysis

Delta$^{15}$N and $\delta^{13}$C were measured following Hobson (1993). Egg yolks were dried at 60°C in an electric oven for 24 h, then grained. Whole-body specimens of euphausiid and 0+ sand lance as well as minced tissue of >1+ sand lance and 0+ greenling were similarly dried and grained. Lipids were extracted from prey tissues using a chloroform:methanol (2:1) solvent rinse, and then dried at 60°C for 24 h to remove any residual solvent (Bligh & Dyer 1959). Samples were kept at −30°C before determination of stable-isotope analyses.

Stable-carbon and nitrogen compositions of the samples were determined using a mass spectrometer (MAT 252, Finnigan MAT, Bremen) coupled online via a Finnigan Conflo II interface with an elemental analyzer (EA 1110, ThermoQuest, Milan). One-milligram subsamples of homogenized materials were loading into tin cups and combusted at 1,000°C, and the resultant CO$_2$ and N$_2$ gases were then analyzed.
Diet during egg formation in seabirds

Pee Dee Belemnite (PDB) collected in South Carolina, United States, and atmospheric N\textsubscript{2} were used as standards following Hobson (1993). Stable-isotope concentrations were expressed in δ notation as parts per thousand according to the equation:

\[ \delta^{15}\text{N} \text{ or } \delta^{13}\text{C} = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1,000 \]

where \( R \) = the corresponding ratio \( {^{13}}\text{C}/^{12}\text{C} \) or \( {^{15}}\text{N}/^{14}\text{N} \). \( R_{\text{standard}} \) for \( {^{13}}\text{C} \) and \( {^{15}}\text{N} \) is that for atmospheric PDB and N\textsubscript{2} (air) standard, respectively. Based on hundreds of measurements of organic standards, the analytical precision (±SD) of these measurements is estimated to be ±0·06‰ for carbon and ±0·11‰ for nitrogen.

The abundance of stable isotopes in various biochemical components of foods will fractionate or change when incorporated into consumer tissues according to the relationship:

\[ D_t = D_d + \Delta d_t \]

where \( D_t \) is the isotopic abundance in the consumer tissue, \( D_d \) the isotopic abundance in the diet, and \( \Delta d_t \) is the isotopic enrichment factors between diet and tissue (Hobson et al. 1994). Once \( \Delta d_t \) is known for a particular trophic relationship, the isotopic signature of the average diet can be inferred from the isotopic abundance measurement of the consumer tissue (e.g. Tieszen et al. 1983). Thus, we can estimate the stable-isotope ratio of the diet consumed by the measured seabird by the equation:

\[ D_d = D_t - \Delta d_t \]

In this study, we defined \( D_d \) as adjusted values of \( \delta^{15}\text{N} \) and \( \delta^{13}\text{C} \) in egg yolk. The enrichment factors for Rhinoceros Auklet, Black-tailed Gull, Slaty-backed Gull and Japanese Cormorant egg yolks were unknown, so the average enrichment factor in lipid-free egg yolks from six avian species was used (\( \delta^{13}\text{C} \): 0.1‰, \( \delta^{15}\text{N} \): 3.4‰; Hobson 1995).

3) Isotopic mixing models

Model variants of the SIAR Bayesian mixing model (Parnell et al. 2010; Parnell & Jackson 2011) in the R environment (Ver. 2.15.1: R Development Core Team 2012) were used to quantify the diets of the four seabirds during the egg formation period from measured stable-isotope ratios. The SIAR model estimates probability distributions of multiple source contributions to a mixture while accounting for the observed variability in source and mixture isotopic signatures, dietary isotopic fractionation, and elemental concentration, using the Markov Chain Monte Carlo method with a 500,000 step length (Polito et al. 2011). Two SIAR model variants with multiple prey sources were used to estimate diet composition for each species/year combination using the \( \delta^{15}\text{N} \) and

![Fig. 2. Values of stable nitrogen and carbon isotope ratios in dietary fishes and krill, and adjusted values of isotopic ratios in seabird egg yolks. RHAU: Rhinoceros Auklet, BTGL: Black-tailed Gull, SBGL: Slaty-backed Gull, JACO: Japanese Cormorant.](image-url)
\[\delta^{13}C\] values of sea bird egg yolk.

For each seabird species, we selected a range of species that were likely to be prey items: krill, 1+ sandlance, 0+ sandlance and 0+ greenling were selected for Rhinoceros Auklets and Black-tailed Gulls; 1+ sandlance, 1+ greenling, 0+ greenling, sculpin, rockfish and flounder were selected for Slaty-backed Gulls and Japanese Cormorants (see Fig. 2). Because 0+ greenlings were split in our data, we divided 0+ greenlings into two groups (0+ greenlings 1 and 2) for analysis.

**RESULTS**

Overall the \(\delta^{15}N\)s of Rhinoceros Auklet and Black-tailed Gull eggs were smaller than those of Slaty-backed Gulls and Japanese Cormorants (Fig. 2, ANOVA, \(F_{4,34}=56.3, P<0.01\), with Scheffe’s post hoc test, \(P<0.01\)), indicating that the trophic levels of the two former species were lower than the two latter species. Delta \(^{13}C\) also varied between species (Fig. 2, ANOVA, \(F_{4,34}=56.3, P<0.01\)). In particular, \(\delta^{13}C\) in Rhinoceros Auklet eggs differed between 2003 and 2005 (Fig. 2, Scheffe’s post hoc test, \(P<0.01\)). Delta \(^{13}C\) and \(\delta^{15}N\) showed large individual variation in Japanese Cormorants (see Fig. 2). Black-tailed Gulls also showed some individual variation in stable-isotope ratios (see Fig. 2). In Rhinoceros Auklets, there was large individual (especially in \(\delta^{13}C\) in 2005) and inter-annual variation in stable-isotope ratios (see Fig. 2). The stable-isotope ratios in Slaty-backed Gulls were less variable than in other seabird species (see Fig. 2).

The adjusted \(\delta^{13}C\) in the egg yolks of Rhinoceros Auklets in 2005 was similar to that of krill, though the \(\delta^{15}N\) was slightly higher (Fig. 2). Delta \(^{13}C\) and \(\delta^{15}N\) levels in Rhinoceros Auklet egg yolks in 2003 were similar to those of 0+ sandlance and 0+ greenling (Fig. 2). Adjusted \(\delta^{15}N\) and \(\delta^{13}C\) in the egg yolks of Black-tailed Gulls were similar to the values of 0+ sandlance and were between the values for krill and 0+ greenling (Fig. 2).

Adjusted \(\delta^{15}N\) and \(\delta^{13}C\) in the egg yolks of Slaty-backed Gulls were close to the values of 1+ sandlance (Fig. 2), whereas those in the egg yolks of Japanese Cormorants varied and appeared to be around those of 1+ sandlance, 1+ and 0+ greenling, sculpin, rockfish, and flounder (Fig. 2).

The SIAR mixing model indicated that: Rhinoceros Auklets mainly fed on krill in 2005, and on 0+ greenling and 0+ sandlance in 2003; Black-tailed

<table>
<thead>
<tr>
<th>Species</th>
<th>1+ Sandlance</th>
<th>1+ Greenling 1</th>
<th>1+ Greenling 2</th>
<th>Sculpin</th>
<th>Rockfish</th>
<th>Flounder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhinoceros Auklet (2003)</td>
<td>15.9 (0–39)</td>
<td>62.0 (53–69)</td>
<td>5.3 (0–16)</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Rhinoceros Auklet (2005)</td>
<td>12.8 (0–32)</td>
<td>36.9 (19–39)</td>
<td>10.9 (0–10)</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Slaty-backed Gull (2003)</td>
<td>13.5 (0–29)</td>
<td>16.7 (0–23)</td>
<td>10.9 (0–23)</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Slaty-backed Gull (2005)</td>
<td>9.9 (0–26)</td>
<td>6.9 (0–10)</td>
<td>5.3 (0–16)</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Japanese Cormorant (2005)</td>
<td>14.3 (0–29)</td>
<td>10.0 (0–24)</td>
<td>6.9 (0–10)</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

95% CI: Bayesian 95% credibility intervals

N.A.: Not applicable
Diet during egg formation in seabirds

Gulls mainly fed on small pelagic fish and small amounts of krill; Slaty-backed Gulls and Japanese Cormorants fed on several kinds of fish species during egg formation (Table 1).

DISCUSSION

Rhinoceros Auklets are generally thought to be piscivorous (e.g. Gaston & Jones 1996), but some studies have indicated that they also feed on substantial amounts of krill in the spring (Ainly & Sanger 1979; Ito et al. 2009). The present study has confirmed the significance of krill in the Rhinoceros Auklet’s diet. Black-tailed Gulls feed mainly on pelagic fishes (e.g. sandlance and anchovy) during chick rearing on Teuri (Deguchi et al. 2004) and Rishiri islands (Kazama et al. 2008), while they are believed to feed on krill around both islands in the spring (Tomita et al. 2009; Kentaro Kazama personal observation). However, the present study demonstrates that Black-tailed Gulls mainly fed on 1+, 0+ sandlance and 0+ greenling and less so on krill. Rhinoceros Auklets and Black-tailed Gulls feed on small pelagic fish that are found in relatively cold water (Nagasawa et al. 1991) and/or krill that make surface swarms in cold water (SST: 4–8°C; Hanamura et al. 1989). Hence, inter-annual variation in sea surface temperatures in the waters surround the breeding colonies might induce variation in prey availability, which in turn causes the proportion of prey invested in egg yolk to vary inter-annually. Relatively large individual variations in stable-isotope ratios were also found in those species, perhaps indicating the existence of dietary or foraging site preference in those species during egg formation.

During chick-rearing Slaty-backed Gulls feed on pelagic fish (1+ sandlance), discarded fish, and seabird chicks, but with considerable individual variation (Watanuki 1987). However, the results of this study indicate that Slaty-backed Gulls during egg formation foraged mainly on 1+ sandlance and greenlings with less individual variation, at least during 2005. Japanese Cormorants mainly fed on the highest trophic level prey (1+ greenling, rock fish, flounder and sculpin) during egg formation. Japanese Cormorants are known to exhibit considerable individual variation in foraging sites, foraging behavior and diet during the chick rearing period (Watanuki et al. 2004; Ishikawa & Watanuki 2004). The present results revealed individual variability in the diet of Japanese Cormorants, which may result from differential foraging site or foraging behavior choices during the period of egg formation and breeding.

The results of the SIAR mixing model also supported these results. Thus, this study provides useful basic dietary information for seabirds during the egg formation period. Additionally, the data revealed some individual and inter-annual variation in diet invested in seabird eggs. It is known that variation in diet invested in eggs may affect the timing of egg laying, egg size and clutch size among seabirds (Sorensen et al. 2009). Future research should focus on clarifying the relationships between those individual or inter-annual variations and the breeding performance of seabirds in order to better understand seabird breeding biology.

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