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STABLE CARBON AND NITROGEN ISOTOPE DISCRIMINATION FACTORS FOR QUANTIFYING SPECTACLED EIDER NUTRIENT ALLOCATION TO EGG PRODUCTION

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Abstract. Nutrient-allocation models based on stable-isotope analysis are used to determine the nutrient sources birds invest in eggs. This approach is particularly useful for birds that migrate between habitats with distinct stable-isotope compositions. A crucial variable is the difference in stable-isotope values of egg tissues relative to diet, so appropriate adjustments can be used in models comparing nutrients from tissues to putative food sources. We established discrimination factors (Δδ) between the diet and eggs of captive Spectacled Eiders (*Somateria fischeri*) fed a controlled diet. Relative to diet, values of $\Delta\delta^{13}C$ were higher for albumen (2.6‰), yolk protein (2.9‰), eggshell (13.0‰), and shell membrane (3.9‰), and lower for whole yolk (–1.6‰) and yolk lipid (–3.5‰). Values of $\Delta\delta^{15}N$ of egg components were higher relative to diet (albumen 3.7‰, yolk protein 4.4‰, shell membrane 4.7‰, and whole yolk 3.5‰). Except for egg proteins, these patterns are generally consistent with published values for other birds. We conclude that choice of discrimination factors could markedly affect estimates of source contributions to eggs and so recommend species-specific estimates. We also provide the first reported discrimination factors between the female's diet and embryonic down feathers ($\Delta\delta^{13}C = 2.1‰$ and $\Delta\delta^{15}N = 5.2‰$). Finally, we determined discrimination factors between lipid and protein in diet sources and eggs, thus enabling consideration of these nutrients separately. Our study enhances the framework for nutrient-allocation modeling in eiders and likely other sea ducks.

Key words: *discrimination factor, egg, eider, feather, sea duck.*

Factores de Discriminación de Isótopos de Carbono y Nitrógeno Estables para Cuantificar la Asignación de Nutrientes de *Somateria fischeri* en la Producción de Huevos

Resumen. Los modelos de asignación de nutrientes basados en análisis de isótopos estables son usados para determinar las fuentes de nutrientes que las aves invierten en los huevos. Este enfoque es particularmente útil para las aves que migran entre hábitats con composiciones distintivas de isótopos estables. Una variable crucial es la diferencia en los valores de isótopos estables de los tejidos de los huevos relativos a la dieta, lo que permite usar ajustes apropiados en los modelos que comparan los nutrientes de los tejidos con las fuentes de alimentos. Establecimos factores de discriminación (Δδ) entre la dieta y los huevos de individuos cautivos de *Somateria fischeri* alimentados con una dieta control. Relativos a la dieta, los valores de $\Delta\delta^{13}C$ fueron superiores para el albumen (2.6‰), la proteína de la yema (2.9‰), la cáscara del huevo (13.0‰) y la membrana de la cáscara (3.9‰), e inferiores para la yema completa (–1.6‰) y los lípidos de la yema (–3.5‰). Los valores $\Delta\delta^{15}N$ de los componentes del huevo fueron superiores relativos a la dieta (albumen 3.7‰, proteína de la yema 4.4‰, membrana de la cáscara 4.7‰ y yema completa 3.5‰). Excepto para las proteínas del huevo, estos hallazgos son generalmente consistentes con los valores publicados para otras aves. Concluimos que la selección de los factores de discriminación podría afectar marcadamente los estimados de las fuentes de contribución a los huevos y por ende permitir recomendar estimados específicos para las especies. Brindamos adicionalmente los primeros informes de factores de discriminación entre la dieta de las hembras y el plumón embrionario ($\Delta^{13}C = 2.1‰$ y $\Delta^{15}N = 5.2‰$). Finalmente, determinamos los factores de discriminación entre lípidos y proteínas en las fuentes de la dieta y los huevos, permitiendo de este modo considerar separadamente estos nutrientes. Nuestro estudio mejora el marco para el modelado de la asignación de nutrientes en *S. fischeri* y probablemente en otros patos marinos.

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INTRODUCTION

Analyses of stable carbon and nitrogen isotopes can be used to evaluate allocation of nutrients for egg production in birds that migrate between isotopically distinct regions for the nonbreeding and breeding stages of the annual cycle (Hobson et al. 1997, 2000, Hobson 2006). With this approach, mixing models can be applied to quantify sources of nutrients used for egg production (Phillips and Gregg 2001, 2003, Moore and Semens 2008). These nutrient-allocation models typically require stable-isotope values for two or more potential nutrient sources and the animal tissue, such as egg components or feathers, corrected for diet to tissue discrimination factors ($\Delta\delta$). However, there are few species-specific data available for quantifying discrimination factors in avian egg components (Hobson 1995), and to our knowledge no information is available for discrimination factors associated with natal down feathers. Experimental studies are needed to determine discrimination factors specific to tissue and species or taxon (Gannes et al. 1997, Hobson 2006, Caut et al. 2008, Bond and Diamond 2011).

Discrimination factors between diet and tissues have been found to vary according to diet composition (DeNiro and Epstein 1978, Ben-David and Schell 2001), species (Hobson 1995), individual physiology (Martínez del Rio and Wolf 2005), age (Minagawa and Wada 1984), body condition (Martínez del Rio and Wolf 2005), and tissue type (Hobson and Clark 1992, Bearhop et al. 2002). Because egg yolk contains proteins and lipids, and lipids have a lower $\delta^{13}C$ (DeNiro and Epstein 1977, McConnaughey and McRoy 1979), it is necessary to determine discrimination factors of the egg's components separately to ascertain differentiation of macronutrient sources (Hobson 1995, 2006). Use of inaccurate discrimination factors may be a source of error in mixing models, so it is important to establish these values for confident stable-isotope modeling of nutrient allocation to reproduction.

Reproduction is energetically demanding for sea ducks such as eiders (Alisauskas and Ankney 1992), yet the proportion of marine-derived nutrients sea ducks allocate to egg production is largely unknown. While sea ducks such as the Common Eider (*Somateria mollissima*) are thought to rely heavily on capital reserves for egg production (Parker and Holm 1990), other studies have reported that sea ducks use mixed (Hobson et al. 2005) or almost entirely income-based breeding strategies (Bond et al. 2007, Oppel 2008). Nutrient-allocation strategies during reproduction of the Spectacled Eider (*Somateria fischeri*), which is listed as threatened in the U.S.(Federal Register 1993), are largely unknown, and the purpose of our study was to advance species-specific methods for nutrient-allocation studies of eiders.

We measured the $\delta^{13}C$ and $\delta^{15}N$ values of experimental diet items, egg components, and down feathers for captive Spectacled Eiders and quantified discrimination factors between whole diet and these tissues. Furthermore, we compared these discrimination factors from eggs of a captive flock of Spectacled Eiders to those reported by Hobson (1995) in a previous study of captive birds. Finally, we measured stable-isotope values of the lipid and protein nutrients in the diet and quantified discrimination factors between the lipid and protein diet sources and egg tissues.

METHODS

CAPTIVE-FEEDING TRIALS

Our research, at the Alaska SeaLife Center in Seward, Alaska, extended from 1 February through the end of egg laying on 30 June 2008. Female Spectacled Eiders ($n = 5$) had hatched in captivity in 2002 and had been housed at the Alaska SeaLife Center since 2003, in an outdoor enclosure all year. Before breeding (February–June 2008), the birds were maintained on a consistent diet of 96% Mazuri Sea Duck diet (Purina Mills, Inc., St. Louis, MO) and 4% of tidewater Atlantic silverside (*Menidia menidia*; harvested near Prince Edward Island, Canada), along with calcium grit. We calculated proportions of the diet on the basis of dry-mass consumption of Mazuri within the previous 24 hr. Mazuri Sea Duck diet consists mostly of ground corn and wheat grains, fish, and pork with a nutrient composition of approximately 6.5% lipid, 21.6% protein, 8.4% fiber, 10.9% ash, 46.6% nitrogen-free extract, and vitamins (www.mazuri.com). We assumed the birds consumed diet components in these proportions, but they were fed in groups so we could not determine an individual's intake. We took samples from five batches of Mazuri and silverside at approximately 1-month intervals (February–June), placed them in airtight plastic bags, and stored them frozen at –20 ºC until analysis.

TISSUE SAMPLING

We monitored the birds daily for egg laying during the breeding season and collected eggs upon observation. The eggs were artificially incubated at 37.4 °C and 60% relative humidity (Grumbach Incubator, Asslar, Germany), and we candled them after 3–5 days to ascertain their fertility. All eggs were then refrigerated at 4 °C and divided into fresh yolk and albumen components within 24 hr. Homogenized samples of Spectacled Eider yolk and albumen were stored separately in polyethylene containers and kept frozen at –20 °C until analysis. Eggshells and shell membranes were rinsed with distilled water, dried, and stored at room temperature, and a section was sampled for analysis.

Five eggs from two females were incubated for 20–23 days. We sampled whole down feathers from the embryos' dorsal tract, rinsed them with ethanol to remove any external substances, and dried and stored them at room temperature until analysis (Knoche 2004).

LABORATORY ANALYSES

Diet items and liquid egg components were dried to a constant mass with a FreeZone 6 Liter Console Freeze Dry System (Labconco Corporation, Kansas City, MO). When

considering macronutrient allocation to eggs, we separated diet and yolk samples into lipid and nonlipid components because of potential mean differences in discrimination factors. To extract lipids from diet items and whole yolk we used a 2:1 ratio of chloroform:methanol solution and followed the methods of Bligh and Dyer (1959) except we used no water because stable isotopes are analyzed as dry material. We used a vortex to mix ~5 mg of dry sample with solution, allowed samples to settle for 24 hr, and extracted lipids manually with a pipette until solvent wash was clear (Oppel et al. 2010). Lipids were kept uncovered under a fume hood until all solvent had evaporated. We powdered all diet items and egg components with a mortar and pestle or grinder, except lipids from diet and yolk, which we analyzed without grinding.

Stable carbon and nitrogen isotopes were analyzed at the Alaska Stable Isotope Facility at the University of Alaska Fairbanks. We weighed all homogenized diet items and tissues (0.01–0.05 mg) into tin cups on a Sartorius M2P electronic microbalance and measured samples via combustion with a continuous-flow isotope-ratio mass spectrometer (Finnigan Delta^{plus}XP CF-IRMS). We express isotopic results in delta (δ) notation relative to international standards ($R_{standard}$ = Vienna PeeDee Belemnite for $\delta^{13}C$, atmospheric air for $\delta^{15}N$) in parts per thousand (‰) according to the following equation $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$, where X denotes either ^{13}C or ^{15}N and R represents the ratio of ^{13}C:^{12}C or ^{15}N:^{14}N, respectively. Multiple ($n = 210$) analyses of a peptone standard (Sigma Chemical Co., Highland, IL; mean $\delta^{13}C$ = –15.82‰ and mean $\delta^{15}N$ = 7.02‰) spaced throughout the run of the samples yielded analytical precisions of ±0.1 for $\delta^{13}C$ and ±0.2 for $\delta^{15}N$, reported as ± 1 SD.

We measured the stable-isotope signatures of each diet item in duplicate; whole diet, dietary lipids, and lipid-free portions of the diet were analyzed separately. We analyzed single measurements of egg components and down feathers from Spectacled Eiders and calculated the mean value and SD for the flock. We then calculated discrimination factors by subtracting the mean isotope signatures of egg components and down feathers (Y) from those values of whole diet (X), described in terms of the difference ($\Delta = \delta Y - \delta X$). We also calculated discrimination factors to account for direct transfer of lipid and protein from diet items to egg components or down feathers. We did this by subtracting the mean isotope signatures of lipid egg yolk (Y) from those values of dietary lipids (X) or stable-isotope signatures of lipid-free egg components (albumen, lipid-free yolk, and shell membrane) or mean isotope signatures of down feathers (Y) from those values of the lipid-free diet (X). To account for variation in diet and tissues, we calculated the SD for the discrimination factors. We report signatures of stable carbon and nitrogen isotopes in the diet items, all egg components, and down feathers and the discrimination factors as means ±1 SD.

STATISTICAL ANALYSES

We used a one-way ANOVA to evaluate interspecific differences in $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ values of egg components (Hobson 1995; this study). For all analyses we used SigmaStat 3.1 (Systat Software, Inc., Point Richmond, CA) and set α at 0.05.

RESULTS

$\delta^{13}C$ AND $\delta^{15}N$ ISOTOPE ANALYSES FOR DIET ITEMS

We determined values of $\delta^{13}C$ and $\delta^{15}N$ and percent carbon and nitrogen concentration for the whole diet, lipid-free diet, and dietary lipids (Table 1). We report only the carbon for the dietary lipids because lipids contain very little nitrogen. Silversides made up only a small proportion of the overall diet (4%). We calculated the stable-isotope signature of the diet from that of the diet item multiplied by the percentage of each diet item eaten per flock (Table 1).

$\Delta\delta^{13}C$ AND $\Delta\delta^{15}N$ FOR EGG COMPONENTS AND DOWN FEATHERS

We calculated discrimination factors for $\delta^{13}C$ and $\delta^{15}N$ (i.e., $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$) from whole diet for whole yolk, lipid-free yolk, albumen, and shell membrane and for down feathers. We report only $\Delta\delta^{13}C$ from whole diet for yolk lipid and eggshell because of the low nitrogen content of these tissues.

We observed similar increases in $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ for protein-based components of the egg (i.e., albumen, lipid-free yolk, and shell membrane) and down feathers over the whole diet (Table 2). Whole yolk increased in $\Delta\delta^{15}N$ relative to whole diet, but whole yolk and yolk lipid decreased in $\Delta\delta^{13}C$ relative to whole diet (Table 2). The $\Delta\delta^{13}C$ for eggshell increased considerably in relation to whole diet (Table 2).

TABLE 1. Signatures of stable carbon and nitrogen isotopes ($\delta^{13}C$ and $\delta^{15}N$, respectively) and percent carbon and nitrogen concentration for the whole diet, lipid-free diet, and dietary lipids fed to captive Spectacled Eiders.

	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Conc. C (%)	Conc. N (%)
Whole diet ($n = 5$)				
Mazuri	–21.1 ± 1.0	6.3 ± 0.6	43.7 ± 1.0	3.6 ± 0.4
Silverside	–18.5 ± 0.9	11.6 ± 0.4	46.6 ± 5.8	11.3 ± 0.9
Diet proportion[a]	–21.0 ± 0.9	6.5 ± 0.5	—	—
Lipid-free diet ($n = 5$)				
Mazuri	–20.4 ± 0.6	6.5 ± 0.5	36.4 ± 5.8	3.3 ± 0.5
Silverside	–17.6 ± 0.6	12.0 ± 0.6	42.9 ± 0.9	13.6 ± 0.4
Diet proportion[a]	–20.3 ± 0.6	6.7 ± 0.5	—	—
Diet lipid ($n = 5$)				
Mazuri	–26.6 ± 0.2	—	63.5 ± 5.0	—
Silverside	–23.4 ± 0.8	—	55.9 ± 4.6	—
Diet proportion[a]	–26.4 ± 0.2	—	—	—

[a]Diet proportion based on isotope signature of diet items multiplied by percentage of each diet item eaten per flock. Diet consists of 96% Mazuri® and 4% Atlantic silverside.

TABLE 2. Average (± 1 SD) discrimination factors (Δδ) of stable carbon and nitrogen isotopes from whole diet to eggs (n = 5) and down feathers (n = 2) for captive Spectacled Eiders and, for comparison, from whole diet to eggs data for captive Japanese Quail (n = 9), Mallards (n = 8), Peregrine Falcons (n = 6), Gyrfalcons (n = 4), and Prairie Falcons (n = 2) (Hobson 1995).

Species	Δ δ	Albumen	Lipid-free yolk	Membrane	Yolk	Yolk lipid	Eggshell	Down feathers
Spectacled Eider	C	2.6 ± 0.4	2.9 ± 0.4	3.9 ± 0.4	–1.6 ± 0.4	–3.5 ± 0.4	13.0 ± 0.4	2.1 ± 0.5
	N	3.7 ± 0.2	4.4 ± 0.3	4.7 ± 0.3	3.5 ± 0.3	—	—	5.2 ± 0.4
Japanese Quail	C	1.6 ± 0.4	0.1 ± 0.3	3.5 ± 0.3	–1.1 ± 0.3	–2.6 ± 0.5	15.6 ± 0.5	
	N	2.4 ± 0.2	3.4 ± 0.3	4.1 ± 0.4	3.4 ± 0.3	—	—	
Mallard	C	1.4 ± 0.6	–0.1 ± 0.5	3.7 ± 0.3	–1.4 ± 0.7	–2.7 ± 0.5	14.3 ± 0.7	
	N	3.0 ± 0.4	3.1 ± 0.4	4.4 ± 0.3	3.4 ± 0.3	—	—	
Peregrine Falcon	C	0.9 ± 0.5	0.0 ± 0.5	2.6 ± 0.5	–2.2 ± 0.5	–3.5 ± 0.5	11.1 ± 0.5	
	N	3.1 ± 0.4	3.5 ± 0.3	3.5 ± 0.4	3.3 ± 0.4	—	—	
Gyrfalcon	C	0.8 ± 0.5	0.1 ± 0.5	—	–1.8 ± 0.5	–3.2 ± 0.4	11.2 ± 0.5	
	N	3.3 ± 0.3	3.6 ± 0.4	—	3.1 ± 0.4	—	—	
Prairie Falcon	C	0.9	0.1	3.0	–1.4	–3.6	11.6	
	N	3.1	3.5	3.2	3.5	—	—	

We provide $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ from whole diet to tissue to allow comparison of previously established discrimination factors from other species of birds (Hobson 1995) with those from our study of the Spectacled Eider (Table 2). Hobson (1995) determined the isotope value for the whole diet only but presented a hypothetical model that separated the macronutrients (e.g., lipid and protein) in the diet from those nutrients in the different egg components. As an expansion of this hypothetical model, we partitioned the whole diet into lipid and nonlipid components and calculated those actual values so that we could quantify discrimination factors to track specific macronutrients used for egg synthesis (e.g., dietary lipids to yolk lipids and lipid-free diet to the protein-based tissues such as lipid-free yolk, albumen, shell membrane, and down feathers; Fig. 1).

COMPARISON OF SPECTACLED EIDER Δδ AND PREVIOUSLY DETERMINED VALUES FOR OTHER SPECIES

Although the $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ values we observed in Spectacled Eider eggs followed overall distribution patterns similar to those documented in other species, we found a significant difference between many of the egg components (Table 2; Hobson 1995). The $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ values for the Spectacled Eider were higher (all $p < 0.001$) than those of other species for albumen (mean increase in $\Delta\delta^{13}C$ of 0.8–1.0 and in $\Delta\delta^{15}N$ of 0.4–1.3), lipid-free yolk (mean increase in $\Delta\delta^{13}C$ of 2.7–3.0 and in $\Delta\delta^{15}N$ of 0.8–1.3), and shell membrane (mean increase in $\Delta\delta^{13}C$ of 0.2–1.3 and in $\Delta\delta^{15}N$ of 0.3–1.5). The $\Delta\delta^{13}C$ value for the Spectacled Eider was different from that of other species (whole yolk $p = 0.019$ and yolk lipid $p < 0.001$), being lower than those for the Japanese Quail (*Coturnix japonica*) and Mallard (*Anas platyrhynchos*) for whole yolk (0.2–0.5‰) and yolk lipid (0.8–0.9‰) and higher than or similar to those for the Prairie Falcon (*Falco mexicanus*), Peregrine Falcon (*F. peregrinus*), and Gyrfalcon (*F. rusticolus*) for whole yolk (0.2–0.6‰) and yolk lipid (0–0.3‰). The average $\Delta\delta^{15}N$ value for whole yolk was either similar to or higher than that in previously studied species (0–0.5‰; $p = 0.073$). Although the average of $\Delta\delta^{13}C$ for eggshells varies widely by species ($p < 0.001$), the average $\Delta\delta^{13}C$ value for calcium carbonate in Spectacled Eider eggshells was lower than that in Japanese Quail and Mallard eggshells (1.3–2.6‰) and higher than that of eggshells of the three falcons (1.4–1.9‰).

DISCUSSION

Our discrimination factors for whole diet to egg tissues of the Spectacled Eider differed by egg component, consistent with discrimination factors in eggs previously reported from other birds (Hobson 1995). We found $\Delta\delta^{13}C$ values for the Spectacled Eider higher than previously reported for the protein portion (albumen, lipid-free yolk, and shell membrane) of eggs of other birds. In particular, the discrimination factor for carbon in lipid-free yolk of the Spectacled Eider differed markedly from that Hobson (1995) reported for other species. It is possible that these differences observed in lipid-free yolk may have been an effect of lipid extraction (Oppel et al. 2010) because methanol (the polar solvent) does not target specific lipids. Some polar structural fats are attached to proteins, and the loss of these amino acids as a result of lipid extraction may increase values of $\delta^{13}C$ and $\delta^{15}N$ (Sotiropoulos et al. 2004, Sweeting et al. 2006). Additionally, the $\Delta\delta^{13}C$ values for eggshells of the Spectacled Eider were similar to those for other species, though averages for other species range widely. Because eggshells consist primarily of calcium carbonate, they can be derived from calcium in the diet or skeletal tissue and carbonate in the blood bicarbonate or CO_2 (Johnson 2000), thus inflating the value for carbon. We

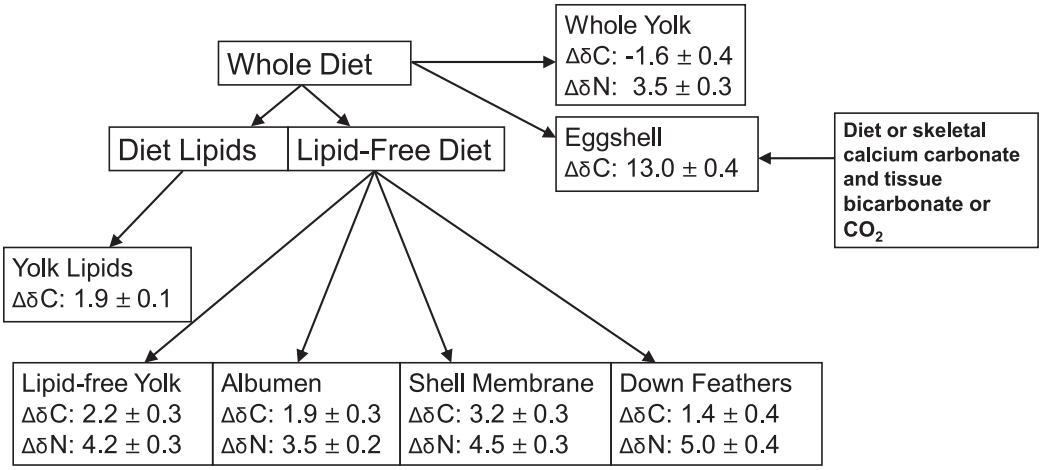

FIGURE 1. As an expansion of the hypothetical model presented by Hobson (1995). The whole diet was partitioned into lipid and nonlipid components, and isotopic discrimination factors ($\Delta\delta$) were determined for dietary lipids to lipids in the egg (yolk lipids), and for diet with lipids removed to the protein-based components (albumen, lipid-free yolk, shell membrane, and down feathers) of the egg and developing embryo.

found a decrease in $\delta^{13}C$ for egg components that contain lipid (i.e., whole yolk and yolk lipid) as previously described. These lower $\delta^{13}C$ values are the result of high lipid concentration in these tissues and oxidation of these lipids during lipid synthesis (DeNiro and Epstein 1977). Finally, $\Delta\delta^{15}N$ values for albumen, whole yolk, lipid-free yolk, and shell membrane were higher than those found by Hobson (1995).

Our results also provide discrimination factors from the female's diet to embryonic down feathers, which have not been previously documented for birds. Feathers consist of proteins (Murphy 1996), and, as might be expected, discrimination factors for embryonic down feathers were similar to those for protein-based components of the egg.

We determined stable-isotope values for the whole diet, diet with lipid removed, and lipid from the diet and therefore discrimination factors from diet macronutrients (i.e., diet lipids and diet with lipids removed) to those lipid (i.e., yolk lipid) and protein portions of the egg or the embryo, respectively (i.e., albumen, lipid-free yolk, shell membrane, and down feathers). Use of macronutrient-specific discrimination factors will further increase the applicability of stable isotopes in studies of nutrient allocation to egg production because these different macronutrients may be derived from sources in isotopically distinct habitats.

Previous studies have shown that differences in isotopic discrimination of tissues may be the result of species-specific physiology (DeNiro and Epstein 1978, 1981, Hobson and Clark 1992, Hobson 1995, Dalerum and Angerbjorn 2005). Isotope discrimination of bird eggs has been reported for only a few species (Hobson 1995), and no data were available previously for sea ducks. Our results have revealed some interspecific differences in the discrimination factors from the whole diet to egg components, mainly for the protein-based portions of the egg. The differences in isotopic discrimination factors we found and those reported by Hobson (1995) indicate that choice of an isotopic discrimination factor could have a marked influence on subsequent inferences about sources of nutrients in tissues (Federer 2009), emphasizing the need for these values to be determined for specific species and tissues (Dalerum and Angerbjorn 2005, Hobson 2006, Caut et al. 2008).

Diet composition is also an important consideration in studies of stable-isotope discrimination (DeNiro and Epstein 1978, 1981, Hobson and Clark 1992, Hobson 1995). Spectacled Eiders are thought to feed on both animal protein and plants while on their breeding grounds and on a diet of animal protein while in their winter range (U.S. Fish and Wildlife Service 1996, Lovvorn et al. 2003). In their comprehensive studies, DeNiro and Epstein (1978, 1981) found much less variation in stable-isotope ratios when the consumer was fed a diet based on animal tissues than when it was fed one based on plants. Hobson and Clark (1992) and Hobson (1995) also reported differences in discrimination factors for birds fed diets of dissimilar composition, but they suggested some of these differences were species-specific. The commercial diet we fed Spectacled Eiders is primarily plant based (~60%) but also contains about 20% or more animal protein (Liz Koutsos, Purina Mills, pers. comm.). Although a natural diet could not be fed to these captive birds, the benefit of the artificial diet for our study is that a commercially manufactured food is likely to be less variable than is a diet from the wild. While we analyzed samples of batches of the commercially manufactured food and found very little variation among the batches, we note that this may also contribute to variation in the discrimination factors. Despite not being able to replicate the diet of wild Spectacled Eiders, we assume that species-specific

discrimination factors are a significant advancement (Hobson and Clark 1992, Hobson 1995, 2006, Dalerum and Angerbjorn 2005, Caut et al. 2008) and believe our data represent values for appropriate for the Spectacled Eider more accurately. Further experiments using artificial and natural diets varying in nutrient composition may be useful for understanding how changes in diet composition may affect isotopic discrimination factors in tissues of the Spectacled Eider, particularly for a species whose diet varies seasonally.

CONSERVATION IMPLICATIONS

The method of nutrient-allocation modeling using stable isotopes has important conservation implications for a threatened species like the Spectacled Eider. This species spends the majority of its annual cycle in offshore marine habitats (Petersen et al. 1995, 1999) and travels to freshwater tundra habitats for reproduction (U.S. Fish and Wildlife Service 1996). Potential factors leading to the decline or affecting population recovery include changes in the marine environment, such as food abundance and availability (U.S. Fish and Wildlife Service 1996, Richman and Lovvorn 2003) and shifts in marine habitats (Lovvorn et al. 2003). Because many sea ducks spend much of the winter and staging periods in marine habitats, it is plausible that their reproductive performance is correlated with the availability and quality of marine resources. However, the nutrient-allocation strategies used by breeding Spectacled Eiders are largely unknown. Tissues such as eggs and down feathers and nest-bowl contents could be useful in understanding the sources and timing of acquisition of nutrients for reproduction. Stable-isotopic discrimination factors for other species have been calculated in one study previously, but our data are the first discrimination factors reported for the Spectacled Eider. While down feathers have been used to infer percentages of nutrient sources used for egg production (Klaassen et al. 2001, 2004), stable-isotopic discrimination factors for down feathers have not been previously calculated, so these data will allow a more precise calculation for understanding proteins used for egg production in wild populations. Furthermore, down feathers as well as eggshells and eggshell membranes can be sampled noninvasively when collected from hatched eggs once the young have left the nest, which is especially important for a threatened population. Building information about strategies of nutrient acquisition and allocation will be useful for evaluating factors affecting population productivity and will contribute to conservation of the threatened Spectacled Eider.

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