Stable carbon and nitrogen isotope discrimination factors from diet to blood plasma, cellular blood, feathers, and adipose tissue fatty acids in Spectacled Eiders (*Somateria fischeri*)

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Abstract: Stable isotope analyses of animal tissues can be used to infer diet through application of mixing models. An important component in a mixing model is the incorporation of stable isotope discrimination factors so that isotopic shifts between diet and tissues built from the diet can be accounted for when comparing tissues to potential food sources. We determined the stable carbon and nitrogen isotopic discrimination factors between lipid-free diet and blood plasma, cellular blood, and adult chest contour feathers for captive female Spectacled Eiders (*Somateria fischeri* (Brandt, 1847)). Mean discrimination factors for blood components and feathers were either similar or slightly larger compared with previously studied species. Additionally, we determined the stable carbon isotope discrimination factors between dietary lipids and adipose tissue fatty acids using three adipose tissue biopsies from captive male Spectacled Eiders that were fed three different diet treatments. Isotopic signatures of adipose tissue fatty acids closely reflected shifts in the diet and were either similar to or increased relative to diet. Our study provides a foundation for research using tissues as end-members in stable isotope nutrient allocation models and foraging ecology studies of Spectacled Eiders, and will provide the most applicable isotope data to date for sea ducks.

Résumé : Les analyses des isotopes stables des tissus animaux peuvent servir à déduire le régime alimentaire par l'utilisation des modèles de mélange. Une composante importante d'un modèle de mélange est l'incorporation des facteurs discriminants des isotopes stables de manière à ce que les changements isotopiques entre le régime et les tissus élaborés à partir de ce régime puissent être expliqués lors de la comparaison des tissus avec leurs sources potentielles de nourriture. Nous avons déterminé les facteurs discriminants pour les isotopes stables de carbone et d'azote entre un régime sans lipides et le plasma sanguin, le sang cellulaire et les plumes de contour de la poitrine chez des femelles de l'eider à lunettes (*Somateria fischeri* (Brandt, 1847)) en captivité. Les facteurs discriminants moyens pour les composantes du sang et les plumes sont ou bien semblables ou alors légèrement plus élevés par rapport à ceux des espèces étudiées antérieurement. De plus, nous avons déterminé les facteurs discriminants des isotopes stables entre les lipides du régime et les acides gras du tissu adipeux à l'aide de trois biopsies du tissu adipeux faites sur des mâles de l'eider à lunettes en captivé nourris de trois régimes alimentaires expérimentaux. Les signatures isotopiques des acides gras des tissus adipeux reflètent avec exactitude les changements de régime et sont ou bien semblables ou alors élevées par comparaison au régime. Notre étude fournit une base pour la recherche qui utilise les tissus comme points terminaux dans les modèles d'allocation des nutriments basés sur les isotopes stables et pour les études écologiques de l'alimentation des eiders à lunettes et elle présente les données isotopiques les plus appropriées à ce jour pour les canards de mer.

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Introduction

Analysis of stable isotopes in avian tissues is a valuable tool for tracking migration, inferring diet, and evaluating foraging ecology and resource allocation (Kelly 2000; Rubenstein and Hobson 2004; Hobson 2006; Inger and Bearhop 2008). Dietary models have been employed to quantitatively assess foraging ecology and nutrient allocation to reproduction (Phillips and Gregg 2001; Post 2002; Moore and Semmens 2008). Avian tissues such as blood

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and feathers can be collected nondestructively and can be used to infer information about diet sources and movement patterns within and between ecosystems (Rubenstein and Hobson 2004). For example, blood components are metabolically active and may be useful for identifying diet and movement within or between habitats because these tissues turnover at different rates (e.g., blood plasma turnover is on a scale of hours or days, whereas cellular blood turnover is approximately 1 month; Hobson and Clark 1993). Tissues that are metabolically inert after a period of growth (e.g., feathers) preserve a record of diet and movement across seasons (Hobson and Clark 1992*a*). Furthermore, studies evaluating nutrient allocation during egg production have employed tissues (e.g., cellular blood, adipose tissue, feathers, liver, muscle) as isotopic end-members with distinct isotopic signatures in cases where dietary sources are not available or are not known (Hobson et al. 2000, 2004, 2005; Klaassen et al. 2001; Gauthier et al. 2003; Schmutz et al. 2006; Bond et al. 2007).

Successful dietary modeling using stable isotope analyses requires knowledge on how the stable isotope signatures of tissues reflect that of the diet, referred to as discrimination factors ($\Delta = \delta$ animal tissue $- \delta$ diet; Ben-David and Schell 2001; Phillips 2001; Caut et al. 2008*a*). Most avian studies have used $\delta^{13}C$ and $\delta^{15}N$ data because these are good indicators of diet or geographical region and trophic position (Peterson and Fry 1987; Knoche et al. 2007). Previous studies have shown that stable carbon isotopes fractionate during tissue synthesis and generally increase or decrease slightly, but are comparable with values found in the diet (DeNiro and Epstein 1978; Peterson and Fry 1987). Stable nitrogen isotopes generally increase approximately 3‰–5‰ relative to values found in the diet (DeNiro and Epstein 1981; Minagawa and Wada 1984; Peterson and Fry 1987). Discrimination factors can vary by species, tissue type, and diet (Hobson and Clark 1992*b*; Hobson 1995; Bearhop et al. 2002). Studies of isotopic discrimination associated with avian blood components suggest that cellular blood increased between 0.5‰ and 1.5‰ for carbon and 3‰ for nitrogen in one study relative to diet, while the range for blood plasma was comparable to diet (–1.5‰ to 0.6‰) for carbon and increased 2.5‰–3.3‰ relative to diet for nitrogen (Hobson and Clark 1993; Pearson et al. 2003; Evans Ogden et al. 2004). Additionally, discrimination factors for feathers ranged from –0.4‰ to 4.3‰ for carbon and 1.1‰ to 5.6‰ for nitrogen (Hobson and Clark 1992*b*; Mizutani et al. 1992; Bearhop et al. 2002; Hobson and Bairlein 2003; Pearson et al. 2003; Knoche 2004; Cherel et al. 2005; Becker et al. 2007). Although these values may also vary across taxa (Dalerum and Angerbjorn 2005; Caut et al. 2008*a*), limited information is currently available regarding discrimination factors in feathers (Knoche 2004) and blood components of sea ducks. Therefore, experimental studies are needed to determine tissue and species- or taxon-specific discrimination values that will further facilitate interpretation of results from field studies (Gannes et al. 1997; Dalerum and Angerbjorn 2005; Caut et al. 2008*a*; Martínez del Rio et al. 2009).

Limited information is available about foraging ecology and resource allocation in sea ducks (Hobson et al. 2005; Bond et al. 2007; Oppel 2008). Spectacled Eiders (*Somateria fischeri* (Brandt, 1847)) are threatened sea ducks (Federal Register 1993) that spend the majority of their lives at sea (Petersen et al. 1999). Shifts in prey types and availability (US Fish and Wildlife Service 1996; Richman and Lovvorn 2003) have been suggested to affect population dynamics and potential changes in marine food resources are important questions for conservation. Furthermore, understanding ecological links between habitats and dietary sources is important for supporting population recovery and management of critical foraging habitats for this threatened species. Determining Spectacled Eider discrimination factors for tissues will provide species-specific tissue corrections useful for stable isotope research and modeling in field studies and may be useful for other sea duck species. The goal of this study is therefore to calculate the stable carbon and nitrogen isotopic discrimination factors between diet items and blood plasma, cellular blood, adult chest contour feathers, and adipose tissue fatty acids for Spectacled Eiders in controlled captive experiments.

Materials and methods

Captive population description and IACUC information

Eleven adult Spectacled Eiders ($n = 5$ females; $n = 6$ males) were held in captivity at the Alaska SeaLife Center (Seward, Alaska) after arrival as subadults in 2003. Additionally, two males arrived as rehabilitated animals in 2003 and 2004. The flock was housed together in an outdoor enclosure year-round. Animal care was reviewed and approved in accordance with the Institutional Animal Care and Use Committee (IACUC) of the Alaska SeaLife Center under protocol Nos. 05-006, 07-011, and 08-004 and complies with current US laws.

Diet regime and tissue sampling for blood and feathers

Birds were maintained on a consistent diet during pre- to post-breeding months (February–July 2008) of approximately 95% Mazuri® Sea Duck Diet (Purina Mills Incorporated, St. Louis, Missouri, USA) and 5% of tidewater Atlantic silverside (*Menidia menidia* (L., 1758); harvested near Prince Edward Island, Canada) along with calcium grit. Diet proportion was calculated based on dry mass consumption of Mazuri® within the previous 24 h. Birds were assumed to have consumed diet items similarly, but were fed in groups, and thus individual intakes could not be determined. The commercial diet consisted of approximately 6.5% lipid, 21.6% protein, 8.4% fiber, 10.9% ash, and 46.6% nitrogen-free extract along with vitamins (http://www.mazuri.com; accessed 15 March 2006).

Blood samples from each female Spectacled Eider ($n = 5$) were collected for a concurrent project weekly for up to 3 weeks prior to egg-laying (5, 14, 20 May) and available aliquots of cellular blood ($n = 5$) and blood plasma ($n = 5$) were archived for stable isotope analysis. Blood was sampled via jugular, tarsal, or wing vein using a 23- or 25-gauge needle and transferred to Li-heparinized tubes (Sarstedt Inc., Newton, North Carolina, USA). Whole blood was centrifuged at 1500*g* for 10 min in a Clay Adams Triac® centrifuge (Becton Dickinson, Franklin Lakes, New Jersey, USA) within 4 h of drawing blood. Blood plasma was separated from all cellular blood components and samples were

frozen in –80 and –20 °C freezer (respectively) until analysis.

Body contour feathers are typically replaced following breeding in early July to mid-August (Petersen et al. 2000). The outer tips of feather samples were obtained from each female Spectacled Eider (n = 5) after completion of breeding in early July. Feathers were rinsed with ethanol to remove any external substances, dried, and stored at room temperature until analysis (Knoche 2004).

Diet regime and tissue sampling for adipose tissues

Starting 19 September 2007, birds were fed a baseline diet (diet 1) for 69 days of approximately 97% Mazuri® and 3% supplements of krill (*Euphausia superba* Dana, 1852; harvested near southwest Atlantic Ocean for krill), Atlantic silverside (same as above), northern razor clam (*Siliqua patula* (Dixon, 1789); harvested near Cook Inlet, Alaska), and mussel (*Mytilus edulis* L., 1758; harvested near Cook Inlet, Alaska), then switched to a diet of 80% Mazuri® and 20% krill for 21 days (diet 2), and finally switched to a diet of 78% Mazuri® and 22% Atlantic silverside (diet 3) for 29 days for another study (Wang et al. 2010).

Adipose tissue samples were collected from captive adult male Spectacled Eiders for another study (Wang et al. 2010) on 27 November 2007 (biopsy 1; n = 8), 18 December 2007 (biopsy 2; n = 8), and 16 January 2008 (biopsy 3; n = 8). Mass was measured prior to each adipose tissue biopsy.

Sampling of dietary items

We sampled the diet items consumed prior to blood and feather collection at approximately 1 month intervals (February–June 2008) from individual batches of Mazuri® (n = 5) and from the same batch of Atlantic silverside (n = 5). We collected one sample of each diet consumed prior to adipose tissue biopsies. Samples were placed in airtight plastic bags and stored frozen at –20 °C until analysis.

Laboratory analysis

Diet items were dried to a constant mass using the FreeZone® 6 L Console Freeze Dry System (Labconco Corporation, Kansas City, Missouri, USA). Samples were then homogenized using a mortar and pestle or grinder, with the exception of dietary lipids that were analyzed directly. Each diet sample was then separated into whole diet, dietary lipids, and lipid-free portions of that sample. Lipids were removed from diet items using a 2:1 ratio of chloroform:methanol solution following methods similar to Bligh and Dyer (1959) except that no water was used because stable isotopes are analyzed as dry material. We used a vortex mixer to mix approximately 5 mg of dry sample with solution, allowed the sample to settle for 24 h, and extracted lipids manually with a pipette until the solvent wash was clear (Oppel et al. 2010). Dietary lipids were kept uncovered under a fume hood until all solvent had evaporated.

Blood components were freeze-dried and we did not extract lipid from the blood components because of small sample volumes and typically low proportions of lipid in bird blood (Wolf et al. 1985; Alonso et al. 1991). Additionally, a previous study suggested no significant difference when comparing stable carbon and nitrogen isotopes in lipid-extracted and whole blood (Bearhop et al. 2000).

Lipids were extracted from adipose tissue using a modified Folch technique (Folch et al. 1957; Iverson et al. 2001). Fatty acid methyl esters (FAMEs) were prepared from lipid extracts (Budge et al. 2006), concentrated with hexane, and stored at –20 °C until analysis. Solvent was evaporated from FAMEs under nitrogen. Samples collected on 27 November 2007 (biopsy 1; n = 8), 18 December 2007 (biopsy 2; n = 5), and 16 January 2008 (biopsy 3; n = 8) in Wang et al. 2010 were used for stable isotope analysis.

Stable isotope analyses and discrimination factors

Stable carbon and nitrogen isotope analysis was conducted at the Alaska Stable Isotope Facility (University of Alaska Fairbanks, Fairbanks, Alaska, USA). All samples were loaded into tin cups (0.01–0.05 mg) and weighed using a Satorius M2P electronic microbalance. Samples were measured via combustion using a continuous flow, isotope ratio mass spectrometer (Finnigan Delta^{plus}XP CF-IRMS Thermo Fischer LLC, San Jose, California, USA). Isotopic analyses are expressed as ratios in delta (δ) notation relative to an international standard ($R_{standard}$ = Vienna PeeDee Belemnite for $\delta^{13}C$ and 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. The mean analytical precision was determined using peptone standards (Sigma Chemical Co., Highland, Illinois, USA; mean $\delta^{13}C$ = –15.82‰ and mean $\delta^{15}N$ = 7.02‰) across separate sample runs (total sample runs, n = 24; total standards across all runs, n = 210; given as 1 SD) and showed 0.1‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$.

Stable isotope signatures of each diet item (dietary lipids and lipid-free portions of the diet were analyzed separately) were measured in duplicate for precision. We took a mean value of individual lipid-free diet samples collected prior to blood and feather collection to calculate the mean and SD of each diet item, but could not measure variation within diet items over time. One sample of each diet item for dietary lipids was collected prior to the adipose tissue biopsies, and thus the mean and SD for dietary lipids could not be determined. Although the majority of the diet was made up of Mazuri®, we accounted for diet items by multiplying the percentage of each diet item eaten per flock by the respective stable isotope signature to calculate the overall stable isotope signature for the diet. Single samples of feathers and adipose tissue fatty acids tissues and either duplicate or triplicate measurements (depending on individual sampling) of the blood components from Spectacled Eiders were analyzed and the mean value and SD were calculated for the flock. Discrimination factors were then calculated by subtracting the mean isotope signatures for feathers or blood components (Y) from those values of the lipid-free diet (X) or isotope signatures of adipose tissue (Y) from those values of the dietary lipids (X), described in terms of the difference in delta ($\Delta = \delta Y - \delta X$). The SD for the discrimination factors were calculated to account for variation in diet and tissues. Stable carbon and nitrogen isotope signatures of the lipid-free diet items and all tissues, and the discrimination factors between lipid-free diet and blood plasma, cellular

Table 1. Stable carbon and nitrogen isotope signatures ($\delta^{13}C$ and $\delta^{15}N$, respectively), C:N ratios (percent carbon concentration to percent nitrogen concentration), and discrimination factors (Δ) for lipid-free diet, blood components (cellular and plasma), and feathers of captive Spectacled Eiders (*Somateria fischeri*).

	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:N ratio	$\Delta^{13}C$ (‰)	$\Delta^{15}N$ (‰)
Lipid-free diet (*n* = 5)					
Mazuri®	–20.4±0.6	6.5±0.5	11.0	—	—
Atlantic silverside	–17.6±0.6	12.0±0.6	3.2	—	—
Diet proportion*	–20.3±0.4	6.7±0.3	9.9	—	—
Tissues (*n* = 5)					
Cellular blood	–18.3±0.1	10.7±0.3	3.2	2.0±0.2	4.0±0.2
Blood plasma	–20.3±0.1	11.6±0.2	5.8	0.0±0.2	4.9±0.2
Feathers	–17.1±0.2	12.3±0.6	3.2	3.2±0.2	5.6±0.3

Note: Values are mean ± 1 SD, except for the C:N ratio column.

*Diet proportion based on isotope signature of diet item multiplied by percentage of each diet item eaten per flock. Diet consists of 96% Mazuri® to 4% Atlantic silverside (*Menidia menidia*).

Table 2. Stable carbon isotopes ($\delta^{13}C$) of dietary lipids from diets 1, 2, and 3, and subsequent adipose tissue fatty acids from biopsies 1, 2, and 3 of captive Spectacled Eiders (*Someteria fischeri*), and discrimination factors ($\Delta^{13}C$) between adipose tissue and dietary lipids.

	$\delta^{13}C$ (‰)		
Diet and biopsy samples	Diet proportion – dietary lipids (*n* = 1)	Adipose tissue fatty acids	$\Delta^{13}C$ (‰) of adipose tissue fatty acids – dietary lipids
1	–26.2*	–25.0±0.3 (*n* = 8)	1.2±0.3 (range 1.0 to 1.6)
2	–27.2*	–26.6±0.1 (*n* = 4†)	0.6±0.1 (range 0.5 to 0.7)
3	–25.1*	–25.1±0.4 (*n* = 8)	0.0±0.4 (range –0.6 to 0.5)

*Diet proportion based on isotope signature of diet item multiplied by percentage of each diet item eaten per flock. Diet consists of –26.3‰ Mazuri®, –20.9‰ Atlantic silverside (*Menidia menidia*), –22.4‰ northern razor clams (*Siliqua patula*), –23.9‰ mussels (*Mytilus edulis*), and –31.1‰ krill (*Euphausia superba*).

†One individual that did not consume the krill in diet 2 was removed from the analysis.

blood, and adult contour feathers, and between dietary lipids and adipose tissue fatty acids are reported as mean ± 1 SD.

Results

$\delta^{13}C$ and $\delta^{15}N$ isotope analyses of diet items

Stable carbon and nitrogen isotope signatures were determined for all diet items (Tables 1 and 2). During prebreeding, diet supplements made up only a small proportion of the total diet (4.0%). The calculated stable carbon and nitrogen isotope signatures, respectively, using diet proportions given to birds were –20.3‰ ± 0.6‰ and 6.7‰ ± 0.5‰ for lipid-free diet (Table 1).

During the nonbreeding season, total diet supplements in diet 1 from Wang et al. 2010 made up approximately 3%, where Atlantic silverside and mussel were the largest proportion (1.2% and 1.0%, respectively) and krill and northern razor clam were the smallest proportions (0.5% and 0.4%, respectively). Diet 2 consisted of 19.8% krill and diet 3 consisted of 21.9% Atlantic silverside. When the diet was switched from diet 1 to diet 2, the stable carbon isotope signature of the dietary lipids decreased, but then increased in diet 3 (Table 2, Fig. 1).

Discrimination factors of adult tissues

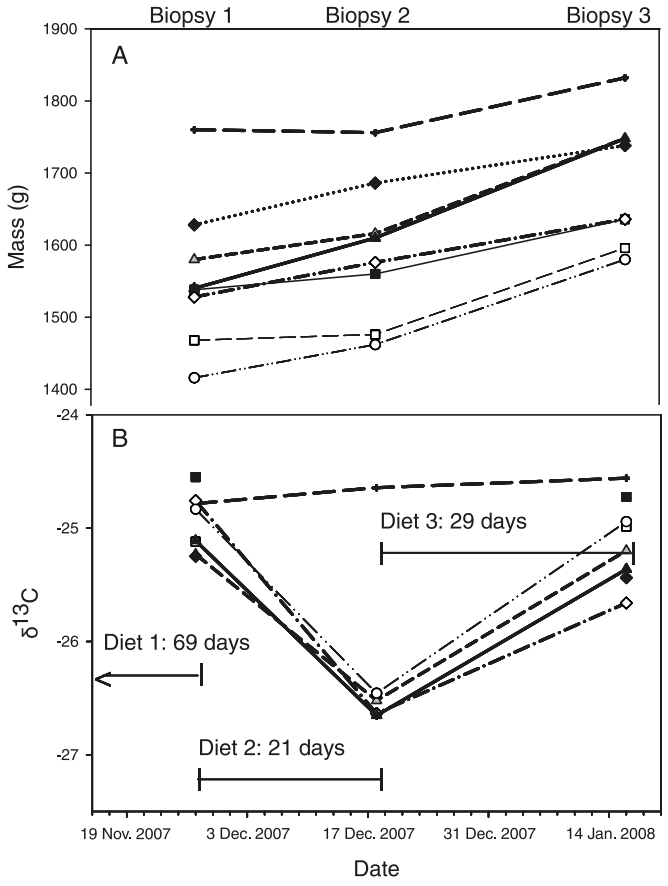
Stable carbon and nitrogen isotopic discrimination factors (Δ) were calculated for blood plasma, cellular blood, and adult contour feathers (Table 1). The mean stable carbon and nitrogen isotope signatures for all tissues increased relative to the lipid-free diet, except the carbon isotope signature of blood plasma remained the same as the diet (Table 1).

The mean stable carbon isotope signature of adipose tissue fatty acids from biopsy 1 was 1.2‰ greater than the dietary lipid from diet 1 (Table 2, Fig. 1). Similarly, the mean stable carbon isotope signature of adipose tissue fatty acids from biopsy 2 increased by 0.6‰ relative to the dietary lipids from diet 2 (Mazuri® and krill) when we excluded one individual that ate relatively little to no krill (Table 2, Fig. 1). The mean stable carbon isotope signature of the adipose tissue fatty acids from biopsy 3 was similar to the isotope signatures from biopsy 1 (Table 2, Fig. 1).

Discussion

We provide the first experimentally derived isotopic discrimination factors between diet and tissue for Spectacled Eiders and any sea duck species, other than discrimination factors for feathers in King Eiders (*Somateria spectabilis* (L., 1758); Knoche 2004). Discrimination factors for blood components and feathers were generally consistent with those of previously studied avian species. We also demonstrate that stable carbon isotopes in adipose tissue fatty acids generally tracked changes in the stable carbon isotope signatures in dietary lipids. These discrimination factors will further facilitate the interpretation of foraging ecology and nutrient allocation models in eiders and other sea ducks.

Fig. 1. (A) Mass of captive male Spectacled Eiders (*Somateria fischeri*) over three biopsy periods illustrating that mass was kept constant or increased for each individual and (B) the stable carbon isotope signature ($\delta^{13}C$) for dietary lipids (diet 1 = 69 days; diet 2 = 21 days; diet 3 = 29 days) and adipose tissue fatty acids (line plots) from each male Spectacled Eider from biopsy 1 (n = 8), biopsy 2 (n = 5), and biopsy 3 (n = 8).



Discrimination factors for blood components from Spectacled Eider

Few avian studies have been conducted for discrimination factors of blood plasma (Hobson and Clark 1993; Pearson et al. 2003; Evans Ogden et al. 2004) and the cellular fraction of blood (Hobson and Clark 1993; Evans Ogden et al. 2004), but no discrimination data are available for blood components of sea ducks. Stable carbon isotope discrimination factors for cellular blood were slightly higher or within the range of previously reported values (0.5‰ to 1.5‰; Hobson and Clark 1993; Evans Ogden et al. 2004), whereas stable nitrogen isotope discrimination factors were slightly higher than the value reported by a single study (3.0‰; Evans Ogden et al. 2004). Stable carbon isotope discrimination factors in blood plasma fell within the previously reported range (–1.5‰ to 0.6‰; Hobson and Clark 1993; Pearson et al. 2003; Evans Ogden et al. 2004), but the discrimination factor for nitrogen was higher than has been previously reported (2.5‰ to 3.3‰; Hobson and Clark 1993; Pearson et al. 2003; Evans Ogden et al. 2004).

Previous studies report variability in the discrimination factors related to different species, individuals, tissues, and diet composition (DeNiro and Epstein 1978, 1981; Hobson and Clark 1992*b*; Hobson 1995; Bearhop et al. 2002; Dalerum and Angerbjorn 2005; Caut et al. 2008*b*; Robbins et al. 2010). Discrimination factors for blood components have only been reported for a few species and have demonstrated either a slight increase or similar isotope signatures for carbon and an increase in isotope signatures for nitrogen relative to the diet (Hobson and Clark 1993; Pearson et al. 2003; Evans Ogden et al. 2004). The data from this study also demonstrate similar patterns in isotopic discrimination of blood components, and carbon and nitrogen isotope values were within the ranges previously reported, except the nitrogen discrimination factor in blood plasma was slightly higher. Individual physiology and temporal energetic demands could also affect isotopic discrimination factors, potentially through change in efficiency or deposition of nutrients to specific tissues (Hobson and Clark 1992*b*; Bearhop et al. 2002). For example, the sample time period occurred during an energetically demanding period directly prior to egg-laying (Alisauskas and Ankney 1992) and the metabolism of stored nutrients could affect the way nutrients are transported to these metabolically active tissues. Furthermore, slight differences in isotopic signatures or diet composition between Mazuri® food batches and Atlantic silverside could affect isotopic discrimination values. For example, studies have shown that when multiple diet items are consumed, the differences in the quality of food, such as proteins, can affect isotope ratio discrimination and assimilation rates (Caut et al. 2008*b*; Robbins et al. 2010). Because Mazuri® made up the majority of the diet, the isotope signature of Mazuri® most accurately reflected the overall diet isotope signature. Finally, small sample sizes of diet items collected may have inhibited the ability to capture variability in isotopic discrimination values; however, variation was relatively low and we consider that it was unlikely to have affected results. Also, a pilot year of sampling from the same flock indicated similar patterns in isotopic discrimination factors (Federer 2009). In general, these findings emphasize the importance of calculating discrimination factors for different species or tissue types (Dalerum and Angerbjorn 2005; Caut et al. 2008*a*).

Discrimination factors for feathers from Spectacled Eider

Extensive research on isotopic discrimination factors for feathers from different species has been previously conducted (Hobson and Clark 1992*b*; Mizutani et al. 1992; Bearhop et al. 2002; Hobson and Bairlein 2003; Pearson et al. 2003; Knoche 2004; Cherel et al. 2005; Becker et al. 2007). Overall, discrimination factors for feathers from Spectacled Eider fell within the range of discrimination factors found for other studies (mean carbon range = –0.4‰ to 4.3‰; mean nitrogen range = 1.1‰ to 5.6‰). However, it is important to recognize that these studies covered a wide range of species, dietary composition, and feather types. The most similar study to ours was from a flock of the taxonomically closely related King Eider, which was fed the same commercial Mazuri® Sea Duck diet at a different facility (Knoche 2004). Discrimination factors for feathers from Spectacled Eider were similar to that from King Eiders in carbon, but nitrogen for Spectacled Eiders was higher relative to that of King Eiders (mean = 4.3‰; Knoche 2004).

This could indicate differences in the way that these individual species used nitrogen during feather development. Another study evaluated discrimination factors in feathers of taxonomically similar penguin species that were fed separate fish diets and found considerable variation for carbon (0.1‰ to 2.9‰) and nitrogen (3.5‰ to 4.8‰; Mizutani et al. 1992; Cherel et al. 2005), but these studies did not evaluate the effects of different diets among these species. Additionally, Mizutani et al. (1992) found that gulls, herons, and egrets that were fed the same fish diet varied in both mean carbon (3.1‰ to 3.6‰) and mean nitrogen (3.9‰ to 5.3‰) values. A further study evaluated different dietary compositions and reported that the percentage of lipid in the diet corresponded linearly to increasing mean stable carbon isotope discrimination factors (1.9‰ to 4.3‰) in warblers (Pearson et al. 2003).

Differences in feather type may also influence isotopic discrimination factors. For instance, Becker et al. (2007) measured isotope signatures in body and primary feathers for murres and found differences for carbon (2.5‰ and 1.9‰, respectively), but essentially no difference for nitrogen. Finally, there is some evidence from compound-specific isotope analysis that individual amino acids can vary extensively (Howland et al. 2003; O'Brien et al. 2005), and therefore it may be important to distinguish specific amino acids that are synthesized for feather growth. However, no information is available for discrimination factors of specific amino acids in bird feather keratin, such as cysteine and methionine (Murphy 1996), and it is unknown to what extent the differential discrimination factors among amino acids would influence bulk isotope analyses. Further investigation of discrimination factors in different feather types and amino acid differentiation to feathers may help us understand specific nutrients used for feather production by Spectacled Eider.

Discrimination factors of spectacled eider adipose tissue fatty acids

Stable carbon isotope signatures for adipose tissue fatty acids of captive Spectacled Eiders were comparable or slightly increased relative to diet. Podlesak and McWilliams (2007) suggested that birds fed a diet high in lipids would have lipid stores with an isotope signature similar to that of the dietary lipid, whereas biosynthesis of lipids from both dietary carbohydrates and lipid may reflect discrimination factors from both of these macronutrients. Therefore, it is possible that birds used different macronutrients to synthesize adipose tissue during the different diet treatments. Diet 1 mainly consisted of Mazuri® with approximately 6.5% fat and 8.4% carbohydrate composition (dry mass proximate analysis reported by Mazuri®). During diets 2 and 3, birds were fed single diet supplements, krill (approximately 20% of the diet) and Atlantic silverside (approximately 22% of the diet), respectively. Both krill and Atlantic silverside had low carbohydrate content and krill had high lipid content (2008 unpublished data from Michelson Laboratories, Inc., Commerce, California, USA), therefore dietary lipid deposition may have been more prevalent during diets 2 and 3, resulting in more similar stable carbon isotope signatures between biopsies 2 and 3 and their respective diets. Overall, the stable carbon isotope signature of adipose tissue fatty acids reflected the stable carbon isotopic shifts in the diet for all individuals, except for one bird that did not switch diets in a similar pattern to the others (i.e., was not observed to consume much krill). This individual's stable carbon isotope signatures in the adipose tissue fatty acids were similar throughout. These findings suggest that isotopic signatures in adipose tissue fatty acids are predictable if dietary macronutrients are known.

Although whole lipid samples from adipose tissue were not available, adipose tissue fatty acids have been used to qualitatively infer changes and quantitatively describe the diets of marine birds, including Spectacled Eiders (Wang et al. 2007, 2009, 2010), and therefore were used as a proxy for lipids to describe diet sources. However, using fatty acids or fatty acid methyl esters instead of lipid samples could potentially bias the resulting $\delta^{13}C$ values and thus any discrimination factors calculated from them. For fatty acid analysis, lipids are extracted then transesterified into their corresponding fatty acid methyl esters. In the process, the esterified glycerol backbone from triacylgerides is removed, which could lead to dilution of the dietary carbon source. Additionally, during transesterification, carbon (from the addition of a methyl group) is added from a nondietary sources (methanol) to fatty acids, which could also bias the resulting $\delta^{13}C$ values. Discrimination factors from diet to adipose tissue fatty acids were calculated using isotope ratios measured from dietary whole lipids and adipose tissue FAMEs, which may also bias our discrimination factor calculations. Mobilization and deposition rates of fatty acids into adipose tissue may also affect the isotope signatures, as complete turnover of dietary fatty acids into adipose tissue had not occurred by the time of biopsies 2 and 3 (Wang et al. 2010). Therefore, adipose tissue fatty acid isotope values and discrimination factors calculated should be interpreted with caution and require further investigation.

Conclusions

This study is the first to evaluate diet to tissue discrimination factors for blood plasma and cellular blood (in two subsequent study years), adult contour feathers, and adipose tissue fatty acids of Spectacled Eider. We found that isotope signatures of blood components and feathers were similar or increased relative to lipid-free diet and these data were comparable with that of other avian species. Data for discrimination factors of blood and feathers are limited, and therefore this information will add to the overall body of knowledge for birds, and in particular for sea ducks. A useful addition to future stable isotope research would be to determine feather molt or growth chronology and isotopic signatures in different feather types under controlled experimental conditions. These data would contribute to the understanding of movement patterns and diet.

We also found that isotope signatures of adipose tissue fatty acids increased relative to dietary lipids and followed this pattern throughout a shift in diet treatments. However, discrimination factors for adipose tissue fatty acids were calculated using the isotope signature of dietary lipids rather than dietary fatty acids, which could bias discrimination factor calculations. Additionally, calculations for discrimination factors of adipose tissue fatty acids of biopsies 2 and 3 may less accurately reflect the correct discrimination factors be-

cause mobilization and deposition rates of fatty acids into the adipose tissue were not complete. These data have not been previously reported, and therefore will provide valuable contributions for discrimination factors of adipose tissue fatty acids, which requires further investigation. Further understanding of micronutrient pathways associated with different tissues and growth would be useful for identifying nutrient limitations for Spectacled Eiders in the wild.

This study highlights the importance of controlled captive experiments to the application of these techniques in field studies. Our results will contribute additional tools to study movement patterns and diet sources or variation in wild Spectacled Eiders, and thus may help to understand factors affecting population recovery.

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References

Alisauskas, R.T., and Ankney, C.D. 1992. The cost of egg laying and its relationship to nutrient reserves in waterfowl. *In* Ecology and management of breeding waterfowl. *Edited by* B.D.J. Batt, A.D. Afton, M.G. Anderson, C.D. Ankney, D.H. Johnson, J.A. Kadlec, and G.L. Krapu. University of Minnesota Press, Minneapolis. pp. 30–61.

Alonso, J.C., Huecas, V., Alonso, J.A., Abelenda, M., Muñoz-Pulido, R., and Puerta, M.L. 1991. Hematology and blood chemistry of adult White Storks (*Ciconia ciconia*). Comp. Biochem. Physiol. A Physiol. **98**(3–4): 395–397. doi:10.1016/0300-9629(91)90421-8.

Bearhop, S., Teece, M.A., Waldron, S., and Furness, R.W. 2000. Influence of lipid and uric acid on $\delta^{13}C$ and $\delta^{15}N$ values of avian blood: implications for trophic studies. Auk, **117**(2): 504–507. doi:10.1642/0004-8038(2000)117[0504:IOLAUA]2.0.CO;2.

Bearhop, S., Waldron, S., Votier, S.C., and Furness, R.W. 2002. Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. Physiol. Biochem. Zool. **75**(5): 451–458. doi:10.1086/342800. PMID:12529846.

Becker, B.H., Newman, S.H., Inglis, S., and Beissinger, S.R. 2007. Diet–feather stable isotope ($\delta^{15}N$ and $\delta^{13}C$) fractionation in Common Murres and other seabirds. Condor, **109**(2): 451–456. doi:10.1650/0010-5422(2007)109[451:DSINAC]2.0.CO;2.

Ben-David, M., and Schell, D.M. 2001. Mixing models in analyses of diet using multiple stable isotopes: a response. Oecologia (Berl.), **127**(2): 180–184. doi:10.1007/s004420000570.

Bligh, E.G., and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**(8): 911–917. PMID:13671378.

Bond, J.C., Esler, D., and Hobson, K.A. 2007. Isotopic evidence for sources of nutrients allocated to clutch formation by Harlequin Ducks. Condor, **109**(3): 698–704. doi:10.1650/8241.1.

Budge, S.M., Iverson, S.J., and Koopman, H.N. 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Mar. Mamm. Sci. **22**(4): 759–801. doi:10.1111/j.1748-7692.2006.00079.x.

Caut, S., Angulo, E., and Courchamp, F. 2008*a*. Caution on isotopic model use for analyses of consumer diet. Can. J. Zool. **86**(5): 438–445. doi:10.1139/Z08-012.

Caut, S., Angulo, E., and Courchamp, F. 2008*b*. Discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$) in an omnivorous consumer: effect of diet isotopic ratio. Funct. Ecol. **22**(2): 255–263. doi:10.1111/j.1365-2435.2007.01360.x.

Cherel, Y., Hobson, K.A., and Hassani, S. 2005. Isotopic discrimination between food and blood and feathers of captive penguins: implications for dietary studies in the wild. Physiol. Biochem. Zool. **78**(1): 106–115. doi:10.1086/425202. PMID:15702469.

Dalerum, F., and Angerbjorn, A. 2005. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia (Berl.), **144**(4): 647–658. doi:10.1007/s00442-005-0118-0.

DeNiro, M.J., and Epstein, S. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta, **42**(5): 495–506. doi:10.1016/0016-7037(78)90199-0.

DeNiro, M.J., and Epstein, S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. Acta, **45**(3): 341–351. doi:10.1016/0016-7037(81)90244-1.

Evans Ogden, L.J., Hobson, K.A., and Lank, D.B. 2004. Blood isotopic ($\delta^{13}C$ and $\delta^{15}N$) turnover and diet–tissue fractionation factors in captive Dunlin (*Calidris alpina pacifica*). Auk, **121**(1): 170–177. doi:10.1642/0004-8038(2004)121[0170:BICANT]2.0.CO;2.

Federal Register. 1993. Final rule to list the spectacled eider as threatened. Fed. Regist. **58**: 27474–27480.

Federer, R.F. 2009. Quantifying diet to tissue isotopic ($\delta^{13}C$ and $\delta^{15}N$) fractionation factors in captive spectacled eiders (*Somateria fischeri*): implications for nutrient allocation and foraging studies. M.Sc. thesis, School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Fairbanks.

Folch, J., Lees, M., and Sloane Stanley, G.H. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. **226**(1): 497–509. PMID: 13428781.

Gannes, L.Z., O'Brien, D.M., and Martínez del Rio, C. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology, **78**(4): 1271–1276. doi:10.1890/0012-9658(1997)078[1271:SIIAEA]2.0.CO;2.

Gauthier, G., Bety, J., and Hobson, K.A. 2003. Are greater snow geese capital breeders? New evidence from a stable-isotope model. Ecology, **84**(12): 3250–3264. doi:10.1890/02-0613.

Hobson, K.A. 1995. Reconstructing avian diets using stable-carbon and nitrogen isotope analysis of egg components: patterns of isotopic fractionation and turnover. Condor, **97**(3): 752–762. doi:10.2307/1369183.

Hobson, K.A. 2006. Using stable isotopes to quantitatively track endogenous and exogenous nutrient allocations to eggs of birds that travel to breed. Ardea, **94**(3): 359–369. Available from

http://nou.natuurinfo.nl/website/ardea/ardea_show_abstract.php?lang=uk&nr=604 [accessed 15 September 2009].

Hobson, K.A., and Bairlein, F. 2003. Isotopic fractionation and turnover in captive garden warblers (*Sylvia borin*): implications for delineating dietary and migratory associations in wild passerines. Can. J. Zool. **81**(9): 1630–1635. doi:10.1139/z03-140.

Hobson, K.A., and Clark, R.G. 1992*a*. Assessing avian diets using stable isotopes I: Turnover of ^{13}C in tissues. Condor, **94**(1): 181–188. doi:10.2307/1368807.

Hobson, K.A., and Clark, R.G. 1992*b*. Assessing avian diets using stable isotopes II: Factors influencing diet–tissue fractionation. Condor, **94**(1): 189–197. doi:10.2307/1368808.

Hobson, K.A., and Clark, R.G. 1993. Turnover of ^{13}C in cellular and plasma fractions of blood: implications for nondestructive sampling in avian dietary studies. Auk, **110**(3): 638–641. Available from http://elibrary.unm.edu/sora/Auk/v110n03/p0638-p0641.pdf [accessed 15 September 2009].

Hobson, K.A., Sirois, J., and Gloutney, M.L. 2000. Tracing nutrient allocation to reproduction with stable isotopes: a preliminary investigation using colonial waterbirds of Great Slave Lake. Auk, **117**(3): 760–774. doi:10.1642/0004-8038(2000)117[0760:TNATRW]2.0.CO;2.

Hobson, K.A., Atwell, L., Wassenaar, L.I., and Yerkes, T. 2004. Estimating endogenous nutrient allocations to reproduction in Redhead Ducks: a dual isotope approach using δD and $\delta^{13}C$ measurements of female and egg tissues. Funct. Ecol. **18**(5): 737–745. doi:10.1111/j.0269-8463.2004.00890.x.

Hobson, K.A., Thompson, J.E., Evans, M.R., Boyd, S., and Sheaffer. 2005. Tracing nutrient allocation to reproduction in Barrow's goldeneye. J. Wildl. Manage. **69**(3): 1221–1228. doi:10.2193/0022-541X(2005)069[1221:TNATRI]2.0.CO;2.

Howland, M.R., Corr, L.T., Young, S.M.M., Jones, V., Jim, S., Van der Merwe, N.J., Mitchell, A.D., and Evershed, R.P. 2003. Expression of the dietary isotope signal in the compound-specific $\delta^{13}C$ values of pig bone lipids and amino acids. Int. J. Osteoarchaeol. **13**(1–2): 54–65. doi:10.1002/oa.658.

Inger, R., and Bearhop, S. 2008. Applications of stable isotope analyses to avian ecology. Ibis, **150**(3): 447–461. doi:10.1111/j.1474-919X.2008.00839.x.

Iverson, S.J., Lang, S.L.C., and Cooper, M.H. 2001. Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. Lipids, **36**(11): 1283–1287. doi:10.1007/s11745-001-0843-0. PMID:11795862.

Kelly, J.F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can. J. Zool. **78**(1): 1–27. doi:10.1139/cjz-78-1-1.

Klaassen, M., Lindström, Å., Meltofte, H., and Piersma, T. 2001. Ornithology: Arctic waders are not capital breeders. Nature (London), **413**(6858): 794. doi:10.1038/35101654. PMID:11677593.

Knoche, M.J. 2004. King eider wing molt: inferences from stable isotope analyses. M.Sc. thesis, Department of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks.

Knoche, M.J., Powell, A.N., Quakenbush, L.T., Wooller, M.J., and Phillips, L.M. 2007. Further evidence for site fidelity to wing molt locations by King Eiders: integrating stable isotope analyses and satellite telemetry. Waterbirds, **30**(1): 52–57. doi:10.1675/1524-4695(2007)030[0052:FEFSFT]2.0.CO;2.

Martínez del Rio, C.M., Wolf, N., Carleton, S.A., and Gannes, L.Z. 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biol. Rev. Camb. Philos. Soc. **84**(1): 91–111. doi:10.1111/j.1469-185X.2008.00064.x. PMID:19046398.

Minagawa, M., and Wada, E. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochim. Cosmochim. Acta, **48**(5): 1135–1140. doi:10.1016/0016-7037(84)90204-7.

Mizutani, H., Fukuda, M., and Kabaya, Y. 1992. ^{13}C and ^{15}N enrichment factors of feathers of 11 species of adult birds. Ecology, **73**(4): 1391–1395. doi:10.2307/1940684.

Moore, J.W., and Semmens, B.X. 2008. Incorporating uncertainty and prior information into stable isotope mixing models. Ecol. Lett. **11**(5): 470–480. doi:10.1111/j.1461-0248.2008.01163.x. PMID:18294213.

Murphy, M.E. 1996. Energetics and nutrition of molt. *In* Avian energetics and nutritional ecology. *Edited by* C. Carey. Chapman and Hill, New York. pp. 158–198.

O'Brien, D.M., Boggs, C.L., and Fogel, M.L. 2005. The amino acids used in reproduction by butterflies: a comparative study of dietary sources using compound-specific stable isotope analysis. Physiol. Biochem. Zool. **78**(5): 819–827. doi:10.1086/431191.

Oppel, S. 2008. King Eider migration and seasonal interactions at the individual level. Ph.D. thesis, Department of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks. Available from http://mercury.bio.uaf.edu/~steffen_oppel/kingeider/Oppel_Phd_thesis2008.pdf [accessed 17 September 2009].

Oppel, S., Federer, R.N., O'Brien, D.M., Powell, A.N., and Hollmén, T.E. 2010. Effects of lipid extraction on stable isotope ratios in avian egg yolk: is arithmetic correction a reliable alternative? Auk, **127**(1): 72–78. doi:10.1525/auk.2009.09153.

Pearson, S.F., Levey, D.J., Greenberg, C.H., and Martínez del Rio, C. 2003. Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. Oecologia (Berl.), **135**: 516–523. doi:10.1007/s00442-003-1221-8.

Petersen, M.R., Larned, W.W., and Douglas, D.C. 1999. At-sea distribution of Spectacled Eiders: a 120-year-old mystery resolved. Auk, **116**(4): 1009–1020. Available from http://alaska.usgs.gov/science/biology/avian_influenza/pdfs/Petersen_At_Sea_Distribution_Spectacled_Eiders_120_Yr_Mystery_Resolved.pdf [accessed 15 September 2009].

Petersen, M.R., Grand, J.B., and Dau, C.P. 2000. Spectacled Eider (*Somateria fischeri*). *In* The birds of North America. No. 547. *Edited by* A. Poole and F. Gill. The Birds of North America Inc., Philadelphia. pp. 1–24.

Peterson, B.J., and Fry, B. 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. **18**(1): 293–320 doi:10.1146/annurev.es.18.110187.001453.

Phillips, D.L. 2001. Mixing models in analyses of diet using multiple stable isotopes: a critique. Oecologia (Berl.), **127**(2): 166–170. doi:10.1007/s004420000571.

Phillips, D.L., and Gregg, J.W. 2001. Uncertainty in source partitioning using stable isotopes. Oecologia (Berl.), **127**(2): 171–179. doi:10.1007/s004420000578.

Podlesak, D.W., and McWilliams, S.R. 2007. Metabolic routing of dietary nutrients in birds: effects of dietary lipid concentration on $\delta^{13}C$ of depot fat and its ecological implications. Auk, **124**(3): 916–925. doi:10.1642/0004-8038(2007)124[916:MRODNI]2.0.CO;2.

Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology, **83**(3): 703–718. doi:10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2.

Richman, S.E., and Lovvorn, J.R. 2003. Effects of clam species dominance on nutrient and energy acquisition by spectacled eiders in the Bering Sea. Mar. Ecol. Prog. Ser. **261**: 283–297. doi:10.3354/meps261283.

Robbins, C.T., Felicetti, L.A., and Florin, S.T. 2010. The impact of protein quality on stable nitrogen isotope ratio discrimination and assimilated diet estimation. Oecologia (Berl.), **162**(3): 571–579. doi:10.1007/s00442-009-1485-8.

Rubenstein, D.R., and Hobson, K.A. 2004. From birds to butterflies: animal movement patterns and stable isotopes. Trends Ecol. Evol. **19**(5): 256–263. doi:10.1016/j.tree.2004.03.017. PMID:16701265.

Schmutz, J.A., Hobson, K.A., and Morse, J.A. 2006. An isotopic assessment of protein from diet and endogenous stores: effects on egg production and incubation behaviour of geese. Ardea, **94**(3): 385–397. Available from http://nou.natuurinfo.nl/website/ardea/ardea_show_abstract.php?lang=uk&nr=606 [accessed 15 September 2009].

US Fish and Wildlife Service. 1996. Spectacled eider recovery plan. US Fish and Wildlife Service, Anchorage, Alaska.

Wang, S.W., Iverson, S.J., Springer, A.M., and Hatch, S.A. 2007. Fatty acid signatures of stomach oil and adipose tissue of northern fulmars (*Fulmarus glacialis*) in Alaska: implications for diet analysis of Procellariiform birds. J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. **177**(8): 893–903. doi:10.1007/s00360-007-0187-y. PMID:17712565.

Wang, S.W., Iverson, S.J., Springer, A.M., and Hatch, S.A. 2009. Spatial and temporal diet segregation in northern fulmars *Fulmarus glacialis* breeding in Alaska: insights from fatty acid signatures. Mar. Ecol. Prog. Ser. **377**: 299–307. doi:10.3354/meps07863.

Wang, S.W., Hollmén, T.E., and Iverson, S.J. 2010. Validating quantitative fatty acid signature analysis to estimate diets of spectacled and Steller's eiders (*Somateria fischeri* and *Polysticta stelleri*). J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. **180**(1): 125–139. doi:10.1007/s00360-009-0393-x. PMID:19652982.

Wolf, S.H., Schreiber, R.W., Kahana, L., and Torres, J.J. 1985. Seasonal, sexual and age-related variation in the blood composition of the brown pelican (*Pelecanus occidentalis*). Comp. Biochem. Physiol. A Physiol. **82**(4): 837–846. doi:10.1016/0300-9629(85)90492-X.