

Eider Research: Eider Status and Trends

Annual Progress Report to the Ocean Alaska Science and Learning Center (OASLC)

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Summary

The eider research project has focused on developing methodologies to evaluate health, body condition, and dietary requirements in free ranging populations throughout the annual cycle. The overall goal of these studies has been to utilize biomarker methodologies to answer ecological questions about habitat quality, nutritional limitations, and stress factors impacting population health and viability.

During 2008-2009, the project has focused on further understanding nutritional health and constraints using the captive flocks of spectacled eiders (*Somateria fischeri*) and Steller's eiders (*Polysticta stelleri*) housed at the Alaska SeaLife Center as experimental models. The program has focused on the validation and development of two diet estimation techniques for use in eiders: bulk stable isotope analysis (SIA) and quantitative fatty acid signature analysis (QFASA). The captive research flocks of both species of threatened eiders have allowed for unique opportunities for method validation and testing. Our next objective is to apply the information gathered from these captive experiments into free ranging populations, to further understand nutritional strategies and limitations in the threatened populations.

The progress of the study is described in detail in two sections. Results from our studies on the development of SIA methodologies are described in the section "Quantifying diet to tissue isotopic ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) fractionation factors in captive spectacled eiders (*Somateria fischeri*): Implications for nutrient allocation and foraging studies", and results from validation studies for QFASA are described in "Estimating diets of two species of threatened sea ducks, the Steller's eider (*Polysticta stelleri*) and the spectacled eider (*Somateria fischeri*): Validation of novel diet assessment techniques". A list of publications and presentations resulting from these studies is provided.

List of Publications, Reports, and Conference Proceedings

- Federer RN (2009) Quantifying diet to tissue isotopic ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) 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, Alaska
- Federer R, Hollmén T, Esler D, Wooller MJ (2008) Stable Isotope Fractionation Factors for Quantifying Nutrient Allocation to Reproduction in Spectacled Eiders (*Somateria fischeri*). 3rd North America Sea Duck Conference, Québec City, Québec
- Federer R, Hollmén T (2008) Evaluating nutrient acquisition and allocation in captive breeding spectacled eiders (*Somateria fischeri*) using stable isotope analyses: MSc thesis. Project completion technical report to the AK Coastal Marine Resources Grant
- Oppel S, Federer RN, O'Brien DM, Powell AN, Hollmén T. Effects of Lipid Extraction on Stable Isotope Ratios in Avian Egg Yolk: Is Arithmetic Correction a Reliable Alternative? *Auk*, *In Press*
- Wang, SW, Hollmén, TE, and Iverson, SJ. (2008). Using fatty acids to estimate diets of threatened spectacled and Steller's eiders. 3rd North America Sea Duck Conference, Québec City, Québec
- Wang, SW, Hollmén, TE, and Iverson, SJ. (2009). Validating quantitative fatty acid signature analysis (QFASA) to estimate diets of threatened spectacled and Steller's eiders. Alaska Marine Science Symposium, Anchorage, Alaska
- Wang, SW, Hollmén, TE, and Iverson, SJ. (2009). Validating quantitative fatty acid signature analysis to estimate diets of spectacled and Steller's eiders (*Somateria fischeri* and *Polysticta stelleri*). *J Comp Phys B*. DOI 10.1007/s00360-009-0393-x

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Eider Research

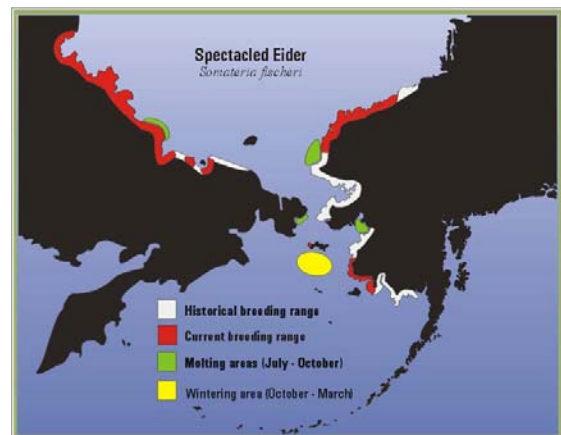
Quantifying diet to tissue isotopic ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) fractionation factors in captive spectacled eiders (*Somateria fischeri*): Implications for nutrient allocation and foraging studies

Rebekka Federer and Tuula Hollmén

Introduction

Several North American sea ducks (Tribe *Mergini*) have declined over recent decades. The spectacled eider (*Somateria fischeri*) is a sea duck species of particular conservation concern. Between the 1970's and 1990's, the breeding population on the Yukon-Kuskokwim Delta declined by up to 96% (Ely et al. 1994; Stehn et al. 1993) leading to their listing as threatened under the Endangered Species Act in 1993 (Federal Register 1993). Potential causes for the decline or threats to recovery include lead poisoning, hunting, predation,

contaminants, disease, and changes in the marine ecosystem, such as shifts in benthic food availability, dietary competition with other benthic feeders, and habitat degradation (Petersen et al. 2000; U.S. Fish and Wildlife Service 1996). The relative importance and extent that these factors affected the decline and pose threats to recovery is unknown (U.S. Fish and Wildlife Service 1996). Some of these factors could affect foraging ecology and nutrient allocation for reproduction, and therefore subsequent reproductive success. Development of methods to evaluate nutrient allocation to reproduction is an important step in understanding critical foraging habitats and factors affecting productivity.



Map courtesy of USFWS

The spectacled eider lives in arctic and subarctic Alaska and Russia using freshwater wetlands and marine habitats throughout the annual cycle (Petersen et al. 2000; U.S. Fish and Wildlife Service 1996). In Alaska, breeding birds inhabit remote northern and western coastal freshwater tundra habitats between May and August (Grand and Flint 1997; Petersen et al. 2000; U.S. Fish and Wildlife Service 1996). These birds molt in areas along the coast of northern and western Alaska and the entire world population is thought to congregate in the winter offshore south of St. Lawrence Island among the Bering Sea ice leads (Petersen et al. 1995; Petersen et al. 1999; U.S. Fish and Wildlife

Service 1996). There is some knowledge on foraging ecology for this species. On breeding grounds, spectacled eiders feed on a range of invertebrates, seeds, and plant material (Dau 1974; Kistchinski and Flint 1974; U.S. Fish and Wildlife Service 1996) and during the winter, bivalves are considered to be the most common food source (Lovvorn et al. 2003; Petersen et al. 1998).

Arctic waterfowl, including eiders, are faced with high reproductive costs during a short breeding season. Acquiring energy and nutrients for egg production can be particularly costly for waterfowl that lay energy-rich eggs and hatch relatively mature (precocial) offspring (Alisauskas and Ankney 1992). In addition, waterfowl like eiders have long incubation periods with few incubation breaks, and therefore must sustain high self maintenance costs (Flint and Grand 1999). The strategy of building nutrient reserves prior to breeding (endogenous reserves) that can subsequently be invested for reproductive events is termed ‘capital’ breeding (Drent and Daan 1980; Jönsson 1997). While many waterfowl species are thought to rely to some extent on nutrient reserves for reproductive costs (e.g., Gauthier et al. 2003; Hobson et al. 2005; Parker and Holm 1990; Schmutz et al. 2006), exclusive reliance on reserves is considered rare (Meijer and Drent 1999). An alternative strategy, in which females rely on foods consumed at the breeding grounds (exogenous resources) for reproduction, is referred to as ‘income’ breeding (Drent and Daan 1980; Jönsson 1997) and has been identified as a breeding strategy used by some female waterfowl (e.g., Bond et al. 2007; Hobson et al. 2004). However, there are costs and benefits of these strategies to female fitness and reproductive success, and most species are thought to lie along a continuum of capital and income strategies (Jönsson 1997). Additionally, evaluation of these strategies during reproduction has mainly focused on the period during egg formation; however, reliance on stored nutrients may also be important during other stages like incubation and brood-rearing.



Spectacled eider female on her nest at the Yukon Kuskokwim Delta (Photos courtesy of the Alaska SeaLife Center and Tasha DiMarzio 2009).

Limited information is available on sources of nutrients used for reproduction in sea ducks, although these data may be important for understanding factors influencing reproductive performance (Grand et al. 2002). Common eiders (*Somateria mollissima*) are thought to rely heavily on endogenous reserves for egg production (Parker and Holm 1990), suggesting that inadequate resources obtained on the wintering/spring staging grounds could have significant effects on reproduction for this species. Barrow’s goldeneye (*Bucephala islandica*) have been shown to utilize some reserves built on marine wintering areas for production of eggs laid early in the clutch; however, local freshwater diet sources contributed the bulk of nutrients for later laid eggs (Hobson et al. 2005). This study emphasized the importance of nutrient sources from both habitats for egg production. Female harlequin ducks (*Histrionicus histrionicus*) and king eiders (*Somateria spectabilis*) have been shown to store endogenous nutrients on wintering

areas prior to spring migration, but little to none of these were found to be allocated to egg formation (Bond et al. 2007; Oppel 2009). No information to date is available for determining strategies of nutrient acquisition and allocation to reproduction in spectacled eiders.

Understanding sources and timing of acquisition of nutrients for reproduction is important for identification of critical habitats of threatened spectacled eiders and other declining sea duck species. Because many sea ducks spend much of the winter and staging periods in marine habitats, it is plausible that reproductive performance is correlated with availability and quality of marine resources. Therefore, carry-over or cross-seasonal effects of habitat quality and food availability in non-breeding habitat may have important consequences for recruitment and subsequently population dynamics (Norris 2005; Sorensen et al. 2009; Webster and Marra 2005; Webster et al. 2002). Because sea ducks are considered K-strategists where a greater emphasis is placed on adult survival in relation to reproductive success (Bellrose 1980; Eadie et al. 1988; Goudie et al. 1994), they may defer reproduction under scenarios of food limitation or poor environmental conditions (Goudie and Jones 2005; Klaassen et al. 2006), including those caused by broad-scale environmental changes (Grebmeier et al. 2006). However, assessment of seasonal interactions between habitats may be limited by the inability to follow individuals throughout their annual cycle (Webster et al. 2002). As a result, the relative influence that dietary or environmental conditions in both marine and freshwater habitats have on reproductive performance is not well understood.

Stable isotope analyses can be used to evaluate foraging ecology and nutrient allocation for egg production in birds that travel between isotopically distinct regions between non-breeding and breeding stages of the annual cycle (Hobson 2006; Inger and Bearhop 2008; Kelly 2000; Rubenstein and Hobson 2004). Using this approach, mixing models can be applied to quantify sources of nutrients used for egg production (e.g., Moore and Semens 2008; Phillips and Gregg 2001). These nutrient allocation models typically require mean isotope signatures for two or more potential nutrient sources and the animal tissue (e.g., egg components or down feathers) corrected for diet to tissue isotopic fractionation ($\Delta\delta = \delta \text{ animal tissue} - \delta \text{ diet}$). Model accuracy relies on variables such as appropriate selection of potential nutrient sources (Ben-David and Schell 2001; Phillips 2001) and accurate estimates of isotopic fractionation factors (Gannes et al. 1997). There are few species-specific data available for isotopic fractionation in avian egg components (Hobson 1995) and no information to our knowledge is available for isotopic fractionation factors associated with natal down feathers. Additionally, avian tissues like blood and feathers can be collected using minimally invasive techniques and in combination with analyses of other tissues can be used to infer information about diet sources and movement patterns within and between ecosystems (Rubenstein and Hobson 2004). For example, metabolically active tissues like blood components may be useful for identifying diet and movement within or between seasons because these tissues turnover at different rates (e.g., blood plasma turnover is on a scale of hours/days and cellular blood over months; Hobson and Clark 1993). Tissues that are metabolically inert after a period of growth (e.g., feathers) may preserve a record of diet and movement across seasons (Hobson and Clark 1992a), particularly if the timing of when certain feather tracts are grown is known.

Further, studies evaluating nutrient allocation during egg production have employed tissues (e.g., cellular blood, fat, feathers, liver, muscle) as isotopic end-members with distinct isotopic signatures in cases where dietary sources are not available or are not known (Bond et al. 2007; Gauthier et al. 2003; Hobson et al. 2004; Hobson et al. 2000; Hobson et al. 2005; Klaassen et al. 2001; Schmutz et al. 2006).

Most mixing models that estimate nutrient allocation to reproduction have utilized carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope values; (see review by Hobson 2006). Both stable isotopes are known as good indicators of diet or geographical region and $\delta^{15}\text{N}$ values are also good indicators of trophic position (Kelly 2000; Peterson and Fry 1987). Generally, the $\delta^{13}\text{C}$ compositions of animal tissues are similar to their diet (DeNiro and Epstein 1978; Peterson and Fry 1987), but may increase or decrease as a result from preferential loss of the more abundant and lighter stable carbon isotope (^{12}C) during respiration, preferential uptake of the relatively rare and heavier stable carbon isotope (^{13}C) during digestion, or the metabolic fractionation of isotopes in different tissue types (e.g., DeNiro and Epstein 1978; Peterson and Fry 1987). However, a decrease in the $\delta^{13}\text{C}$ can occur in tissues that contain lipid because of the oxidation in lipids during lipid synthesis (DeNiro and Epstein 1977). The $\delta^{15}\text{N}$ composition of tissues generally reflects the diet within approximately 3-5‰ (DeNiro and Epstein 1981; Minagawa and Wada 1984; Peterson and Fry 1987). The reason for the increase in $\delta^{15}\text{N}$ is unclear, but may result from the preferential excretion of the lighter isotope (^{14}N) or the preferential absorption of the heavy isotope (^{15}N) in the tissue (Martínez del Río et al. 2009). The isotopic differences between diet and tissues has been found to vary according to diet composition, species, individual physiology, age, body condition, and tissue type (Bearhop et al. 2002; Ben-David and Schell 2001; DeNiro and Epstein 1978; DeNiro and Epstein 1981; Hobson and Clark 1992b; Minagawa and Wada 1984). Because egg yolk contains proteins and lipids, and lipids exhibit a relatively lower $\delta^{13}\text{C}$ (DeNiro and Epstein 1977; McConnaughey and McRoy 1979), it is necessary to determine the isotopic fractionation of the individual egg components separately to ascertain information about potential differentiation of macronutrient sources (Hobson 1995; Hobson 2006). Use of inaccurate isotopic fractionation factors may be a source of error in mixing models, and therefore, experimental studies have been suggested to determine tissue and species/taxon specific isotopic fractionation factors (Caut et al. 2008; Dalerum and Angerbjörn 2005; Gannes et al. 1997; Hobson 2006).

The purpose of this study was to establish carbon and nitrogen isotopes (i.e., $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of experimental diet items, egg components, down feathers and adult tissues including contour feathers, blood components (i.e., blood plasma and cellular blood), and adipose tissue for captive spectacled eiders and to quantify isotopic fractionation factors for these tissues. Further, the values for spectacled eider egg components were compared to a previous captive study by Hobson (1995) which



Spectacled eider female on her nest at the Alaska SeaLife Center (Photo courtesy of the Alaska SeaLife Center 2009)

determined egg isotopic fractionation factors for captive mallards (*Anas platyrhynchos*), Japanese quail (*Coturnix japonica*), prairie falcons (*Falco mexicanus*), peregrine falcons (*Falco peregrinus*), and gyrfalcons (*Falco rusticolis*) to assess how these values from different species can affect the outcome of the nutrient allocation model. These data were also incorporated into previously published sea duck model to evaluate whether changes were evident in percentage of nutrient allocation to reproduction.

Methodology

Samples for this project were collected during 2006-2008. A summary of sample collection methodologies follows.

Study Species

Eleven birds (females = 5, males = 6) were reared in captivity and arrived at the Alaska SeaLife Center (Seward, Alaska) as sub-adults in 2003, and 2 adult males arrived as rehabilitated animals in 2003 and 2004. The flock was housed together in an outdoor enclosure, and beginning in 2007, the different pairs were separated during the breeding season to confirm parentage of eggs and collect full clutch information.

Feeding Protocols and Diet Collection

During the breeding season, research was conducted 1 February through egg laying (30 June) in 2006 and 2007 and through post-breeding (31 July) in 2008. The captive flock of spectacled eiders was fed a diet of approximately 95% Mazuri® Sea Duck Diet (Purina Mills Incorporated, St. Louis, Missouri) and 5% natural food supplements, along with calcium grit during pre-breeding (ratios calculated by dry mass). In 2006 and 2007, birds were provided five alternating natural food supplements (Antarctic krill, tidewater silversides, Arctic razor clams, Pacific blue mussels, and common squid) each once a week, and estimated consumption was recorded. In 2008, we modified the diet to include only one natural food supplement (tidewater silversides) quantified at 5% dry weight based on the consumption of Mazuri® within the previous 24 hours. Samples of each diet item were collected in all years. To evaluate sample variation of diet items, samples of each diet item in 2008 were collected at approximately one month intervals (February-June; n=5).

During the non-breeding season, research was conducted 1 September 2007 to 31 January 2008. Birds were fed a baseline diet for 69 days of approximately 97% Mazuri® and 3% natural food supplements (Antarctic krill, tidewater silversides, Arctic razor clams, and Pacific blue mussels), then switched to a diet of 80% Mazuri® and 20% Krill for 21 days, and finally switched to a diet of 78% Mazuri® and 22% silversides for 29 days (ratios calculated as dry weight). Samples of each diet item were collected to estimate isotopic dietary shifts. All breeding and non-breeding diet samples were stored at -20°C until analysis.



Separation of spectacled eider egg components from captive birds at the Alaska SeaLife Center (Photos courtesy of the ASLC 2008 and 2009)

Egg Component and Down Feather Collection

Females were monitored daily for egg laying during the breeding season in 2006, 2007, and 2008 and eggs were collected upon observation ($n = 37, 39,$ and 50 , respectively). All eggs were then refrigerated at 4°C and divided into fresh yolk and albumen components within 24 hours. Homogenized spectacled eider yolk and albumen samples 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 in 2008 were incubated until 20-23 days, at which time embryos were observed to be inviable. Whole down feathers were collected from the back tract of inviable embryos. Feathers were rinsed with ethanol to remove any external substances, dried, and stored at room temperature until analysis (Knoche 2004).

Simulation Model to Evaluate $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ of Egg Components in Different Species

We simulated a single-isotope, two-end member mixing model (e.g., Phillips and Gregg 2001) to examine model sensitivity when we applied different diet to tissue isotopic fractionation factors (i.e., derived from different avian species, Hobson 1995 and this study). Stable isotopes signatures (calculated separately for carbon and nitrogen) of two nutrient sources (i.e., terrestrial and marine) were incorporated into the model as end-members. Then, stable isotope signatures of five egg components were adjusted for diet to egg isotopic fractionation and applied to the model as the mixture. All model components were kept constant, except the diet to egg isotopic fractionation values were replaced for each species. Marine ($\delta^{13}\text{C} = -22\text{‰}$, $\delta^{15}\text{N} = 7\text{‰}$) and terrestrial ($\delta^{13}\text{C} = -27\text{‰}$, $\delta^{15}\text{N} = 3\text{‰}$) nutrient source end-members in the model were based on Kelly (2000). Each egg component was assigned a $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively (albumen = -23‰ , 8.0‰ ; lipid-free yolk -23‰ , 8.5‰ ; whole yolk -25‰ , 7.5‰ ; and shell membrane -22‰ , 9.0‰) or $\delta^{13}\text{C}$ (yolk lipid -27‰) that was between the two nutrient source end members after adjustment for isotopic fractionation. The model was calculated using the isotopic fractionation values for the spectacled eider from this study and those presented by Hobson (1995) for the mallard $n = 8$, Japanese quail $n = 9$, prairie falcons $n = 2$, peregrine falcons $n = 6$, and gyrfalcons $n = 4$. Sample size and statistical parameters were set at $n = 10$ and $\text{SD} = 0.5\text{‰}$ for both nutrient sources and egg components. The model was then used to estimate mean proportional contribution from the terrestrial nutrient source used for egg synthesis.

Blood Components

Samples from spectacled eider females were collected for a concurrent project weekly for up to 4 weeks prior to egg laying in 2007 (2 May 2007, 8 May 2007, 15 May 2007, 22 May 2007) and 2008 (5 May 2008, 14 May 2008, 20 May 2008) and available aliquots of cellular blood (2007 $n = 15$ and 2008 $n = 13$) and blood plasma (2007 $n = 12$ and 2008 $n = 12$) were archived for stable isotope analysis.

Adult Contour Feathers

We collected adult chest contour feathers during post breeding in 2008 from each spectacled eider female (n = 5). Feathers were rinsed with ethanol to remove any external substances, dried in the hood, and stored at room temperature until analysis (Knoche 2004).

Adipose Tissues

Adipose tissue samples were collected from all spectacled eider males for a concurrent project (Wang et al. 2009) on 27 November 2007 (n = 8), 18 December 2007 (n = 8), and 1/16/08 (n = 8). Mass was measured prior to each adipose tissue biopsy to ensure that the birds were not losing mass as a result of the different diets. Aliquots of fatty acid methyl esters (FAME) from samples collected on 27 November 2007 (n = 8), 18 December 2007 (n = 5), and 16 January 2008 (n = 8) were used for stable isotope analysis.



Loading samples in tin cups (right) and measuring for stable isotope analysis using the Isotope Ratio Mass Spectrometer (left) (Photos courtesy of ASLC and ASIF 2008)

Laboratory Analyses

Diet samples, egg components, embryonic down feathers, and adult tissues (contour feathers, blood components, and adipose tissue) were processed at the Alaska SeaLife Center (Seward, Alaska) and the Alaska Stable Isotope Facility (Fairbanks, Alaska). Diet lipids, feathers, and adipose tissues were measured directly because these contain very little water content. Diet samples and all other tissues were dried using a FreeZone® 12 Liter Console Freeze Dry System (Labconco Corp., Kansas City, Missouri) and homogenized to a fine powder with a grinder or mortar and pestle. Diet samples and whole yolk were separated into lipid and non-lipid components due to potential differences in

macronutrient allocation into eggs. Lipids were removed from diet samples using a 2:1 chloroform:methanol solution and rinsing 4 to 5 times and allowing solvents to evaporate in the hood (Bligh and Dyer 1959; Oppel et al. 2010). Diet samples were duplicate measurements and all other tissues were single measurements.

The carbon and nitrogen stable isotope signatures of samples were processed by loading 0.1-0.4 mg of each sample into tin cups and combusting them at 1020°C. Isotopic ratios were measured using the Elemental Analysis-Isotope Ratio Mass Spectrometry, Delta^{plus} XP system (Thermo Fisher LLC, San Jose, California). All isotope values are expressed in delta (δ) notation, a ratio of heavier to lighter isotope relative to the universal standard in parts per thousand (‰):

$$\delta X (\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where X is ¹³C or ¹⁵N and R is the corresponding ratio ¹³C/¹²C or ¹⁵N/¹⁴N. The standards for carbon and nitrogen are Pee Dee Belemnite and atmospheric nitrogen, respectively.

Calculations

The stable carbon and nitrogen isotope signature for the diet was calculated by multiplying dry mass of daily diet ratio (Mazuri®:natural food supplement) by the respective isotope signatures. Mean and standard deviations for whole diet, lipid free diet, and diet lipids were reported ($\bar{X} \pm SD$). Stable carbon and nitrogen isotope fractionation factors are reported as the mean difference between whole diet (X) and tissues (Y), such that ($\delta Y - \delta X$).

Incorporating Spectacled Eider $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ of Egg Components Into Sea Duck Models

A mixing model approach applied in two previously published studies (Bond et al. 2007; Hobson et al. 2005) was used to evaluate relative contributions of marine nutrient reserves allocated to egg production in two sea duck species, the harlequin duck and Barrow's goldeneye. However, the models were re-analyzed to examine the influence of replacing the isotopic fractionation factors between diet and egg components calculated for falcon species ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N = 0.9\text{‰}$ and 3.4‰ in albumen and 0‰ and 3.4‰ in lipid-free yolk, and $\Delta\delta^{13}C = 0\text{‰}$ in yolk lipids; Hobson 1995) with those values calculated using captive spectacled eiders ($\Delta\delta^{13}C$ and $\delta^{15}N = 1.8\text{‰}$ and 3.8‰ in albumen and 1.6‰ and 4.3‰ in lipid-free yolk, and $\Delta\delta^{13}C = 1.9\text{‰}$ in yolk lipids; Federer 2009). Because these models only considered two nutrient sources, a single-isotope linear mixing model approach using the following mass balance equation was applied for egg component mixture (M) and potential marine and freshwater (A and B) nutrient sources (Phillips and Gregg 2001):

$$\delta_M = f_A\delta_A + f_B\delta_B, 1 = f_A + f_B$$

where δ is the mean isotopic signature and f is the proportion of each source that was determined in the mixture. The following equation was then used to calculate relative mean marine (A) and freshwater source (B) contributions (Phillips and Gregg 2001):

$$f_A = \frac{\delta_M - \delta_B}{\delta_A - \delta_B}$$

Bond et al. (2007) evaluated carbon and nitrogen isotopes independently for lipid-free yolk ($n = 23$, $\delta^{13}C = -25.5 \pm 2.4$, $\delta^{15}N = 6.9 \pm 1.2$) and albumen ($n = 23$, $\delta^{13}C = -25.1 \pm 2.1$, $\delta^{15}N = 5.8 \pm 1.2$). Mean isotope signature for invertebrates collected from freshwater breeding streams (non-lipid $n = 9$, $\delta^{13}C = -25.4 \pm 2.4$, $\delta^{15}N = 2.2 \pm 1.3$) and cellular blood from birds captured just after arrival on breeding habitat ($n = 60$, $\delta^{13}C = -13.9 \pm 1.1$, $\delta^{15}N = 14 \pm 1.3$) describe the end-members for protein-comprised egg yolk (i.e., lipid-free yolk) and albumen. Only the carbon isotope was evaluated for yolk lipids ($n = 23$, $\delta^{13}C = -29.8 \pm 2.3$) because of low nitrogen content in lipids. The lipid portion of invertebrates from freshwater breeding streams ($n = 5$, $\delta^{13}C = -29.7 \pm 3.2$) and abdominal fat from birds captured on marine areas ($n = 18$, $\delta^{13}C = -19.7 \pm 1.3$) describe the end-members for yolk lipids.

Hobson et al. (2005) evaluated relative source contributions for eight nests (1st, 4th, and 8th laid eggs from each nest) among five different wetland systems. This study utilized

only carbon isotope data in the analysis for lipid-free yolk, albumen, and yolk lipids because trophic variability in the $\delta^{15}\text{N}$ values of local food webs was too high to employ a dual-isotope approach. Freshwater invertebrates were collected in four of the five wetland systems. The isotope signatures of prey items among wetlands #8, 11, and 42 were not significantly different from each other and therefore these data were combined; however, isotope signatures for prey items in wetland #115 were significantly different and therefore were evaluated separately. There were no prey items collected for wetland #41, so the freshwater nutrient source end-member for the nests from this wetland were assigned the same end-member as wetlands #8, 11, and 42. The non-lipid ($n = 12$, #115 = $-24.6\text{‰} \pm 1.2$; $n=34$, #'s 8, 11, 42 = $-27.1\text{‰} \pm 0.5$) and lipid portion of freshwater invertebrates ($n = 12$, #115 = $-28.0\text{‰} \pm 1.2$; $n=34$, #'s 8, 11, 42 = $-30.5\text{‰} \pm 0.5$) similarly represented freshwater source, while muscle ($n = 9$, $-19.9\text{‰} \pm 1.2$) and abdominal fat ($n = 5$, $-25.3\text{‰} \pm 0.5$) were used to represent non-lipid and lipid portions of the marine nutrient source, under the assumption that these reserves were built on the coast during the non-breeding period. A model was run for the 1st, 4th, and 8th laid egg (for each egg component) from eight nests among five different wetlands.

Results

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Isotope Analyses for Diet Items

During the breeding season, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were determined for individual diet items consumed from 2006-2008 (Federer 2009). Natural food supplements made up only a small proportion of the overall diet in each year (2006 = 3.0%, 2007 = 5.9%, and 2008 = 4.0%), thus would be expected to have only a small influence on the results. Percent dry mass of each diet item was considered in all calculations (Mazuri = 92-94%; silversides = 22-25%, clams and mussels = 23%, squid = 18-20%, and krill = 17-19%). Dietary proportions were incorporated into the final estimate of the diet isotopic ratios in each year to calculate a single diet value for diet lipid, whole diet, and lipid-free diet (Table 1).

Table 1. Average ($\pm$ SD) carbon and nitrogen stable isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) for all years during the breeding season based on proportional source contribution (Mazuri®:natural food) for diet lipids, whole diet, and lipid-free diet.

Year	Diet Lipid $\delta^{13}\text{C}$ (‰)	Whole Diet $\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Lipid-free Diet $\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
2006 (n=2)	-26.4 ± 0.2	-21.3 ± 0.1	5.7 ± 0.2	-21.0 ± 0.5	6.3 ± 0.4
2007 (n=2)	-26.2 ± 0.1	-21.3 ± 0.3	7.3 ± 0.9	-20.0 ± 0.3	7.1 ± 0.7
2008 (n=10)	-26.4 ± 0.2	-21.0 ± 0.9	6.5 ± 0.5	-20.3 ± 0.6	6.7 ± 0.5

While birds were on the baseline diet during the non-breeding season, natural food supplements made up approximately 3% of diet consumed. The two subsequent diet switches following the baseline diet included Mazuri® along with a single natural food supplement. Krill made up 19.8% during the first diet switch and silversides were 21.9% of the second diet switch. Proportions of diet items were then multiplied by the respective stable isotope signatures in both years to calculate a carbon stable isotope signature for diet lipids (Table 2). When the diet was switched from the baseline diet to Mazuri® and krill (diet switch 1), the carbon stable isotope signature of the diet decreased, but then

increased similar to that of the baseline diet when switched to Mazuri® and silversides diet ratio (diet switch 2; Table 2).

Table 2. Average ($\pm$ SD) carbon stable isotopes ($\delta^{13}\text{C}$) of diet lipids (baseline diet and 2 diet switches) during the non-breeding season and subsequent adipose tissue biopsies collected over 3 time periods (*one fat sample from a male that did not eat krill during the 1st diet switch was excluded).

Diet Samples	Lipid Diet $\delta^{13}\text{C}$	Biopsy Samples	Adipose Tissue $\delta^{13}\text{C}$
Baseline Diet	-26.2 ± 0.1 (n = 2)	1	-25.0 ± 0.3 (1.2, n=8)
Diet 1	-27.2 ± 0.1 (n = 2)	2	-26.6 ± 0.1 (0.6, n=4*)
Diet 2	-25.1 ± 0.1 (n = 2)	3	-25.1 ± 0.4 (0.0, n=8)

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Isotope Fractionation Factors for Egg Components and Down Feathers

Isotopic fractionation factors for carbon and nitrogen (i.e., $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$) were calculated for whole yolk, lipid-free yolk, albumen, and shell membrane egg components in each year and for down feathers in 2008 (Table 3). Only $\Delta\delta^{13}\text{C}$ was reported for yolk lipids and eggshell due to the low nitrogen content of these tissues. We determined similar increases in $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ for albumen, yolk proteins (denoted by lipid-free yolk), and shell membrane components and down feathers in relation to whole diet. The $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ for shell membrane were consistently higher than albumen and lipid-free yolk across all years and the $\Delta\delta^{15}\text{N}$ for down feathers was higher than albumen, lipid-free yolk, and membrane. Whole yolk increased in $\Delta\delta^{15}\text{N}$, but whole yolk and yolk lipids decreased in $\Delta\delta^{13}\text{C}$ relative to whole diet. The $\Delta\delta^{13}\text{C}$ increased considerably for eggshell in relation to whole diet. Isotopic differences ($\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$, respectively) observed among years for each tissue were minimal in albumen (1.0‰ and 1.6‰), lipid-free yolk (1.1‰ and 1.5‰), shell membrane (1.2‰ and 1.4‰), and whole yolk (0.9‰ and 0.9‰) and for $\Delta\delta^{13}\text{C}$ in yolk lipid (1.0‰) and eggshell (1.5‰).

Table 3. Isotopic fractionation factors (average $\pm$ SD) between whole diet and egg components and down feathers for captive spectacled eiders.

Year	δ	Albumen	Lipid-free Yolk	Membrane	Yolk	Yolk Lipid	Eggshell	Down Feathers
2006	C	2.1 ± 0.3	1.8 ± 0.3	3.0 ± 0.3	-2.2 ± 0.3	-3.6 ± 0.4	12.3 ± 0.8	
(n=37)	N	5.0 ± 0.4	5.4 ± 0.5	5.8 ± 0.5	4.0 ± 0.6			
2007	C	3.1 ± 0.2	2.5 ± 0.7	4.2 ± 0.3	-1.3 ± 0.4	-2.5 ± 0.6	13.8 ± 0.7	
(n=39)	N	3.4 ± 0.3	3.9 ± 0.6	4.4 ± 0.4	3.1 ± 0.6			
2008	C	2.7 ± 0.1	2.9 ± 0.1	3.9 ± 0.2	-1.6 ± 0.3	-3.5 ± 0.3	13.0 ± 0.7	2.2 ± 0.4
(n=50 eggs)	N	3.6 ± 0.3	4.3 ± 0.4	4.6 ± 0.4	3.5 ± 0.3			5.3 ± 0.4
(n=5 feathers)								
2006-2008								
Average	C	2.6 ± 0.5	2.5 ± 0.6	3.7 ± 0.6	-1.6 ± 0.5	-3.2 ± 0.6	13.1 ± 0.9	
(n=126)	N	4.0 ± 0.8	4.5 ± 0.8	4.9 ± 0.7	3.5 ± 0.6			

Simulation Model to Evaluate $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ of Egg Components in Different Species

Overall, the differences observed among years in spectacled eider egg isotopic fractionation were comparable to or less than (depending on the tissue type) that observed among species (Hobson 1995 and this study) for $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ (respectively) in albumen (1.8‰ and 1.6‰), lipid-free yolk (2.6‰ and 1.4‰), shell membrane (1.1‰ and 1.7‰), and whole yolk (1.1‰ and 1.5‰) and for $\Delta\delta^{13}\text{C}$ in yolk lipid (1.0‰) and eggshell (4.5‰). The mean $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ among years for spectacled eiders were either similar or increased for albumen, lipid-free yolk, and shell membrane relative to other species. The $\delta^{13}\text{C}$ for lipid-free yolk was similar to that of the diet for previously studied species, but increased relative to the diet similarly to the other protein-comprised egg components (i.e., albumen and shell membrane) for spectacled eiders. The mean $\Delta\delta^{13}\text{C}$ among years for whole yolk and yolk lipid were similar relative to other species where the spectacled eider isotopic fractionation values decreased relative to those for the quail and mallard and increased or were similar to those for falcon species. The mean $\Delta\delta^{15}\text{N}$ among years for whole yolk were either similar or increased slightly relative to previously studied species. While there was a wide mean range in the $\Delta\delta^{13}\text{C}$ of eggshells among species, the mean $\Delta\delta^{13}\text{C}$ among years for spectacled eider eggshells decreased relative to those for the quail and mallard and increased relative to those for falcon species.

Results from mixing models indicated that estimated contributions from the terrestrial source using our simulated data ranged from 20-52% based on $\delta^{13}\text{C}$ values and 10-43% based on the $\delta^{15}\text{N}$ values, depending on the isotopic fractionation factors used from different species (Figure 1). The models showed that the proportional contribution of carbon from a terrestrial source differed up to 36% for albumen, 52% for lipid-free yolk, 22% for both shell membrane and whole yolk, and 20% for yolk lipids when using factors derived from other species relative to our spectacled eider values (Figure 1). Similarly, the proportional contribution of $\delta^{15}\text{N}$ from a terrestrial source differs up to 40% for albumen, 35% for lipid-free yolk, 43% for shell membrane, and 10% for whole yolk when using factors derived from other species relative to our spectacled eider values (Figure 1).

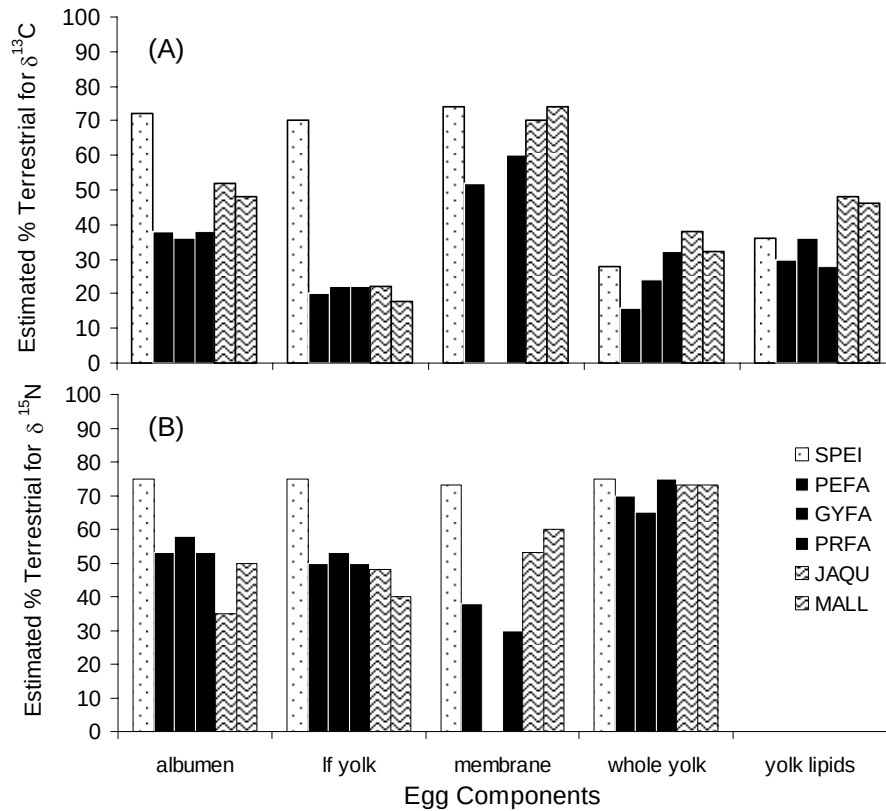


Figure 1. Theoretical mixing models (described in text) illustrate the estimated percentages of terrestrial nutrient sources allocated to each egg component using carbon (A) and nitrogen (B) stable isotopes. Whole diet to tissue isotopic fractionation factors from different species were substituted in the model to demonstrate how the choice of isotopic fractionation factors can influence the model. Black bars indicate a model using isotopic fractionation factors from three falcon species (fed a carnivore diet) and zig-zag shading indicate a model using these values from Japanese quail and mallards (fed an herbivore diet, Hobson 1995), while dots indicate a model using our spectacled eider isotopic fractionation factors from this study.

Differentiating Macronutrient Sources in $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$

Because we calculated isotope values for whole diet and protein (lipid-free) and lipid portions of the diet, we were able to provide $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ from whole diet to tissue to allow comparison of previously established isotopic fractionation factors from other avian species (Hobson 1995) with those from spectacled eider values from this study. In the Hobson (1995) study, only the isotope signature for the whole diet was determined, but a hypothetical model was presented that separated the macronutrients (e.g., lipid and protein) in the diet to those nutrients in the different egg components. As an expansion of this hypothetical model, we have quantified isotopic fractionation factors useful for delineating specific macronutrient in the diet to those used for egg synthesis (e.g., diet lipids to egg yolk lipids, whole diet to whole yolk, and lipid-free diet to the protein-comprised egg components like lipid-free yolk, albumen, and shell membrane or newly developed tissues of embryos like down feathers; Figure 2).

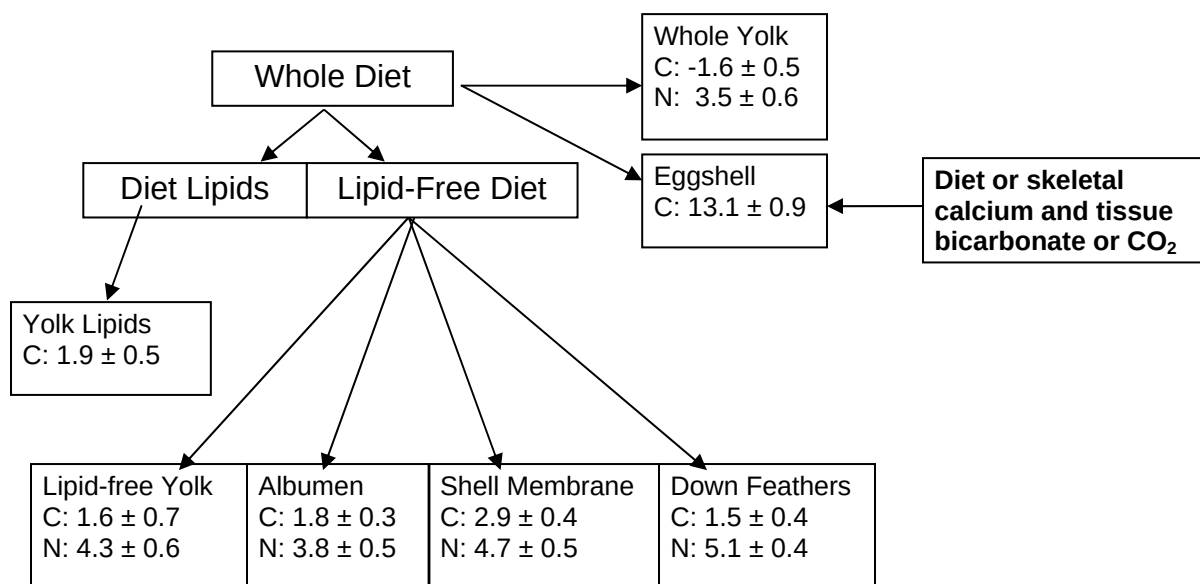


Figure 2. As an expansion from the Hobson (1995) hypothetical model, isotopic fractionation factors were determined for whole diet to whole yolk (containing both proteins and lipids) and eggshell (containing calcium from diet or skeletal tissue and carbonate from bicarbonate or CO₂), diet lipids to lipids in the egg (yolk lipids), and diet with lipids removed to those protein-comprised components (albumen, lipid-free yolk, shell membrane, and down feathers) of the egg and developing embryo.

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Isotope Fractionation Factors in Adult Tissues

Carbon and nitrogen stable isotopic fractionation factors ($\Delta\delta = \delta$ animal tissue - δ lipid-free diet) were calculated for blood components in 2007 and 2008 and adult contour feathers in 2008 (Table 4). The mean carbon stable isotope signature from the adipose tissue from biopsy 1 was 1.2‰ greater than the baseline lipid diet (Table 2 and Figure 3). Similarly, the mean carbon stable isotope signature from the adipose tissue of biopsy 2 increased by 0.6‰ relative to the diet lipids from the Mazuri® and krill ratio when we excluded one individual that did not eat the krill during the first diet switch (Figure 3). The mean carbon stable isotope signature of the adipose tissue from biopsy 3 was the same as the Mazuri® and silverside diet (Table 2 and Figure 3).

Table 4. Average ($\pm$ SD) carbon and nitrogen stable isotopic fractionation factors ($\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$) from lipid-free diet to blood components and feathers for captive spectacled eiders in 2007 and 2008.

Year	$\Delta\delta$	Cellular Blood	$\Delta\delta$	Blood Plasma	$\Delta\delta$	Feathers
2007	^{13}C	0.9 ± 0.2 (n=15)	^{13}C	-0.2 ± 0.2 (n=12)		
	^{15}N	4.0 ± 0.4 (n=15)	^{15}N	4.5 ± 0.2 (n=12)		
2008	^{13}C	2.0 ± 0.1 (n=13)	^{13}C	$0.0 \pm 0.$ (n=12)	^{13}C	3.2 ± 0.2 (n=5)
	^{15}N	4.0 ± 0.3 (n=13)	^{15}N	4.9 ± 0.5 (n=12)	^{15}N	5.6 ± 0.6 (n=5)
Average	^{13}C	1.4 ± 0.6	^{13}C	-0.1 ± 0.4		
	^{15}N	4.0 ± 0.3	^{15}N	4.7 ± 0.4		

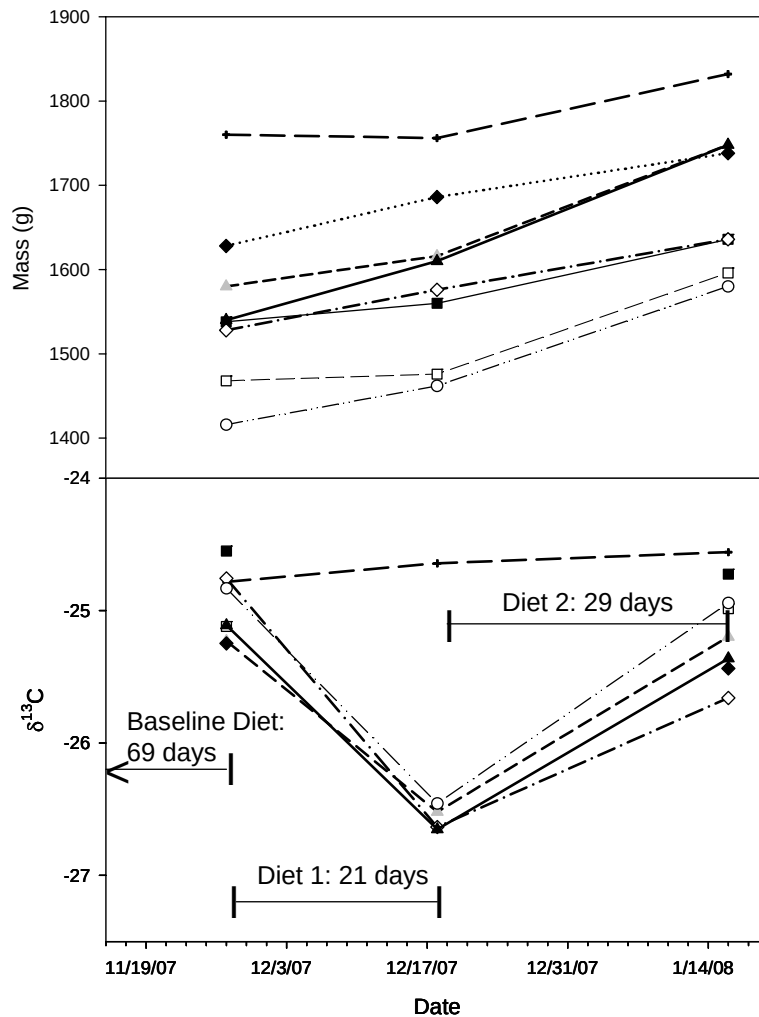


Figure 3. Mass of 8 male birds over three biopsy time periods (A) illustrating that weight was kept constant or increasing for each individual. Straight lines (B) indicate the carbon stable isotope signature ($\delta^{13}\text{C}$) for diet lipids the corresponding time period that birds were fed a diet (baseline diet = 69 days, diet switch 1 = 21 days, and diet switch 2 = 29 days). Individual line plots indicate a different male bird (same individual for graph A and B) and subsequent $\delta^{13}\text{C}$ of adipose tissue for biopsy 1 (male = 8), biopsy 2 (male = 5), and biopsy 3 (male = 8). Note that one individual did not switch diet in a similar pattern (i.e., would not eat krill) that constituted the change in diet prior to biopsy 2.

Incorporating Spectacled Eider $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ of Egg Components Into Sea Duck Models

In both models, there was a decrease in the percentage points of marine nutrient contribution to egg production when the spectacled eider diet to egg isotopic fractionation factors were applied relative to previous estimates. For the harlequin duck model (Table 5), marine nutrient sources were estimated to contribute a lower percentage in both isotopes for albumen ($\delta^{13}\text{C} = 8\%$, $\delta^{15}\text{N} = 4\%$) and lipid-free yolk ($\delta^{13}\text{C} = 14\%$, $\delta^{15}\text{N} = 8\%$), and in carbon for yolk lipids ($\delta^{13}\text{C} = 19\%$) relative to previous estimates. Model results yield a nutrient source contribution between 0-100% with a 95% CI, and therefore

are given as estimates (as described by Ben-David and Schell 2001; Phillips 2001). Because some of the egg components fell outside of the marine nutrient source end-member considered for this study, these values were estimated at 0%. For the carbon mixing model, each egg component was estimated to derive 0% from marine-derived nutrient sources (95% CI: albumen = 0-6%, lipid-free yolk = 0-5%, yolk lipids = 0-30%). While the previous model using falcon values also indicated 0% marine-derived nutrient source contribution in carbon, the 95% CI's were slightly different (albumen = 0-13%, lipid-free yolk = 0-17%, and yolk lipids = 0-41%). Similarly, 0% of albumen (95% CI = 0-8%) and 3% lipid-free yolk (95% CI = 0-12%) were estimated to originate from marine-derived sources for the nitrogen model. Previous estimates for the nitrogen model suggested that 2% albumen (95% CI = 0-11%) and 11% lipid-free yolk (95% CI = 0-19%) were derived from marine-derived sources.

Table 5. Mean and 95% CI difference in marine nutrient source contribution when isotopic fractionation factors ($\Delta\delta$) were applied from spectacled eider (SPEI) and falcon species (FALC) for both carbon and nitrogen isotopes in the Bond et al (2007) model.

$\Delta\delta$	δ	Egg Component	Mean	95% CI
SPEI	$\delta^{13}\text{C}$	Albumen	0	0-6
SPEI	$\delta^{13}\text{C}$	Lipid-free Yolk	0	0-5
SPEI	$\delta^{13}\text{C}$	Yolk lipid	0	0-30
SPEI	$\delta^{15}\text{N}$	Albumen	0	0-8
SPEI	$\delta^{15}\text{N}$	Lipid-free Yolk	3	0-12
FALC	$\delta^{13}\text{C}$	Albumen	0	0-13
FALC	$\delta^{13}\text{C}$	Lipid-free Yolk	0	0-17
FALC	$\delta^{13}\text{C}$	Yolk lipid	0	0-41
FALC	$\delta^{15}\text{N}$	Albumen	2	0-11
FALC	$\delta^{15}\text{N}$	Lipid-free Yolk	11	0-19

For the Barrow's goldeneye carbon model (Table 6), percentage of marine nutrient sources allocated to the eggs in each nest decreased when spectacled eider isotopic fractionation factors were used in relation to those values from falcon species for albumen ($\delta^{13}\text{C}$ = 19% for nests on wetland #115 and 13% for nests on wetland #'s 8, 11, 42, 41), lipid-free yolk ($\delta^{13}\text{C}$ = 35% for nests on wetland #115 and 23% for nests on wetland #'s 8, 11, 42, 41), and yolk lipids ($\delta^{13}\text{C}$ = 71% for nests on wetland #115 and 37% for nests on wetlands #'s 8, 11, 42, 41). Values that fell outside of the range of the marine end-member were reported as 100% and the freshwater end-member as 0% following Hobson et al. (2005). Both freshwater and marine nutrients were allocated to eggs for nests among wetland #'s 8, 11, 42, 41, but most nests had a pattern of higher marine-derived nutrient allocation to eggs laid early on in the clutch (1st egg per nest given as mean range: 0-78% for albumen, 0-96% for lipid-free yolk, and 0-73% for yolk lipids) and a decrease in marine-derived nutrients to later laid eggs (4th and 8th egg given as mean range: 0-21% and 0-15% for albumen, 0-29% and 0-25% for lipid-free yolk, and

0% in both for yolk lipids). The previous model indicated a similar pattern of higher marine-derived nutrients contributed to early laid eggs and less marine-derived nutrients in later laid eggs, but these values were higher in comparison to our results (1st, 4th, 8th egg given as mean range among wetlands #'s 8, 11, 41, and 42 = 0-90%, 0-34%, 0-28% for albumen, 0-100%, 0-51%, and 0-47% for lipid-free yolk, and 0-100%, 0-23%, and 0-9% for yolk lipids). For nests on wetland #115, we found 0% marine-derived nutrient sources for 1st, 4th, and 8th eggs in all egg components when using spectacled eider isotopic fractionation values in the model. Similarly, the previous model indicated that 0% marine-derived nutrient sources were used to build albumen and yolk lipids for 1st, 4th, and 8th eggs, but some marine nutrients were allocated to lipid-free yolk (22% for 1st egg, 18% for 4th egg, and 9% for 8th egg).

Table 6. Difference in mean marine nutrient source contribution from the 1st, 4th, and 8th eggs per nest in wetland #115 and difference in mean range marine nutrient source contribution from the 1st, 4th, and 8th eggs per nest among wetlands #'s 8, 11, 41, 42 when isotopic fractionation factors ($\Delta\delta$) were applied from spectacled eider (SPEI) and falcon species (FALC) for stable carbon isotopes ($\delta^{13}\text{C}$) in the Barrow's goldeneye model.

$\Delta\delta$	δ	Egg Component	Wetland #115 Mean (1 st , 4 th , 8 th)	Wetland #'s 8, 11, 41, 42 Mean Range (1 st , 4 th , 8 th)
SPEI	$\delta^{13}\text{C}$	Albumen	0, 0, 0	0-78, 0-21, 0-15
SPEI	$\delta^{13}\text{C}$	Lipid-free Yolk	0, 0, 0	0-96, 0-29, 0-25
SPEI	$\delta^{13}\text{C}$	Yolk lipid	0, 0, 0	0-73, 0, 0
FALC	$\delta^{13}\text{C}$	Albumen	0, 0, 0	0-90, 0-34, 0-28
FALC	$\delta^{13}\text{C}$	Lipid-free Yolk	0, 0, 0	0-100, 0-51, 0-47
FALC	$\delta^{13}\text{C}$	Yolk lipid	22, 18, 9	0-100, 0-23, 0-9

Discussion

The research has quantified stable isotope fractionation factors for various tissues in spectacled eiders. There are very few data available on isotopic fractionation between the diet and tissues of sea duck species (Knoche 2004). These data will contribute to the body of knowledge of isotopic fractionation in avian tissues, and will be particularly useful for modeling foraging ecology and nutrient allocation in threatened spectacled eiders and other sea ducks.

These are the first isotopic fractionation factors developed for eggs of any sea duck species, and therefore are likely to be the most applicable for modeling nutrient allocation in sea ducks. The isotopic fractionation values for diet to egg components of spectacled eiders differed among egg components, consistent with previously reported isotopic fractionation factors in eggs for other birds (Hobson 1995). This emphasizes the importance of calculating isotopic fractionation values for individual egg components separately. Isotopic fractionation factors of eggs were quantified over three consecutive years using the same individuals and these data demonstrate a consistent pattern of

isotopic fractionation for each individual egg component relative to the diet. While these values were generally consistent with egg components in other avian species (Hobson 1995), this study suggests an increase in the isotopic fractionation of protein-comprised egg components in spectacled eiders. These results suggest higher carbon isotopic fractionation factors for spectacled eiders than were previously reported in other avian species for the protein-comprised egg components (albumen, lipid-free yolk, and shell membrane, Hobson 1995). Nitrogen isotope fractionation factors for albumen, whole yolk, lipid-free yolk, and shell membrane were slightly higher, but similar to those found in previous studies (Hobson 1995). Additionally, we confirmed a wide mean range and high carbon isotope fractionation factors for eggshells (Hobson 1995). Because eggshells consist primarily of calcium carbonate, nutrient sources can be derived from calcium in the diet or skeletal tissue and carbonate in the blood bicarbonate or CO_2 (Johnson 2000), thus leading to a highly elevated carbon isotope signature. Finally, our results demonstrate a decrease in $\delta^{13}\text{C}$ values for avian egg components that contain lipid (i.e., whole yolk and yolk lipid) as was previously found (Hobson 1995). These lower carbon isotope signatures are the result of oxidation in lipids during lipid synthesis (DeNiro and Epstein 1977).

Simulated sensitivity models indicated that the choice of isotopic fractionation values from different species could considerably alter estimates of the percentages of nutrient source contributions to egg production. However, this study also provides isotopic signatures in diet and tissues for both lipid and protein macronutrients (e.g., $\Delta\delta$ = yolk lipids-diet lipids) and published nutrient allocation models use these values rather than those values subtracted from whole diet. This enables consideration of different nutrient sources and the strategies of nutrient allocation to particular egg components. When spectacled eider isotopic fractionation factors were incorporated into previously published sea duck models that used values from falcon, there was little difference in the conclusion of the model. The largest difference was observed when there was not a difference of more than 3-5‰ between end-members of potential nutrient sources.

This research was also the first to our knowledge to have calculated isotopic fractionation values for embryonic down feathers for any bird. Feathers are comprised of proteins (Murphy 1996), and as might be expected, isotopic fractionation values for down feathers were similar to other protein-comprised egg components. Down feather tissues could provide a novel approach to evaluate protein source contribution in nutrient allocation modeling (Klaassen et al. 2001), and therefore this tissue can be a useful understanding proteins used for egg production in the field. Further, these tissues as well as eggshells and eggshell membranes can be sampled non-invasively when collected from nest bowl contents once the female and ducklings have left the nest, which can be very important when evaluating species of conservation concern.

Additional tissues, including adult contour feathers, blood components, and adipose tissue, will be useful end-members representative of diet sources in nutrient allocation modeling and for understanding foraging ecology. No information is available for the isotopic fractionation for the blood components of sea ducks and only few avian studies have been conducted for blood plasma (Evans Ogden et al. 2004; Hobson and Clark

1993; Pearson et al. 2003) and the cellular fraction of blood (Evans Ogden et al. 2004; Hobson and Clark 1993). Carbon stable isotope fractionation associated with cellular blood was slightly higher or within the range of previously reported values (0.5-1.5‰; Hobson and Clark 1993; Evans Ogden et al. 2004) between the two years (2007 = 0.9‰, 2008 = 2.0‰), while nitrogen stable isotope fractionation was higher (4.0‰ for both years) than the value reported by a single study (3.0‰, Evans Ogden et al. 2004). Carbon stable isotope fractionation in blood plasma (2007 = 0.2‰, 2008 = 0‰) 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 nitrogen stable isotope fractionation was higher (2007 = 4.5‰, 2008 = 4.9‰) than has been previously reported (2.5-3.3‰; Hobson and Clark 1993; Pearson et al. 2003; Evans Ogden et al. 2004). There was little mean difference in isotope fractionation of blood components from the same individuals between the years (blood plasma = 0.2‰ for carbon and 0.4‰ for nitrogen, cellular blood = 1.1‰ for carbon and 0‰ for nitrogen). Although concurrent sample years have not been previously evaluated, these data indicate that individual physiology may vary over years, such as assimilation efficiency and deposition of nutrients to specific tissues (Bearhop et al. 2002; Hobson and Clark 1992b).

Extensive research on the isotopic fractionation of feathers from different species has been previously conducted (Bearhop et al. 2002; Becker et al. 2007; Cherel et al. 2005; Hobson and Bairlein 2003; Hobson and Clark 1992b; Knoche 2004; Mizutani et al. 1992; Pearson et al. 2003). Overall, isotopic fractionation values for spectacled eider feathers (mean carbon = 3.2‰ and nitrogen = 5.6‰) fell within the range found for isotopic fractionation shown in other studies (mean carbon range = -0.4‰ to 4.3‰ and nitrogen range = 1.1-5.6‰). 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 eiders which were fed the same commercial diet (Mazuri® Sea Duck Diet) at a different facility (Knoche 2004). Isotopic fractionation in spectacled eider feathers were similar to king eiders in carbon (mean = 2.8‰), but nitrogen for spectacled eiders was 1.3‰ higher in relation to king eiders (mean = 4.3‰; Knoche 2004). Another study evaluated isotopic fractionation factors in feathers of taxonomically similar penguin species that were fed different fish diets and found considerable variation for carbon (0.1-2.9‰) and nitrogen (3.5-4.8‰, Mizutani et al. 1992; Cherel et al. 2005), but these studies did not evaluate the effects of different diets among these species. However, another study found that different species (gull, heron, and egret) that were fed the same fish diet varied in both mean carbon (3.1-3.6‰) and nitrogen (3.9-5.3‰, Mizutani et al. 1992). Further studies have evaluated different dietary compositions and reported that percent lipid in diet corresponded linearly with increasing mean carbon stable isotope fractionation (1.9-4.3‰) in warblers (Pearson et al. 2003). Additionally, differences in the carbon isotope signatures, but little to no difference for nitrogen isotopes, have been reported between feather types (Becker et al. 2007). 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 isotopic fractionation of specific amino acids in bird feather keratin, such as cysteine and methionine (Murphy

1996), and it is unknown to what extent differential isotopic fractionation among amino acids would influence bulk isotope analyses. Further investigation of isotopic fractionation in different feather types and amino acid differentiation to feathers may help us understand specific nutrients used for spectacled eider feather production.

Isotopic fractionation processes between dietary lipids and adipose tissue has not been previously reported to our knowledge for birds. While lipid samples from adipose tissue were not available, FAMES have been used to describe diets and foraging of birds (Wang et al. 2007; Wang et al. 2009a), and therefore can be used as a proxy for lipids to describe potential diet sources. Carbon stable isotope signatures for adipose tissue of captive spectacled eiders were comparable or slightly increased relative to diet (0-1.2‰). A recent study suggests that birds fed a high lipid diet 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 isotopic fractionation from both of these lipid and protein macronutrients (Podlesak and McWilliams 2007). Therefore, it is possible that birds used different macronutrients to synthesize adipose tissue during the different diet treatments. In this study, the baseline diet mainly consisted of Mazuri® with approximately 6.5% fat and 8.4% carbohydrate composition (dry weight proximate analysis reported by Mazuri®). During diet switch 1 and 2, birds were fed single natural food supplements, krill (~20% of the diet) and silversides (~22% of the diet), respectively. Both natural food supplements had low carbohydrate content and krill had high lipid content (Michelson Laboratories, Inc. 2008, unpublished data), therefore dietary lipid deposition may have been more prevalent during the single natural food treatments that exhibited a closer carbon stable isotope signature for adipose tissue to that of the diet. It is also possible that birds were building and burning reserves or that lipid tissue had simply not completed turnover from the diet lipids. Overall, the carbon stable isotope signature of adipose tissue reflected the carbon stable isotopic shifts in the diet for all individuals, except for one bird that did not switch diets in a similar pattern (i.e., would not eat the krill). This bird turned out to be an interesting case study because this individual ate the baseline diet, then only the Mazuri® during the first diet switch, and finally the Mazuri® and silversides diet ratio and, interestingly, carbon stable isotope signatures in the adipose tissue were similar throughout. This chance finding suggests that isotopic signatures in adipose tissue are predictable if dietary macronutrients are known. These diet switches were also confirmed using fatty acid analysis (Wang et al. 2009b).

Conclusions

Overall, this research has identified some differences in the isotopic fractionation factors between spectacled eider diet and tissues in comparison to previously studied species. This study also reports some differences among years using the same individuals suggesting that physiological variation within and among individuals or species, such as assimilation efficiency and deposition of nutrients to specific tissues, may play an important role in the variation of isotopic fractionation processes. Previous studies report variability in the isotopic fractionation factors related to different species, individuals, tissues, and diet composition (Bearhop et al. 2002; Dalerum and Angerbjorn 2005;

DeNiro and Epstein 1978; DeNiro and Epstein 1981; Hobson 1995; Hobson and Clark 1992b). These findings emphasize the importance of calculating isotopic fractionation factors for different species/taxonomic groups or tissue types (Caut et al. 2008; Dalerum and Angerbjorn 2005). These findings will be a useful contribution for understanding nutrient allocation and foraging ecology in eiders and other sea duck species. Methodologies developed in this study provide new tools for further studies on resource allocation strategies in other species of sea duck, including the threatened Steller's eider (Federal Register 1997).

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Eider Research

Estimating diets of two species of threatened sea ducks, the Steller's eider (*Polysticta stelleri*) and the spectacled eider (*Somateria fischeri*): validation of novel diet assessment techniques

Shiway Wang and Tuula Hollmén

Introduction

The spectacled eider (*Somateria fischeri*) and Steller's eider (*Polysticta stelleri*) were listed as threatened under the provisions of the U.S. Endangered Species Act in the 1990s (Federal Register 1993, 1997). Changes in the marine environment and available food resources may be limiting the recovery of both species (U.S. Fish and Wildlife Service 1996, 2002). Eiders winter and stage in marine habitats, and their breeding outcome likely depends on availability of adequate marine resources, but information about timing and sources of critical nutrient acquisition to reproduction is lacking for the threatened eider species in the North Pacific (Petersen et al. 2000; Fredrickson 2001). Determining the diet preferences and foraging habitat associations of these eiders in relation to seasonal and life history stages will provide information to help identify and characterize critical habitats of these threatened breeding populations. Given limitations of traditional methods of diet analysis such as stomach contents analyses, our overall goal was to validate quantitative fatty acid (FA) signature analysis (QFASA) to estimate the diets of spectacled and Steller's eiders.

Fatty acid signatures have been used to study foraging ecology and food webs in marine ecosystems in three ways. First, FA signatures of consumer fat stores alone can be used to qualitatively infer spatial and temporal patterns in diets of free ranging animals. This qualitative analysis of FAs in lipids from adipose tissue, stomach oil, and blood plasma has been used in several marine bird species (Raclot et al. 1998; Dahl et al. 2003; Connan et al. 2005, 2007a, b; Käkälä et al. 2005, 2006, 2007, 2009; Iverson et al. 2007; Wang et al. 2007, 2009; Williams et al. 2008). Second, the existence of unique FAs found in a consumer can occasionally be traced to specific prey species and thus used for identification of forage items (Budge et al. 2006, 2007). Finally, using consumer FA signatures, along with a comprehensive database of prey FA signatures, and accounting for consumer FA metabolism, it is possible to estimate the proportions of different prey in the diet using QFASA (Iverson et al. 2004). QFASA estimates the diet by calculating the weighted mixture of FA signatures of diet items that most closely resembles that of the consumer's adipose tissue after accounting for metabolism effects, and then uses the relative fat content of each diet item to translate the signature mix into a diet estimate. This approach has been validated in several marine mammals (e.g., Iverson et al. 2004,

2006; Nordstrom et al. 2008) and most recently, seabirds in the Bering Sea (Iverson et al. 2007). Additionally, K  kel   et al. (2009) showed that several FAs from plasma of herring gulls (*Larus argentatus*) can quantitatively reflect dietary changes. Because FAs provide information over an extended period of time (e.g., 2–4 weeks) versus the most recent meal as with most traditional methods of diet sampling (Barrett et al. 2007), it may be possible to get information about diets during non-breeding seasons (e.g., describe the diet during migration by sampling birds at arrival to breeding sites). However, in order to do this, controlled feeding studies must be performed to determine the period of time over which the diet can be back calculated. This non-lethal technique of FA signature analysis may be particularly useful in estimating the diets of species of conservation concern.

In order to account for consumer metabolism, calibration coefficients (CCs) are calculated for individual FAs by feeding consumers a known diet until complete turnover of FAs is thought to have occurred, at which point consumer adipose tissue FA composition should be as similar to that of diet as it ever will be. These CCs (the ratio of a given FA in the consumer relative to that in the long-term diet) are then used in the QFASA model to weight individual FAs in subsequent diet estimation studies (Iverson et al. 2004, 2006, 2007). Related to the concept of CCs, is selecting appropriate subset(s) of FAs for use in diet estimations, as not all FAs provide equal information about diet due to consumer metabolism, FA origin (predominantly dietary versus biosynthesis), and levels found in tissue (for instance, trace levels may not be correctly identified) (Iverson et al. 2004). Finally, rates of FA turnover in adipose tissue may also be affected by consumer metabolism (e.g., Williams et al. 2009). Thus, in order to interpret the time frame of the estimated diet, turnover rates of dietary FAs in consumer adipose tissue should be estimated in a controlled experimental study. In this study, we developed CCs for individual FAs to account for eider lipid metabolism. We used simulation studies using the captive eider diet to evaluate the reliability with which diet items could be distinguished in the QFASA model. Finally, we tested the QFASA model, FA subsets, and CCs by estimating the diets of experimentally fed captive eiders.

Materials & Methods

Samples for this study were collected during 2007-2008. A summary of sample collection methodologies follows.

Captive Feeding Trials

The feeding study took place between 19 September 2007 and 16 January 2008 with 8 adult male spectacled and 8 adult male Steller’s eiders housed at the Alaska SeaLife Center (ASLC) in Seward, Alaska. Birds were fed a diet of approximately 95% Mazuri® sea duck formula (Purina Mills, St. Louis, MO, USA) and the remaining 5% from supplements of Antarctic krill (*Euphausia superba*), Atlantic silverside (*Menidia menidia*), Atlantic surf clam (*Spisula solidissima*), blue mussel (*Mytilus edulis*), and California market squid (*Loligo opalescens*). Birds were segregated by species and housed in outdoor pens at ambient conditions and natural saltwater habitats, with females of the same species.



Photo 1: Feeding of captive birds.

Sixty-nine days before the start of the feeding trials, the diet composition of spectacled eiders consisted of approximately 88% Mazuri®, 3% krill, 4% silverside, 1% clam, and 4% mussel (Diet 1); for Steller's eiders the diet consisted of approximately 88% Mazuri®, 1% krill, 3% silverside, 1% clam, and 7% mussel (Diet 1). Mazuri® was given every day while supplements of clam, krill, mussel and silverside were fed on a weekly rotating schedule. Feeding trials started on

Day 0, and all eiders were biopsied (see below). Spectacled eiders were switched to Diet 2 which consisted of 56% krill and 44% Mazuri®, while Steller's eiders were switched to Diet 2 which consisted of 66% krill and 34% Mazuri® for 21 days. After the Day 21 biopsy, spectacled eiders were switched to Diet 3 which consisted of 52% silverside and 48% Mazuri®, and Steller's eiders were switched to Diet 3 which consisted of 66% silverside and 34% Mazuri® for 29 days. Final biopsies were taken on Day 50. Krill and silverside were fed in the morning to ensure that these diet items would be consumed. Krill and silverside were sometimes hand fed to the birds for training purposes and the remaining allotment placed in feeding bowls accessible to all birds in the pen. Mazuri® was placed in accessible feeding bowls in the afternoon. Daily amounts of each diet item fed and consumed per flock were recorded. Subsamples of all diet items were collected twice each week throughout the feeding study, placed in airtight plastic bags, and stored frozen until analysis.

Biopsy Sampling

A live biopsy technique according to Iverson et al. (2007) with addition of a local anesthetic was used to obtain synsacral adipose tissue samples from captive eiders. Between 0.02 and 0.15 g of adipose tissue was excised using forceps and a scalpel blade, and placed in aluminum foil. Samples were placed in chloroform containing 0.01% BHT (an antioxidant) in glass centrifuge tubes with Teflon-lined caps. Lipid extraction and transesterification was performed the same day the biopsy was taken.



Photo 2: lipid extractions

Laboratory Analysis

Lipids were extracted from eider adipose tissue samples and homogenates of whole individual mussel (without shell, $n = 15$) and silverside ($n = 39$) using a modified Folch technique (Folch et al. 1957, Iverson et al. 2001). Individual krill and Mazuri® pellets were pooled into samples so that the combined sample weight was

approximately 1.5 g; between two and six individual krill were pooled per sample ($n = 39$) and between 14 and 17 individual Mazuri® pellets were pooled per sample ($n = 23$). Clams were already shelled and pre-cut into 2.0 – 4.0 g pieces and shipped to ASLC in frozen blocks. Each pre-cut piece was analyzed as an individual sample ($n = 15$). FA methyl esters (FAME) were prepared using an acidic transesterification (Budge et al. 2006). The presence of fatty alcohols resulting from the transesterification of wax esters in diet items was determined using thin layer chromatography. In order to account for wax esters in diets, the alcohols of which are deposited as their corresponding FA in the adipose tissue (Budge and Iverson 2003), wax ester alcohols were converted to their respective FAs according to Budge et al. (2006). FA methyl esters were quantified using temperature-programmed gas liquid chromatography on a Perkin Elmer Autosystem II Capillary FID gas chromatograph fitted with a 30m x 0.25 mm id column coated with 50% cyanopropyl-methylpolysiloxane (DB-23) and linked to a computerized integration system (Varian Galaxie software) according to Iverson et al. (2002). Each chromatogram was manually assessed for correct peak identification and reintegrated, where necessary.

Qualitative Analysis of Diet Items and Eider FA

Multivariate analyses were conducted using discriminant function analysis (DFA) followed by classification using a jack-knifing procedure (leave-one-out cross-validation) to assess how well FAs separate 1) diet items, 2) eider biopsies by species, diet and biopsy date, and 3) diet items and eider biopsies. Given restrictions on variables used in DFA ($n-1$ of smallest sample size), we selected 14 diet item FAs which had the highest variance and overall means of the 72 total FAs identified (14:0, 16:0, 16:1n-7, 18:0, 18:1n-9, 18:1n-7, 18:2n-6, 18:3n-3, 18:4n-3, 20:1n-9, 20:1n-7, 20:5n-3, 22:5n-3, 22:6n-3), and 7 eider adipose tissue FAs with the highest observed variance across eider groupings (14:0, 16:0, 16:1n-7, 18:0, 18:1n-9, 18:2n-6, 22:6n-3), and 7 FAs with the highest observed variance and means across all diet items and eider samples (14:0, 16:0, 16:1n-7, 18:1n-9, 18:2n-6, 20:5n-3, 22:6n-3). The subsets of FAs used in these analyses are also known to be derived dominantly or solely from dietary intake (Iverson et al. 2004). Proportional representation of each FA was recalculated for this subset and arcsine square-root transformed prior to analysis. We used quadratic DFA because the within-group covariance matrices in our dataset were not homogenous (Bartlett's test, $P < 0.001$), which may result in poorer separation between groups, although there is little evidence that moderate violation significantly alters cross-validated classification success (McGarigal et al. 2000).

CC and FA Subset Selection

An integral part of the QFASA model is the use of CCs to account for predator lipid metabolism by weighting individual FAs according to their tissue deposition relative to diet (Iverson et al. 2004, 2006, 2007). We calculated eider CCs by dividing levels of individual FA in eiders from biopsy 1 (Day 0) by levels of those same FA in Diet 1, which was fed to the eider for 69 days before this first biopsy (sensu Iverson et al. 2004). Not all FAs provide equal information about diet due to consumer metabolism, origin (predominantly dietary versus biosynthesis), and levels found in tissue (trace levels may not be correctly identified) (Iverson et al. 2004). Therefore, it is important to select an appropriate FA subset when using QFASA to estimate diet. Twenty-five new FA subsets

were developed based on two published subsets that include FAs that could arise from dietary origin alone (Dietary, 33 FAs) or include the Dietary subset along with 8 FAs whose levels in a consumer are also influenced by diet (Extended Dietary, 41 FAs) (Iverson et al. 2004). FAs were omitted from the published subsets in order of highest variability observed in CCs (e.g., Nordstrom et al. 2008). Twenty-seven subsets of the 72 FAs identified were tested in our model optimization exercise. Relative proportions of individual FA in each new subset were normalized to a sum of 100%. Subsets were used in the QFASA model and performance was evaluated using all biopsies and all diets from all eiders. The FA subset that provided the lowest overall sum of the squared errors (SSE) of diet was determined to be the optimal subset for our QFASA model, where $SSE = (\text{actual proportion of diet item} - \text{model estimate of diet item})^2$ summed across all diet items for Diet 1, 2, and 3.

QFASA Model

To evaluate the reliability with which diet items could be distinguished in the model, we first performed a number of simulations using the captive eider diet database. These simulations were performed without CCs to assess the ability to estimate diet composition based only on differentiating and quantifying diet items by their FA signatures (sensu Iverson et al. 2004). We constructed five pseudo diets: Diets 1-3 were based on the actual Diets 1-3 that the eiders consumed. Diet 4 represented a marine diet without the commercially processed Mazuri®. Diet 5 represented a diet comprised of only bivalves, which are the primary diet item for spectacled eiders wintering in the Bering Sea (Lovvorn et al. 2003). Simulations were used to evaluate how the accuracy of our estimates was affected by diets and FA subsets. Simulations were performed 1000 times for each constructed diet. Prey-on-prey simulations were also performed to determine how well each individual diet item could be differentiated as itself from all other diet items in the diet library. These were done by taking each diet item in the library and dividing the samples into two sets: a modeling set and a simulation set. The selected diet item was modeled with the modeling set and all other diet items in the prey library. The simulation was performed 1000 times for each diet item. Details of the simulation procedures are provided in Appendix B in Iverson et al. (2004).

The diets of captive eiders were estimated using the QFASA model developed by Iverson et al. (2004). QFASA estimates the diet by estimating the weighted the mixture of FA signatures of diet items that most closely resemble that of the eider's adipose tissue after accounting for metabolism effects, and then uses the relative fat content of each diet item to translate the signature mix into a diet estimate. Calculated CCs were also used in the model to account for eider lipid metabolism.

Results

Qualitative Separation of DietItems and EiderBiopsies

The FA signatures of the five diet items fed to captive eiders differed markedly (Fig. 1a). For example, compared to the other diet items Mazuri® was extremely high in 18:2n-6, which is relatively rare in marine systems and probably reflected corn in the commercially made sea duck pellets. Mazuri® was also lower in 20:5n-3 and 22:6n-3,

which are relatively abundant in marine systems. Levels of 14:0, 16:0, and 18:1n-7 were higher in krill than other prey items. The two bivalves, clams and mussels, were much lower in 18:1n-9 and higher in 20:1n-7 than other diet items. Silversides had the highest levels of 22:5n-3 and 22:6n-3. These differences in levels of prey FAs were reflected in the eider biopsy signatures at Day 0, 21, and 50 (Figs. 1b,c). Although Mazuri® remained a large proportion of the diet throughout the experiment, the influence of krill and then silverside FAs was evident at Days 21 and 50. For example, levels of Mazuri® were relatively higher in 18:0, 18:1n-9, and 18:2n-6, all of which dropped after the introduction of krill and silverside. Likewise, the levels of krill were relatively higher in 20:5n-3 and 22:6n-3; and levels of silverside were relatively higher in 20:5n-3, 22:5n-3, and 22:6n-3, all of which increased in biopsies at Days 21 and 50 after the introduction of krill and silverside, respectively. Finally, silverside was relatively higher in 16:1n-7, 22:5n-3, 22:6n-3 and lower in 14:0, 16:0, 18:1n-7, 18:4n-3, and 20:5n-3 than krill and these changes in FA levels were reflected in the Day 50 biopsies after the birds had been switched to increased amounts of silverside. Thus, changes in dietary FAs were tracked in eider adipose tissue (Fig. 1).

Diet items were clearly differentiated using DFA. The 14 FAs selected for the DFA of diet items accounted for 84.4% of the total FAs identified in all diet items. Discriminant function (DF) 1 and DF 2 accounted for 90.8% of the variation (Fig. 2a). Diet samples were correctly classified to species with 100% of original grouped cases and 80% of cross-validated grouped cases (mussels were misclassified as silversides using the jack-knife procedure).

Eider adipose tissue samples were also clearly differentiated using DFA. The 7 FAs selected for the eider DFA accounted for 86.2% of the total FA identified in all eider biopsies. DFs 1 and 2 accounted for 93.2% of the total variance (Fig. 2b). Eider biopsies were correctly classified to biopsy day but also to species with 97.9% of both original and cross-validated grouped cases. Thus, both species behaved in a similar manner in response to diet shifts, but additionally, the slight differences in the diet composition between the species were detected (Fig. 2b). One spectacled eider was observed to not consume much krill during Days 0-20 and consistent with this, his Day 21 biopsy was more similar to his Day 0 biopsy (Fig. 2b).

A DFA of diet items and eider biopsies combined using the 7 FAs selected across all diet items and eider samples confirmed that eider biopsy FA signatures were most similar to Mazuri® FA signatures, which was consistent with Mazuri® being the major diet item throughout the experiment (Fig. 2c). However, after 21 days of increased krill in their diet, the Day 21 biopsy signatures had shifted towards the krill signatures. Similarly, after 29 days of increased silverside in their diet, the eider Day 50 biopsy signatures had shifted towards the silverside signatures (Fig. 2c). DFs 1 and 2 accounted for 88.9% of the variation (Fig. 2c). Diet items and eider biopsies were correctly classified to their own groups with 100% of original grouped cases and 45.5% cross-validated grouped cases (jack-knife procedure).

Calibration coefficients (CCs) and FA subset selection

The CC values estimated from the eiders after sixty-nine days on the same diet (Day 0) ranged from 0.03 for 22:2d7, 13 and 22:2d7,15 to 4.26 for 21:5n-3 (Fig. 3). CC values of 1.0 indicate a 1:1 ratio of FA deposition from diet (Fig. 3). CCs were generally similar between the two eiders, except for three CCs (those for 16:1n-9, iso 17:0, 21:5n-3) that differed substantially. However, 16:1n-9 and iso 17:0 are not included in either the Dietary or Extended Dietary FA sets, and 21:5n-3 is not included in any of the modified FA subsets used in the diet modeling. CCs calculated for eiders differed from those calculated for common murre (*Uria aalge*) chicks. The CCs which exhibited the greatest deviance between eider and murre included the same three FAs mentioned above and most of the typical marine “dietary” FAs, that is, the long-chain mono- (20:1 and 22:1 isomers) and polyunsaturated FAs, except 20:5n-3 and 22:6n-3 (Fig. 3).

The performance of FA subsets in the QFASA model was dependent on eider species and diet (Table 1). Of the 25 other FA subsets modified from the published Dietary and Extended Dietary FA subsets, test 18 (Reduced A) and test 21 (Reduced B) FA subset had the lowest total SSE across all diets and were determined to be the best FA subsets for modeling diets for spectacled and Steller’s eiders, respectively (Table 1). The Dietary and Extended Dietary FA subsets from Iverson et al. (2004) were also used in the simulations and modeling for comparison.

QFASA Model

The five specified pseudo diets were well-estimated using QFASA simulations (Table 2). The Reduced A, B, Dietary, and Extended Dietary FA subsets produced similar results, however the Extended Dietary subset estimated proportions of individual species within each diet generally closer to the true values (Table 2). Furthermore, the prey-on-prey simulations showed that the diet items in the captive eider diet database were correctly identified as themselves 93% - 100% out of 1000 simulations (Fig. 4). Mussels had the lowest identification rate with the widest distribution, while clams and Mazuri® had the highest identification rate (Fig. 4). Although mussels were misclassified as other diet items (< 7% as clam, krill, and Mazuri®), in general the diet items in this study were reliably distinguished from one another.

We used the Reduced A, B, Dietary, and Extended Dietary FA subsets in the model along with species-specific CCs calculated for spectacled and Steller’s eiders. For comparison, we also modeled eider diets using the Extended Dietary subset and the common murre chick CCs from Iverson et al. (2007). Our results showed that the QFASA model estimated the relative proportion of diet items in Diet 1 at Day 0 for both eider species generally very well no matter which subset and CCs were used, although exact levels varied among subsets and CCs (Fig. 5). From Day 0 to 20, spectacled eiders consumed a diet of 56% krill and 44% Mazuri®, and Steller’s eiders consumed a diet of 66% krill and 34% Mazuri®. The model correctly predicted a substantial proportion of krill coming into the diet by Day 21 (Fig. 5), with the predicted dietary percentages of krill averaging 24% in spectacled eiders and about 33% in Steller’s eiders. From Day 21 to 49, spectacled eiders consumed a diet of 52% silverside and 48% Mazuri®, and Steller’s eiders consumed a diet of 66% silverside and 34% Mazuri®. Again, the model accurately predicted a substantial proportion of silverside coming into the diet at Day 50, with some

residual krill remaining from Diet 2. The percentage of silverside predicted in diet averaged 21% in spectacled eiders and 36% in Steller's eiders. Commensurate with the increases in krill or silverside at both Day 21 and 50, the model correctly estimated a predictable decrease in Mazuri® in Diets 2 and 3. Compared to the actual diets fed, the lower levels of krill predicted in Diet 2 and the lower levels of silverside along with residual krill in Diet 3, suggests that complete turnover of FA from a switch in diet (Diet 2 and Diet 3) was not complete at either 21 or 29 days.

The Extended Dietary FA subset and eider CCs overestimated the proportion of mussel in Diet 2 (Day 21) and Diet 3 (Day 50) (Fig. 5). Birds were not fed mussel in Diets 2 or 3 and the amount of mussel fed in Diet 1 was relatively lower than the other prey items (4% in spectacled, 7% in Steller's eiders). However, the amount of mussel predicted using the Extended Dietary FA subset and eider CCs, especially in Diet 3 (Day 50) was much greater than the amount that was consumed by either eider species (Fig. 5). Additionally, the Extended Dietary FA subset and common murre chick CCs overestimated the amount of silverside in Diet 2 (Day 21) (Fig. 5).

Table 1. Mean estimates of diet simulations over 1000 simulation runs for each of the five pseudo diets using the Reduced A, B, Dietary and Extended Dietary fatty acid (FA) subsets. Dietary and Extended Dietary subsets from Iverson et al. (2004).

Diet	Species	Specified Diet	Reduced A		Reduced B		Dietary FA		Extended Dietary FA	
			Estimate	1 SD	Estimate	1 SD	Estimate	1 SD	Estimate	1 SD
1	Mazuri	0.80	0.82	0.012	0.81	0.013	0.82	0.004	0.80	0.005
	krill	0.05	0.03	0.020	0.04	0.021	0.03	0.014	0.05	0.003
	silverside	0.05	0.05	0.002	0.06	0.002	0.04	0.006	0.05	0.004
	clam	0.05	0.05	0.027	0.05	0.027	0.06	0.008	0.05	0.005
	mussel	0.05	0.04	0.006	0.04	0.006	0.06	0.016	0.05	0.005
2	Mazuri	0.70	0.78	0.011	0.77	0.012	0.79	0.004	0.70	0.003
	krill	0.30	0.20	0.017	0.21	0.018	0.21	0.005	0.30	0.004
	silverside	0.00	0.00	0.001	0.00	0.001	0.00	0.003	0.00	0.002
	clam	0.00	0.02	0.021	0.02	0.021	0.00	0.001	0.00	0.001
	mussel	0.00	0.00	0.005	0.00	0.005	0.00	0.002	0.00	0.002
3	Mazuri	0.70	0.68	0.005	0.67	0.006	0.75	0.006	0.70	0.006
	krill	0.00	0.00	0.001	0.00	0.002	0.00	0.004	0.00	0.003
	silverside	0.30	0.31	0.002	0.33	0.002	0.24	0.008	0.29	0.007
	clam	0.00	0.00	0.004	0.00	0.004	0.00	0.003	0.00	0.003
	mussel	0.00	0.00	0.002	0.00	0.002	0.00	0.002	0.00	0.003
4	Mazuri	0.00	0.01	0.010	0.01	0.014	0.00	0.003	0.00	0.003
	krill	0.25	0.20	0.033	0.20	0.032	0.18	0.021	0.25	0.011
	silverside	0.25	0.30	0.007	0.30	0.008	0.21	0.016	0.25	0.013
	clam	0.25	0.29	0.030	0.28	0.034	0.31	0.018	0.25	0.018
	mussel	0.25	0.21	0.019	0.20	0.020	0.29	0.027	0.25	0.021
5	Mazuri	0.00	0.01	0.016	0.02	0.022	0.00	0.005	0.00	0.006
	krill	0.00	0.04	0.039	0.05	0.040	0.01	0.017	0.00	0.004
	silverside	0.00	0.00	0.005	0.00	0.005	0.00	0.006	0.00	0.005
	clam	0.50	0.54	0.035	0.53	0.039	0.50	0.024	0.50	0.027
	mussel	0.50	0.40	0.025	0.39	0.027	0.48	0.032	0.49	0.027

Table 2. Sum of the squared errors (SSE) for 7 of 27 fatty acid (FA) subsets for Diets 1, 2 and 3 for spectacled and Steller's eiders. $SSE = (\text{actual proportion of diet item} - \text{model estimate of diet item})^2$ summed across all diet items. SSE for Diets 1, 2 and 3 separately. Total = sum of SSE of Diets 1, 2 and 3. Dietary and Extended Dietary FA subsets are from Iverson et al. (2004). Values in **bold italicized** print indicate the lowest SSE. Test 18 (Reduced A) and test 21 (Reduced B) FA subsets had the lowest overall SSE for spectacled and Steller's eiders, respectively, and were determined to be the subsets that give the best overall model estimates of diet. See Appendix 1 in publication for FAs included in each subset.

FA subset	Diet 1		Diet 2		Diet 3		Total	
	spectacled	Steller's	spectacled	Steller's	spectacled	Steller's	spectacled	Steller's
Extended Dietary	0.00008	0.00003	0.29729	0.44574	0.16514	0.22901	0.46251	0.67478
test13	0.00018	0.00001	0.31072	0.48584	0.17783	0.24764	0.48873	0.73350
Dietary	0.00055	0.00027	0.16345	0.15921	0.17475	0.15286	0.33875	0.31234
test17	0.00054	0.00039	0.16258	0.15927	0.17276	0.15154	0.33588	0.31121
test18 (Reduced A)	0.00070	0.00040	0.15881	0.13921	0.17627	0.15590	0.33578	0.29550
test21 (Reduced B)	0.00080	0.00053	0.15889	0.13954	0.17739	0.15539	0.33709	0.29546
test23	0.00098	0.00052	0.15878	0.13949	0.17940	0.15574	0.33916	0.29575

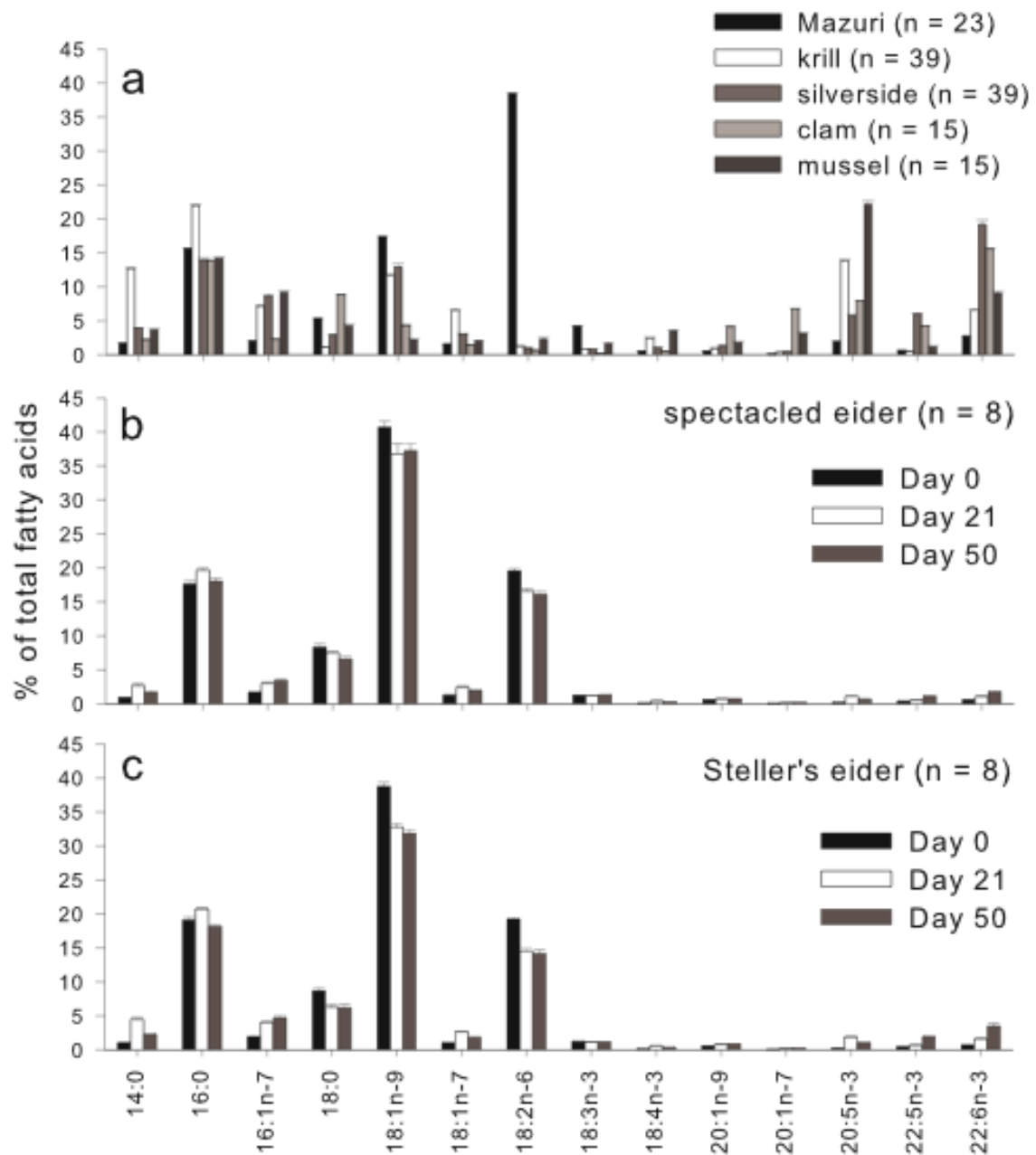


Figure 1. Select fatty acids (FAs, 14 out of 72 identified) with the largest overall variance and mean of total FAs and that illustrate characteristic differences in patterns: (a) among the 5 diet items fed to captive eiders, (b) in adipose tissue of spectacled eiders at Day 0, 21, and 50, and (c) in adipose tissue of Steller's eiders at Day 0, 21, and 50. Means + 1 SE.

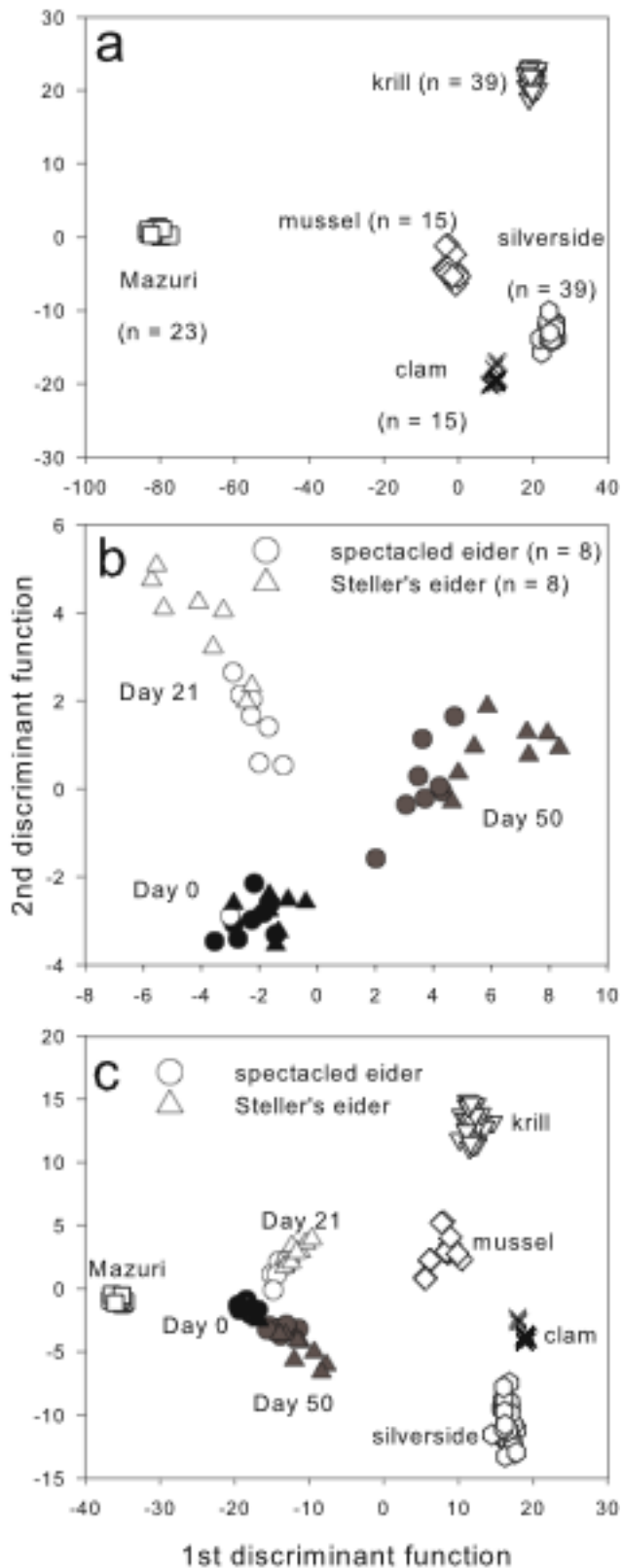


Figure 2. Discriminant function analysis plots for: (a) Diet items based on 14 fatty acids (FAs) (14:0, 16:0, 16:1n-7, 18:0, 18:1n-9, 18:1n-7, 18:2n-6, 18:3n-3, 18:4n-3, 20:1n-9, 20:1n-7, 20:5n-3, 22:5n-3, 22:6n-3). Discriminant functions 1 and 2 accounted for 78.2% and 12.6% of the total variance, respectively. (b) Eider biopsies based on 7 FAs (14:0, 16:0, 16:1n-7, 18:0, 18:1n-9, 18:2n-6, 22:6n-3). Discriminant functions 1 and 2 accounted for 67.3% and 25.9% of the total variance, respectively. (c) Diet items and eider biopsies based on 7 FAs (14:0, 16:0, 16:1n-7, 18:1n-9, 18:2n-6, 20:5n-3, 22:6n-3). Discriminant functions 1 and 2 accounted for 74.4% and 14.5% of the total variance, respectively.

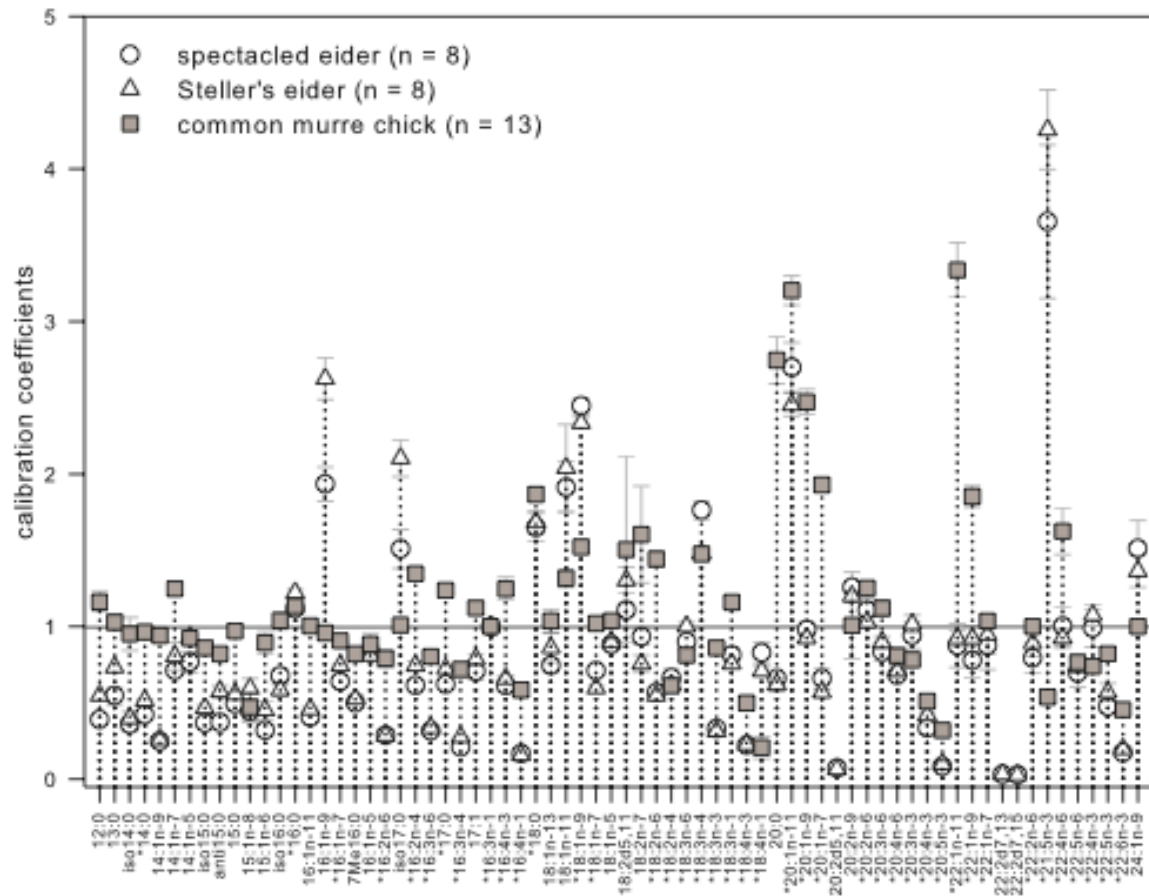


Figure 3. Spectacled and Steller's eider calibration coefficients (CCs) calculated from birds fed on a constant diet for 69 days (means $\pm$ 1 SE). Average common murre CCs determined from a previous study (Iverson et al. 2007) are presented for comparison. * in front of fatty acids (FAs) denotes FAs used in simulations and modeling. The 1:1 line denotes the degree to which a given FA in the bird is present in the same amount as that consumed in diet.

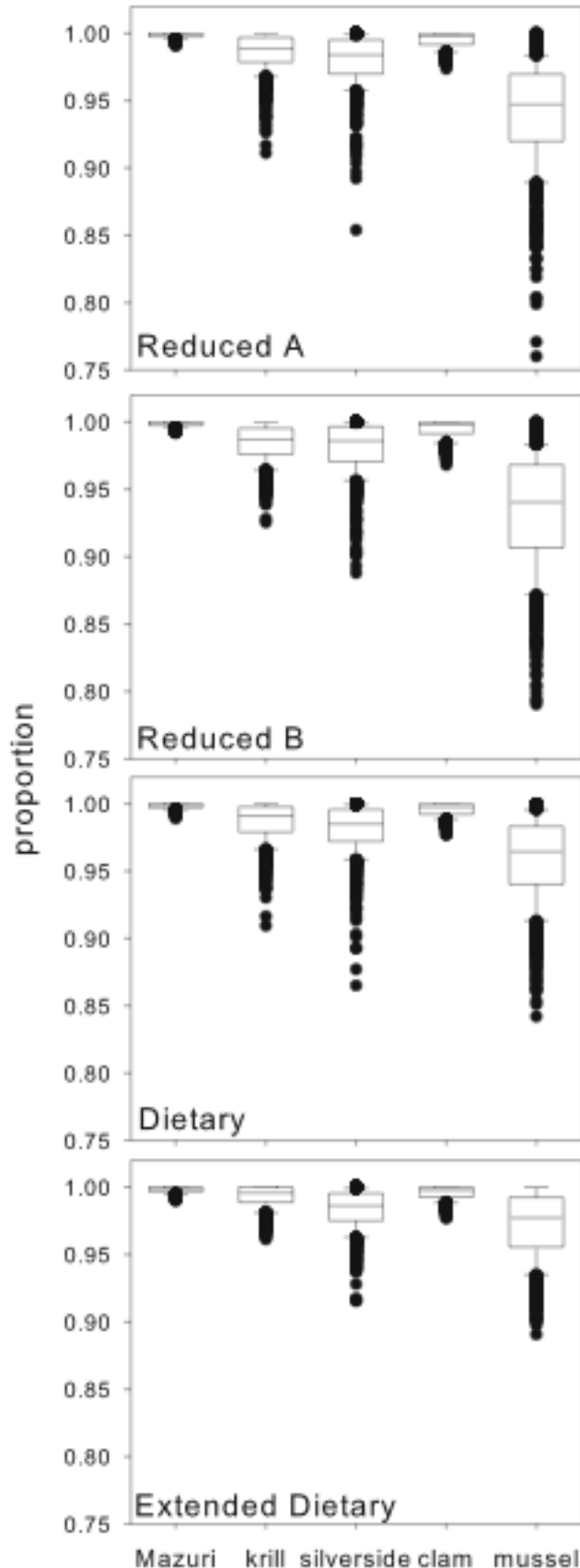


Figure 4. Results for prey-on-prey simulation using the Reduced A, B, Dietary, and Extended Dietary fatty acid (FA) subsets which shows the ability of the QFASA procedure to identify the FA profile for a given food item from the FA profiles of the other food items in the diet library. Dietary and Extended Dietary subsets from Iverson et al. (2004). Estimates are represented in box plots, as the median (middle horizontal bar), the 25th percentile (lower bar), and the 75th percentile (top bar) of the data distribution (i.e., the box contains 50% of the data). Dots represent outliers defined as being any value greater (or less) than 1.5 times the interquartile range (75th percentile – 25th percentile) above the 75th (or below the 25th) percentile. Results are presented as the proportion of prey item correctly identified as itself from all other prey items in 1000 trials. For Reduced A FA subset: 100% of Mazuri®, 99% of krill, 98% of silversides, 100% of clams, and 94% of mussels were correctly identified as themselves. For Reduced B FA subset: 100% of Mazuri®, 98% of krill, 98% of silversides, 99% of clams, and 93% of mussels were correctly identified as themselves. For Dietary FA subset: 100% of Mazuri®, 99% of krill, 98% of silversides, 100% of clams, and 96% of mussels were correctly identified as themselves. For Extended Dietary FA subset: 100% of Mazuri®, 99% of krill, 98% of silversides, 100% of clams, and 97% of mussels were correctly identified as themselves.

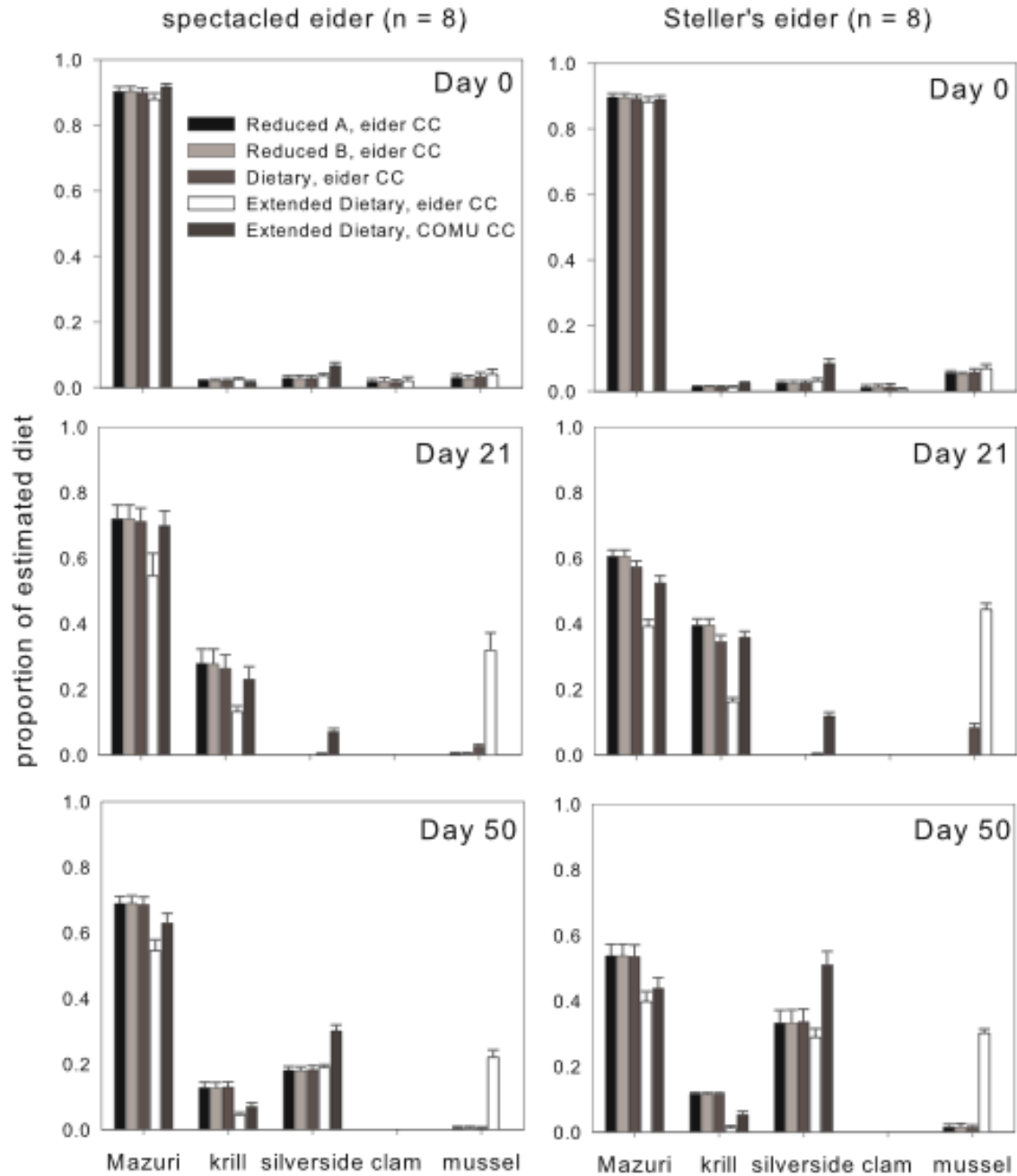


Figure 5. QFASA estimates using the Reduced A, B, Dietary, Extended Dietary fatty acid (FA) subsets for Diets 1, 2, and 3 at Days 0, 21 and 50 for spectacled and Steller's eiders. Species-specific calibration coefficients (CCs) were used in the model. Common murre CCs were also used with the Extended Dietary FA subset for comparison. Dietary and Extended Dietary FA subsets and common murre CCs from Iverson et al. (2007). Actual diets consumed for spectacled eiders Diet 1 (Day 0): 88% Mazuri®, 3% krill, 4% silverside, 1% clam, 4% mussel; Diet 2 (Day 21): 44% Mazuri®, 56% krill,; Diet 3 (Day 50): 48% Mazuri®, 52% silverside. Actual diets consumed for Steller's eiders Diet 1: 88% Mazuri®, 1% krill, 3% silverside 1% clam, 7% mussel; Diet 2: 34% Mazuri®, 66% krill; Diet 3: 34% Mazuri®, 66% silverside. Means + 1 SE.

Discussion

The results from this captive feeding study demonstrated that for both species of eiders, changes in diet were clearly discriminated using adipose tissue FAs. Captive eider diet items were differentiated from each other using both multivariate DFA and more rigorous QFASA simulations. Our results also demonstrated that the response of adipose tissue FAs in both species of eiders to dietary intake was predictable. Relative dietary percentages of these five diet items were accurately quantified by QFASA when those percentages had been constant for 10 weeks. Three weeks after a shift between diets with very distinct FA profiles, the shift was reflected in tissue FA but the estimated dietary percentages were lower from the true percentages in diet, reflecting an integration of previous diet in the estimations and confirming that turnover was not complete within the 21-29 day study periods. Most dietary subsets used in QFASA yielded very similar estimates of dietary percentages, although one subset appreciably underestimated the percentages of Mazuri® and krill and overestimated the percentage for mussels. Comparison of results for a suite of dietary subsets should identify such problems. CCs for the two eider species fed a diet of 88% Mazuri with supplements of krill, silversides, and bivalves were generally very similar and yielded similar results in QFASA estimates. The few instances where CCs differed substantially between the two species (16:1n-9, iso 17:0, 21:5n-3), were cases where the FAs were present in only trace amounts in diet items and eider adipose tissue and thus CCs were variably estimated. CCs for common murre chicks fed entirely Atlantic silversides appeared to differ from those of the eiders, however, when the murre CCs were applied to eiders, dietary estimates remained similar except for slower loss of silverside at Day 21 and more rapid incorporation of FAs from silversides at Day 50.

Conclusions

We conclude that applying minimally invasive QFASA techniques to determine the diets of spectacled and Steller's eiders during different life history stages can provide useful information about their diets and help identify critical habitats to support conservation of these threatened breeding populations. Nevertheless, we advise researchers to apply our QFASA results with some caution in that the captive eider CCs, turnover rates, and FA subsets may require further consideration in application to wild birds. Further work is required to calculate complete turnover rates of FAs from a natural diet, investigate the sensitivity of CCs and FA subsets to diets, and of the QFASA model to the CCs and FA subsets used. However, despite this sensitivity our study showed that QFASA accurately estimated Diet 1 and accurately indicated diet switches in captive eiders, and that diets can be estimated over an extended time period. Thus, our understanding of diet can be back-tracked to more than a month in a captive feeding eider. Regardless of model limitations, this study provides an important basis for further validation studies and interpretation of adipose tissue FA data from wild populations.

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