

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

By

Rebekka Federer

RECOMMENDED:

Dr. Matthew Wooller

Dr. Daniel Esler

Dr. Tuula Hollmén, Advisory Committee Chair

Dr. Katrin Iken
Head, Program in Marine Science and Limnology

APPROVED:

Dr. Denis Wiesenburg
Dean, School of Fisheries and Ocean Sciences

Dr. Lawrence Duffy, Dean of the Graduate School

Date

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

A
THESIS

Presented to the Faculty
of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

By

Rebekka N. Federer, B.S.

Fairbanks, Alaska

August 2009

Abstract

The spectacled eider (*Somateria fischeri*) was listed as threatened under the Endangered Species Act in 1993, and potential threats to population recovery include changes in the marine prey abundance and availability. Therefore, development of diet assessment techniques has been listed as an eider recovery task. Stable isotopes have been used to evaluate foraging ecology and nutrient allocation to reproduction in birds. Application of this technique requires knowledge of how stable isotope signatures of animal tissues differ from their diet, referred to as isotopic fractionation, and these values can be determined experimentally using captive populations. I established stable isotopic fractionation factors for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from diet to egg components, down feathers, contour feathers, cellular blood, blood plasma, and fat of captive spectacled eiders. Sensitivity analyses indicate that choice of isotopic fractionation values from eggs of different species could considerably alter model conclusions. Therefore, I incorporated isotopic fractionation factors from spectacled eider eggs into two published sea duck nutrient allocation models that previously used these values from falcons (*Falco* spp.) to assess differences in model conclusions. Results from these studies provide further resources to understand foraging and nutrient transfer in eiders and may offer more accurate estimates for sea duck models.

Table of Contents

	Page
Signature Page.....	i
Title Page.....	ii
Abstract.....	iii
Table of Contents.....	iv
List of Figures.....	vii
List of Tables.....	viii
List of Appendices.....	x
Dedication.....	xi
Acknowledgements.....	xii
General Introduction.....	1
Sea Duck Declines and Spectacled Eider Background.....	1
Thesis Background.....	3
Thesis Outline and Goals.....	8
References.....	11
Chapter 1 Carbon and Nitrogen Stable Isotope Fractionation Factors for Quantifying Spectacled Eider (<i>Somateria fischeri</i>) Nutrient Allocation to Egg Production.....	18
1.1 Abstract.....	18
1.2 Introduction.....	19
1.3 Materials and Methods.....	22
1.3.1 Study Species.....	22
1.3.2 Diet and Reproductive Tissue Collection.....	22
1.3.3 Laboratory Analyses.....	24
1.3.4 Calculations and Statistical Analyses.....	25
1.4 Results.....	27
1.4.1 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Isotope Analyses for Diet Items.....	27
1.4.2 $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ for Egg Components and Down Feathers.....	27
1.4.3 Species Comparison for Isotopic Egg Fractionation and Theoretical Modeling.....	29

1.4.4	Differentiating Macronutrient Sources in $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$	30
1.5	Discussion.....	31
1.5.1	Spectacled Eider Egg and Down Isotopic Fractionation	31
1.5.2	Potential Factors Contributing to Variation in Spectacled Eider $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$	32
1.5.3	Species Differences in $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$	34
1.5.4	Differentiating Macronutrient Sources	37
1.6	Acknowledgements.....	37
1.7	References.....	38
Chapter 2 Stable Carbon and Nitrogen Isotope Fractionation Factors from Diet to Cellular Blood, Blood Plasma, Feathers, and Subcutaneous Fat in Spectacled Eiders (<i>Somateria</i> <i>fischeri</i>)		50
2.1	Abstract.....	50
2.2	Introduction.....	51
2.3	Materials and Methods.....	54
2.3.1	Study Species.....	54
2.3.2	Diet and Adult Tissue Collection.....	55
2.3.3	Laboratory Analyses	57
2.3.4	Calculations and Statistical Analyses	58
2.4	Results.....	59
2.4.1	$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Isotope Analyses for Diet Items	59
2.4.2	$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Isotope Fractionation Factors in Adult Tissues.....	60
2.5	Discussion.....	60
2.5.1	Stable Isotope Fractionation of Spectacled Eider Blood Components .	61
2.5.2	Stable Isotope Fractionation of Spectacled Eider Feathers.....	62
2.5.3	Stable Isotope Fractionation of Spectacled Eider Subcutaneous Fat....	63
2.5.4	Conclusions and Future Recommendations.....	64
2.6	Acknowledgements.....	65
2.7	References.....	66

Chapter 3 Evaluating Sea Duck Nutrient Allocation to Reproduction Model Outcomes Using Diet to Egg Isotopic Fractionation Factors ($\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$) Derived from Captive Spectacled Eiders (<i>Somateria fischeri</i>)	73
3.1 Abstract	73
3.2 Introduction.....	74
3.3 Materials and Methods.....	77
3.4 Results.....	78
3.5 Discussion	80
3.6 Acknowledgements.....	83
3.7 References.....	84
3.8 Appendices.....	88
General Conclusions and Future Directions	91
Thesis Conclusions	91
Future Directions	94
References.....	98

List of Figures

	Page
Figure 1.1 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.	46
Figure 1.2 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 CO ₂), 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.....	47
Figure 2.1 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 subcutaneous fat 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 the diet prior to biopsy 2.	72

List of Tables

	Page
Table 1.1 Captive female spectacled eider start and end lay dates, and number of eggs and down feathers from embryos in sampling years 2006, 2007, and 2008.....	43
Table 1.2 Average ($\pm$ SD) carbon and nitrogen stable isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) for all years based on proportional source contribution (Mazuri®:natural food) for diet lipids, whole diet, and lipid-free diet.	43
Table 1.3 Whole diet to egg component isotopic fractionation factors (mean $\text{‰} \pm$ SD) for captive spectacled eiders. Results of ANOVA tests for significant differences among years for each isotope and Bonferroni multiple comparison t-tests are indicated by superscripts where means that have a superscript in common are not significantly different ($p>0.05$).	44
Table 1.4 Whole diet to egg isotopic fractionation factors (mean $\text{‰} \pm$ SD) for spectacled eiders and other avian species described in Hobson 1995. ANOVA statistics reported for all species and only significant differences from spectacled eiders (SPEI) are listed in parentheses (Japanese quail=JAQU, 2 clutches for mallard=MALL 1&2, peregrine falcon=PEFA, gyrfalcon=GYFA, and prairie falcon=PRFA; see Hobson 1995 for differences between other species).	45
Table 2.1 Average ($\pm$ SD) carbon and nitrogen stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of pre- and post-breeding diet in 2007 and 2008 based on proportional source contribution (Mazuri®:natural food) for diet lipids, whole diet, and lipid-free diet.....	71
Table 2.2 Average ($\pm$ SD) carbon stable isotopes ($\delta^{13}\text{C}$) of diet lipids (baseline diet and 2 diet switches) and subsequent subcutaneous fat biopsies collected over 3 time periods. Isotopic fractionation factors between diet and subcutaneous fat along with sample size (n) are noted in parentheses (*one fat sample from a male that did not eat krill during the 1 st diet switch was excluded).	71
Table 2.3 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. Blood components that have the same superscript in	

common for each isotope indicate no significant difference between the subsequent sample years.....	71
Table 3.1 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 ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes.....	87
Table 3.2 Difference in mean marine nutrient source contribution from the 1 st , 4 th , and 8 th eggs per nest in wetland #115 and difference in mean range marine nutrient source contribution from the 1 st , 4 th , and 8 th 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 ($\delta^{13}\text{C}$).....	87

List of Appendices

	Page
Appendix 1.1 Natural food supplements offered to captive spectacled eiders and approximate harvesting location.	48
Appendix 1.2 Average ($\pm$ SD) stable carbon and nitrogen isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) for whole diet, lipid-free diet and diet lipids of Mazuri® sea duck diet and natural food supplements offered to spectacled eiders in 2006.	48
Appendix 1.3 Average ($\pm$ SD) stable carbon and nitrogen isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) for whole diet, lipid-free diet and diet lipids of Mazuri® sea duck diet and natural food supplements offered to spectacled eiders in 2007.	49
Appendix 1.4 Average ($\pm$ SD) stable carbon and nitrogen isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) for whole diet, lipid-free diet and diet lipids of Mazuri® sea duck diet and silversides offered to spectacled eiders in 2008.	49
Appendix 3.1 Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope signatures for nutrient sources or tissue end-members and harlequin duck egg components (Bond et al. 2007) used in the mixing model (Phillips and Gregg 2001).	88
Appendix 3.2 Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope signatures for nutrient sources or tissue end-members and Barrow's goldeneye egg components (Hobson et al. 2005) used in the mixing model (Phillips and Gregg 2001).	88

Dedication

To my grandparents (Papa and Nannie),

George (died April 2007) and Arlene Dorn,

both of which enjoyed science, education, teaching, art, creativity, and traveling, and
always encouraged lifelong learning. They have been an inspiration.

Acknowledgements

First, I would like want to thank my committee members Tuula Hollmén (committee chair), Daniel Esler, and Matthew Wooller. I was very lucky to have such an exceptional committee with a high degree of excellence in their scientific fields. All contributed valuable comments and guidance during this project. I am especially grateful to Tuula who made this thesis possible. She has offered support and encouragement during the course of this program and has been an excellent scientific mentor throughout my professional development.

Working in collaboration, the Alaska SeaLife Center (ASLC) and the University of Alaska Fairbanks (UAF) have provided me with a valuable graduate education experience. I am thankful to the ASLC Eider Research Staff (Ann Riddle, Dave Safine, and Shiway Wang) and Avian Husbandry Staff (Heidi Cline, Tasha DiMarzio, Gwen Gerdson, Michael Grue, Sadie Ulman, and Christina Haskins along with the many volunteers and interns), all which have been excellent to work with and have offered their friendship and support. I would also like to thank Veterinary Staff, Pam Tuomi, Carrie Goertz, and Millie Gray from the ASLC and Dan Mulcahy from United States Geological Survey, for monitoring the health of the birds and providing support during tissue collection. Their collaborative efforts have provided invaluable assistance and input, sharing of samples from concurrent projects, and countless hours of avian care and data collection. Additionally, friends and co-workers at the ASLC have provided administrative support (Tara Riemer Jones), statistical advice (Daniel Hennen), and comments on earlier drafts of the manuscripts (Pamela Parker and Mandy Keogh). I am ever grateful for the friendships and partnerships that have developed with my fellow UAF graduate students Abigail Ellsworth, Mandy Keogh, Jeanette Nienaber, Jared Guthridge, Steffen Oppel, Chris Latty, Brook McFarland, Caroline Jezierski, Beate Litz, Benjamin Daly, Mary Ann Bozza, Sara Whiteside, Jason Waite, Susan Inglis, Brenna McConnell, and Amy Tippery. The UAF School of Fisheries and Ocean Sciences (SFOS)

Staff and Seward Marine Center Staff have also offered a tremendous amount of support, especially Madeline Scholl, Christina Neumann, Katie Straub, Shannon Atkinson, Phyllis Shoemaker, Linda Lasota, Nici Murawsky, and Jennifer Elhard. I would like to thank UAF SFOS Dean Denis Wiesenburg, Associate Dean Michael Castellini, and Program Head Katrin Iken and my UAF instructors Rolf Grading, Dean Stockwell, Michael Castellini, Katrin Iken, Diane O'Brien, Jo-Ann Mellish, Lori Polasek, Brenda Konar, Brenda Norcross, and Julie McIntyre. In addition to my core coursework, I benefited from a course in Animal Stable Isotope Ecology taught by Diane O'Brien, who contributed valuable input and discussion specific to this thesis. Thanks to Steve MacLean and Stacey Buckelew and field mates Jennifer Curl, Sarah Abel, and Jonathan Giffard, for their collaboration in my field course study on Rat Island. Tim Howe and Norma Haubenstock from the Alaska Stable Isotope Facility at UAF provided laboratory assistance for the stable isotope analyses and were always welcoming when I visited the campus.

I am thankful to the funding agencies that made this thesis possible. Primary funding was made available from the United States Fish and Wildlife Service (#70181-6-G413), Ocean Alaska Science and Learning Center (H9845060030), and National Park Foundation (07AKCMRG007 and 08AKCMRG004). Thanks especially to Peter Armato with Kenai Fjords National Park for his support and collaboration in continuing education. Also, I thank the Dieter Family Marine Science Research Scholarship, Francis "Bud" Fay Memorial Scholarship, and Chancellor's Annual Giving Student Initiative, for their research and education support during the last year of my thesis. Finally, I would like to thank the University of Alaska Fairbanks Graduate School and Alaska Bird Conference for the travel money granted for conference attendance.

Thanks to my family, especially my mom and dad Michael and Miriam Dorn, siblings Chris, Rachel, and Angela, aunts and uncles, and grandparents, and the multitude of friends who have kept me sane during this project. Family and friends have been key in lending support and an ear when I needed it. Finally, but most of all, I am extremely

grateful to my husband and friend, Terry Federer, who has provided love and support in my personal and professional goals and Joker the dog who has made sure I did not go one day without going for a walk. Terry has been incredibly patient with me and in the sacrifices that were made to see this degree to fruition. Thanks to Terry for your companionship, keeping our lives organized, for making me smile, and for making sure that I got outside to play and enjoy life.

General Introduction

Sea Duck Declines and Spectacled Eider Background

Several North American sea duck (Tribe *Mergini*) species have declined over the past few decades, and factors contributing to the declines remain largely unknown (Goudie et al. 1994; Henny et al. 1995; Sea Duck Joint Venture 2008; Stehn et al. 1993; Suydam et al. 2000). Sea ducks inhabit remote locations during much of their annual cycle, and therefore little is known about basic life history parameters for many of these species. For example, the spectacled eider (*Somateria fischeri*) 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). Spectacled eiders are thought to molt along the coast of northern and western Alaska and the entire world population 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).

The spectacled eider 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), and as a result the species was listed as threatened under the Endangered Species Act in 1993 (Federal Register 1993). Potential causes for the decline or threats to recovery are unclear, but 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). Recent aerial and ground-plot surveys in western Alaska estimate that numbers have stabilized or are increasing slightly in western Alaska (Petersen et al. 2000). The current nesting population is estimated at <5,000 females (Fischer et al. 2008), a significant decline from an estimated 50,000 breeding pairs in the 1970's (Dau and Kistchinski 1977). Survey efforts for estimating the nesting populations in northern Alaska (1992-2008) suggest approximately 6,200 breeding pairs are present (Larned et al. 2008).

Little is known about at-sea distribution and foraging ecology, which constrains understanding of causes of population declines. Given that the global population inhabits a small wintering range, spectacled eiders could be particularly vulnerable to environmental change in the wintering region (U.S. Fish and Wildlife Service 1996). In fact, there is evidence of a major ecosystem shift in the Northern Bering Sea (Grebmeier et al. 2006). Of relevance to spectacled eiders, changing environmental conditions may have contributed to a switch in dominant clam species between the 1960's and 1980's (Lovvorn et al. 2003; National Research Council 1996; Richman and Lovvorn 2003; Sirenko and Koltun 1992). Additionally, increases in some marine mammal populations and commercial fishing activity may have caused increased competition with the spectacled eider (Fay et al. 1989; U.S. Fish and Wildlife Service 1996). Furthermore, shifting ice conditions may be influencing flight energetics (Bump and Lovvorn 2004) and the ability of spectacled eiders to forage on the Bering shelf habitat (Lovvorn et al. 2003). Quantity and quality of food on nonbreeding areas is thought to influence body condition and subsequent reproductive performance of some waterfowl (Anteau and Afton 2004; Devries et al. 2008), but the relationships among diet and reproductive success and the extent to which these may influence spectacled eider population dynamics are unknown. Development of techniques to identify diet sources has been listed as a recovery task by the eider recovery team to further understand nutrient requirements and habitat relationships for this threatened species.

Thesis Background

Reproductive Energetic Strategies-Arctic waterfowl including eiders are faced with meeting 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 yield relatively mature (precocial) offspring (Alisauskas and Ankney 1992; King 1973). In addition, waterfowl like eiders incur long incubation periods with few incubation breaks, and therefore must sustain high self maintenance costs (Flint and Grand 1999). To compensate for high reproduction costs, some female waterfowl may build nutrient reserves for later use. 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 (Alisauskas and Ankney 1994; Barzen and Serie 1990; Esler et al. 2001; Gauthier et al. 2003; Hobson et al. 2005; Parker and Holm 1990; Schmutz et al. 2006; Tome 1984), 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 (Bond et al. 2007; Hobson et al. 2004; MacCluskie and Sedinger 2000). 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. The trade-offs associated with breeding strategies are considered an important part of species life history evolution (Stearns 1989).

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*) have been shown to store endogenous nutrients on wintering areas prior to spring migration, but little to none of these were allocated to egg formation (Bond et al. 2007). This study suggested that endogenous resources were used for other reproductive costs, such as self-maintenance during incubation or brood-rearing. 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 nonbreeding 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. Building information about strategies of nutrient acquisition and allocation will be useful for evaluating factors affecting population productivity and will contribute to conservation of threatened spectacled eiders and potentially other declining sea ducks.

Stable Isotopes and Nutrient Allocation Mixing Models -Methods for evaluating nutrient allocation to reproduction have previously relied on indirect approaches, such as proximate composition analyses of female somatic tissue (Alisauskas and Ankney 1992). However, the quantity of nutrient reserves that are directly invested in egg nutrients versus female maintenance requirements remain unclear from these analyses, potentially resulting in overestimation of endogenous nutrients transferred to eggs (Meijer and Drent 1999). Moreover, lethal methods such as carcass proximate analysis are not usually feasible options when studying a species of conservation concern.

The development of stable isotope techniques has provided an opportunity to quantitatively assess nutrient allocation to egg production (Hobson 2006) and to evaluate cross-seasonal interactions (Kelly 2000; Webster et al. 2002), particularly for species that travel between isotopically dissimilar habitats (e.g., marine versus freshwater; Peterson and Fry 1987). Using mixing models (Phillips 2001; Phillips and Gregg 2001; Phillips and Koch 2002), stable isotope signatures from the adult diet or tissue (i.e., blood components, fat, feathers, muscle) can be analyzed to estimate relative contributions of various nutrient sources to tissues like egg components or down feathers (Bond et al. 2007; Gauthier et al. 2003; Hobson et al. 2004; Hobson et al. 2005; Klaassen et al. 2001; Schmutz et al. 2006). In addition to being valuable endpoints in nutrient allocation models, stable isotope analyses of tissues also can be useful for establishing information

about foraging ecology and movement patterns within and among seasons, and therefore can provide an important link to seasonal interactions throughout the annual cycle (Klaassen et al. 2001; Rubenstein and Hobson 2004). For example, metabolically active tissues like blood plasma and cellular blood have a short turnover rate, and can therefore identify diet sources and variability within a season (Hobson and Clark 1993). Feathers, on the other hand, are metabolically inert after a period of growth and may provide a record about diet and movement patterns across seasons (Hobson and Clark 1992a). Further, because sea ducks undergo both primary feather and body feather molt during separate stages of their annual cycle, knowledge of diet and movement patterns can be assessed across separate habitats (Bellrose 1980). Accordingly, stable isotope analyses using a combination of tissues can enable assessment of nutrient sources useful for reproduction and seasonal interactions, and therefore can be useful for evaluating factors that may influence fitness and productivity (Kelly 2000; Webster et al. 2002).

Quantification of Isotopic Fractionation Factors - Successful applications of stable isotope analyses to nutrient allocation models, diet evaluation, and cross-seasonal movements are dependent on the accuracy of model components, such as appropriate selection of food sources (Ben-David and Schell 2001; Phillips 2001), and accurate parameter estimates for isotopic fractionation factors, tissue turnover, and metabolic routing (Gannes et al. 1997). A poorly characterized component in models of this type is the isotopic increase that occurs between the diet and tissue, referred to as isotopic fractionation (i.e., $\Delta\delta = \delta \text{ tissue} - \delta \text{ diet}$; see Martínez del Río et al. 2009). Stable isotopic fractionation factors for carbon generally increase slightly relative to diet due to the loss of the lighter and more abundant isotope (^{12}C) to respiration and therefore the uptake of the heavier and relatively rare isotope (^{13}C) in body tissues (DeNiro and Epstein 1978; Peterson and Fry 1987). However, carbon stable isotope fractionation factors also can be less than that of the diet in tissues that contain lipid because of oxidation during lipid synthesis (DeNiro and Epstein 1977). Nitrogen stable isotopic fractionation generally increases approximately 3-5‰ relative to the diet (DeNiro and Epstein 1981; Peterson

and Fry 1987). The reason for the increase in $\delta^{15}\text{N}$ is still unclear, but may result from the preferential excretion of the lighter isotope (^{14}N) in the excretory material or the preferential absorption of the heavy isotope (^{15}N) in the tissue (Martínez del Río et al. 2009). However, isotopic fractionation values can vary according to a number of factors, including diet, species, individual physiology, tissue type, age, and body condition (Bearhop et al. 2002; DeNiro and Epstein 1978; DeNiro and Epstein 1981; Hobson and Clark 1992b; Hobson and Montevecchi 1991; Martínez del Río and Wolf 2005; Minagawa and Wada 1984). Previously published studies have cautioned that utilizing fixed isotopic fractionation factors can cause large errors in mixing models, and therefore suggest that more data on isotopic fractionation factors should be established for specific species/taxon and tissues (Caut et al. 2008; Dalerum and Angerbjörn 2005; Hobson 2006; Martínez del Río et al. 2009). Currently, there are few species-specific data available for diet to egg component fractionation information (Hobson 1995) and no information to our knowledge is available for diet to natal down feather fractionation. Additionally, no isotopic fractionation studies have been previously conducted for spectacled eider tissues and few isotopic fractionation data are available for other sea duck species (Knoche 2004). Establishing isotopic fractionation for spectacled eider tissues will provide better estimations for source contributions to egg production in nutrient allocation models as well as tissue adjustments for evaluating diet sources/variability and tracking movement patterns within and among seasons.

Captive Research Facility-The Alaska SeaLife Center, a marine research facility in Seward, Alaska, houses a captive population of spectacled eiders. Eleven birds were reared in captivity and arrived at the facility as sub-adults in 2003 (males = 6, females = 5). Two additional wild adult males arrived as rehabilitated birds in 2003 and 2004. The flock is housed together in an outdoor enclosure with a natural saltwater habitat and was separated into pairs during the breeding season to facilitate identification of parentage and complete nest information. Birds are fed *ad libitum* with a commercial Mazuri® Sea Duck Diet (Purina Mills Incorporated, St. Louis, Missouri) formulated for sea ducks and

a small proportion (~5%) of natural food supplements. This captive flock is monitored regularly for health and body condition and is important for establishing baselines for wild populations as well as a large scope of research initiatives, including reproductive biology and physiology, foraging ecology and nutrition, disease ecology and epidemiology, contaminants, predation, and conservation (www.alaskasealife.org). Captive spectacled eiders provide an important reference for refining stable isotope techniques and establishing isotopic fractionation factors for this species. Data obtained from these captive birds will provide a purposeful foundation in stable isotope research and modeling for spectacled eiders in their natural environment and additionally may be useful for other sea duck species.

Thesis Outline and Goals

The goal of this thesis was to improve the utility of carbon and nitrogen stable isotope applications using captive spectacled eiders. Stable isotopes provide a unique opportunity to understand diet sources, migration patterns, and nutrient transfer across habitats that may otherwise be difficult to discern in sea ducks. Captive populations play an important role for development of these methods because stable isotope signatures in body tissue are increased relative to the diet (i.e., isotopic fractionation), and these data must be experimentally determined from a known, calibrated diet and are therefore impossible to derive in the field. Diet to tissue isotopic fractionation factors have never been assessed for spectacled eiders, and therefore these data will provide the foundation for studies conducted in the natural environment and nutrient allocation modeling for this species. This thesis also contributes preliminary assessments of model outcomes when spectacled eider isotopic fractionation factors are incorporated into sea duck models from the literature that used stable isotopes to address nutrient allocation to reproduction. Stable isotope applications may serve as a valuable assessment tool for understanding nutrient sources used for reproduction, diet, and movements throughout the annual cycle, and

therefore may have important implications for habitat management and conservation plans for threatened spectacled eiders. The following objectives constitute this thesis:

- 1) To establish stable carbon and nitrogen isotope signatures for experimental diet items, egg components, down feathers, and adult tissues (i.e., cellular blood, blood plasma, adult contour feathers, and subcutaneous fat).
- 2) To characterize stable isotope fractionation factors between diet and tissue (i.e., $\delta\Delta = \delta \text{ animal tissue} - \delta \text{ diet}$).
- 3) To simulate a theoretical nutrient allocation model to test the effects of model outcome when using isotopic fractionation factors between diet and egg components established for different bird species.
- 4) To provide a preliminary assessment of model outcome when spectacled eider diet to egg isotopic fractionation factors are incorporated into previously published sea duck models.

Chapters 1 and 2 were prepared for manuscript submission, and therefore I have acknowledged my co-authors in those sections. In Chapter 1, I established the diet to egg isotopic fractionation factors over three consecutive years using the same captive flock of spectacled eiders. These values have not been previously characterized for any sea duck. I then simulated a theoretical nutrient allocation model to test the effects of model outcome when using diet to egg isotopic fractionation factors established for different bird species. In Chapter 1, I have also established isotopic fractionation factors between diet and down feathers for spectacled eiders, which to my knowledge have not been previously established for any bird species. I also provided supplemental appendices with stable isotope signatures of natural food supplements used in this study. In Chapter 2, I provide data on isotopic fractionation factors between diet and several spectacled eider tissues, including blood plasma, cellular blood, adult contour feathers, and subcutaneous fat. In Chapter 3, I provide a preliminary assessment of incorporating the spectacled eider diet to egg isotopic fractionation factors into previously published sea duck models.

Finally, I offer concluding remarks for this thesis and suggestions for future research directions.

References

- Alisauskas RT, Ankney CD (1992) The cost of egg laying and its relationship to nutrient reserves in waterfowl. In: Batt BDJ et al. (eds) Ecology and Management of Breeding Waterfowl. University of Minnesota Press, Minneapolis, MN, USA, pp 30-61
- Alisauskas RT, Ankney CD (1994) Nutrition of breeding female ruddy ducks: The role of nutrient reserves. *Condor* 96:878-897
- Anteau MJ, Afton AD (2004) Nutrient reserves of lesser scaup (*Aythya affinis*) during spring migration in the Mississippi flyway: A test of the spring condition hypothesis. *Auk* 121:917-929
- Barzen JA, Serie JR (1990) Nutrient reserve dynamics of breeding canvasbacks. *Auk* 107:75-85
- Bearhop S, Waldron S, Votier SC, Furness RW (2002) Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. *Physiological and Biochemical Zoology* 75:451-458
- Bellrose FC (1980) Ducks, geese, and swans of North America. Stackpole Books, Harrisburg, PA, USA. 540 Pp
- Ben-David M, Schell DM (2001) Mixing models in analyses of diet using multiple stable isotopes: A response. *Oecologia* 127:180-184
- Bond JC, Esler D, Hobson KA (2007) Isotopic evidence for sources of nutrients allocated to clutch formation by harlequin ducks. *Condor* 109:698-704
- Bump JK, Lovvorn JR (2004) Effects of lead structure in Bering Sea pack ice on the flight costs of wintering spectacled eiders. *Journal of Marine Systems* 50:113-139
- Caut S, Angulo E, Courchamp F (2008) Caution on isotopic model use for analyses of consumer diet. *Canadian Journal of Zoology* 86:438-445
- Dalerum F, Angerbjorn A (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. *Oecologia* 144:647-658
- Dau CP (1974) Nesting biology of the spectacled eider *Somateria fischeri* (Brandt) on the Yukon Kuskokwim Delta, Alaska. Master of Science Thesis. University of Alaska Fairbanks, Fairbanks, Alaska, USA

- Dau CP, Kistchinski AA (1977) Seasonal movements and distribution of the spectacled eider. *Wildfowl* 28:65-75
- DeNiro MJ, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* 197:261-263
- DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42:495-506
- DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* 45:341-351
- Devries JH, Brook RW, Howerter DW, Anderson MG (2008) Effects of spring body condition and age on reproduction in mallards (*Anas platyrhynchos*). *Auk* 125:618-628
- Drent RH, Daan S (1980) The prudent parent: Energetic adjustments in avian breeding. *Ardea* 68:225-252
- Eadie JM, Kehoe FP, Nudds TD (1988) Pre-hatch and post-hatch brood amalgamation in the North American Anatidae: A review of hypotheses. *Canadian Journal of Zoology* 66:1709-1721
- Ely CR, Dau CP, Babcock CA (1994) Decline in a population of spectacled eiders nesting on the Yukon-Kuskokwim Delta, Alaska. *Northwestern Naturalist* 75:81-87
- Esler D, Grand JB, Afton AD (2001) Intraspecific variation in nutrient reserve use during clutch formation by lesser scaup. *Condor* 103:810-820
- Fay FH, Kelly BP, Sease JL (1989) Managing the exploitation of Pacific walruses: A tragedy of delayed response and poor communication. *Marine Mammal Science* 5:1-16
- Fischer JB, Stehn RA, Walters G (2008) Nest Population Size and Potential Production of Geese and Spectacled Eiders on the Yukon-Kuskokwim Delta, Alaska, 2008. U.S. Fish and Wildlife Service unpublished report. Anchorage, AK, USA. 34 Pp
- Federal Register (1993) Final rule to list the spectacled eider as threatened. 58:27474-27480
- Flint PL, Grand JB (1999) Incubation behavior of spectacled eiders on the Yukon-Kuskokwim Delta, Alaska. *Condor* 101:413-416
- Gannes LZ, O'Brien DM, Martínez del Río C (1997) Stable isotopes in animal ecology: Assumptions, caveats, and a call for more laboratory experiments. *Ecology* 78:1271-1276

Gauthier G, Bety J, Hobson KA (2003) Are greater snow geese capital breeders? New evidence from a stable-isotope model. *Ecology* 84:3250-3264

Goudie RI, Kondratyev AV, Brault S, Petersen MR, Conant B, Vermeer K (1994) The status of sea duck in the north Pacific rim: Toward their conservation and management. In: Transactions of the 59th North American Wildlife and Natural Resources Conference Proceedings 59:27-49

Goudie RI, Jones IL (2005) Feeding behavior of harlequin ducks (*Histrionicus histrionicus*) breeding in Newfoundland and Labrador: A test of the food limitation hypothesis. *Bird Behavior* 17:9-18

Grand JB, Flint PL (1997) Productivity of nesting spectacled eiders on the lower Kashunuk River, Alaska. *Condor* 99:926-932

Grand JB, Franson JC, Flint PL, Petersen MR (2002) Concentrations of trace elements in eggs and blood of spectacled and common eiders on the Yukon-Kuskokwim Delta, Alaska, USA. *Environmental Toxicology and Chemistry* 21:1673-1678

Grebmeier JM, Overland JE, Moore SE, Farley EV, Carmack EC, Cooper LW, Frey KE, Helle JH, McLaughlin FA, McNutt SL (2006) A major ecosystem shift in the northern Bering Sea. *Science* 311:1461-1464

Henny CJ, Rudis DD, Roffe TJ, Robinson-Wilson E (1995) Contaminants and sea ducks in Alaska and the circumpolar region. *Environmental Health Perspectives* 103:41-49

Hobson KA (1995) Reconstructing avian diets using stable-carbon and nitrogen isotope analysis of egg components: Patterns of isotopic fractionation and turnover. *Condor* 97:752-762

Hobson KA (2006) Using stable isotopes to quantitatively track endogenous and exogenous nutrient allocations to eggs of birds that travel to breed. *Ardea* 94:359-369

Hobson KA, Clark RG (1992a) Assessing avian diets using stable isotopes I: Turnover of ¹³C in tissues. *Condor* 94:181-188

Hobson KA, Clark RG (1992b) Assessing avian diets using stable isotopes II: Factors influencing diet-tissue fractionation. *Condor* 94:189-197

Hobson KA, Clark RG (1993) Turnover of ¹³C in cellular and plasma fractions of blood: Implications for nondestructive sampling in avian dietary studies. *Auk* 110:638-641

Hobson KA, Montevecchi WA (1991) Stable isotopic determinations of trophic relationships of great auks. *Oecologia* 87:528-531

- Hobson KA, Atwell L, Wassenaar LI, Yerkes T (2004) Estimating endogenous nutrient allocations to reproduction in redhead ducks: A dual isotope approach using δD and $\delta^{13}\text{C}$ measurements of female and egg tissues. *Functional Ecology* 18:737-745
- Hobson KA, Thompson JE, Evans MR, Boyd S (2005) Tracing nutrient allocation to reproduction in Barrow's goldeneye. *Journal of Wildlife Management* 69:1221-1228
- Jönsson KI (1997) Capital and income breeding as alternative tactics of resource use in reproduction. *Oikos* 78:57-66
- Kelly JF (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology* 78:1-27
- King JR (1973) Energetics of reproduction in birds. In: Farmer DS (ed) *Breeding Biology of Birds*. National Academy of Sciences, Washington D.C., USA, pp 78-107
- Kistchinski AA, Flint VE (1974) On the biology of the spectacled eider. *Wildfowl* 25:5-15
- Klaassen M, Lindstrom A, Meltofte H, Piersma T (2001) Arctic waders are not capital breeders. *Nature* 413:794-794
- Klaassen M, Abraham KF, Jefferies RL, Vrtiska M (2006) Factors affecting the site of investment, and the reliance on savings for arctic breeders: The capital-income dichotomy revisited. *Ardea* 94:371-384
- Knoche MJ (2004) King eider wing molt: Inferences from stable isotope analyses. Master of Science Thesis. University of Alaska Fairbanks, Fairbanks, Alaska, USA
- Larned WW, Stehn RS, Platte RM (2008) Waterfowl breeding population survey Arctic Coastal Plain, Alaska 2008. U.S. Fish and Wildlife Service unpublished report. Anchorage, Alaska. 42 Pp
- Lovvorn JR, Richman SE, Grebmeier JM, Cooper LW (2003) Diet and body condition of spectacled elders wintering in pack ice of the Bering Sea. *Polar Biology* 26:259-267
- MacCluskie MC, Sedinger JS (2000) Nutrient reserves and clutch-size regulation of northern shovelers in Alaska. *Auk* 117:971-979
- Martínez del Río C, Wolf BO (2005) Mass-balance models for animal isotopic ecology. In: Stark JM, Wang T (eds) *Physiological and Ecological Adaptations to Feeding in Vertebrates*. Science Publishers, Enfield, NH, USA, pp 141-174

- Martínez del Río C, Wolf N, Carleton SA, Gannes LZ (2009) Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews* 84:91-111
- Meijer T, Drent R (1999) Re-examination of the capital and income dichotomy in breeding birds. *Ibis* 141:399-414
- Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochimica et Cosmochimica Acta* 48:1135-1140
- National Research Council (1996) *The Bering Sea Ecosystem*. National Academy Press, Washington D.C., USA. 308 Pp
- Norris DR (2005) Carry-over effects and habitat quality in migratory populations. *Oikos* 109:178-186
- Parker H, Holm H (1990) Patterns of nutrient and energy expenditure in female common eiders nesting in the high Arctic. *Auk* 107:660-668
- Petersen MR, Douglas DC, Mulcahy DM (1995) Use of implanted satellite transmitters to locate spectacled eiders at-sea. *Condor* 97:276-278
- Petersen MR, Piatt JF, Trust KA (1998) Foods of spectacled eiders *Somateria fischeri* in the Bering Sea, Alaska. *Wildfowl* 49:124-128
- Petersen MR, Larned WW, Douglas DC (1999) At-sea distribution of spectacled eiders: A 120-year-old mystery resolved. *Auk* 116:1009-1020
- Petersen MR, Grand JB, Dau CP (2000) Spectacled eider (*Somateria fischeri*). In: Poole A, Gill F (eds) *The Birds of North America* No 547. The Birds of North America Inc, Philadelphia, PA, USA, pp 1-24
- Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18:293-320
- Phillips DL (2001) Mixing models in analyses of diet using multiple stable isotopes: A critique. *Oecologia* 127:166-170
- Phillips DL, Gregg JW (2001) Uncertainty in source partitioning using stable isotopes. *Oecologia* 127:171-179
- Phillips DL, Koch PL (2002) Incorporating concentration dependence in stable isotope mixing models. *Oecologia* 130:114-125

Richman SE, Lovvorn JR (2003) Effects of clam species dominance on nutrient and energy acquisition by spectacled eiders in the Bering Sea. *Marine Ecology Progress Series* 261:283-297

Rubenstein DR, Hobson KA (2004) From birds to butterflies: Animal movement patterns and stable isotopes. *Trends in Ecology and Evolution* 19:256-263

Schmutz JA, Hobson KA, Morse JA (2006) An isotopic assessment of protein from diet and endogenous stores: Effects on egg production and incubation behaviour of geese. *Ardea* 94:385-397

Sea Duck Joint Venture (2008) Sea Duck Joint Venture Strategic Plan 2008 – 2012. Available at <http://seaduckjv.org> or U.S. Fish and Wildlife Service, Anchorage, Alaska or Canadian Wildlife Service, Sackville, New Brunswick. 103 Pp

Sirenko BI, Koltun VM (1992) Characteristics of benthic biocenoses of the Chukchi and Bering Seas. In: Nagel P (ed) Results of the third joint US-USSR Bering and Chukchi Seas expedition (BERPAC), summer 1988, USFWS, Washington D.C., USA, pp 251-261

Sorensen MC, Hipfner JM, Kyser TK, Norris DR (2009) Carry-over effects in a Pacific seabird: Stable isotope evidence that pre-breeding diet quality influences reproductive success. *Journal of Animal Ecology* 78:460-467

Stearns SC (1989) Trade-offs in life-history evolution. *Functional Ecology* 3:259-268

Stehn RA, Dau CP, Conant B, Butler Jr. WI (1993) Decline of spectacled eiders nesting in western Alaska. *Arctic* 46:264-277

Suydam RS, Dickson DL, Fadely JB, Quakenbush LT (2000) Population declines of king and common eiders of the Beaufort Sea. *Condor* 102:219-222

Tome MW (1984) Changes in nutrient reserves and organ size of female ruddy ducks breeding in Manitoba. *Auk* 101:830-837

U.S. Fish and Wildlife Service (1996) Spectacled eider recovery plan. Anchorage, Alaska. 157 Pp

Webster MS, Marra PP (2005) The importance of understanding migratory connectivity and seasonal interactions. In: Greenburg R, Marra PP (eds) *Birds of Two Worlds: The Ecology and Evolution of Migration*. John Hopkins University, Baltimore, MD, USA, pp 199-209

Webster MS, Marra PP, Haig SM, Bensch S, Holmes RT (2002) Links between worlds: Unraveling migratory connectivity. *Trends in Ecology and Evolution* 17:76-83

Chapter 1 Carbon and Nitrogen Stable Isotope Fractionation Factors for Quantifying Spectacled Eider (*Somateria fischeri*) Nutrient Allocation to Egg Production¹

1.1 Abstract

Nutrient allocation models based on stable isotope analysis have been useful for determining sources of nutrients invested in egg production by birds, and therefore are important for identifying nutritional constraints on reproductive performance. This approach is particularly useful for birds that travel between habitats with distinct stable isotope (e.g., carbon and nitrogen) compositions like marine and freshwater habitats. Spectacled eiders (*Somateria fischeri*) are threatened sea ducks that spend the majority of the annual cycle in the marine environment and migrate to freshwater habitats to breed. Because reproduction is energetically demanding for waterfowl species like eiders, we expect that nutrients may be transported from the non-breeding habitat for egg production. However, foraging ecology and nutrients used for egg production in sea ducks is poorly understood and changes occurring in their marine habitat could be affecting fitness and reproductive success. An important consideration in nutrient allocation models using stable isotopes, however, are the differences in the stable isotope signatures of the consumer tissue relative to the diet, referred to as isotopic fractionation (i.e., $\Delta\delta = \delta \text{ animal tissue} - \delta \text{ diet}$), so that these can be accounted for when comparing isotope signatures of egg components to putative sources. We established stable carbon and nitrogen isotopic fractionation factors between whole diet and egg components of captive spectacled eiders in 2006-2008. Results indicated similar isotopic fractionation patterns with minimal variation among tissues and years. The $\delta^{13}\text{C}$ values were higher relative to whole diet for albumen, yolk protein, eggshell, and shell membrane and lower

¹In preparation for submission to *Oecologia* as Federer R, Hollmén T, Esler D, and Wooller M.J, Stable Carbon and Nitrogen Isotope Fractionation Factors for Quantifying Spectacled Eider (*Somateria fischeri*) Nutrient Allocation to Egg Production

relative to whole diet for whole yolk and yolk lipid. The $\delta^{15}\text{N}$ of the egg components analyzed were higher relative to whole diet. These data are generally consistent with previously published isotopic fractionation factors for other species, but also identify differences in isotopic fractionation factors for egg proteins. A theoretical sensitivity model indicates that choice of isotopic fractionation factors from different species could considerably alter predicted percentages of nutrient source contributions to reproduction. Our study also provides the first carbon and nitrogen isotopic fractionation values for embryonic down feathers, which can additionally serve as a useful tissue for identifying protein sources used for egg production. Finally, we determined isotopic fractionation factors for macronutrients in diet sources and egg components, thus enabling consideration of these nutrients separately. Our study enhances the framework for nutrient allocation modeling for spectacled eiders, and may be useful for other sea duck models.

1.2 Introduction

Stable isotope analyses (e.g., carbon and nitrogen) can be used to evaluate nutrient allocation for egg production in birds that travel between isotopically distinct regions to breed (Hobson 2006; Hobson et al. 1997; Hobson et al. 2000). Using this approach, mixing models can be applied to quantify nutrients used for egg production (Phillips and Gregg 2001; Phillips and Gregg 2003; Phillips and Koch 2002). These nutrient allocation models typically require mean isotope signatures for two or more potential nutrient sources and the animal tissue (i.e., 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 appropriate selection of nutrient sources (Ben-David and Schell 2001; Phillips 2001) and accurate estimates of isotopic fractionation factors, tissue turnover, and metabolic routing (Gannes et al. 1997). Currently, 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 in natal down feathers.

Accordingly, experimental studies are needed to determine tissue and species/taxon specific isotopic fractionation factors (Caut et al. 2008; Dalerum and Angerbjorn 2005; Gannes et al. 1997; Hobson 2006).

Most mixing models that evaluate nutrient allocation to reproduction have utilized carbon ($\delta^{13}\text{C}$) and nitrogen stable isotopes ($\delta^{15}\text{N}$; see review by Hobson 2006). Both stable isotopes are good indicators of diet or geographical region and $\delta^{15}\text{N}$ are also good indicators of trophic position (Kelly 2000; Peterson and Fry 1987). Generally, the $\delta^{13}\text{C}$ compositions of animal tissues slightly increase or decrease, but are comparable to their diet (DeNiro and Epstein 1978; Peterson and Fry 1987). The increase of the $\delta^{13}\text{C}$ in tissues may 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 (DeNiro and Epstein 1978; Peterson and Fry 1987; Rau et al. 1983; Tieszen et al. 1983). 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 still unclear, but may result from the preferential excretion of the lighter isotope (^{14}N) in urea or the preferential absorption of the heavy isotope (^{15}N) in the tissue (Martínez del Río et al. 2009). The isotopic fractionation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from diet into 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 1992; Hobson and Montevecchi 1991; Jardine et al. 2006; Martínez del Río and Wolf 2005; 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 inappropriate isotopic fractionation factors may be a source of possible error in dietary mixing models, and therefore it is essential to establish the isotopic fractionation factors between diet and tissue for confident application of stable isotope models.

Limited information is available about nutrient allocation to reproduction in sea ducks (Bond et al. 2007; Hobson et al. 2005; Lawson 2006). Spectacled eiders (*Somateria fischeri*) are sea ducks that were listed as threatened under the Endangered Species Act in 1993 (Federal Register 1993). Potential factors affecting population recovery include changes in the marine environment, such as food abundance and availability or food competition (Fay et al. 1989; Richman and Lovvorn 2003; U.S. Fish and Wildlife Service 1996). These factors may influence foraging ecology and nutrient allocation strategies for reproduction in spectacled eiders, and therefore subsequent reproductive success. This species spends the majority of the annual cycle in offshore marine habitats (Petersen et al. 1995; Petersen et al. 1999) and travels to freshwater tundra habitats for reproduction (U.S. Fish and Wildlife Service 1996). Reproduction is energetically demanding for waterfowl species like eiders (Alisauskas and Ankney 1992), and therefore we speculate that this species may transport nutrients from marine habitats for reproduction, but it is unknown how much of these marine-derived nutrients are used for egg production and female maintenance requirements. Because marine and freshwater habitats have distinct isotopic compositions (Kelly 2000; McConnaughey and McRoy 1979; Michener and Schell 1994; Minagawa and Wada 1984; Montoya 2007; Saupe et al. 1989), stable isotopes could contribute to our understanding of where and when spectacled eiders obtain nutrients for egg production.

Understanding nutrient acquisition and allocation strategies for reproduction may provide important information for management of critical foraging habitats for spectacled eiders. The purpose of this study was to establish $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of experimental diet items, egg components, and down feathers for captive spectacled eiders and to evaluate

isotopic fractionation factors for these tissues. Further, we compared these differences in isotopic egg fractionation factors from our captive spectacled eiders to a previous captive study conducted by Hobson (1995) that 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*). Our spectacled eider egg isotopic fractionation results will serve as important values to determine accurate results from nutrient allocation models for spectacled eiders.

1.3 Materials and Methods

1.3.1 Study Species

Research was conducted from February 1 through egg laying during 2006, 2007, and 2008 (5/19-6/30, 5/23-6/30, and 5/20-6/30, respectively; Table 1.1) at the Alaska SeaLife Center in Seward, Alaska. Eleven birds (females = 5, males = 6) were reared in captivity and arrived at the Alaska SeaLife Center 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 and collect full clutch information.

1.3.2 Diet and Reproductive Tissue Collection

Feeding Protocols and Diet Collection - 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). Mazuri® Sea Duck Diet is a commercially available diet formulated for maintaining sea ducks and consists of mostly ground corn and wheat grains, fish, and pork with a nutrient composition of approximately 6.5% lipid, 21.6% protein, 8.4% fiber, 10.9% ash, and 46.6% nitrogen-free extract along with vitamins

(www.mazuri.com). Daily consumption of Mazuri® was recorded for the flock and amount consumed per individual was calculated from those values. In 2006 and 2007, birds were offered five alternating natural food supplements (Antarctic krill, *Euphausia superba*; tidewater silversides, *Menidia menidia*; Arctic razor clams, *Siliqua patula*; Pacific blue mussels, *Mytilus trossulus*; and common squid, *Loligo opalescens*; Appendix 1.1) on a weekly schedule and estimated consumption was recorded. In 2008, we simplified the diet to include only one natural food supplement (tidewater silversides; Appendix 1.1) quantified at 5% dry weight based on the consumption of Mazuri® within the previous 24 hours. All diet samples were collected and stored at –20°C until analysis.

Egg Component and Down Feather Collection - Females were monitored daily during the breeding season in 2006, 2007, and 2008 and fresh eggs were collected upon observation ($n = 37, 39$, and 50 , respectively; Table 1.1). Eggs were artificially incubated at 37.4°C and 60% relative humidity (Grumbach Incubator, Asslar, Germany) for 6-8 days in 2006 and 3-5 days in 2007 and 2008 to document fertility status by candling. Five eggs in 2008 were incubated longer until embryos were found dead within 20-23 days of incubation (Table 1.1). Eggs were then refrigerated at 4°C and divided into fresh yolk and albumen components within 24 hours. Field preservation methods require boiling eggs rather than fresh egg separation, and studies have suggested that isotope signatures do not differ between these treatments (Gloutney and Hobson 1998). We confirmed that fresh eggs are comparable to boiled eggs (using a one-way analysis of variance (ANOVA)) by measuring the isotopic signatures of 5 boiled and 5 fresh chicken egg yolks ($\delta^{13}\text{C}$, $F = 2.269$, $df = 9$, $p = 0.170$; $\delta^{15}\text{N}$, $F = 0.433$, $df = 9$, $p = 0.529$) and albumen ($\delta^{13}\text{C}$, $F = 1.638$, $df = 9$, $p = 0.237$; $\delta^{15}\text{N}$, $F = 1.004$, $df = 9$, $p = 0.346$). 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. Whole down feathers were collected from the back tract of embryos. Feathers

were rinsed with ethanol to remove any external substances, dried, and stored at room temperature until analysis (Knoche 2004; Knoche et al. 2007).

1.3.3 Laboratory Analyses

Diet items and liquid egg components were dried to a constant mass using a FreeZone® 6 Liter Console Freeze Dry System (Labconco Corporation, Kansas City, Missouri). All samples were separated into lipid and non-lipid components because of potential differences in macronutrient allocation to eggs. We applied both manual lipid extraction and lipid soxhlet extraction techniques to separate lipid-free diet samples in 2006 and 2007, and found no significant differences (using a one-way ANOVA) for nitrogen ($F=0.0385$, $df=3$, $p=0.863$; $F=0.0308$, $df=3$, $p=0.877$, respectively) or carbon isotopic composition ($F=0.692$, $df=3$, $p=0.493$; $F=9.846$, $df=3$, $p=0.088$) between methods. We could not retrieve lipids using the lipid soxhlet apparatus because of dissolution of the lipids in the solvent, and therefore, lipids were extracted from the diet items and whole yolk using a 2:1 ratio of chloroform:methanol solution (Bligh and Dyer 1959). We used a vortex to mix ~5 mg of dry sample with solution, allowed samples to settle for 24 hours, and extracted lipids manually with a pipette until the solvent wash was clear (Steffen Oppel, personal communication). Lipids were kept uncovered under a fume hood until all the solvent had evaporated. All whole or lipid-extracted samples were then homogenized using a mortar and pestle or grinder.

Stable carbon and nitrogen isotope analysis was conducted at the Alaska Stable Isotope Facility at the University of Alaska Fairbanks (Fairbanks, Alaska). All homogenized diet items and tissues were weighed (0.01-0.05 mg) into tin cups using a Satorius M2P electronic microbalance (Goettingen, Germany). Each diet item was analyzed in duplicate for all years. To evaluate for sample variation in 2008, we analyzed samples from five batches of Mazuri® (based on ~monthly shelf life) and five samples from the same batch of silversides from February 1 to end of egg laying. Each egg and its components and

down feathers were analyzed in single measurements. The stable carbon and nitrogen isotope combustion of these samples were then measured using continuous flow isotope ratio mass spectrometry (Finnigan Delta^{plus}XP CF-IRMS). Isotopic compositions of samples are expressed relative to international standards (R_{standard} =Vienna PeeDee Belemnite for $\delta^{13}\text{C}$, atmospheric air for $\delta^{15}\text{N}$) in delta (δ) notation and in parts per thousand (‰) according to the following equation: $\delta X\text{‰} = ([R_{\text{sample}}/R_{\text{standard}}]-1) \times 1000$, with X denoting either ^{13}C or ^{15}N , and R representing the ratio of $^{13}\text{C}:^{12}\text{C}$ or $^{15}\text{N}:^{14}\text{N}$, respectively. The average analytical precision (defined here as ± 1 SD) was determined using peptone standards (Sigma Chemical Co., Highland, Illinois, mean: $\delta^{13}\text{C} = -15.82\text{‰}$ and $\delta^{15}\text{N} = 7.02\text{‰}$) across separate sample runs (total sample runs $n = 24$, total standards across all runs $n = 210$) and showed $0.1\text{‰} \pm 0.06$ for $\delta^{13}\text{C}$ and $0.2\text{‰} \pm 0.1$ for $\delta^{15}\text{N}$.

1.3.4 Calculations and Statistical Analyses

Average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for diet (± 1 SD) were calculated by multiplying average diet proportion (Mazuri®:natural food supplement) by the respective isotope signatures. The average proportion of total Mazuri®:natural food supplements was calculated from February 1 to the end of egg laying. Independent proportions per natural food supplement were calculated by dividing the number of days each natural food was offered by the total number of days, and then multiplied by the average proportion per year. Isotopic fractionation factors were then calculated by subtracting the carbon or nitrogen isotope signatures from individual egg components or down feathers (Y) from those of the diet (X), described in terms of the difference in delta ($\Delta\delta = \delta Y - \delta X$), and reported as mean and ± 1 SD.

Assumptions of equal variance (Levene median test) and normality (Kolmogorov-Smirnov) of the residuals were assessed. A one-way ANOVA (F statistic) was used to evaluate 1) inter-annual differences (2006-2008) in $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ of spectacled eider egg components and 2) average interspecies differences in $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ (we report

differences from spectacled eiders, but see Hobson (1995) for results between other avian species). A Bonferroni t-test was then used to identify groups that were not significantly different. If equal variance or normality failed, a one-way ANOVA on Ranks (Kruskal-Wallis test; H statistic) was conducted followed by a Dunn's pairwise multiple comparison (Q-test) to separate groups that were not significantly different. All analyses were conducted using SigmaStat 3.1 and SigmaPlot 9.0 (Systat Software Inc., Point Richmond, California) with α set at 0.05.

Finally, we employed a theoretical single-isotope, two-end member mixing model by Phillips and Gregg (2001) to examine model sensitivity when we applied different diet to egg isotopic fractionation factors (i.e., derived from different avian species, Hobson 1995 and this study). We applied stable isotope signatures (calculated separately for carbon and nitrogen) of two nutrient sources (i.e., terrestrial and marine) and stable isotope signatures of five egg components that were then adjusted for diet to egg isotopic fractionation. All model components were kept constant, except the diet to egg isotopic fractionation values were replaced for each species. Theoretical 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). For the model to perform appropriately, 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 clutch 1 $n=8$, 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.

1.4 Results

1.4.1 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Isotope Analyses for Diet Items

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were determined for individual diet items used from 2006-2008 (Appendix 1.2-1.4). Percent dry mass of each diet item was considered in all calculations (Mazuri® 92% in 2006 and 94% in both 2007 and 2008; 2006, 2007, and 2008 silversides = 25%, 24%, and 22%, respectively; 2006-2007, clams and mussels = 23% in both years; squid = 18% and 20% in 2006 and 2007, respectively; and krill = 17% and 19% in 2006 and 2007, respectively). Although there was a wide range of isotopic signatures among diet items, the total natural food supplements made up a very small proportion of the overall diet in each year (2006 = 3.0%, 2007 = 5.9%, and 2008 = 4.0%). Thus, natural food supplements would be expected to have only minimal influence on the results, particularly because isotopic signatures of all diet items were incorporated into the final estimate of the diet isotopic ratios. Further partitions to individual natural food contribution suggested that krill had the highest proportion (1.1%) while mussels and clams had the lowest proportion (0.4%) in 2006. In 2007, silversides and krill had the highest proportional contribution (2.8% and 1.1%) while clams and mussels had the lowest (0.4% and 0.5%). In 2008 when only silverside was offered, the proportion consumed was 4.0% silversides. Dietary proportions were then multiplied by the respective isotope signatures in all years to calculate a single diet value for diet lipids, whole diet, and lipid-free diet (Table 1.2). We reported the $\delta^{13}\text{C}$ only for diet lipids because lipids contain very little nitrogen.

1.4.2 $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ 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. Only $\Delta\delta^{13}\text{C}$ was reported for yolk lipids and

eggshell due to the low nitrogen concentrations associated with these tissues. One yolk sample each from 2006 and 2008 and two albumen samples from 2008 were not available for analysis. We excluded 2 samples from the analysis (1 lipid-free yolk from 2006 and 1 albumen from 2008) that showed evidence of lipid contamination (i.e., notably low $\delta^{13}\text{C}$ and a high C:N ratio).

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 (Table 1.3). The $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ for shell membrane were consistently higher among years and the $\Delta\delta^{15}\text{N}$ for down feathers was higher than the other protein-comprised egg components (Table 1.3). 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 (Table 1.3). The $\Delta\delta^{13}\text{C}$ increased considerably for eggshell in relation to whole diet (Table 1.3).

We found a statistical difference among years in $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ for all spectacled eider egg components, with the exception of $\Delta\delta^{13}\text{C}$ in yolk lipids ($Q=6.825$ and 6.269 , $p < 0.05$) and $\Delta\delta^{15}\text{N}$ in lipid-free yolk ($Q=7.944$ and 6.218 , $p < 0.05$) between two of the three years (Table 1.3). Although there was a statistical significance, among year comparisons have not been previously determined, and from a biological perspective these differences observed were minimal for the $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$, respectively, in albumen (1.0‰ and 1.6‰, respectively), 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 1.3). Additionally, there was a minimal difference among individuals in 2007 for $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$, respectively (albumen, 0.1‰ and 0.2‰; lipid-free yolk, 1.0‰ and 0.5‰; shell membrane, 0.3‰ and 0.4‰; whole yolk, 1.1‰ and 1.3‰) and for $\Delta\delta^{13}\text{C}$ (yolk lipid, 1.2‰; eggshell, 1.1‰) and in 2008 for $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ (albumen, 0.2‰ and 0.4‰; lipid-free yolk, 0.7‰ and 0.6‰; shell membrane, 0.2‰ and 0.6‰; whole yolk, 0.2‰ and 0.6‰) and for $\Delta\delta^{13}\text{C}$ (yolk lipid, 0.4‰; eggshell, 1.0). Overall, the differences

observed among years in spectacled eider egg isotopic fractionation was comparable to or less than (depending on the tissue type) that observed among species for $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ (albumen, 1.8‰ and 1.6‰; lipid-free yolk, 2.6‰ and 1.4‰; shell membrane, 1.1‰ and 1.7‰; whole yolk, 1.1‰ and 1.5‰) and for $\Delta\delta^{13}\text{C}$ (yolk lipid, 1.0‰; eggshell, 4.5‰).

1.4.3 Species Comparison for Isotopic Egg Fractionation and Theoretical Modeling

The mean $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ among years for spectacled eiders were either comparable or increased for albumen, lipid-free yolk, and shell membrane relative to other species (Table 1.4). 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 (Table 1.4). The mean $\Delta\delta^{13}\text{C}$ among years for whole yolk and yolk lipids were comparable 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 (Table 1.4). The mean $\Delta\delta^{15}\text{N}$ among years for whole yolk were either comparable or increased slightly relative to previously studied species (Table 1.4). 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 (Table 1.4).

Among all species, the $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ in each egg components were significantly different, except for $\Delta\delta^{15}\text{N}$ in whole yolk (Table 1.4). We performed post-hoc tests to identify significant differences from spectacled eiders and found that 1) the $\Delta\delta^{13}\text{C}$ for albumen, lipid-free yolk, and eggshell were significantly different from all other species, 2) the $\Delta\delta^{15}\text{N}$ for albumen and lipid-free yolk, and $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ for shell membrane were significantly different from some species, and 3) there were no significant differences between spectacled eiders and other species in the $\Delta\delta^{13}\text{C}$ or $\Delta\delta^{15}\text{N}$ for whole

yolk and the $\Delta\delta^{13}\text{C}$ for yolk lipids (Table 1.4). See Hobson (1995) for significant differences observed among other avian species.

Theoretical 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 1.1). The models show that the proportional contribution of $\delta^{13}\text{C}$ from a terrestrial source differs 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 1.1). Similarly, the proportional contribution of $\delta^{15}\text{N}$ from a terrestrial source differs up 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 1.1).

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

Thus far, we have described $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ from whole diet to tissue so that we could compare previously established isotopic fractionation factors from other avian species that were determined in the Hobson (1995) study 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 1.2).

1.5 Discussion

Our study provides stable isotope fractionation factors for egg components and embryonic down feathers for spectacled eiders. These are the first isotopic fractionation factors developed for eggs from sea ducks and to our knowledge the first values for down feathers in any avian species, and therefore are likely to be the most applicable for modeling nutrient allocation in other sea duck species. Our data demonstrate minimal mean difference among consecutive years using egg tissues from the same spectacled eiders, and below we discuss potential causes for this variation. Furthermore, this study provides isotopic signatures in diet and tissues for both lipid and protein macronutrients, which enables consideration of different nutrient sources and the strategies of nutrient allocation to particular egg components. This study will contribute knowledge for reproductive energetics in spectacled eiders, and thus increase our understanding of potential factors influencing foraging ecology and reproductive success.

1.5.1 *Spectacled Eider Egg and Down Isotopic Fractionation*

The isotopic fractionation values for diet to egg components of spectacled eiders differs per nutrient composition of each egg component, 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. We measured isotopic fractionation factors of eggs over three consecutive years using the same individuals and found a consistent pattern of isotopic fractionation for each individual egg component relative to the diet. Our results suggest higher carbon isotopic fractionation factors for spectacled eiders than were previously established 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 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).

The results from this study provide isotope fractionation values for diet to embryonic down feathers, which to our knowledge have not been previously documented in the literature for birds. 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. Using down feather tissues is a novel approach to evaluate protein source contribution in nutrient allocation modeling (Klaassen et al. 2001), but it is important to first incorporate an accurate isotopic fractionation factor so that the appropriate source contributions can be estimated.

1.5.2 Potential Factors Contributing to Variation in Spectacled Eider $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$

Studies of stable isotopic fractionation in egg components have not been previously considered over consecutive years and using the same individuals. We found minimal annual and individual differences in isotopic fractionation factors in the egg components using the same spectacled eiders and this may be important to consider in future studies. Mean $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ among egg components varied annually over the three years by 0.9-1.5‰ and 0.9-1.6‰, respectively. Additionally, we observed minimal differences among individual females and their egg components for $\Delta\delta^{13}\text{C}$ (0.1-1.2‰ in 2007 and 0.2-1.0‰ in 2008) and $\Delta\delta^{15}\text{N}$ (0.2-1.3‰ in 2007 and 0.4-0.6‰ in 2008). Other studies also report variation in the same species feeding on the same diet (Ambrose and Norr

1993; Bearhop et al. 2002; DeNiro and Epstein 1978; DeNiro and Epstein 1981; Hobson and Clark 1993; Tieszen et al. 1983). These studies suggest that physiological characteristics within 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 factors.

Diet composition is also an important consideration in isotopic fractionation studies (DeNiro and Epstein 1978; DeNiro and Epstein 1981; Hobson 1995; Hobson and Clark 1992). A comprehensive study by DeNiro and Epstein (1978) and DeNiro and Epstein (1981) noted that much less variation existed when the consumer was fed an animal protein based diet in comparison to a plant based diet. Additional studies also have reported differences in isotopic fractionation factors for birds that were fed diets with dissimilar compositions, but some of these differences may have also been species-related (Hobson 1995; Hobson and Clark 1992). For example, in the Hobson (1995) study, isotopic fractionation factors for eggs components were determined for Japanese quail and mallards that were fed an herbivore diet and three falcon species that were fed a carnivore diet and isotopic fractionation factors were more similar for species that were fed the same diet. In our study, the commercial diet formulated for spectacled eiders is primarily plant based (~60%), but also contains about 20% or more fish and pork (Liz Koutsos, Purina Mills Incorporated, personal communication). Therefore, some of the differences we observed among years or individuals may have been because of the diet composition. This model may be useful for species that feed on both animal protein and plant based diets in the wild. For example, spectacled eiders are thought to feed on both animal protein and plant diets on the breeding grounds and mainly on an animal protein diet while on the wintering grounds (Lovvorn et al. 2003; U.S. Fish and Wildlife Service 1996). Therefore temporal variation in the diet composition may be an important consideration when incorporating isotopic fractionation factors into nutrient allocation models.

Additionally, natural food supplements that varied in diet composition and isotope signatures could contribute to isotopic differences observed in the egg tissues. However, we expect that these would be small differences because these only made up a small proportion of the diet and we accounted for different isotopic signatures in our diet estimate. Although small sample sizes of diet analyzed in 2006 and 2007 may have limited our ability to capture isotopic variation in the diet in those years, we still observed similar patterns in 2008 when the diet was simplified and larger sample size was collected.

It is also important to consider that analytical error within instruments and measurements over different time periods may contribute to variation in isotopic fractionation (Jardine and Cunjak 2005; Mill et al. 2008). For example, studies have detected differences of 0.2‰ while measuring laboratory standards at two different time periods and suggested a greater probability of identifying significant differences when analyzing sample sizes greater than 30 (Jardine and Cunjak 2005). Therefore, analytical precision may account for some of the variation observed in stable isotope studies. Additionally, Mill et al. (2008) suggested a variation of 2.1‰ in nitrogen and 0.5‰ in carbon for the same cod muscle tissue measured in eight different instruments. In our study, we accounted for this variation by processing frequent laboratory standards and conducted stable isotope signatures at the same facility. This inherent variability may be important to consider when making comparisons between studies conducted during different time periods and at different laboratories.

1.5.3 *Species Differences in $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$*

Many studies have shown that differences in isotopic fractionation of tissues may be the result of species-specific physiology (Dalerum and Angerbjorn 2005; DeNiro and Epstein 1978; DeNiro and Epstein 1981; Hobson 1995; Hobson and Clark 1992). Avian egg isotope fractionation has only been reported for a few species (Hobson 1995) and no data

were available previously for sea ducks. Our results have identified some important differences in the isotopic fractionation factors for egg components among species, mainly for the protein-comprised portions of the egg. For example, $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ in albumen, lipid-free yolk, and shell membrane increased for spectacled eiders in relation to other avian species (Hobson 1995). However, there were similarities in $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ in whole yolk and for $\Delta\delta^{13}\text{C}$ in yolk lipids for spectacled eiders in comparison to other avian species (Hobson 1995). There was a wide range in $\Delta\delta^{13}\text{C}$ of eggshells among the different species considered in the Hobson (1995) study and our spectacled eider values fell within that range. Eggshell composition can be derived from a variety of sources including dietary calcium or skeletal tissue and blood bicarbonate or CO_2 (Johnson 2000). The similarities that we observed between species may have been the result of differences in diet composition. Birds fed an herbivore diet had a carbon isotopic fractionation factor that was higher relative to those values determined for spectacled eiders and birds fed a carnivore diet were lower than those values that were determined for spectacled eiders. Because the spectacled eider diet consisted of both animal protein and plant, this may suggest an isotopic fractionation factor for carbon stable isotopes in whole yolk, yolk lipids, and eggshell that is representative of an omnivore diet. Nitrogen stable isotopes among those tissues essentially overlapped. Finally, our theoretical sensitivity test indicated that choice of isotopic fractionation factors could considerably alter the conclusions of mixing models for percentages of source contributions in nutrient allocation to reproduction. This further emphasizes the need to determine these values for individual species and tissues (Caut et al. 2008; Dalerum and Angerbjorn 2005; Hobson 2006). This study suggests that we can improve model accuracy by using species-specific isotopic fractionation factors, and increased accuracy of these models will yield more applicable results regarding nutrient sources used for egg production.

Interestingly, the largest difference observed between spectacled eider diet to egg isotopic fractionation and those values for previously studied species were for lipid-free yolk (Hobson 1995). Our data suggest that carbon isotope fractionation of lipid extracted yolk

increased by a mean of 4.1‰ in comparison to whole yolk, while Hobson (1995) reported a much lower value (1.2-1.5‰ for quail and mallards and 1.5-2.2‰ for falcon species). A general increase in the carbon isotope fractionation factor relative to whole yolk values was expected because of the removal of lipids that exhibit lower isotope signatures. However, the degree of difference relative to the values previously reported by Hobson (1995) was notable. On the other hand, our results indicate a similar decrease in the carbon isotope fractionation factor that Hobson (1995) reported for yolk lipids in comparison to whole yolk. The isotopic fractionation of spectacled eider whole yolk in relation to yolk lipids averaged 1.6‰ among the years, while quail and mallards ranged 1.3-1.5‰ and falcon species ranged 1.3-2.2‰. These results suggest that spectacled eiders may use some different lipid or protein sources for egg formation in relation to those previously studied species. Variations in lipid, amino acid, and carbohydrate content of the egg yolk and albumen have been reported between precocial (i.e., *Anseriformes*) and altricial species (Bucher 1987; Roca et al. 1984), which may be influencing these relationships. Further, variations in these sources could be reflected in isotope studies as recent compound specific isotope analysis have suggested that isotope signatures in individual amino acids and fatty acids can differ (Budge et al. 2008; Howland et al. 2003; O'Brien et al. 2005). This finding warrants further investigation into specific micronutrients (i.e., amino acids and fatty acids) used for spectacled eider egg production and compound specific isotope analysis.

Nitrogen isotopic fractionation factors were comparable or slightly higher for spectacled eiders relative to those previously reported for other avian species. In our study, lipid-extracted yolk was approximately 1‰ higher in comparison to whole yolk, while nitrogen was similar between lipid and lipid-extracted yolk in the Hobson (1995) study. It is possible that the increase in isotope signature observed in nitrogen for lipid-free yolk may have been an effect of lipid extraction because methanol (the polar solvent) does not target specific lipids. Some polar structural fats are attached to proteins, and the loss of these amino acids as a result of lipid extraction may increase the $\delta^{15}\text{N}$ (Sotiropoulos et al.

2004; Sweeting et al. 2006). Further investigation of specific nutrients, like lipoproteins, that may be important for spectacled eider egg production would be valuable.

1.5.4 Differentiating Macronutrient Sources

As an expansion of a previously established hypothetical model (Hobson 1995), this study has determined isotopic fractionation values from whole diet to whole yolk and eggshell and from diet macronutrients (i.e., diet lipids and diet with lipids removed) to those lipid (i.e., yolk lipids) and protein portions of the egg or the newly developing embryo (i.e., albumen, lipid-free yolk, shell membrane, and down feathers). Use of macronutrient-specific isotopic fractionation factors will further increase the applicability of stable isotopes in studies of nutrient allocation to egg production because these different macronutrients may be derived from nutrient sources that are from isotopically distinct habitats.

1.6 Acknowledgements

Financial support for this research was provided by the United States Fish and Wildlife Service (#70181-6-G413), Ocean Alaska Science and Learning Center (H9845060030), and National Park Foundation (07AKCMRG007 and 08AKCMRG004). Samples were prepared at Alaska SeaLife Center and the Alaska Stable Isotope Facility at the University of Alaska Fairbanks. Thanks to many Alaska SeaLife Center staff members, Ann Riddle for lab support; Heidi Cline, Tasha DiMarzio, Gwen Gerdson, Mike Grue, Christina Haskins, and Sadie Ulman for their assistance with care of the captive spectacled eiders; and Daniel Hennen who provided advice on statistical analysis. Also we appreciate comments from Mandy Keogh and Pam Parker on earlier drafts of this manuscript. Tim Howe conducted the stable isotope analyses and assisted with interpretation of the results, and Norma Haubenstock provided valuable lab support from the Alaska Stable Isotope Facility.

1.7 References

- Alisauskas RT, Ankney CD (1992) The cost of egg laying and its relationship to nutrient reserves in waterfowl. In: Batt BDJ et al. (eds) *Ecology and Management of Breeding Waterfowl*. University of Minnesota Press, Minneapolis, MN, USA, pp 30-61
- Ambrose SH, Norr L (1993) Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. In: *Prehistoric human bone: Archeology at the molecular level*. Springer-Verlag, New York, NY, USA, pp 1-37
- Bearhop S, Waldron S, Votier SC, Furness RW (2002) Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. *Physiological and Biochemical Zoology* 75:451-458
- Ben-David M, Schell DM (2001) Mixing models in analyses of diet using multiple stable isotopes: A response. *Oecologia* 127:180-184
- Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37:911-917
- Bond JC, Esler D, Hobson KA (2007) Isotopic evidence for sources of nutrients allocated to clutch formation by harlequin ducks. *Condor* 109:698-704
- Bucher TL (1987) Patterns in the mass-independent energetics of avian development. *Journal of Experimental Zoology* 1:139-150
- Budge SM, Wooller MJ, Springer AM, Iverson SJ, McRoy CP, Divoky GJ (2008) Tracing carbon flow in an arctic marine food web using fatty acid-stable isotope analysis. *Oecologia* 157:117-129
- Caut S, Angulo E, Courchamp F (2008) Caution on isotopic model use for analyses of consumer diet. *Canadian Journal of Zoology* 86:438-445
- Dalerum F, Angerbjorn A (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. *Oecologia* 144:647-658
- DeNiro MJ, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* 197:261-263
- DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42:495-506

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* 45:341-351

Fay FH, Kelly BP, Sease JL (1989) Managing the exploitation of Pacific walruses: A tragedy of delayed response and poor communication. *Marine Mammal Science* 5:1-16

Federal Register (1993) Final rule to list the spectacled eider as threatened. 58:27474-27480

Gannes LZ, O'Brien DM, Martínez del Rio C (1997) Stable isotopes in animal ecology: Assumptions, caveats, and a call for more laboratory experiments. *Ecology* 78:1271-1276

Gloutney ML, Hobson KA (1998) Field preservation techniques for the analysis of stable-carbon and nitrogen isotope ratios in eggs. *Journal of Field Ornithology* 69:223-227

Hobson KA (1995) Reconstructing avian diets using stable-carbon and nitrogen isotope analysis of egg components: Patterns of isotopic fractionation and turnover. *Condor* 97:752-762

Hobson KA (2006) Using stable isotopes to quantitatively track endogenous and exogenous nutrient allocations to eggs of birds that travel to breed. *Ardea* 94:359-369

Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes II: Factors influencing diet-tissue fractionation. *Condor* 94:189-197

Hobson KA, Clark RG (1993) Turnover of ^{13}C in cellular and plasma fractions of blood: Implications for nondestructive sampling in avian dietary studies. *Auk* 110:638-641

Hobson KA, Hughes KD, Ewins PJ (1997) Using stable-isotope analysis to identify endogenous and exogenous sources of nutrients in eggs of migratory birds: Applications to Great Lakes contaminants research. *Auk* 114:467-478

Hobson KA, Montevecchi WA (1991) Stable isotopic determinations of trophic relationships of great auks. *Oecologia* 87:528-531

Hobson KA, Sirois J, Gloutney ML (2000) Tracing nutrient allocation to reproduction with stable isotopes: A preliminary investigation using colonial waterbirds of Great Slave Lake. *Auk* 117:760-774

Hobson KA, Thompson JE, Evans MR, Boyd S (2005) Tracing nutrient allocation to reproduction in Barrow's goldeneye. *Journal of Wildlife Management* 69:1221-1228

Howland MR, Corr LT, Young SMM, Jones V, Jim S, Van Der Merwe NJ, Mitchell AD, Evershed RP (2003) Expression of the dietary isotope signal in the compound-specific $\delta^{13}\text{C}$ values of pig bone lipids and amino acids. *International Journal of Osteoarchaeology* 13:54-65

Jardine TD, Cunjak RA (2005) Analytical error in stable isotope ecology. *Oecologia* 144:528-533

Jardine TD, Kidd KA, Fisk AT (2006) Applications, considerations, and sources of uncertainty when using stable isotope analysis in ecotoxicology. *Environmental Science and Technology* 40:7501-7511

Johnson AL (2000) Reproduction in the female. In: Whittow GC (ed) *Sturkie's Avian Physiology*, 5th edition. Academic Press, New York, NY, USA, pp 569-596

Kelly JF (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology* 78:1-27

Klaassen M, Lindstrom A, Meltote H, Piersma T (2001) Arctic waders are not capital breeders. *Nature* 413:794

Knoche MJ (2004) King eider wing molt: Inferences from stable isotope analyses. Master of Science Thesis. University of Alaska Fairbanks, Fairbanks, Alaska, USA

Knoche MJ, Powell AN, Quakenbush LT, Wooller MJ, Phillips LM (2007) Further evidence for site fidelity to wing molt locations by king eiders: Integrating stable isotope analyses and satellite telemetry. *Waterbirds* 30:52-57

Lawson S (2006) Comparative reproductive strategies between long-tailed ducks and king eiders at Karrak Lake, Nunavut: Use of energy resources during the nesting season. Master of Science Thesis. University of Saskatchewan, Saskatoon, Canada

Lovvorn JR, Richman SE, Grebmeier JM, Cooper LW (2003) Diet and body condition of spectacled elders wintering in pack ice of the Bering Sea. *Polar Biology* 26:259-267

Martínez del Río C, Wolf BO (2005) Mass-balance models for animal isotopic ecology. In: Stark JM, Wang T (eds) *Physiological and Ecological Adaptations to Feeding in Vertebrates*. Science Publishers, Enfield, NH, USA, pp 141-174

Martínez del Río C, Wolf N, Carleton SA, Gannes LZ (2009) Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews* 84:91-111

McConnaughey T, McRoy CP (1979) Food-web structure and the fractionation of carbon isotopes in the Bering Sea. *Marine Biology* 53:257-262

- Michener RH, Schell DM (1994) Stable isotope ratios as tracers in marine aquatic food webs. In: Lajtha K, Michener RH (eds) *Stable Isotopes in Ecology and Environmental Science*. Blackwell Scientific Publications, Oxford, UK, pp 138-157
- Mill AC, Sweeting CJ, Barnes C, Al-Habsi SH, MacNeil MA (2008) Mass-spectrometer bias in stable isotope ecology. *Limnology and Oceanography Methods* 6:34-39
- Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochimica et Cosmochimica Acta* 48:1135-1140
- Montoya JP (2007) Natural abundance of ^{15}N in marine planktonic ecosystems. In: Michener R, Lajtha K (eds) *Stable Isotopes in Ecology and Environmental Science*, 2nd edition. Blackwell Publishing, Malden, MA, USA
- Murphy ME (1996) Energetics and nutrition of molt. In: Carey C (ed) *Avian Energetics and Nutritional Ecology*. Chapman & Hill, New York, NY, USA, pp 158-198
- O'Brien DM, Boggs CL, Fogel ML (2005) The amino acids used in reproduction by butterflies: A comparative study of dietary sources using compound-specific stable isotope analysis. *Physiological and Biochemical Zoology* 78:819-827
- Petersen MR, Douglas DC, Mulcahy DM (1995) Use of implanted satellite transmitters to locate spectacled eiders at-sea. *Condor* 97:276-278
- Petersen MR, Larned WW, Douglas DC (1999) At-sea distribution of spectacled eiders: A 120-year-old mystery resolved. *Auk* 116:1009-1020
- Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18:293-320
- Phillips DL (2001) Mixing models in analyses of diet using multiple stable isotopes: A critique. *Oecologia* 127:166-170
- Phillips DL, Gregg JW (2001) Uncertainty in source partitioning using stable isotopes. *Oecologia* 127:171-179
- Phillips DL, Gregg JW (2003) Source partitioning using stable isotopes: Coping with too many sources. *Oecologia* 136:261-269
- Phillips DL, Koch PL (2002) Incorporating concentration dependence in stable isotope mixing models. *Oecologia* 130:114-125

Rau GH, Mearns AJ, Young DR, Olson RJ, Schafer HA, Kaplan IR (1983) Animal $^{13}\text{C}/^{12}\text{C}$ correlates with trophic level in pelagic food webs. *Ecology* 64:1314-1318

Richman SE, Lovvorn JR (2003) Effects of clam species dominance on nutrient and energy acquisition by spectacled eiders in the Bering Sea. *Marine Ecology Progress Series* 261:283-297

Roca P, Sainz F, Gonzalez M, Alemany M (1984) Structure and composition of the eggs from several avian species. *Comparative Biochemistry and Physiology* 77A:307-310

Saupe SM, Schell DM, Griffiths WB (1989) Carbon-isotope ratio gradients in western arctic zooplankton. *Marine Biology* 103:427-432

Sotiropoulos MA, Tonn WM, Wassenaar LI (2004) Effects of lipid extraction on stable carbon and nitrogen isotope analyses of fish tissues: Potential consequences for food web studies. *Ecology of Freshwater Fish* 13:155-160

Sweeting CJ, Polunin NVC, Jennings S (2006) Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. *Rapid Communications in Mass Spectrometry* 20:595-601

Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}\text{C}$ analysis of diet. *Oecologia* 57:32-37

U.S. Fish and Wildlife Service (1996) Spectacled eider recovery plan. Anchorage, Alaska. 157 Pp

Table 1.1 Captive female spectacled eider start and end lay dates, and number of eggs and down feathers from embryos in sampling years 2006, 2007, and 2008.

	Female color and band number				
	Blue 10018	Black 10012	Pink 10015	White 10019	Yellow 10024
2006					
start lay date	19-May (individual start not known)				
end lay date	30-Jun (individual end not known)				
n = eggs	37 (#/individual not known)				
2007					
start lay date	7-Jun	27-May	22-May	23-May	29-May
End lay date	14-Jul	3-Jun	29-May	31-May	30-Jun
n = eggs	6	6	6	7	14
2008					
start lay date	26-May	27-May	20-May	20-May	2-June
End lay date	3-June	23-June	29-May	30-May	30-June
n = eggs	6	15	7	8	14
n = down feathers from embryos	-	-	2	3	-

Table 1.2 Average ($\pm$ SD) carbon and nitrogen stable isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) for all years based on proportional source contribution (Mazuri®:natural food) for diet lipids, whole diet, and lipid-free diet.

Year	Lipid Diet	Whole Diet		Lipid-free Diet	
	$\delta^{13}\text{C}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
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

Table 1.3 Whole diet to egg component isotopic fractionation factors (mean ‰ ± SD) for captive spectacled eiders. Results of ANOVA tests for significant differences among years for each isotope and Bonferroni multiple comparison t-tests are indicated by superscripts where means that have a superscript in common are not significantly different (p>0.05).

Year	δ	Albumen	Lipid-free Yolk	Membrane	Yolk	Yolk Lipid	Eggshell	Down Feathers
2006	C	2.1 ± 0.3 ^a	1.8 ± 0.3(35) ^a	3.0 ± 0.3 ^a	-2.2 ± 0.3(36) ^a	-3.6 ± 0.4(36) ^a	12.3 ± 0.8 ^a	
(n=37*)	N	5.0 ± 0.4 ^a	5.4 ± 0.5(36) ^a	5.8 ± 0.5 ^a	4.0 ± 0.6(36) ^a			
2007	C	3.1 ± 0.2 ^b	2.5 ± 0.7 ^b	4.2 ± 0.3 ^b	-1.3 ± 0.4 ^b	-2.5 ± 0.6 ^b	13.8 ± 0.7 ^b	
(n=39)	N	3.4 ± 0.3 ^b	3.9 ± 0.6 ^b	4.4 ± 0.4 ^b	3.1 ± 0.6 ^b			
2008	C	2.7 ± 0.1(47) ^c	2.9 ± 0.1(49) ^c	3.9 ± 0.2 ^c	-1.6 ± 0.3(49) ^c	-3.5 ± 0.3(49) ^a	13.0 ± 0.7 ^c	2.2 ± 0.4 (5)
(n=50*)	N	3.6 ± 0.3(48) ^c	4.3 ± 0.4(49) ^b	4.6 ± 0.4 ^c	3.5 ± 0.3(49) ^c			5.3 ± 0.4 (5)
2006-2008								
Mean	C	2.6 ± 0.5(123)	2.5 ± 0.6(123)	3.7 ± 0.6	-1.6 ± 0.5(124)	-3.2 ± 0.6(124)	13.1 ± 0.9	
(n=126*)	N	4.0 ± 0.8(124)	4.5 ± 0.8(124)	4.9 ± 0.7	3.5 ± 0.6(124)			
ANOVA C:	F	-	-	-	66.801	-	51.802	
	p	-	-	-	<0.001	-	<0.001	
N:	F	251.246	-	-	-			
	p	<0.001	-	-	-			
Ranks C:	H	99.488	65.792	91.428	-	56.83	-	
	p	<0.001	<0.001	<0.001	-	<0.001	-	
N:	H	-	68.092	75.483	34.81			
	P	-	<0.001	<0.001	<0.001			

*sample size unless otherwise noted for specific egg components in parentheses

Table 1.4 Whole diet to egg isotopic fractionation factors (mean ‰ ± SD) for spectacled eiders and other avian species described in Hobson 1995. ANOVA statistics reported for all species and only significant differences from spectacled eiders (SPEI) are listed in parentheses (Japanese quail=JAQU, 2 clutches for mallard=MALL 1&2, peregrine falcon=PEFA, gyrfalcon=GYFA, and prairie falcon=PRFA; see Hobson 1995 for differences between other species).

Species (n)	δδ	Albumen	Lipid-free Yolk	Membrane	Yolk	Yolk Lipid	Eggshell
SPEI (126)	C	2.6 ± 0.5 (all spp.)	2.5 ± 0.6 (all spp.)	3.7 ± 0.6 (PEFA)	-1.6 ± 0.5 (not significant)	-3.2 ± 0.6 (not significant)	13.1 ± 0.9 (all spp.)
	N	4.0 ± 0.8 (JAQU, MALL 1&2)	4.5 ± 0.8 (JAQU, MALL 1&2)	4.9 ± 0.7 (JAQU, MALL 2, PEFA, PRFA)	3.5 ± 0.6 (not significant)		
JAQU (9)	C	1.6 ± 0.4	0.1 ± 0.3	3.5 ± 0.3	-1.1 ± 0.3	-2.6 ± 0.5	15.6 ± 0.5
	N	2.4 ± 0.2	3.4 ± 0.3	4.1 ± 0.4	3.4 ± 0.3		
MALL 1 (8)	C	1.4 ± 0.6	-0.1 ± 0.5	3.7 ± 0.3	-1.4 ± 0.7	-2.7 ± 0.5	14.3 ± 0.7
	N	3.0 ± 0.4	3.1 ± 0.4	4.4 ± 0.3	3.4 ± 0.3		
MALL 2 (8)	C	1.4 ± 0.6	0.2 ± 0.5	3.4 ± 0.4	-1.3 ± 0.7	-2.6 ± 0.5	14.6 ± 0.7
	N	2.8 ± 0.4	3.2 ± 0.4	4.0 ± 0.3	3.0 ± 0.3		
PEFA (6)	C	0.9 ± 0.5	0.0 ± 0.5	2.6 ± 0.5	-2.2 ± 0.5	-3.5 ± 0.5	11.1 ± 0.5
	N	3.1 ± 0.4	3.5 ± 0.3	3.5 ± 0.4	3.3 ± 0.4		
GYRF (4)	C	0.8 ± 0.5	0.1 ± 0.5		-1.8 ± 0.5	-3.2 ± 0.4	11.2 ± 0.5
	N	3.3 ± 0.3	3.6 ± 0.4		3.1 ± 0.4		
PRFA (2)	C	0.9	0.1	3.0	-1.4	-3.6	11.6
	N	3.1	3.5	3.2	3.5		
ANOVA C:	F	35.143	86.591	5.265	3.546	4.060	45.374
	p	<0.001	<0.001	<0.001	0.003	<0.001	<0.001
	N:	F	11.938	12.383	12.343	1.404	
	p	<0.001	<0.001	<0.001	0.216		

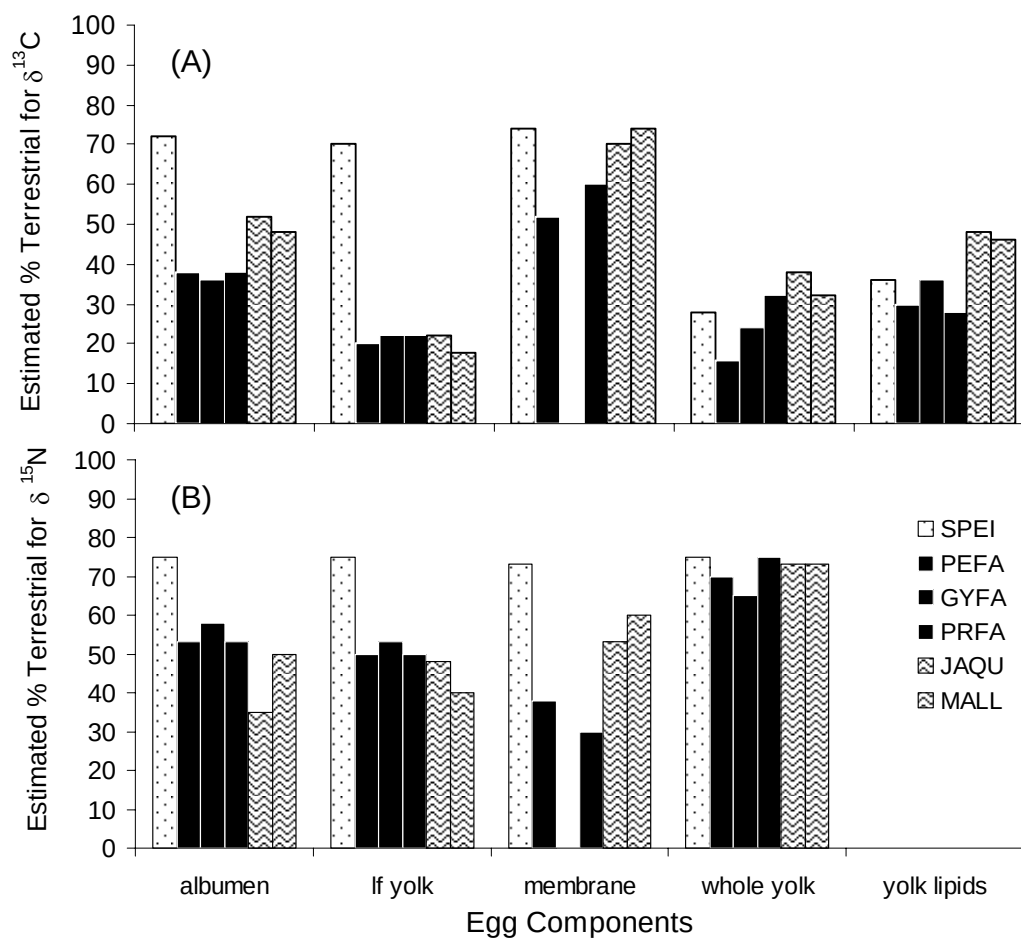


Figure 1.1 Theoretical mixing models (described in text) illustrate the estimated percentages of terrestrial nutrient sources allocated to each egg component using carbon (A) and nitrogen (B) stable isotopes. Whole diet to tissue isotopic fractionation factors from different species were substituted 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.

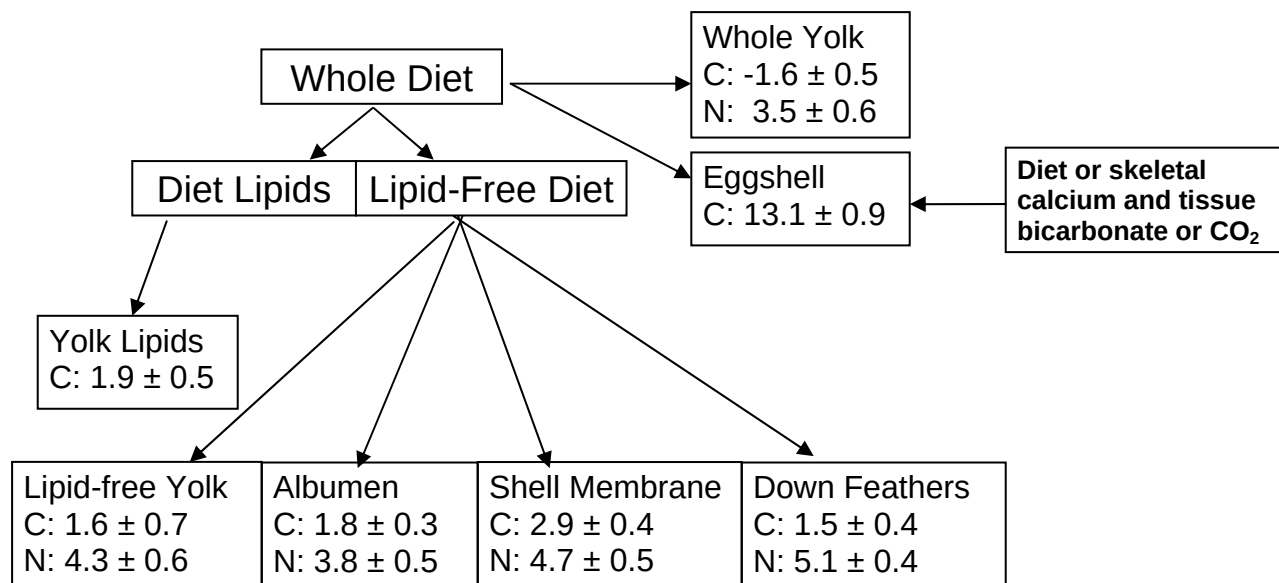


Figure 1.2 As an expansion from the Hobson (1995) hypothetical model, isotopic fractionation factors were determined for whole diet to whole yolk (containing both proteins and lipids) and eggshell (containing calcium from diet or skeletal tissue and carbonate from bicarbonate or CO₂), diet lipids to 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.

Appendix 1.1 Natural food supplements offered to captive spectacled eiders and approximate harvesting location.

Common Name	Scientific Name	Location
common squid	<i>Loligo opalescens</i>	Catalina Island, California
Pacific blue mussels	<i>Mytilus trossulus</i>	Cook Inlet (Sadie/Halibut Cove), Alaska
Tidewater silversides	<i>Menidia menidia</i>	Prince Edward Island, Canada
Antarctic krill	<i>Euphausia superba</i>	Southwest Atlantic Ocean
Arctic razor clam	<i>Siliqua patula</i>	Cook Inlet, Alaska

Appendix 1.2 Average ($\pm$ SD) stable carbon and nitrogen isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) for whole diet, lipid-free diet and diet lipids of Mazuri® sea duck diet and natural food supplements offered to spectacled eiders in 2006.

2006	Diet Items	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Diet items whole-diet	Mazuri® original (n=2)	-21.4 ± 0.3	6.3 ± 0.8
	Silversides (n=2)	-19.1 ± 0.1	10.4 ± 0.2
	Razor Clams (n=2)	-16.5 ± 0.1	9.0 ± 0.1
	Squid (n=2)	-18.4 ± 0.1	12.9 ± 0.2
	Mussels (n=2)	-18.3 ± 0.2	7.0 ± 0.2
	Krill (n=2)	-26.1 ± 0.1	3.8 ± 0.2
Diet items (lipid-free)	Mazuri® original (n=2)	-21.1 ± 0.5	6.3 ± 0.4
	Silversides (n=2)	-18.9 ± 0.2	12.1 ± 0.4
	Razor Clams (n=2)	-16.3 ± 0.2	9.9 ± 0.3
	Squid (n=2)	-16.9 ± 0.1	13.2 ± 0.3
	Mussels (n=2)	-17.7 ± 0.1	8.2 ± 0.2
	Krill (n=2)	-22.4 ± 0.3	5.4 ± 0.8
Diet items (lipid only)	Mazuri® original (n=2)	-26.4 ± 0.2	-
	Silversides (n=2)	-24.0 ± 0.1	-
	Razor Clams (n=2)	-21.8 ± 0.1	2.5 ± 0.3
	Squid (n=2)	-22.9 ± 0.5	8.7 ± 0.2
	Mussels (n=2)	-23.2 ± 0.1	5.5 ± 0.4
	Krill (n=2)	-30.7 ± 0	-

Appendix 1.3 Average ($\pm$ SD) stable carbon and nitrogen isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) for whole diet, lipid-free diet and diet lipids of Mazuri® sea duck diet and natural food supplements offered to spectacled eiders in 2007.

2007	Diet Items	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Diet items whole-diet	Mazuri® (n=2)	-21.4 ± 0.4	7.2 ± 0.9
	Silversides (n=2)	-17.2 ± 1.0	10.3 ± 0.1
	Razor Clams (n=2)	-18.0 ± 0	13.8 ± 0.3
	Squid (n=2)	-19.9 ± 0.1	11.9 ± 0.4
	Mussels (n=2)	-19.5 ± 0.1	7.2 ± 0.2
	Krill (n=2)	-26.5 ± 0.6	4.1 ± 0.1
Diet items (lipid-free)	Mazuri® (n=2)	-20.0 ± 0.3	6.9 ± 0.7
	Silversides (n=2)	-16.8 ± 0.4	11.6 ± 0.8
	Razor Clams (n=2)	-17.7 ± 0.4	14.1 ± 0.1
	Squid (n=2)	-17.2 ± 0.1	10.0 ± 0.3
	Mussels (n=2)	-18.2 ± 0	7.8 ± 0.1
	Krill (n=2)	-23.9 ± 0.1	4.4 ± 0.1
Diet items (lipid only)	Mazuri® (n=2)	-26.3 ± 0.1	-
	Silversides (n=2)	-20.9 ± 0.1	-
	Razor Clams (n=2)	-22.4 ± 0.6	4.8 ± 0.5
	Squid (n=2)	-24.0 ± 0.1	7.3 ± 0.4
	Mussels (n=2)	-23.9 ± 0.5	2.6 ± 0.5
	Krill (n=2)	-31.1 ± 0.1	-

Appendix 1.4 Average ($\pm$ SD) stable carbon and nitrogen isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) for whole diet, lipid-free diet and diet lipids of Mazuri® sea duck diet and silversides offered to spectacled eiders in 2008.

2008	Diet Items	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Diet items whole-diet	Mazuri® original (n=10)	-21.1 ± 1.0	6.3 ± 0.6
	Silversides (n=10)	-18.5 ± 0.9	11.6 ± 0.4
Diet items (lipid-free)	Mazuri® original (n=10)	-20.4 ± 0.6	6.5 ± 0.5
	Silversides (n=10)	-17.6 ± 0.6	12.1 ± 0.6
Diet items (lipid only)	Mazuri® original (n=10)	-26.6 ± 0.2	-
	Silversides (n=10)	-23.4 ± 0.8	-

Chapter 2 Stable Carbon and Nitrogen Isotope Fractionation Factors from Diet to Cellular Blood, Blood Plasma, Feathers, and Subcutaneous Fat in Spectacled Eiders (*Somateria fischeri*)²

2.1 Abstract

Spectacled eiders (*Somateria fischeri*) are threatened sea ducks that spend the majority of their lives at-sea. Little is known about marine prey items consumed by this species because of their remote locations during the nonbreeding season. However, this attribute has important implications for conservation because marine habitat changes, specifically shifts in prey types and availability, have been speculated to affect population dynamics. Stable isotope analyses of various avian tissues have been used to infer diet because feathers can preserve a record of diet from the habitat where they were grown and blood components can offer more recent information about diet sources and variability. Results from stable isotope analyses can be applied to foraging ecology studies using mixing models to calculate diet source contributions. Additionally, researchers have utilized stable isotope signatures of tissues like fat and cellular blood in nutrient allocation mixing models to represent lipid and protein reserves, respectively. The isotope signatures of tissues generally increase relative to the diet, referred to as stable isotope fractionation factors (i.e., $\Delta\delta = \delta \text{ consumer tissue} - \delta \text{ diet}$), and these values must be quantified to enable the comparison of tissues relative to putative nutrient sources. Diet to tissue isotopic fractionation factors have been determined for some birds, but only few data are available for isotopic fractionation in sea duck feathers and no previous data exist for isotopic fractionation of sea duck blood components and fat. Studies using captive animals fed controlled diets are important for interpretation of stable isotopes in animal

²prepared for submission to *Oecologia* as Federer R, Hollmén T, Esler D, and Wooller MJ, Stable Carbon and Nitrogen Isotope Fractionation Factors from Diet to Blood Components, Feathers, and Subcutaneous Fat in Spectacled Eiders (*Somateria fischeri*)

tissues, as isotopic fractionation factors cannot be accurately determined from field research. We determined the stable carbon and nitrogen isotopic fractionation factors between lipid-free diet and blood components of captive female spectacled eiders in 2007 and 2008 and adult chest contour feathers in 2008. Average carbon and nitrogen stable isotope fractionation factors for blood components and feathers were either similar or increased slightly in comparison to previously studied bird species. Additionally, we determined the isotopic fractionation factors from diet lipids to subcutaneous fat during three different diets and three subsequent fat biopsies for captive male spectacled eiders. Isotopic signatures of subcutaneous fat closely reflected the shifts in the diet and were either equivalent or increased relative to diet providing evidence that changes in diet composition, tissue turnover, or the building and burning of nutrient reserves could influence isotopic fractionation factors. Our study provides a foundation for research using tissues as end-members in stable isotope nutrient allocation models and foraging ecology studies in spectacled eiders, and likely offers more accurate estimations of diet to tissue isotopic fractionation factors for other sea duck models.

2.2 Introduction

Analysis of stable isotopes in avian tissues has become a valuable tool for tracking migration, inferring diet, and evaluating foraging ecology and resource allocation (Hobson 2006; Inger and Bearhop 2008; Kelly 2000; Rubenstein and Hobson 2004). This is because isotopic signatures in consumer tissues generally reflect that of the diet (DeNiro and Epstein 1978; DeNiro and Epstein 1981; Minagawa and Wada 1984; Peterson and Fry 1987), and therefore function as food web tracers within and between ecosystems (Webster et al. 2002). Models can be employed to quantitatively assess foraging ecology and nutrient allocation to reproduction (Phillips and Gregg 2001; Post 2002). This technique may be a particularly useful tool for sea ducks, like spectacled eiders (*Somateria fischeri*), that inhabit offshore marine locations most of the year (Petersen et al. 1999) and travel to isotopically distinct regions to breed (Peterson and Fry

1987). Avian tissues like blood and feathers can be collected nondestructively 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. 2000; Hobson et al. 2004; Hobson et al. 2005; Klaassen et al. 2001; Schmutz et al. 2006).

Successful dietary modeling using stable isotope analyses requires knowledge on how the stable isotope signatures of tissues reflect that of the diet ($\delta\Delta = \delta \text{ animal tissue} - \delta \text{ diet}$), referred to as isotopic fractionation (Ben-David and Schell 2001; Caut et al. 2008; Phillips 2001). Most avian studies have used $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data because these are good indicators of diet or geographical region and trophic position (Peterson and Fry 1987). Previous studies have shown that stable carbon isotopes fractionate during tissue synthesis and generally increase or decrease slightly, but are comparable to diet (DeNiro and Epstein 1978; Peterson and Fry 1987). The $\delta^{13}\text{C}$ in tissues is thought to increase due to the respiration of the lighter and more abundant carbon isotope (^{12}C) and uptake of the heavier and relatively rare stable carbon isotope (^{13}C) in the body tissue (DeNiro and Epstein 1978; Peterson and Fry 1987; Rau et al. 1983; Tieszen et al. 1983). Conversely, a decrease in the $\delta^{13}\text{C}$ can occur in tissues that contain lipid because of oxidation during lipid synthesis (DeNiro and Epstein 1977). Nitrogen stable isotopes generally increase approximately 3-5‰ relative to the diet (DeNiro and Epstein 1981; Minagawa and Wada

1984; Peterson and Fry 1987). The reason for the increase in $\delta^{15}\text{N}$ is still unclear, but may result from the preferential excretion of the lighter stable isotope (^{14}N) in the excretory material or the preferential absorption of the heavy stable isotope (^{15}N) in the tissue (Martínez del Río et al. 2009). These isotopic fractionation factors can vary by tissue type because of different nutrient compositions (Bearhop et al. 2002; Hobson 1995; Hobson and Clark 1992b). Studies of isotopic fractionation associated with avian blood components suggest that cellular blood increased a range of 0.5-1.5‰ for carbon and 3‰ in one study for nitrogen relative to diet, while the range for blood plasma were comparable to diet (-1.5‰-0.6‰) for carbon and increased 2.5-3.3‰ relative to diet for nitrogen (Evans Ogden et al. 2004; Hobson and Clark 1993; Pearson et al. 2003). Additionally, feathers ranged from -0.4‰-4.3‰ for carbon and 1.1-5.6‰ for nitrogen (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). While these values may also vary across taxa (Caut et al. 2008; Dalerum and Angerbjorn 2005), limited to no information is currently available regarding isotopic fractionation factors in sea duck feathers (Knoche 2004) and blood components. Therefore, experimental studies are needed to determine tissue and species/taxon specific isotopic fractionation values that will further facilitate interpretation of results from field studies (Caut et al. 2008; Dalerum and Angerbjorn 2005; Gannes et al. 1997).

Spectacled eiders are threatened sea ducks (Federal Register 1993) and population recovery may be influenced by changes in the marine environment, including temporal variation in prey type and abundance (Richman and Lovvorn 2003; U.S. Fish and Wildlife Service 1996). Relatively little is known about the migratory patterns and diet for this species during the nonbreeding season (Lovvorn et al. 2003; Petersen et al. 1999), and stable isotope methods may provide insight for understanding these biological traits. The purpose of this study was to experimentally establish the carbon and nitrogen stable isotopic fractionation factors between diet items and tissues (i.e., cellular blood, blood plasma, adult chest contour feathers, and subcutaneous fat) for captive spectacled eiders.

Isotopic fractionation factors in blood components were measured in two consecutive years (2007 and 2008), which enabled us to evaluate variation between years. Our spectacled eider isotopic fractionation factors for tissues provide appropriate tissue corrections useful for stable isotope research and modeling in field studies and may be useful for other sea duck species. Understanding ecological links between habitats and dietary sources is important for supporting population recovery and management of critical foraging habitats for spectacled eiders.

2.3 Materials and Methods

We determined diet to tissue isotopic fractionation factors by comparing stable isotope signatures of various tissues to those of the diet. Diet was held relatively stable during the pre-breeding months (February-May) in 2007 and pre- to post-breeding months in 2008 (February-July), and lipid-free diet signatures were contrasted to those of cellular blood and blood plasma collected in May. Carbon and nitrogen stable isotope signatures from feather samples collected post-breeding in 2008 were also compared to stable isotope signatures of lipid-free diet. For another study during the nonbreeding period (September-January), birds were fed three diets with different carbon stable isotope signatures, and at the end of each diet treatment a subcutaneous lipid biopsy was taken to contrast with the stable isotopic signatures of the preceding diet. Details are presented below.

2.3.1 Study Species

Research was conducted during pre-breeding between February and May in 2007 and pre- through post-breeding between February and July in 2008, and from September 2007 to January 2008 at the Alaska SeaLife Center (Seward, Alaska), where a captive flock of spectacled eiders has been housed since 2003. Eleven birds (females = 5, males = 6) were reared in captivity and arrived 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 separated into pairs during the breeding season.

2.3.2 Diet and Adult Tissue Collection

Feeding Protocols and Diet Collection - In this study, the pre-breeding diet was the same as used in the study of stable isotope fractionation factors to egg components (Federer et al. in prep.). Throughout housing at the Alaska SeaLife Center, birds were held on a consistent diet of approximately 95% Mazuri® Sea Duck Diet (Purina Mills Incorporated, St. Louis, Missouri) and 5% natural food supplements (diet proportion calculated by dry weight). The commercial diet consisted of approximately 6.5% lipid, 21.6% protein, 8.4% fiber, 10.9% ash, and 46.6% nitrogen-free extract along with vitamins (www.mazuri.com). Prior to sampling in 2007, five natural food supplements (Antarctic krill, *Euphausia superba*; tidewater silversides, *Menidia menidia*; Arctic razor clams, *Siliqua patula*; Pacific blue mussels, *Mytilus trossulus*; and common squid, *Loligo opalescens*) were offered on a weekly schedule along with calcium grit during pre-breeding. Daily consumption of Mazuri® and estimates for each natural food supplement were recorded. We simplified the diet in 2008 for more than 3 months prior to tissue collection (February 1 through July 31) to include only silversides as a supplement to Mazuri®, quantified at 5% based on consumption of the commercial diet within the previous 24 hours. In 2008, we analyzed carbon and nitrogen stable isotope signatures of samples from five batches of Mazuri® (new batches were based on shelf life and samples were collected ~monthly between February 5 and June 28) and five samples of silversides from the same batch. Samples were stored at –20°C until analysis.

During the non-breeding season in 2007 and 2008, birds were fed a baseline diet for 69 days prior to the start of the experiment 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). Diet items were collected prior to fat biopsies to estimate isotopic dietary shifts during the diet switch experiment.

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 (5/2/07, 5/8/07, 5/15/07, 5/22/07) and 2008 (5/5/08, 5/14/08, 5/20/08) 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. Blood was sampled via jugular or tarsal vein in 2007 and either jugular, tarsal, or wing vein in 2008 using a 23 or 25-gauge needle and transferred to Li- Heparinized tubes (Sarstedt Inc., Newton, North Carolina). Whole blood was centrifuged at 1500 rpm for 10 minutes in a Clay Adams Compact II® centrifuge (Becton Dickinson, Franklin Lakes, New Jersey) within four hours of the blood draw. Blood plasma was separated from cellular blood and samples were frozen in –80°C and –20°C freezer (respectively) until analysis.

Feathers - Captive spectacled eiders molt their adult contour feather after breeding between late May to late June and new feather growth occurs subsequently (Heidi Cline, personal communication). We collected new growth 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 (as described by Knoche 2004).

Subcutaneous Fat - Samples from all spectacled eider males were collected for a concurrent project during three biopsy dates 11/27/2007 (n = 8), 12/18/2007 (n = 5), and 1/16/08 (n = 8) and available aliquots were archived for stable isotope analysis. Mass was measured prior to each fat biopsy to ensure that the birds were not losing mass as a result of the different diets. Lipids were extracted from fat samples using a modified Folch technique (Folch et al. 1957; Iverson et al. 2001). Fatty acid methyl esters were prepared

from lipid extracts (Budge et al. 2006), concentrated with hexane, and stored at -20°C until analysis.

2.3.3 Laboratory Analyses

Diet items and blood components were dried to a constant mass using the FreeZone® 6 Liter Console Freeze Dry System (Labconco Corporation, Kansas City, Missouri). We did not extract lipid from the blood components because of small sample volumes and typically low proportions of lipid in bird blood (Alonso et al. 1991; Wolf et al. 1985). Additionally, isotopic studies have suggested no significant difference when comparing carbon and nitrogen stable isotopes in lipid extracted and whole blood (Bearhop et al. 2000). Samples were then homogenized using a mortar and pestle or grinder. Lipids were extracted from diet items using a 2:1 ratio of chloroform:methanol solution (Bligh and Dyer 1959). We used a vortex to mix ~ 5 mg of dry sample with solution, allowed the sample to settle for 24 hours, and extracted lipids manually with a pipette until the solvent wash was clear (Steffen Oppel, personal communication). Lipids were kept uncovered under a fume hood until all solvent had evaporated. Feathers (outer tip) and subcutaneous fat were subsampled and analyzed directly.

Carbon and nitrogen stable isotope analysis was conducted at the Alaska Stable Isotope Facility (University of Alaska Fairbanks, Fairbanks, Alaska). All samples were loaded into tin cups (0.01-0.05 mg) and weighed using a Satorius M2P electronic microbalance. Samples were measured via combustion using a continuous flow isotope ratio mass spectrometer Finnigan Delta^{plus}XP CF-IRMS (Thermo Fischer LLC, San Jose, California). Single samples of spectacled eider tissues and the duplicate measurements of diet items were used in the analysis. Isotopic analyses are expressed as ratios in delta (δ) notation relative to an international standard (R_{standard} = Vienna PeeDee Belemnite for $\delta^{13}\text{C}$, atmospheric air for $\delta^{15}\text{N}$) in parts per thousand (‰) according to the following equation, $\delta X\text{‰} = ([R_{\text{sample}}/R_{\text{standard}}]-1) \times 1000$ with X denoting either ^{13}C or ^{15}N , and R

representing the ratio of $^{13}\text{C}:^{12}\text{C}$ or $^{15}\text{N}:^{14}\text{N}$, respectively. The average analytical precision (defined here as ± 1 SD) was determined using peptone standards (Sigma Chemical Co., Highland, Illinois, mean: $\delta^{13}\text{C} = -15.82\text{‰}$ and $\delta^{15}\text{N} = 7.02\text{‰}$) across separate sample runs (total sample runs $n = 24$, total standards across all runs $n = 210$) and showed $0.1\text{‰} \pm 0.06$ for $\delta^{13}\text{C}$ and $0.2\text{‰} \pm 0.1$ for $\delta^{15}\text{N}$.

2.3.4 *Calculations and Statistical Analyses*

Stable isotope signatures for diet (mean $\pm$ SD) were calculated by multiplying average diet proportion (Mazuri®:natural food supplement) by the respective isotope signatures. In 2007, the average proportion of total Mazuri®:natural food supplements was calculated during February 1 to the end of sample collection. Independent proportions per natural food supplement were calculated by dividing the number of days each natural food was offered by the total number of days, and then multiplied by the average proportion per year. The proportion of Mazuri®:silversides in 2008 was directly measured. Isotopic fractionation factors were then calculated by subtracting the feather or blood components (Y) from the lipid-free diet (X) and subcutaneous fat (Y) from the lipid diet (X), described in terms of the difference in delta ($\Delta\delta = \delta Y - \delta X$).

We report carbon and nitrogen stable isotope signatures of the diet items and the isotopic fractionation factors between lipid-free diet and blood components, adult contour feathers, and subcutaneous fat samples as mean ± 1 SD. Assumptions of equal variance (Levene median test) and normality (Kolmogorov-Smirnov) of the residuals were assessed. A one-way analysis of variance (ANOVA; F statistic) was used to evaluate differences between concurrent sample years (2007 and 2008) in isotopic fractionation factors of blood components. If equal variance or normality failed, a one-way ANOVA on Ranks (Kruskal-Wallis test; H statistic) was performed. All analyses were conducted using SigmaStat 3.1 and SigmaPlot 9.0 (Systat Software Inc., Point Richmond, California) with α set at 0.05.

2.4 Results

2.4.1 $\delta^{13}C$ and $\delta^{15}N$ Isotope Analyses for Diet Items

Stable isotope signatures of individual diet items were reported previously (Federer et al. in prep.). During pre-breeding in both years, natural food supplements made up only a small proportion of the diet. In 2007, total natural food supplements comprised 5.8% of the diet; silversides and krill had the highest proportional contribution (2.8% and 1.1%) and razor clams and mussels had the lowest (0.4% and 0.5%). Only silversides were used in the 2008 diet and made up 4.0% of the diet. Proportions of diet items were then multiplied by the respective stable isotope signatures in both years to calculate a carbon and nitrogen stable isotope signature for whole diet and lipid-free diet and carbon stable isotope signature for diet lipids (Table 2.1).

Natural food supplements in the non-breeding baseline diet made up approximately 3%, where silversides and mussels were the largest proportion (1.2% and 1.0%) and krill and razor clams were the smallest proportions (0.5% and 0.4%). The two subsequent diet switches following the baseline diet included Mazuri® along with single natural food supplements. 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 2.2). 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 2.2, Figure 2.1).

2.4.2 $\delta^{13}\text{C}$ 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 2.3). The isotopic fractionation from lipid-free diet to cellular blood was significantly different between 2007 and 2008 for carbon ($F = 422.314$, $df = 27$, $p < 0.001$), but not for nitrogen. Lipid-free diet to blood plasma isotopic fractionation did not differ significantly between 2007 and 2008 for nitrogen or carbon. Feathers were sampled in 2008 only and lipid-free diet to feather isotopic fractionation measured $3.2\text{‰} \pm 0.2$ for carbon and $5.6\text{‰} \pm 0.6$ for nitrogen.

The mean carbon stable isotope signature from the subcutaneous fat from biopsy 1 was 1.2‰ greater than the baseline lipid diet (Table 2.2 and Figure 2.1). Similarly, the mean carbon stable isotope signature from the subcutaneous fat 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 (Table 2.2 and Figure 2.1). The mean carbon stable isotope signature of the subcutaneous fat from biopsy 3 was the same as the Mazuri® and silverside diet (Table 2.2 and Figure 2.1).

2.5 Discussion

We provide the first experimentally-derived isotopic fractionation factors between diet and tissue for spectacled eiders and any sea duck species, other than isotopic fractionation factors for feathers in king eiders (*Somateria spectabilis*; Knoche 2004). For blood components, isotopic fractionation factors were mainly consistent between 2007 and 2008 with a small degree of annual variability. Diet to tissue fractionation of both feathers and blood components were generally consistent with those of previously studied species. We also demonstrate that carbon stable isotopes in subcutaneous fat generally tracked changes in the carbon stable isotope signatures of the diet. These data provide

useful isotopic fractionation factors to facilitate the interpretation of foraging ecology and nutrient allocation modeling using stable isotope analysis in spectacled eiders and may be useful for other sea duck models.

2.5.1 Stable Isotope Fractionation of Spectacled Eider Blood Components

A few avian studies have been conducted on isotope fractionation in 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), but no information is available for blood components of sea ducks. 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).

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). We observed a small mean difference in isotope fractionation of blood components from the same individuals between years. There was only a 0.2‰ mean difference for carbon stable isotope fractionation of blood plasma and 0.4‰ for nitrogen between the concurrent years. Carbon stable isotope fractionation associated with cellular blood had a mean difference between 2007 and 2008 of 1.1‰, but there was no mean difference between years for nitrogen stable isotope fractionation. 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). For example, the sample time period occurred during a energetically demanding period directly prior to egg laying (Alisauskas and Ankney 1992) and metabolism of stored nutrients may have contributed to the differences observed between the sampling years. Further, slight differences in isotopic signatures or diet composition between Mazuri® food batches and natural food supplements may have also contributed to variability between the years, but we expect that these would be small differences because these only made up a small proportion of the diet and we accounted for different isotopic signatures in our diet estimate. These findings emphasize the importance of calculating isotopic fractionation factors for different species or tissue types (Caut et al. 2008; Dalerum and Angerbjorn 2005).

2.5.2 Stable Isotope Fractionation of Spectacled Eider Feathers

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 % lipid in diet corresponded linearly with increasing mean carbon stable isotope fractionation (1.9-4.3‰) in warblers (Pearson et al. 2003). Differences in feather type may also influence isotopic fractionation. For instance, Becker et al. (2007) measured isotope signatures in body and primary feathers for murres and found differences for carbon (2.5‰ and 1.9‰, respectively), but essentially no difference for nitrogen. Finally, there is some evidence from compound specific isotope analysis that individual amino acids can vary extensively (Howland et al. 2003; O'Brien et al. 2005), and therefore it may be important to distinguish specific amino acids that are synthesized for feather growth. However, no information is available for 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.

2.5.3 *Stable Isotope Fractionation of Spectacled Eider Subcutaneous Fat*

Isotopic fractionation processes between dietary lipids and subcutaneous fat tissue has not been previously reported to our knowledge for sea ducks. Carbon stable isotope signatures for subcutaneous fat 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 macronutrients (Podlesak and McWilliams 2007). Therefore, it is possible that birds used different macronutrients to synthesize subcutaneous fat during the different diet treatments. In our 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 subcutaneous fat 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 fat 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 subcutaneous fat were similar throughout. This finding suggests that isotopic signatures in fat are predictable if dietary macronutrients are known.

2.5.4 Conclusions and Future Recommendations

Stable isotope applications are currently among the most valuable tools for tracking movement and evaluating diet sources of birds, particularly for sea ducks that spend most of their lives at-sea like the threatened spectacled eiders. Few sea duck isotopic fractionation data have been generated (spectacled eider egg components, Federer et al. in prep. and king eider feathers, Knoche 2004). This study was the first to evaluate

spectacled eider diet to tissue isotopic fractionation factors for blood plasma and cellular blood (in two subsequent study years), adult contour feathers, and subcutaneous fat. In the future, a useful addition to stable isotope research would be to determine feather molt/growth chronology in captive spectacled eiders as well as isotopic signatures in different feather types. These data will contribute to the understanding of movement patterns and diet. Additionally, further understanding of micronutrient pathways associated with different tissues and growth will be useful for identifying nutrient limitations for spectacled eiders in the wild. This study highlights the importance of captive studies to application of these techniques in field studies. Our results will contribute additional tools to study movement patterns and diet sources/variation in wild spectacled eiders, and thus may help to understand factors affecting population recovery.

2.6 Acknowledgements

We thank the United States Fish and Wildlife Service (#70181-6-G413), Ocean Alaska Science and Learning Center (H9845060030), and National Park Foundation (07AKCMRG007 and 08AKCMRG004) for their financial support in this research. This project would not have been possible without the assistance of the Alaska SeaLife Center avian husbandry staff Heidi Cline, Tasha DiMarzio, Gwen Gerdson, Michael Grue, Christina Haskins, and Sadie Ulman for care of the birds and rigorous record keeping. Additionally, we thank the Alaska SeaLife Center research staff David Safine, Shiway Wang, and Ann Riddle and the veterinary staff Pam Tuomi, Carrie Goertz, and Millie Gray along with Dan Mulcahy from United States Geological Survey for their collaboration in sample collection and laboratory analyses. Further, we thank Daniel Hennen from the Alaska SeaLife Center for statistical advice. Samples were analyzed at the Alaska SeaLife Center and the Alaska Stable Isotope Facility, University of Alaska Fairbanks. Tim Howe conducted the stable isotope analyses and Norma Haubenstock provided laboratory assistance at the Alaska Stable Isotope Facility.

2.7 References

- Alisauskas RT, Ankney CD (1992) The cost of egg laying and its relationship to nutrient reserves in waterfowl. In: Batt BDJ et al. (eds) Ecology and Management of Breeding Waterfowl. University of Minnesota Press, Minneapolis, MN, USA, pp 30-61
- Alonso JC, Huecas V, Alonso JA, Abelenda M, Munoz-Pulido R, Puerta ML (1991) Hematology and blood chemistry of adult white storks (*Ciconia ciconia*). Comparative Biochemistry and Physiology 98A:395-397
- Bearhop S, Teece MA, Waldron S, Furness RW (2000) Influence of lipid and uric acid on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of avian blood: Implications for trophic studies. Auk 117:504-507
- Bearhop S, Waldron S, Votier SC, Furness RW (2002) Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. Physiological and Biochemical Zoology 75:451-458
- Becker BH, Newman SH, Inglis S, Beissinger SR (2007) Diet-feather stable isotope ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) fractionation in common murrelets and other seabirds. Condor 109:451-456
- Ben-David M, Schell DM (2001) Mixing models in analyses of diet using multiple stable isotopes: A response. Oecologia 127:180-184
- Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911-917
- Bond JC, Esler D, Hobson KA (2007) Isotopic evidence for sources of nutrients allocated to clutch formation by harlequin ducks. Condor 109:698-704
- Budge SM, Iverson SJ, Koopman HN (2006) Studying trophic ecology in marine ecosystems using fatty acids: A primer on analysis and interpretation. Marine Mammal Science 22:759-801
- Caut S, Angulo E, Courchamp F (2008) Caution on isotopic model use for analyses of consumer diet. Canadian Journal of Zoology 86:438-445
- Cherel Y, Hobson KA, Hassani S (2005) Isotopic discrimination between food and blood and feathers of captive penguins: Implications for dietary studies in the wild. Physiological and Biochemical Zoology 78:106-115
- Dalerum F, Angerbjörn A (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia 144:647-658

DeNiro MJ, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* 197:261-263

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42:495-506

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* 45:341-351

Evans Ogden LJ, Hobson KA, Lank DB (2004) Blood isotopic ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) turnover and diet-tissue fractionation factors in captive dunlin (*Calidris alpina pacifica*). *Auk* 121:170-177

Federal Register (1993) Final rule to list the spectacled eider as threatened. 58:27474-27480

Federer R (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. Master of Science Thesis. University of Alaska Fairbanks, Fairbanks, Alaska, USA

Folch J, Lees M, Sloane-Stanly GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 226:497-509

Gannes LZ, O'Brien DM, Martínez del Rio C (1997) Stable isotopes in animal ecology: Assumptions, caveats, and a call for more laboratory experiments. *Ecology* 78:1271-1276

Gauthier G, Bety J, Hobson KA (2003) Are greater snow geese capital breeders? New evidence from a stable-isotope model. *Ecology* 84:3250-3264

Hobson KA (1995) Reconstructing avian diets using stable-carbon and nitrogen isotope analysis of egg components: patterns of isotopic fractionation and turnover. *Condor* 97:752-762

Hobson KA (2006) Using stable isotopes to quantitatively track endogenous and exogenous nutrient allocations to eggs of birds that travel to breed. *Ardea* 94:359-369

Hobson KA, Atwell L, Wassenaar LI, Yerkes T (2004) Estimating endogenous nutrient allocations to reproduction in redhead ducks: A dual isotope approach using δD and $\delta^{13}\text{C}$ measurements of female and egg tissues. *Functional Ecology* 18:737-745

- Hobson KA, Bairlein F (2003) Isotopic fractionation and turnover in captive garden warblers (*Sylvia borin*): Implications for delineating dietary and migratory associations in wild passerines. *Canadian Journal of Zoology* 81:1630-1635
- Hobson KA, Clark RG (1992a) Assessing avian diets using stable isotopes I: Turnover of ^{13}C in tissues. *Condor* 94:181-188
- Hobson KA, Clark RG (1992b) Assessing avian diets using stable isotopes II: Factors influencing diet-tissue fractionation. *Condor* 94:189-197
- Hobson KA, Clark RG (1993) Turnover of ^{13}C in cellular and plasma fractions of blood: Implications for nondestructive sampling in avian dietary studies. *Auk* 110:638-641
- Hobson KA, Sirois J, Gloutney ML (2000) Tracing nutrient allocation to reproduction with stable isotopes: A preliminary investigation using colonial waterbirds of Great Slave Lake. *Auk* 117:760-774
- Hobson KA, Thompson JE, Evans MR, Boyd S (2005) Tracing nutrient allocation to reproduction in Barrow's goldeneye. *Journal of Wildlife Management* 69:1221-1228
- Howland MR, Corr LT, Young SMM, Jones V, Jim S, Van Der Merwe NJ, Mitchell AD, Evershed RP (2003) Expression of the dietary isotope signal in the compound-specific $\delta^{13}\text{C}$ values of pig bone lipids and amino acids. *International Journal of Osteoarchaeology* 13:54-65
- Inger R, Bearhop S (2008) Applications of stable isotope analyses to avian ecology. *Ibis* 150:447-461
- Iverson SJ, Lang SLC, Cooper MH (2001) Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. *Lipids* 36:1283-1287
- Kelly JF (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology* 78:1-27
- Klaassen M, Lindstrom A, Meltote H, Piersma T (2001) Arctic waders are not capital breeders. *Nature* 413:794
- Knoche MJ (2004) King eider wing molt: Inferences from stable isotope analyses. Master of Science Thesis. University of Alaska Fairbanks, Fairbanks, Alaska, USA
- Lovvorn JR, Richman SE, Grebmeier JM, Cooper LW (2003) Diet and body condition of spectacled elders wintering in pack ice of the Bering Sea. *Polar Biology* 26:259-267

Martínez del Río C, Wolf N, Carleton SA, Gannes LZ (2009) Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews* 84:91-111

Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochimica et Cosmochimica Acta* 48:1135-1140

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

Murphy ME (1996) Energetics and nutrition of molt. In: Carey C (ed) *Avian Energetics and Nutritional Ecology*. Chapman & Hill, New York, NY, USA, pp 158-198

O'Brien DM, Boggs CL, Fogel ML (2005) The amino acids used in reproduction by butterflies: A comparative study of dietary sources using compound-specific stable isotope analysis. *Physiological and Biochemical Zoology* 78:819-827

Pearson SF, Levey DJ, Greenberg CH, Martínez del Río C (2003) Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. *Oecologia* 135:516-523

Petersen MR, Larned WW, Douglas DC (1999) At-sea distribution of spectacled eiders: A 120-year-old mystery resolved. *Auk* 116:1009-1020

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18:293-320

Phillips DL (2001) Mixing models in analyses of diet using multiple stable isotopes: A critique. *Oecologia* 127:166-170

Phillips DL, Gregg JW (2001) Uncertainty in source partitioning using stable isotopes. *Oecologia* 127:171-179

Podlesak DW, McWilliams SR (2007) Metabolic routing of dietary nutrients in birds: Effects of dietary lipid concentration on $\delta^{13}\text{C}$ of depot fat and its ecological implications. *Auk* 124:916-925

Post DM (2002) Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology* 83:703-718

Rau GH, Mearns AJ, Young DR, Olson RJ, Schafer HA, Kaplan IR (1983) Animal $^{13}\text{C}/^{12}\text{C}$ correlates with trophic level in pelagic food webs. *Ecology* 64:1314-1318

- Richman SE, Lovvorn JR (2003) Effects of clam species dominance on nutrient and energy acquisition by spectacled eiders in the Bering Sea. *Marine Ecology Progress Series* 261:283-297
- Rubenstein DR, Hobson KA (2004) From birds to butterflies: Animal movement patterns and stable isotopes. *Trends in Ecology and Evolution* 19:256-263
- Schmutz JA, Hobson KA, Morse JA (2006) An isotopic assessment of protein from diet and endogenous stores: Effects on egg production and incubation behaviour of geese. *Ardea* 94:385-397
- Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}\text{C}$ analysis of diet. *Oecologia* 57:32-37
- U.S. Fish and Wildlife Service (1996) Spectacled eider recovery plan. Anchorage, Alaska. 157 Pp
- Webster MS, Marra PP, Haig SM, Bensch S, Holmes RT (2002) Links between worlds: Unraveling migratory connectivity. *Trends in Ecology and Evolution* 17:76-83
- Wolf SH, Schreiber RW, Kahana L, Torres JJ (1985) Seasonal, sexual and age-related variation in the blood composition of the brown pelican (*Pelecanus occidentalis*). *Comparative Biochemistry and Physiology* 82A:837-846

Table 2.1 Average ($\pm$ SD) carbon and nitrogen stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of pre- and post-breeding diet in 2007 and 2008 based on proportional source contribution (Mazuri®:natural food) for diet lipids, whole diet, and lipid-free diet.

Year	Lipid Diet	Whole Diet		Lipid-free Diet	
	$\delta^{13}\text{C}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
2007 (n=2)	-26.2 ± 0.1	-21.3 ± 0.3	7.2 ± 0.9	-20.0 ± 0.3	7.0 ± 0.6
2008 (n=10)	-26.4 ± 0.2	-21.0 ± 0.9	6.5 ± 0.5	-20.3 ± 0.6	6.7 ± 0.5

Table 2.2 Average ($\pm$ SD) carbon stable isotopes ($\delta^{13}\text{C}$) of diet lipids (baseline diet and 2 diet switches) and subsequent subcutaneous fat biopsies collected over 3 time periods. Isotopic fractionation factors between diet and subcutaneous fat along with sample size (n) are noted in parentheses (*one fat sample from a male that did not eat krill during the 1st diet switch was excluded).

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

Table 2.3 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. Blood components that have the same superscript in common for each isotope indicate no significant difference between the subsequent sample years.

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

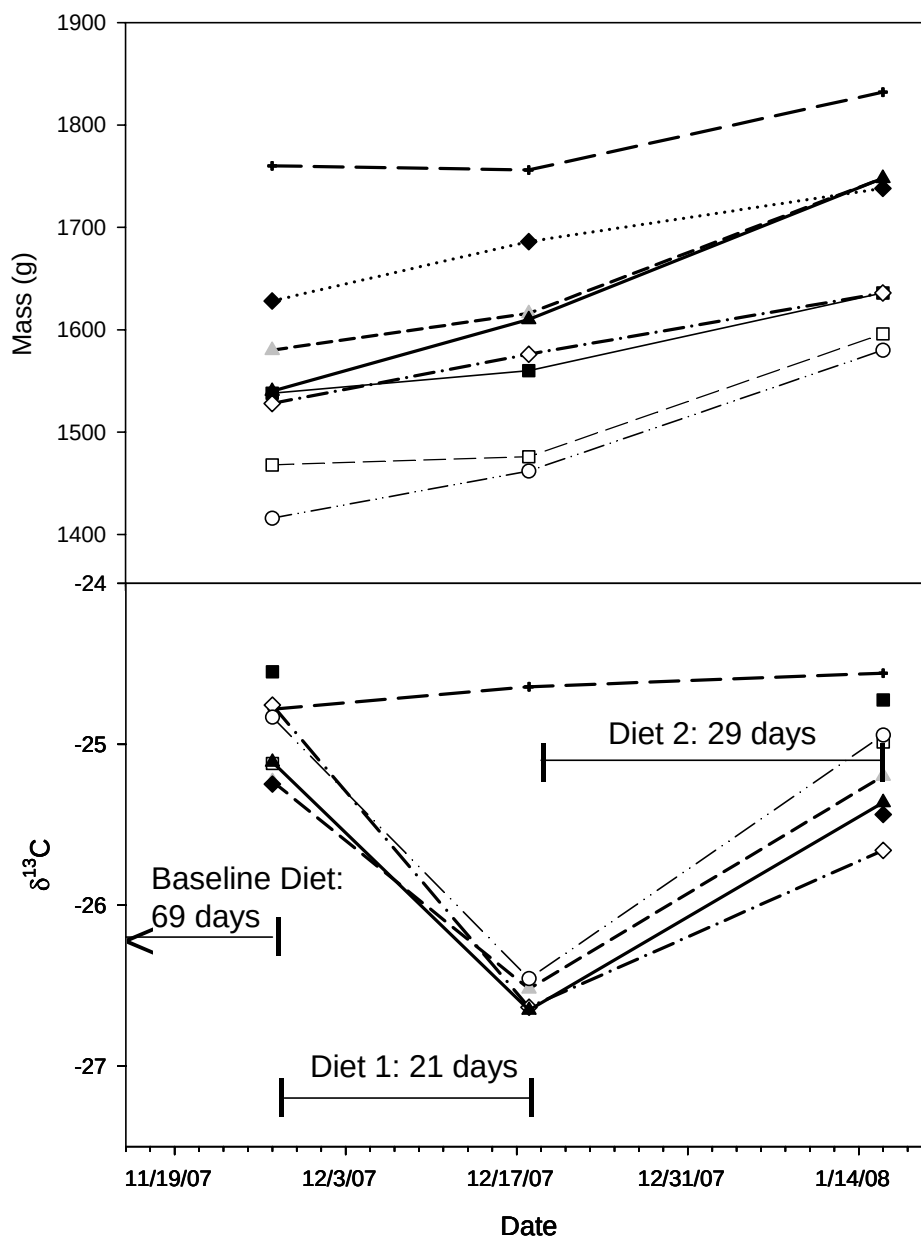


Figure 2.1 Mass of 8 male birds over three biopsy time periods (A) illustrating that weight was kept constant or increasing for each individual. Straight lines (B) indicate the carbon stable isotope signature ($\delta^{13}\text{C}$) for diet lipids the corresponding time period that birds were fed a 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 subcutaneous fat 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 the diet prior to biopsy 2.

Chapter 3 Evaluating Sea Duck Nutrient Allocation to Reproduction Model

Outcomes Using Diet to Egg Isotopic Fractionation Factors ($\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$) Derived from Captive Spectacled Eiders (*Somateria fischeri*)

3.1 Abstract

Stable isotopes provide a tool to build knowledge about waterfowl reproductive nutrient acquisition and allocation strategies. For instance, nutrient allocation mixing models use stable isotope signatures of isotopically distinct nutrient sources (e.g., freshwater and marine) and egg components to evaluate relative nutrient source contributions to egg production. However, the isotopic increase between diet and tissue, referred to as isotopic fractionation (i.e., $\Delta\delta = \delta \text{ tissue} - \delta \text{ diet}$), needs to be incorporated into the model as it is an important variable influencing the model outcome. Previous reproductive nutrient allocation studies of sea ducks have corrected for isotopic fractionation by using available diet to egg isotopic fractionation factors from captive raised falcons (*Falco* spp.), but factors may vary among species and thus previously used values may not accurately represent those for waterfowl. The purpose of this study was to incorporate newly derived diet to egg isotopic fractionation factors determined from spectacled eiders into two published sea duck (harlequin duck, *Histrionicus histrionicus* and Barrow's goldeneye, *Bucephala islandica*) nutrient allocation models and to consider the effects of these values on the model outcome. We observed an increase in the percentage points of freshwater nutrient sources that were allocated to egg production when using the spectacled eider isotopic fractionation values as compared to using those from falcons. Overall, the general model conclusions were similar when either the falcon or eider values were applied. The largest difference was observed when the distance between the isotope signatures of the nutrient sources (i.e., freshwater and marine end-members) were close to one another. This study provides an assessment of sea duck nutrient allocation model outcomes when isotopic fractionation factors derived from the spectacled eider are incorporated. These models have considerable potential to improve our understanding of

nutrient sources used during reproduction, particularly when we can apply better estimates of model variables, and therefore further facilitate interpretation of the model outcome.

3.2 Introduction

Arctic breeding waterfowl incur high breeding costs because they lay large energy-rich eggs during a short breeding season (Alisauskas and Ankney 1992). Stable isotope applications have been useful for evaluating the capital (somatic reserves) versus income (locally derived nutrients) continuum of strategies for acquiring nutrients for reproduction, particularly for species that travel across isotopically distinct regions to breed (Bond et al. 2007; Gauthier et al. 2003; Hobson 2006; Hobson et al. 2004; Hobson et al. 2005; Schmutz et al. 2006). Many waterfowl species, for example, spend wintering/staging periods in marine or estuarine environments that exhibit higher carbon and nitrogen isotope signatures relative to those in their freshwater breeding grounds (Peterson and Fry 1987). Stable isotope signatures in these habitats are reflected in the food sources and subsequently consumer tissues (DeNiro and Epstein 1978; DeNiro and Epstein 1981), and therefore have considerable potential to identify sources of nutrients invested in egg production (Hobson 2006). Using a mixing model formula, stable isotope compositions from two or more isotopically distinct nutrient sources (e.g., freshwater and marine) and a mixture (e.g., egg components) can be applied to estimate average percentage contributions of stored or consumed resources for egg production (Phillips and Gregg 2001). For example, mixing models that evaluated breeding strategies in brant (*Branta bernicla*) and emperor geese (*Anser canagicus*) showed that these species used both stored and locally acquired nutrients for protein in egg yolk, with a higher contribution of nutrient reserves in earlier laid eggs (Schmutz et al. 2006). Similarly, another model evaluated 1st, 4th, and 8th eggs within the same clutch across 5 different wetlands, and showed that nutrients stored from marine nonbreeding areas contributed to early laid Barrow's goldeneye (*Bucephala islandica*) eggs, but local freshwater nutrients

contributed the largest proportion in later laid eggs (Hobson et al. 2005). A model for greater snow geese (*Chen caerulescens atlantica*) evaluated first laid eggs and indicated that nutrients for egg production were mainly derived from freshwater sources (Gauthier et al. 2003). Other waterfowl models (e.g., harlequin duck, *Histrionicus histrionicus* and redhead, *Aythya americana*) have indicated a high degree of reliance on freshwater nutrients from the breeding habitat for egg production (Bond et al. 2007; Hobson et al. 2004). These models can offer valuable insights as to where and when nutrients are obtained for egg production in sea ducks and are a useful tool for understanding nutrient transfer across habitats in migratory birds.

Results from diet mixing models are estimates that rely on accurate measurements of nutrient resources and tissues, statistical parameters, isotopic fractionation factors, tissue turnover, and metabolic routing (Ben-David and Schell 2001; Caut et al. 2008; Dalerum and Angerbjorn 2005; Gannes et al. 1997; Martínez del Rio et al. 2009; Phillips 2001; Phillips and Gregg 2001). For example, putative food sources that are collected from breeding streams may or may not accurately represent what the organism is consuming, and therefore studies could benefit from simultaneous confirmation of diet. In addition, the isotope signature of tissues must be adjusted for isotopic fractionation or the difference in isotope signature between the tissue and the diet (i.e., $\Delta\delta = \delta \text{ egg component} - \delta \text{ diet}$). Isotopic fractionation must be experimentally determined and isotopic fractionation factors between diet and egg components have only been calculated for a few bird species (Federer et al. in prep.; Hobson 1995). Because isotopic fractionation factors are important parameters in these models, researchers have suggested that these should be determined for specific species/taxon and individual tissues (Caut et al. 2008; Dalerum and Angerbjorn 2005; Gannes et al. 1997; Hobson 2006; Martínez del Rio et al. 2009). Isotopic fractionation processes can be even more complex when considering nutrient transfer from stored reserves to tissue and thus far these values are not known (i.e., $\Delta\delta = \delta \text{ egg component} - \delta \text{ somatic reserves}$). To date, researchers have collected animal tissues with a long turnover period, like cellular blood/muscle and fat (Hobson

and Clark 1992; Hobson and Clark 1993), and assumed diet to egg isotopic fractionation factors for falcon species (*Falco* spp.) that were fed muscle from Japanese quail (*Coturnix japonica*), reasoning that values determined from this experiment should resemble an animal feeding off their own reserves (Hobson 1995). However, this has not been experimentally measured. Another important consideration in nutrient allocation modeling is that macronutrients (i.e., lipids and proteins) may preferentially differentiate during tissue synthesis. For example, dietary items may be preferentially routed to particular tissues without mixture of these nutrients in a body pool and could impact fractionation processes within and among tissues (Martínez del Río and Wolf 2005; Martínez del Río et al. 2009; Schwarcz 1991).

Nutrient allocation mixing models have considerable potential to increase our understanding of nutrient sources used during reproduction. The purpose of this study was to re-analyze model outcomes for existing stable isotope studies of sea duck nutrient allocation to eggs when diet to egg isotopic fractionation factors that were experimentally determined for a captive sea duck species, the spectacled eider (*Somateria fischeri*; Federer et al. in prep.) were incorporated, and to compare the results with outcomes based on those values derived from falcons (Hobson 1995). I focused this preliminary assessment on two published sea duck studies evaluating nutrient allocation in the harlequin duck (Bond et al. 2007) and Barrow's goldeneye (Hobson et al. 2005). Because these sea duck species are taxonomically close to spectacled eiders, model outcomes could be assumed to more accurately represent nutrient sources used for egg production. Additionally, sea ducks are a current conservation concern because numbers of many species have declined over the past few decades and factors contributing to the decline remain unclear (Henny et al. 1995; Sea Duck Joint Venture 2008; Stehn et al. 1993; Suydam et al. 2000). Further knowledge on how diet to egg isotopic fractionation factors affect model outcome will contribute overall to nutrient allocation modeling, and will further our understanding of breeding strategies used by waterfowl species.

3.3 Materials and Methods

I followed the mixing model approach applied in two previously published studies (Bond et al. 2007; Hobson et al. 2005) 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, I re-analyzed the models and examined the influence of replacing the isotopic fractionation factors between diet and egg components calculated for falcon species ($\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N} = 0.9\text{‰}$ and 3.4‰ in albumen, 0‰ and 3.4‰ in lipid-free yolk and $\Delta\delta^{13}\text{C} = 0\text{‰}$ in yolk lipids; Hobson 1995) with those values calculated using captive spectacled eiders ($\Delta\delta^{13}\text{C}$ and $\delta^{15}\text{N} = 1.8\text{‰}$ and 3.8‰ in albumen, 1.6‰ and 4.3‰ in lipid-free yolk and $\Delta\delta^{13}\text{C} = 1.9\text{‰}$ in yolk lipids; Federer et al. in prep.). Because these models only considered two nutrient sources, I employed a single-isotope linear mixing model approach using the following mass balance equation for egg component mixture (M) and putative marine and freshwater (A and B) 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 ($n = 23$, $\delta^{13}\text{C} = -25.5 \pm 2.4$, $\delta^{15}\text{N} = 6.9 \pm 1.2$) and albumen ($n = 23$, $\delta^{13}\text{C} = -25.1 \pm 2.1$, $\delta^{15}\text{N} = 5.8 \pm 1.2$; Appendix 4.1). Mean isotope signature for invertebrates collected from freshwater breeding streams (non-lipid $n = 9$, $\delta^{13}\text{C} = -25.4 \pm 2.4$, $\delta^{15}\text{N} = 2.2 \pm 1.3$) and cellular blood from birds captured just after arrival on breeding habitat ($n = 60$, $\delta^{13}\text{C} = -13.9 \pm 1.1$, $\delta^{15}\text{N} = 14 \pm 1.3$) describe the end-members for protein-comprised egg yolk (i.e., lipid-free yolk) and albumen (Appendix 4.1; Bond et al. 2007). Only the carbon isotope was evaluated for yolk lipids ($n = 23$, $\delta^{13}\text{C} = -29.8 \pm 2.3$) because of low nitrogen

content in lipids (Bond et al. 2007). The lipid portion of invertebrates from freshwater breeding streams ($n = 5$, $\delta^{13}\text{C} = -29.7 \pm 3.2$) and abdominal fat from birds captured on marine areas ($n = 18$, $\delta^{13}\text{C} = -19.7 \pm 1.3$) describe the end-members for yolk lipids (Appendix 4.1; Bond et al. 2007).

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 nonbreeding period (Appendix 4.2; Hobson et al. 2005). A model was run for the 1st, 4th, and 8th laid egg (for each egg component) from eight nests among five different wetlands (Appendix 4.2).

3.4 Results

In both models, I found 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, I estimated that marine nutrient sources contributed 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, I estimated that 0% of each egg component were derived from marine-derived nutrient sources (95% CI: albumen = 0-6%, lipid-free yolk = 0-5%, yolk lipids = 0-30%, Table 3.1). 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, I estimated that 0% albumen (95% CI = 0-8%) and 3% lipid-free yolk (95% CI = 0-12%) originated from marine-derived sources for the nitrogen model (Table 3.1). 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 3.1).

For the Barrow's goldeneye carbon model, 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). I report values that fell outside of the range of the marine end-member 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; Table 3.2). 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, I found 0% marine-derived nutrient sources for 1st, 4th, and 8th eggs in all egg components. 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).

3.5 Discussion

Establishing more precise model parameters will enhance model performance, and will build knowledge towards nutrient allocation and breeding strategies utilized by waterfowl species. This study provides an assessment of the influence that substituting diet to egg isotopic fractionation factors determined for falcons (Hobson 1995) with those for spectacled eiders (Federer et al. in prep.) has on breeding strategy estimates in two published sea duck models (Bond et al. 2007; Hobson et al. 2005). Generally the overall model conclusions using isotopic fractionation factors for either species were similar, but when spectacled eider values were applied, there was a lower marine nutrient source contribution towards egg production in both studies. The largest difference was observed when putative nutrient source end-members were closest together. For example, in the Hobson et al. (2005) study, the lipid end-members for nests from wetland #115 were <3‰ from one another and marine nutrients that were contributed towards yolk lipid decreased by 71% when using isotopic fractionation factors from the spectacled eider.

Consequently, some of the isotope signatures for egg components fell outside the end-member nutrient sources resulting in model uncertainty. Sensitivity analyses have suggested that by increasing the distance between the nutrient source end-members (e.g., from 2‰ to 4‰), the uncertainty of the proportional contribution can be reduced by half (Phillips and Gregg 2001). In Bond et al. (2007), all nutrient source end-members were $\geq 10\text{‰}$ and marine nutrients that were contributed to egg components decreased only minimally from 4% in nitrogen for albumen and up to 19% in carbon for yolk lipids when using isotopic fractionation factors from the spectacled eider. It is therefore important to recognize that small differences of nutrient source end-members can pose potential sources of large error in these mixing models.

Accurate selection of nutrient source end-members is also an important component in nutrient allocation modeling. Sea ducks inhabit freshwater ponds during the breeding season (Sea Duck Joint Venture 2008) that are potentially accessible for collecting putative dietary sources. To strengthen the end-member for freshwater nutrients, studies may benefit from concurrent conventional studies (i.e., regurgitates, stomach analyses). For remotely located non-breeding habitats, stable isotope analyses of tissues may provide a valuable option to infer diet choices (Inger and Bearhop 2008). In published sea duck models, tissues with longer turnover times, such as cellular blood/muscle and fat (Hobson and Clark 1992; Hobson and Clark 1993) are used to represent non-lipid and lipid portions (respectively) of somatic reserves from the marine nutrient sources (Bond et al. 2007; Hobson et al. 2005). However, data on isotopic fractionation between somatic reserves and egg components are still not available and current models must assume values determined for a falcon species feeding on quail muscle (Bond et al. 2007; Gauthier et al. 2003; Hobson et al. 2005; Schmutz et al. 2006). This assumption could be tested in future experimental studies.

Researchers have emphasized the need for species/taxon and tissue-specific isotopic fractionation factors (Caut et al. 2008; Dalerum and Angerbjorn 2005; Gannes et al.

1997). However, relatively little information for only a narrow taxonomic group is known about isotopic fractionation between diet and egg components, and variation has been observed within and among these species (Federer et al. in prep.; Hobson 1995). For example, averages differed among different species for carbon and nitrogen isotopic fractionation factors in albumen (up to 1.8‰ and 1.6‰, respectively), lipid-free yolk (up to 2.6‰ and 1.4‰), and whole yolk (up to 1.1‰ and 0.5‰; Federer et al. in prep.). Additionally, the same individuals of spectacled eiders that were measured over 3 subsequent years exhibited some annual average differences for carbon and nitrogen among egg components (albumen 1.0‰ and 1.6‰; lipid-free yolk 1.1‰ and 1.5‰; whole yolk 0.9‰ and 0.9‰; Federer et al. in prep.). Therefore, variation in isotopic fractionation factors may exist even among species in the same taxonomic group, and therefore these values ideally would be experimentally measured for the harlequin duck and Barrow's goldeneye.

Furthermore, isotopic fractionation can be influenced by preferential differentiation of nutrients to specific egg components (Martínez del Río and Wolf 2005; Martínez del Río et al. 2009; Schwarcz 1991). Diet to egg isotopic fractionation factors are calculated averages of bulk isotopic analyses. However, specific amino acids and fatty acids may be preferentially separated to eggs, and these micronutrients could have different isotope signatures, as found in examples from other study species and systems like the bone collagen from pigs (Howland et al. 2003), eggs from butterflies (O'Brien et al. 2005), and within marine ecosystems (Budge et al. 2008). Compound-specific isotope analysis for egg components has not yet been determined and may provide refined insight towards nutrient limitations and potential factors affecting productivity. Finally, dietary composition from the natural environment is usually different than diet supplied for experimental studies. Therefore, it may be important to establish experimental isotopic fractionation studies using food items with similar diet composition as those consumed in the wild.

3.6 Acknowledgements

Thanks go to the United States Fish and Wildlife Service (#70181-6-G413), Ocean Alaska Science and Learning Center (H9845060030), and National Park Foundation (07AKCMRG007 and 08AKCMRG004).for their financial support throughout this research. Additionally, I am grateful to Keith Hobson and Jeanine Bond for their assistance and collaboration in the nutrient allocation modeling data used in this paper.

3.7 References

- Alisauskas RT, Ankney CD (1992) The cost of egg laying and its relationship to nutrient reserves in waterfowl. In: Batt BDJ et al. (eds) Ecology and Management of Breeding Waterfowl. University of Minnesota Press, Minneapolis, MN, USA, pp 30-61
- Ben-David M, Schell DM (2001) Mixing models in analyses of diet using multiple stable isotopes: A response. *Oecologia* 127:180-184
- Bond JC, Esler D, Hobson KA (2007) Isotopic evidence for sources of nutrients allocated to clutch formation by harlequin ducks. *Condor* 109:698-704
- Budge SM, Wooller MJ, Springer AM, Iverson SJ, McRoy CP, Divoky GJ (2008) Tracing carbon flow in an arctic marine food web using fatty acid-stable isotope analysis. *Oecologia* 157:117-129
- Caut S, Angulo E, Courchamp F (2008) Caution on isotopic model use for analyses of consumer diet. *Canadian Journal of Zoology* 86:438-445
- Dalerum F, Angerbjorn A (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. *Oecologia* 144:647-658
- DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42:495-506
- DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* 45:341-351
- Federer R (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. Master of Science Thesis. University of Alaska Fairbanks, Fairbanks, Alaska, USA
- Gannes LZ, O'Brien DM, Martínez del Río C (1997) Stable isotopes in animal ecology: Assumptions, caveats, and a call for more laboratory experiments. *Ecology* 78:1271-1276
- Gauthier G, Bety J, Hobson KA (2003) Are greater snow geese capital breeders? New evidence from a stable-isotope model. *Ecology* 84:3250-3264
- Henny CJ, Rudis DD, Roffe TJ, Robinson-Wilson E (1995) Contaminants and sea ducks in Alaska and the circumpolar region. *Environmental Health Perspectives* 103:41-49

- Hobson KA (1995) Reconstructing avian diets using stable-carbon and nitrogen isotope analysis of egg components: Patterns of isotopic fractionation and turnover. *Condor* 97:752-762
- Hobson KA (2006) Using stable isotopes to quantitatively track endogenous and exogenous nutrient allocations to eggs of birds that travel to breed. *Ardea* 94:359-369
- Hobson KA, Atwell L, Wassenaar LI, Yerkes T (2004) Estimating endogenous nutrient allocations to reproduction in redhead ducks: A dual isotope approach using δD and $\delta^{13}C$ measurements of female and egg tissues. *Functional Ecology* 18:737-745
- Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes I: Turnover of ^{13}C in tissues. *Condor* 94:181-188
- Hobson KA, Clark RG (1993) Turnover of ^{13}C in cellular and plasma fractions of blood: Implications for nondestructive sampling in avian dietary studies. *Auk* 110:638-641
- Hobson KA, Thompson JE, Evans MR, Boyd S (2005) Tracing nutrient allocation to reproduction in Barrow's goldeneye. *Journal of Wildlife Management* 69:1221-1228
- Howland MR, Corr LT, Young SMM, Jones V, Jim S, Van Der Merwe NJ, Mitchell AD, Evershed RP (2003) Expression of the dietary isotope signal in the compound-specific $\delta^{13}C$ values of pig bone lipids and amino acids. *International Journal of Osteoarchaeology* 13:54-65
- Inger R, Bearhop S (2008) Applications of stable isotope analyses to avian ecology. *Ibis* 150:447-461
- Martínez del Río C, Wolf BO (2005) Mass-balance models for animal isotopic ecology. In: Stark JM, Wang T (eds) *Physiological and Ecological Adaptations to Feeding in Vertebrates*. Science Publishers, Enfield, NH, USA, pp 141-174
- Martínez del Río C, Wolf N, Carleton SA, Gannes LZ (2009) Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews* 84:91-111
- O'Brien DM, Boggs CL, Fogel ML (2005) The amino acids used in reproduction by butterflies: A comparative study of dietary sources using compound-specific stable isotope analysis. *Physiological and Biochemical Zoology* 78:819-827
- Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18:293-320
- Phillips DL (2001) Mixing models in analyses of diet using multiple stable isotopes: A critique. *Oecologia* 127:166-170

Phillips DL, Gregg JW (2001) Uncertainty in source partitioning using stable isotopes. *Oecologia* 127:171-179

Schmutz JA, Hobson KA, Morse JA (2006) An isotopic assessment of protein from diet and endogenous stores: Effects on egg production and incubation behaviour of geese. *Ardea* 94:385-397

Schwarcz HP (1991) Some theoretical aspects of isotope paleodiet studies. *Journal of Archaeological Science* 18:261-275

Sea Duck Joint Venture (2008) Sea Duck Joint Venture Strategic Plan 2008 – 2012. Available at <http://seaduckjv.org> or U.S. Fish and Wildlife Service, Anchorage, Alaska or Canadian Wildlife Service, Sackville, New Brunswick. 103 Pp

Stehn RA, Dau CP, Conant B, Butler Jr. WI (1993) Decline of spectacled eiders nesting in western Alaska. *Arctic* 46:264-277

Suydam RS, Dickson DL, Fadely JB, Quakenbush LT (2000) Population declines of king and common eiders of the Beaufort Sea. *Condor* 102:219-222

Table 3.1 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 ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes.

$\Delta\delta$	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

Table 3.2 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 ($\delta^{13}\text{C}$).

$\Delta\delta$	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

3.8 Appendices

Appendix 3.1 Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope signatures for nutrient sources or tissue end-members and harlequin duck egg components (Bond et al. 2007) used in the mixing model (Phillips and Gregg 2001).

Isotope	Number	Diet Source or Tissue End-Member	mean $\pm$ SD
$\delta^{13}\text{C}$	60	Marine: RBC (non-lipid)	-13.9 ± 1.1
$\delta^{15}\text{N}$	60	Marine: RBC (non-lipid)	14 ± 1.3
$\delta^{13}\text{C}$	18	Marine: Fat (lipid)	-19.7 ± 1.3
$\delta^{13}\text{C}$	9	Freshwater: invertebrates (non-lipid)	-25.4 ± 2.4
$\delta^{15}\text{N}$	9	Freshwater: invertebrates (non-lipid)	2.2 ± 1.3
$\delta^{13}\text{C}$	5	Freshwater: invertebrates (lipid)	-29.7 ± 3.2
Egg Components			
Isotope	Number	(not adjusted for isotopic fractionation)	mean $\pm$ SD
$\delta^{13}\text{C}$	23	Albumen	-25.1 ± 2.1
$\delta^{15}\text{N}$	23	Albumen	5.8 ± 1.2
$\delta^{13}\text{C}$	23	Lipid-free Yolk	-25.5 ± 2.4
$\delta^{15}\text{N}$	23	Lipid-free Yolk	6.9 ± 1.2
$\delta^{13}\text{C}$	23	Yolk Lipids	-29.8 ± 2.3

Appendix 3.2 Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope signatures for nutrient sources or tissue end-members and Barrow's goldeneye egg components (Hobson et al. 2005) used in the mixing model (Phillips and Gregg 2001).

Isotope	Number	Diet Source or Tissue End-Member	mean $\pm$ SD
$\delta^{13}\text{C}$	9	Marine: Muscle (non-lipid)	-19.9 ± 1.2
$\delta^{13}\text{C}$	5	Marine: Fat (lipid)	-25.3 ± 0.5
$\delta^{13}\text{C}$	12	Freshwater: # 115 amphipods (non-lipid)	-24.6 ± 1.2
$\delta^{13}\text{C}$	12	Freshwater: # 115 amphipods (lipid)	-28.0 ± 1.2
$\delta^{13}\text{C}$	34	Freshwater: # 8,11,42 amphipods (non-lipid)	$-27.1 \pm \sim 0.5$
$\delta^{13}\text{C}$	34	Freshwater: # 8,11,42 amphipods (lipid)	$-30.5 \pm \sim 0.5$
Wetland Egg Components			
Isotope	(egg #)	(not adjusted for isotopic fractionation)	$\delta^{13}\text{C}$
$\delta^{13}\text{C}$	# 42 (1)	Albumen	-25.5
$\delta^{13}\text{C}$	# 42 (4)	Albumen	-25.3
$\delta^{13}\text{C}$	# 42 (8)	Albumen	-24.5
$\delta^{13}\text{C}$	# 42 (1)	Lipid-free Yolk	-25.0
$\delta^{13}\text{C}$	# 42 (4)	Lipid-free Yolk	-24.8
$\delta^{13}\text{C}$	# 42 (8)	Lipid-free Yolk	-24.1

Appendix 3.2 (continued) Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope signatures for nutrient sources or tissue end-members and Barrow's goldeneye egg components (Hobson et al. 2005) used in the mixing model (Phillips and Gregg 2001).

Isotope	Wetland (egg #)	Egg Components (not adjusted for isotopic fractionation)	$\delta^{13}\text{C}$
$\delta^{13}\text{C}$	# 42 (1)	Yolk lipids	-30.9
$\delta^{13}\text{C}$	# 42 (4)	Yolk lipids	-31.4
$\delta^{13}\text{C}$	# 42 (8)	Yolk lipids	-30.4
$\delta^{13}\text{C}$	# 41 (1)	Albumen	-22.1
$\delta^{13}\text{C}$	# 41 (4)	Albumen	-24.0
$\delta^{13}\text{C}$	# 41 (8)	Albumen	-24.2
$\delta^{13}\text{C}$	# 41 (1)	Albumen	-22.0
$\delta^{13}\text{C}$	# 41 (4)	Albumen	-25.3
$\delta^{13}\text{C}$	# 41 (8)	Albumen	-25.8
$\delta^{13}\text{C}$	# 41 (1)	Albumen	-24.0
$\delta^{13}\text{C}$	# 41 (4)	Albumen	-23.9
$\delta^{13}\text{C}$	# 41 (8)	Albumen	-24.8
$\delta^{13}\text{C}$	# 41 (1)	Lipid-free Yolk	-22.8
$\delta^{13}\text{C}$	# 41 (4)	Lipid-free Yolk	-23.4
$\delta^{13}\text{C}$	# 41 (8)	Lipid-free Yolk	-23.7
$\delta^{13}\text{C}$	# 41 (1)	Lipid-free Yolk	-21.2
$\delta^{13}\text{C}$	# 41 (4)	Lipid-free Yolk	-24.1
$\delta^{13}\text{C}$	# 41 (8)	Lipid-free Yolk	-25.5
$\delta^{13}\text{C}$	# 41 (1)	Lipid-free Yolk	-23.8
$\delta^{13}\text{C}$	# 41 (4)	Lipid-free Yolk	-24.2
$\delta^{13}\text{C}$	# 41 (8)	Lipid-free Yolk	-24.6
$\delta^{13}\text{C}$	# 41 (1)	Yolk lipids	-28.4
$\delta^{13}\text{C}$	# 41 (4)	Yolk lipids	-29.3
$\delta^{13}\text{C}$	# 41 (8)	Yolk lipids	-30.0
$\delta^{13}\text{C}$	# 41 (1)	Yolk lipids	-27.9
$\delta^{13}\text{C}$	# 41 (4)	Yolk lipids	-30.1
$\delta^{13}\text{C}$	# 41 (8)	Yolk lipids	-31.2
$\delta^{13}\text{C}$	# 41 (1)	Yolk lipids	-32.3
$\delta^{13}\text{C}$	# 41 (4)	Yolk lipids	-32.1
$\delta^{13}\text{C}$	# 41 (8)	Yolk lipids	-32.5
$\delta^{13}\text{C}$	# 11 (1)	Albumen	-26.5
$\delta^{13}\text{C}$	# 11 (4)	Albumen	-26.8
$\delta^{13}\text{C}$	# 11 (8)	Albumen	-26.6

Appendix 3.2 (continued) Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope signatures for nutrient sources or tissue end-members and Barrow's goldeneye egg components (Hobson et al. 2005) used in the mixing model (Phillips and Gregg 2001).

Isotope	Wetland (egg #)	Egg Components (not adjusted for isotopic fractionation)	$\delta^{13}\text{C}$
$\delta^{13}\text{C}$	# 11 (1)	Lipid-free Yolk	-26.2
$\delta^{13}\text{C}$	# 11 (4)	Lipid-free Yolk	-26.0
$\delta^{13}\text{C}$	# 11 (8)	Lipid-free Yolk	-26.2
$\delta^{13}\text{C}$	# 11 (1)	Yolk lipids	-30.0
$\delta^{13}\text{C}$	# 11 (4)	Yolk lipids	-30.2
$\delta^{13}\text{C}$	# 11 (8)	Yolk lipids	-30.7
$\delta^{13}\text{C}$	# 8 (1)	Albumen	-19.7
$\delta^{13}\text{C}$	# 8 (4)	Albumen	-27.1
$\delta^{13}\text{C}$	# 8 (8)	Albumen	-28.3
$\delta^{13}\text{C}$	# 8 (1)	Albumen	-27.9
$\delta^{13}\text{C}$	# 8 (4)	Albumen	-27.5
$\delta^{13}\text{C}$	# 8 (8)	Albumen	-31.4
$\delta^{13}\text{C}$	# 8 (1)	Lipid-free Yolk	-18.6
$\delta^{13}\text{C}$	# 8 (4)	Lipid-free Yolk	-23.4
$\delta^{13}\text{C}$	# 8 (8)	Lipid-free Yolk	-27.8
$\delta^{13}\text{C}$	# 8 (1)	Lipid-free Yolk	-27.7
$\delta^{13}\text{C}$	# 8 (4)	Lipid-free Yolk	-27.8
$\delta^{13}\text{C}$	# 8 (8)	Lipid-free Yolk	-30.5
$\delta^{13}\text{C}$	# 8 (1)	Yolk lipids	-24.8
$\delta^{13}\text{C}$	# 8 (4)	Yolk lipids	-30.1
$\delta^{13}\text{C}$	# 8 (8)	Yolk lipids	-33.5
$\delta^{13}\text{C}$	# 8 (1)	Yolk lipids	-34.5
$\delta^{13}\text{C}$	# 8 (4)	Yolk lipids	-36.2
$\delta^{13}\text{C}$	# 8 (8)	Yolk lipids	-35.3
$\delta^{13}\text{C}$	# 115 (1)	Albumen	-28.4
$\delta^{13}\text{C}$	# 115 (4)	Albumen	-24.4
$\delta^{13}\text{C}$	# 115 (8)	Albumen	-24.2
$\delta^{13}\text{C}$	# 115 (1)	Lipid-free Yolk	-23.6
$\delta^{13}\text{C}$	# 115 (4)	Lipid-free Yolk	-23.8
$\delta^{13}\text{C}$	# 115 (8)	Lipid-free Yolk	-24.2
$\delta^{13}\text{C}$	# 115 (1)	Yolk lipids	-29.3
$\delta^{13}\text{C}$	# 115 (4)	Yolk lipids	-33.3
$\delta^{13}\text{C}$	# 115 (8)	Yolk lipids	-30.1

General Conclusions and Future Directions

Thesis Conclusions

Spectacled eiders (*Somateria fischeri*) were listed as threatened in 1993 due to significant declines in the western Alaska breeding population between the 1970's and 1990's (Federal Register 1993). Reasons for the decline and threats to the recovery are not clear; however, changes in the marine habitat, including reduction in sea ice extent and shifts in benthic communities, are considered potential factors affecting quality and abundance of available food sources (Grebmeier et al. 2006; U.S. Fish and Wildlife Service 1996). Limited information is available about foraging ecology and habitat requirements for this species (Lovvorn et al. 2003) because much of the annual cycle is spent in offshore marine habitats (Petersen et al. 1995; Petersen et al. 1999), and therefore the relative importance of marine nutrient resources to fitness and reproductive success is not well understood. Moreover, reported reproductive problems and low productivity in spectacled eiders (Grand et al. 2002) has further emphasized the need to understand relative nutrient requirements during reproduction. The recent progress in stable isotope techniques has provided an opportunity to evaluate diet sources, migratory patterns, nutrient acquisition and allocation breeding strategies, and seasonal interactions that may otherwise be difficult to discern in this threatened sea duck species (Hobson 2005; Hobson 2006; Kelly 2000; Rubenstein and Hobson 2004; Webster et al. 2002).

Stable isotope signatures of tissues generally increase relative to the consumer diet (DeNiro and Epstein 1978; DeNiro and Epstein 1981), which is commonly referred to as isotopic fractionation ($\Delta\delta = \delta \text{ animal tissue} - \delta \text{ diet}$); however, these values can vary between species, tissue type, and diet and tissue composition (Caut et al. 2008; Dalerum and Angerbjorn 2005; Gannes et al. 1997). When using stable isotopes in mixing models, isotopic fractionation factors need to be quantified experimentally and appropriately adjusted to enable proper comparison of isotope signatures in egg components to putative

nutrient sources. However, these values have not been previously established for spectacled eiders or any other sea ducks, and therefore these results provide a foundation for dietary studies and nutrient allocation modeling in this group of birds. In this thesis, I have established stable isotope signatures and refined methods using a captive flock of spectacled eiders held at the Alaska SeaLife Center. Specific objectives for this thesis were to 1) evaluate the carbon and nitrogen isotope signatures in diet items, egg components, down feathers, and adult tissues (i.e., cellular blood, blood plasma, adult contour feathers, and subcutaneous fat), 2) characterize the stable isotope fractionation factors between diet and tissues, 3) evaluate the sensitivity of the model by comparing the diet to egg isotopic fractionation factors of spectacled eiders in relation to the same values for other avian species, and 4) provided a preliminary assessment of model outcome when diet to egg isotopic fractionation factors from spectacled eiders are incorporated into other published sea duck models. These objectives were arranged in Chapters as outlined below.

In Chapter 1, I characterized carbon and nitrogen stable isotope signatures for diet items ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for whole diet and lipid-free diet, $\delta^{13}\text{C}$ for dietary lipids) and egg components ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for whole yolk, lipid-free yolk, albumen, and shell membrane, $\delta^{13}\text{C}$ for yolk lipids and eggshell) and associated isotopic fractionation factors using captive spectacled eiders over three consecutive years. These values had not been previously characterized for spectacled eiders or other sea duck species. Additionally, these values had not been previously measured over multiple years in the same group of study animals. Data among years demonstrated similar isotopic fractionation with minimal mean variation. Relative to diet, isotope fractionation factors increased for carbon in albumen, lipid-free yolk, shell membrane, and eggshell and decreased for whole yolk and yolk lipids. For nitrogen, all isotopic fractionation factors of egg components increased relative to diet. These findings follow similar isotopic fractionation patterns as those determined for previously studied species (Hobson 1995). However, some important differences in these values were identified between species. Sensitivity

tests conducted in Chapter 1 demonstrated that model results could vary considerably when using isotopic fractionation values from different species. In Chapter 1, I also measured carbon and nitrogen stable isotope signatures for a small sample size of embryonic down feathers from 2008 and the related isotopic fractionation factors. These data are the first isotopic fractionation factors characterized for down feathers and we found that down feathers increased similarly to other protein-dominated tissues relative to diet. Therefore, down feathers could serve as a valuable tissue for nutrient allocation studies. Finally, because I calculated isotope signatures for lipid and protein portions of the diet, I expanded a model by Hobson (1995) that illustrates isotopic fractionation factors between macronutrients in the diet to those in the tissue. I include an appendix to Chapter 1 that includes supplemental data for carbon and nitrogen stable isotope signatures of individual natural food items and locations. These data provide information about potential experimental food items for future nutrient allocation modeling studies in spectacled eiders.

In Chapter 2, I evaluated carbon and nitrogen stable isotope signatures and isotopic fractionation factors between lipid-free diet and adult tissues, including cellular blood, blood plasma, and adult contour feathers. These data have not been previously measured for spectacled eiders, and only limited data exist for diet to feather isotopic fractionation in sea ducks (Knoche 2004). Similar to previous avian isotope fractionation studies, the carbon stable isotope composition of blood plasma was similar to that of the diet, but increased for cellular blood and adult contour feathers relative to diet items. The nitrogen stable isotope compositions of blood plasma, cellular blood, and adult feathers were also greater than that of the diet. Additionally, I evaluated the carbon isotope signatures from subcutaneous fat biopsies in relation to a baseline diet and two subsequent diet shifts. The carbon isotope signature of the first subcutaneous fat biopsy was greater than that of the baseline diet. The two subsequent fat biopsies closely reflected those of the diet shifts isotopically, except for in one individual that would not eat the krill natural food supplement offered after the first diet switch. These data indicate that carbon isotope

signatures of subcutaneous fat generally follow that of the lipids from the diet and offer isotopic fractionation factors that will be useful in dietary modeling studies for spectacled eiders.

In Chapter 3, I provide a preliminary assessment of model outcome when incorporating spectacled eider diet to egg isotopic fractionation into current sea duck models (Bond et al. 2007; Hobson et al. 2005). To accomplish this, all model variables remained the same, except the stable isotope fractionation values that were previously applied from falcons (*Falco* spp.) were replaced with those determined for spectacled eiders. Our results demonstrated a similar pattern of nutrient allocation in the egg tissues in relation to the original model, but shifted toward greater percentage points of freshwater nutrient sources that were allocated to egg production for both the harlequin duck (*Histrionicus histrionicus*) and Barrow's goldeneye (*Bucephala islandica*). The largest differences between our results and the original model were observed when nutrient source end-members (i.e., marine and freshwater) were close to one another. It is important to recognize that sometimes model uncertainty occurred (i.e., the isotope signature of the tissue was outside the isotopic range of nutrient sources) when either of the isotopic fractionation values were applied. This could indicate that isotopic fractionation values from spectacled eider or falcon species may not be a good representative for the sea duck species considered in this study or that other model variables need to be further refined to evaluate model performance and outcome (i.e., further complexities in isotopic fractionation, selection of diet sources and distance between these nutrient source end-members, and appropriate statistical data for mean, standard deviation, and sample size).

Future Directions

There are several future directions for the refinement of stable isotope technique in both captive and field studies, some of which are outlined below.

Future captive studies - In this thesis, I have established the first diet to tissue isotopic fractionation factors for various tissues in threatened spectacled eiders that will have a useful application in dietary and nutrient allocation studies. Few data are available for other sea ducks species (Knoche 2004), and therefore further studies could expand isotopic fractionation data to other species in this marine group.

To my knowledge, this thesis provided the first data for diet to down feather isotopic fractionation factors for birds, which will be useful addition for future nutrient allocation studies. Further studies will strengthen this data set and provide information between years and among individuals. In addition, isotopic fractionation factors are suggested to differ in growing individuals (Martínez del Rio and Wolf 2005; Williams et al. 2007), and therefore identifying these values in feathers and other tissues during duckling growth stages would be a useful contribution to dietary studies. Documenting information on feather molt and growth chronology of duckling, juvenile, and adult feather tracts would provide valuable information for sampling in the natural environment.

Further efforts in compound-specific isotope analysis (i.e., amino acids and fatty acids) of diet and egg components may be useful to identify whether specific nutrients are preferentially routed to egg tissues. For example, studies have suggested that differences occur in lipid, amino acid, and carbohydrate composition of egg yolk and albumen in precocial species (i.e., *Anseriformes*) in comparison to altricial species (Bucher 1987; Roca et al. 1984). This may provide an explanation for the species or tissue related differences in the isotopic fractionation factors that were observed in Chapter 1. In addition, studies have identified differences in the stable isotope signatures of specific amino acids and fatty acids in pig bones (Howland et al. 2003), amino acids in butterfly eggs (O'Brien et al. 2005), and fatty acids in marine ecosystems (Budge et al. 2008). Further, differences have been reported in amino acid (Murphy 1996) and fatty acid profiles (Pappas et al. 2007; Speake et al. 1998; Surai et al. 1999) of eggs among avian species; however, these individual compounds have not been measured isotopically in

birds, with the exception of fatty acid stable isotope analysis in black guillemots (*Cepphus grylle*; Budge et al. 2008). Dietary composition may also influence isotopic routing of preferential nutrient sources, and therefore may influence isotopic fractionation factors (Martínez del Río et al. 2009; Pearson et al. 2003). Although eiders feed on bivalves, invertebrates, and vegetation in the wild (Lovvorn et al. 2003; Petersen et al. 2000), the content of commercial sea duck feed is comprised of mainly corn and wheat grains, fish, and pork. Future experimental isotopic fractionation studies might benefit by utilizing food sources with a similar dietary composition as those consumed in the natural environment.

Additionally, isotopic fractionation between somatic reserves and egg components are not known. Therefore, current nutrient allocation models assume isotopic fractionation factors between diet and egg components from the carnivore model (Hobson 1995), reasoning that falcons feeding on the muscle tissue of Japanese quail (*Coturnix japonica*) closely resembles the animal feeding on their own reserves (Bond et al. 2007; Gauthier et al. 2003; Hobson et al. 2005; Schmutz et al. 2006). The isotope analysis of avian tissues with a longer turnover time, like cellular blood/muscle and fat (Hobson and Clark 1992; Hobson and Clark 1993), represent the nonlipid and lipid nutrient reserves obtained from the marine environment, respectively. An experimental study to measure this isotopic fractionation value between reserves and tissue is necessary to test this assumption; however, the complexity of this type of study (i.e., isotopic diet shift, food restriction, sampling tissues during a critical egg development period) may require a study design using a surrogate species.

Future field studies – Spectacled eider eggs from non-viable or abandoned nests and nest bowl contents from hatched nests would be an appropriate compliment to this captive dataset and could be sampled in combination with ongoing field studies. Additional sampling of diet items and tissues (i.e., feathers, blood components, and fat) will provide valuable source endpoint information in the nutrient allocation model. These data are an

important next step to understanding where and when spectacled eiders build nutrients for egg production.

Further, there is very little information available about diet selection and habitat requirements for this species (Lovvorn et al. 2003). Tissue collection like those described in the previous paragraph will be useful for understanding diet sources and variability and migratory patterns across the annual cycle (Rubenstein and Hobson 2004). These data also will contribute to identification of important seasonal interactions between nonbreeding and breeding seasons.

References

- Bond JC, Esler D, Hobson KA (2007) Isotopic evidence for sources of nutrients allocated to clutch formation by harlequin ducks. *Condor* 109:698-704
- Bucher TL (1987) Patterns in the mass-independent energetics of avian development. *Journal of Experimental Zoology* 1:139-150
- Budge SM, Wooller MJ, Springer AM, Iverson SJ, McRoy CP, Divoky GJ (2008) Tracing carbon flow in an arctic marine food web using fatty acid-stable isotope analysis. *Oecologia* 157:117-129
- Caut S, Angulo E, Courchamp F (2008) Caution on isotopic model use for analyses of consumer diet. *Canadian Journal of Zoology* 86:438-445
- Dalerum F, Angerbjorn A (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. *Oecologia* 144:647-658
- DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42:495-506
- DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* 45:341-351
- Federal Register (1993) Final rule to list the spectacled eider as threatened. 58:27474-27480
- Gannes LZ, O'Brien DM, Martínez del Río C (1997) Stable isotopes in animal ecology: Assumptions, caveats, and a call for more laboratory experiments. *Ecology* 78:1271-1276
- Gauthier G, Bety J, Hobson KA (2003) Are greater snow geese capital breeders? New evidence from a stable-isotope model. *Ecology* 84:3250-3264
- Grand JB, Franson JC, Flint PL, Petersen MR (2002) Concentrations of trace elements in eggs and blood of spectacled and common eiders on the Yukon-Kuskokwim Delta, Alaska, USA. *Environmental Toxicology and Chemistry* 21:1673-1678
- Grebmeier JM, Overland JE, Moore SE, Farley EV, Carmack EC, Cooper LW, Frey KE, Helle JH, McLaughlin FA, McNutt SL (2006) A major ecosystem shift in the northern Bering Sea. *Science* 311:1461-1464

- Hobson KA (1995) Reconstructing avian diets using stable-carbon and nitrogen isotope analysis of egg components: Patterns of isotopic fractionation and turnover. *Condor* 97:752-762
- Hobson KA (2005) Stable isotopes and the determination of avian migratory connectivity and seasonal interactions. *Auk* 122:1037-1048
- Hobson KA (2006) Using stable isotopes to quantitatively track endogenous and exogenous nutrient allocations to eggs of birds that travel to breed. *Ardea* 94:359-369
- Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes I: Turnover of ^{13}C in tissues. *Condor* 94:181-188
- Hobson KA, Clark RG (1993) Turnover of ^{13}C in cellular and plasma fractions of blood: Implications for nondestructive sampling in avian dietary studies. *Auk* 110:638-641
- Hobson KA, Thompson JE, Evans MR, Boyd S (2005) Tracing nutrient allocation to reproduction in Barrow's goldeneye. *Journal of Wildlife Management* 69:1221-1228
- Howland MR, Corr LT, Young SMM, Jones V, Jim S, Van Der Merwe NJ, Mitchell AD, Evershed RP (2003) Expression of the dietary isotope signal in the compound-specific $\delta^{13}\text{C}$ values of pig bone lipids and amino acids. *International Journal of Osteoarchaeology* 13:54-65
- Kelly JF (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology* 78:1-27
- Knoche MJ (2004) King eider wing molt: Inferences from stable isotope analyses. Master of Science Thesis. University of Alaska Fairbanks, Fairbanks, Alaska, USA
- Lovvorn JR, Richman SE, Grebmeier JM, Cooper LW (2003) Diet and body condition of spectacled elders wintering in pack ice of the Bering Sea. *Polar Biology* 26:259-267
- Martínez del Rio C, Wolf BO (2005) Mass-balance models for animal isotopic ecology. In: Stark JM, Wang T (eds) *Physiological and Ecological Adaptations to Feeding in Vertebrates*. Science Publishers, Enfield, NH, USA, pp 141-174
- Martínez del Rio C, Wolf N, Carleton SA, Gannes LZ (2009) Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews* 84:91-111
- Murphy ME (1996) Energetics and nutrition of molt. In: Carey C (ed) *Avian Energetics and Nutritional Ecology*. Chapman & Hill, New York, NY, USA, pp 158-198

- O'Brien DM, Boggs CL, Fogel ML (2005) The amino acids used in reproduction by butterflies: A comparative study of dietary sources using compound-specific stable isotope analysis. *Physiological and Biochemical Zoology* 78:819-827
- Pappas AC, Karadas F, Wood NAR, Speake BK (2007) Metabolic fates of yolk lipid and individual fatty acids during embryonic development of the coot and moorhen. *Comparative Biochemistry and Physiology* 147B:102-109
- Pearson SF, Levey DJ, Greenberg CH, Martínez del Río C (2003) Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. *Oecologia* 135:516-523
- Petersen MR, Douglas DC, Mulcahy DM (1995) Use of implanted satellite transmitters to locate spectacled eiders at-sea. *Condor* 97:276-278
- Petersen MR, Larned WW, Douglas DC (1999) At-sea distribution of spectacled eiders: A 120-year-old mystery resolved. *Auk* 116:1009-1020
- Petersen MR, Grand JB, Dau CP (2000) Spectacled eider (*Somateria fischeri*). In: Poole A, Gill F (eds) *The Birds of North America* No 547. The Birds of North America Inc, Philadelphia, PA, USA, pp 1-24
- Roca P, Sainz F, Gonzalez M, Alemany M (1984) Structure and composition of the eggs from several avian species. *Comparative Biochemistry and Physiology* 77A:307-310
- Rubenstein DR, Hobson KA (2004) From birds to butterflies: Animal movement patterns and stable isotopes. *Trends in Ecology and Evolution* 19:256-263
- Schmutz JA, Hobson KA, Morse JA (2006) An isotopic assessment of protein from diet and endogenous stores: Effects on egg production and incubation behaviour of geese. *Ardea* 94:385-397
- Speake BK, Murray AMB, Noble RC (1998) Transport and transformations of yolk lipids during development of the avian embryo. *Progress in Lipid Research* 37:1-32
- Surai PF, Speake BK, Noble RC, Mezes M (1999) Species-specific differences in the fatty acid profiles of the lipids of the yolk and of the liver of the chick. *Journal of the Science of Food and Agriculture* 79:733-736
- U.S. Fish and Wildlife Service (1996) Spectacled eider recovery plan. Anchorage, Alaska. 157 Pp
- Webster MS, Marra PP, Haig SM, Bensch S, Holmes RT (2002) Links between worlds: Unraveling migratory connectivity. *Trends in Ecology and Evolution* 17:76-83

Williams CT, Buck CL, Sears J, Kitaysky AS (2007) Effects of nutritional restriction on nitrogen and carbon stable isotopes in growing seabirds. *Oecologia* 153:11-18