Stable Isotope ($\delta^{15}N$ and $\delta^{13}C$) and Fatty Acid Profiles in Eggs of Three Species of Marine Birds Nesting Sympatrically in the High Arctic

By

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ABSTRACT

Stable isotope (SI) and fatty acid (FA) profiles were examined in early and late-laid eggs of three marine bird species; Arctic Terns (*Sterna paradisaea*), Common Eiders (*Somateria mollissima*) and Long-tailed Ducks (*Clangula hyemalis*), nesting on a Canadian High Arctic island. Differences in stable nitrogen isotope and FA patterns were observed between early and late-laid eggs. These differences may have reflected diet shifts during the period of egg formation or changes in the use of endogenous versus exogenous resources for egg formation. I also examined inter-specific differences in egg SI and FA composition and found consistent differences among all three species. These differences were consistent with literature on their foraging habits. My research has generated baseline information that can be compared to future data to identify and understand potential changes in the Arctic marine environment.

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CHAPTER ONE

General Introduction

Introduction

In the High Arctic marine environment, numerous marine organisms survive and reproduce by relying on the abundant food that becomes available during the short spring and summer months (Welch et al. 1992; CAFF 2001; Stirling 1997; Wassmann 2011). Nesting marine birds extensively utilize this relatively ice free season in the High Arctic (Mallory et al. 2006; Latour et al. 2008).

The Arctic marine environment has currently been experiencing rapid change, i.e., increasing levels of some environmental contaminants (e.g. mercury), increased offshore oil and gas activities, expansion of shipping, commercial fisheries (Huntington 2009), and sea-ice reduction (Polyak et al. 2010). An iron ore mining company (Baffinland Iron Mines Corporation), for instance, has looked at shipping 18-million tonnes annually year round through Nunavut waters, which would involve breaking sea-ice during the sea-ice season (Eegeesiak 2010). These changes could potentially affect the marine environment, including the food supply of marine birds, which could in turn affect the resources available for the formation of their eggs. Changes in food supply might affect the availability of essential nutrients for marine birds to produce viable eggs which could reduce reproductive success and, ultimately, impact their populations. Thus, it is important to characterize the current biochemical composition of marine bird eggs to be able to identify future changes. Such information may assist in protecting marine bird populations.

Food Web Studies

Various methods have been used to examine the feeding ecology, and in turn the trophic interactions, of marine organisms (Tremblay et al. 2006; Müller-Navarra 2008). Both direct (e.g. observations of prey delivery, stomach content analysis) and indirect techniques (e.g. biochemical approaches such as stable isotope and fatty acid analysis) (Barrett et al. 2007) have been successfully used to elucidate marine food web interactions. For instance, long-term observations of Thick-billed Murre (*Uria lomvia*) parents feeding their chicks at northern Hudson Bay colonies showed a shift from Arctic to subarctic fish, suggesting changes in fish populations near the colony (Gaston et al. 2003). Hebert et al. (2009) utilized Herring Gull (*Larus argentatus*) egg nitrogen and carbon isotopes and fatty acid (FA) tracers to reveal temporal changes in utilization of terrestrial and aquatic prey, likely due to changes in fish abundance. Increasingly, studies are using FAs and stable isotopes (SI) alongside direct methods to examine the feeding ecology of numerous organisms such as marine birds (Dahl et al. 2003; Hebert et al. 2006; Budge et al. 2008). Fatty acids and SIs have provided insights into marine bird trophic relationships (Hobson et al. 1994; Hebert et al. 2008), diet composition (Wang et al. 2007; Harding et al. 2008) and have complemented conventional feeding ecology studies (Barrett et al. 2007; Tierney et al. 2008).

Stable Isotopes

Stable isotopes are atoms that occur in a chemically stable form (i.e., not radioactive) that vary in their neutron quantity, and hence their mass (Peterson and Fry 1987; Sulzman 2007; Hobson 2008; Inger and Bearhop 2008). For instance, nitrogen atoms occur as either the heavier ^{15}N or lighter ^{14}N isotope, with ^{15}N atoms containing 15 neutrons and ^{14}N atoms containing 14 neutrons. Stable isotopes exist for many elements such as hydrogen, carbon, nitrogen, oxygen and sulphur (Inger and Bearhop 2008). In ecological studies, stable isotopes are typically expressed in delta notation (δ) and the units as per mil (‰) and defined as $\delta=((Rx-Rstd))/Rstd) * 1000$, where 'R' is the ratio of the abundance of the heavy (^{15}N or ^{13}C) to the light (^{14}N or ^{12}C) isotope in a sample (x) and in a standard reference material (std) (i.e., atmospheric AIR for nitrogen and Pee Dee Belemnite limestone for carbon). One of the ways in which SIs are useful in ecology stems from the degree to which they can be used to track the movement of energy and nutrients through food webs (Peterson and Fry 1987; Forero and Hobson 2003; Inger and Bearhop 2008; Wolf et al. 2009).

Stable Carbon Isotopes

Stable carbon isotopes (^{13}C and ^{12}C) have primarily been used to identify food sources in feeding ecology studies (France and Peters 1997; Michener and Kaufman 2007). Marine organisms feeding in nearshore benthic locations tend to have less negative $\delta^{13}C$ values relative to those feeding in offshore pelagic

areas or in freshwater ecosystems (Hobson et al. 1994; France 1995). Consequently, marine birds feeding in inshore benthic locations also have less negative $\delta^{13}C$ values in their tissues compared to pelagic marine birds (Hobson 1993; Hobson et al. 1994; Thompson et al. 1999).

Stable Nitrogen Isotopes

Nitrogen isotopes (^{15}N and ^{14}N) are commonly used to elucidate trophic interactions amongst marine organisms since $\delta^{15}N$ values generally increase by approximately 3 to 5‰ at each successive trophic level (Post 2002; Barrett et al. 2007). Thus, marine birds feeding on higher trophic level organisms have greater $\delta^{15}N$ values in their tissues compared to lower trophic level marine birds (Hobson 1993; Hebert et al. 1999; Thompson et al. 1999).

Fatty Acids

Fatty acids (FAs) are components of lipids (i.e. triacyglycerols, wax esters and phospholipids) that contain carbon atom chains with an acid (carboxyl, COOH) group at one end and a methyl (CH_3) terminal group at the other end (Iverson 2009; Williams and Buck 2010). They occur as saturated (no double bonds between carbon atoms) or unsaturated (mono- and poly-unsaturated with one or more carbon-carbon double bonds) forms and are required for growth and development (Parrish 2009; the fatty acid common name, acronym and structure can be found in Table 1.1). Certain FAs are considered to be essential FAs,

such as the poly-unsaturated fatty acids (PUFAs, e.g. arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid) since they cannot be produced by the organism to meet their nutritional requirements and have to be obtained through the diet (Arts et al. 2001; Kainz et al. 2004, Parrish 2009). The functions of essential FAs vary widely, from transmitting neural signals, regulating hormonal processes, maintaining metabolic processes and reproduction, to adapting to cold weather environments by ensuring membrane fluidity (Arts et al. 2001; Groscolas et al. 2003; Kainz et al. 2004). These essential FAs tend to remain unmodified as they pass from producer to consumer (Kainz et al. 2004; Williams and Buck 2010).

Table 1.1. Fatty acid common name, acronym and structure.

FA Common Name	Acronym	FA Structure
Myristic acid	n/a	14:0
Pentadecanoic acid	n/a	15:0
Palmitic acid	n/a	16:0
Heptadecanoic acid	n/a	17:0
Stearic acid	n/a	18:0
Behenic acid	n/a	22:0
Myristoleic acid	n/a	14:1n-5
Palmitoleic acid	n/a	16:1n-7
Elaidic acid	n/a	18:1n-9t
Oleic acid	n/a	18:1n-9
Eicosenoic acid	n/a	20:1n-9
Erucic acid	n/a	22:1n-9
Nervonic acid	n/a	24:1n-9
Eicosadienoic acid	n/a	20:2n-6
Eicosatrienoic acid	ETA	20:3n-6
Linoleic acid	n/a	18:2n-6
γ-Linolenic acid	GLA	18:3n-6
Arachidonic acid	ARA	20:4n-6
α-Linolenic acid	ALA	18:3n-3
Eicosapentaenoic acid	EPA	20:5n-3
Docosapentaenoic acid	DPA	22:5n-3
Docosahexaenoic acid	DHA	22:6n-3
Saturated FA	SAFA	no double bonds
Mono-unsaturated FA	MUFA	1 double bond
Poly-unsaturated FA	PUFA	>1 double bond
Omega-3 FA	n-3	1st double bond 3 C from methyl group
Omega-6 FA	n-6	1st double bond 6 C from methyl group
Omega-3/Omega-6 FA	n-3/n-6	

In marine environments, FAs are predominantly found consisting of 14 to 24 carbon atom chains and, although some modification of prey FA patterns occurs during trophic transfer (i.e. trophic fractionation), FA patterns in predators generally reflect the patterns in their prey (Iverson et al. 2002, 2004; Budge et al. 2008; Wang et al. 2010). As such, FAs have been used to infer the diets of predators through qualitative and quantitative approaches (Ramirez et al. 2009; Bianchi and Canuel 2011). One qualitative approach uses the FA patterns in predators to obtain information on individual and/or population FA patterns that may be related to spatial differences, temporal changes, or inter-specific differences in diet (Williams and Buck 2010). Such baseline information is useful in identifying environmental change. Another qualitative method involves identifying FA biomarkers that are characteristic of certain prey types that can be used to deduce potential prey item(s) in predators (Käkelä et al. 2007; Ramirez et al. 2009). A third method, quantitative fatty acid signature analysis, makes use of predator and prey FA profiles (also taking into account the trophic fractionation of FAs) to create a statistical model to estimate predator diet composition (Budge and Iverson 2003; Iverson et al. 2004; Bianchi and Canuel 2011).

In a controlled lab experiment, Iverson et al. (2004) showed that FAs could be used to quantitatively deduce the diet of seals. Käkelä et al. (2006, 2007) used FA biomarkers to examine intra- and inter-specific differences in the diets of marine birds. Fatty acids have also been applied extensively in marine bird

feeding ecology studies (Tierney et al. 2008; Käkelä et al. 2009; Wang et al. 2009; Williams and Buck 2010).

Integrated Use of FAs & SIs

Fatty acids and SIs have also been used together to determine marine bird feeding patterns (Hebert et al. 2006, 2008, 2009; Oppel et al. 2010; Ronconi et al. 2010; Wang et al. 2010; Wold et al. 2011). In general, SIs have been used as a broad tool (e.g. carbon isotopes to locate general feeding locations and nitrogen isotopes for determining trophic relationships; Hebert et al. 1999; Thompson et al. 1999; Nisbet et al. 2002; Karnovsky et al. 2008) and FAs have been used to identify more specific types of prey (Käkelä et al. 2009; Williams and Buck 2010).

Both groups of biochemical tracers provide opportunities to examine marine bird feeding ecology over various time frames, by examining these tracers in different bird tissues (Tieszen et al. 1983; Barrett et al. 2007; Williams and Buck 2010). For example, Hobson and Clark (1992) found that carbon isotopes in different tissues of Japanese Quail (*Coturnix japonica*) reflected their controlled diet integrated over periods of a few days (e.g. liver) to several months (e.g. bone collagen). Likewise, the FAs of certain tissues may also reflect the diet of consumers over different time scales (Käkelä et al. 2009). Wang et al. (2007) suggested that the stomach oil FAs of Northern Fulmars (*Fulmarus glacialis*) may be indicative of recent diet compared to FAs in Northern Fulmar adipose tissue. In a captive study, Käkelä et al. (2005) showed that the FA

signature of Herring Gull plasma reflected a completely new diet within approximately five days. The FA composition of marine bird eggs will also reflect the diet of the laying female but this may be complicated by the laying strategy of particular species (i.e., income (exogenous resources) or capital (endogenous resources) breeder) (Williams and Buck 2010). For instance, Emperor Penguins (*Aptenodytes forsteri*) utilize endogenous reserves for egg synthesis (Speake et al. 1999) so resources acquired at a previous time are incorporated into the egg. Other birds are thought to rely on a mix of endogenous and exogenous resources for egg synthesis but this may vary annually (Gauthier et al. 2003). Nonetheless, examination of the FA profiles of eggs has revealed important potential changes in diet (Hebert et al. 2009).

Analysis of marine bird tissues has been extensively used to monitor and study the marine environment (Furness and Camphuysen 1997; Mallory et al. 2010), including the analysis of SIs and/or FAs in eggs (Hobson et al. 1997; Royle et al. 1999; Thompson et al. 1999; Surai et al. 2001; Hebert et al. 2006, 2009; Jacobs et al. 2009). In lab experiments, Baucells et al. (2000) manipulated the diet of female Leghorn Chickens (*Gallus domesticus*), which was then reflected in their eggs. PUFAs, in particular, increased following the addition of fish oils to the diet. PUFAs are critical for developing bird embryos (Royle et al. 1999; Speake et al. 1996; Surai et al. 2001; Speake and Deans 2004). In most vertebrates, PUFAs like omega-3 and omega-6 (n-3 and n-6, respectively) FAs cannot be produced *de novo* (Surai et al. 2001) so have to be obtained from the

diet (Royle et al. 1999; Rubio-Rodríguez et al. 2010; Williams and Buck 2010). Likewise, Hebert et al. (1999) used the SIs of Herring Gull eggs to show intra-specific differences in isotopic signatures of gulls breeding at different colonies, likely reflecting spatial differences in gull trophic position and feeding ecology.

The Current Study

In the summer of 2008, I collected marine bird eggs at a remote High Arctic island (hereafter referred to as Tern Island; 75°49'N, 96°18'W; Fig. 1) north of the community of Resolute Bay, Nunavut. My objectives were to examine the early versus late-laid egg and inter-species egg SI and FA profiles for three marine birds that breed in the High Arctic. Specifically, I selected eggs from Arctic Terns (*Sterna paradisaea*), Common Eiders (*Somateria mollissima*) and Long-tailed Ducks (*Clangula hyemalis*) because these three species can all be found nesting in the same location, but are known to differ in their prey and possibly in the resources used for egg production (i.e., endogenous versus exogenous). I investigated the degree to which SI and FA patterns varied according to laying sequence. This is important in that it will inform future monitoring activities, i.e. is it necessary to consider laying order of eggs as part of future collections? I also determined if SI and FA patterns in eggs could be used to distinguish species. The characterization of SI and FA patterns in eggs from these species provides a baseline against which future dietary change can be assessed.

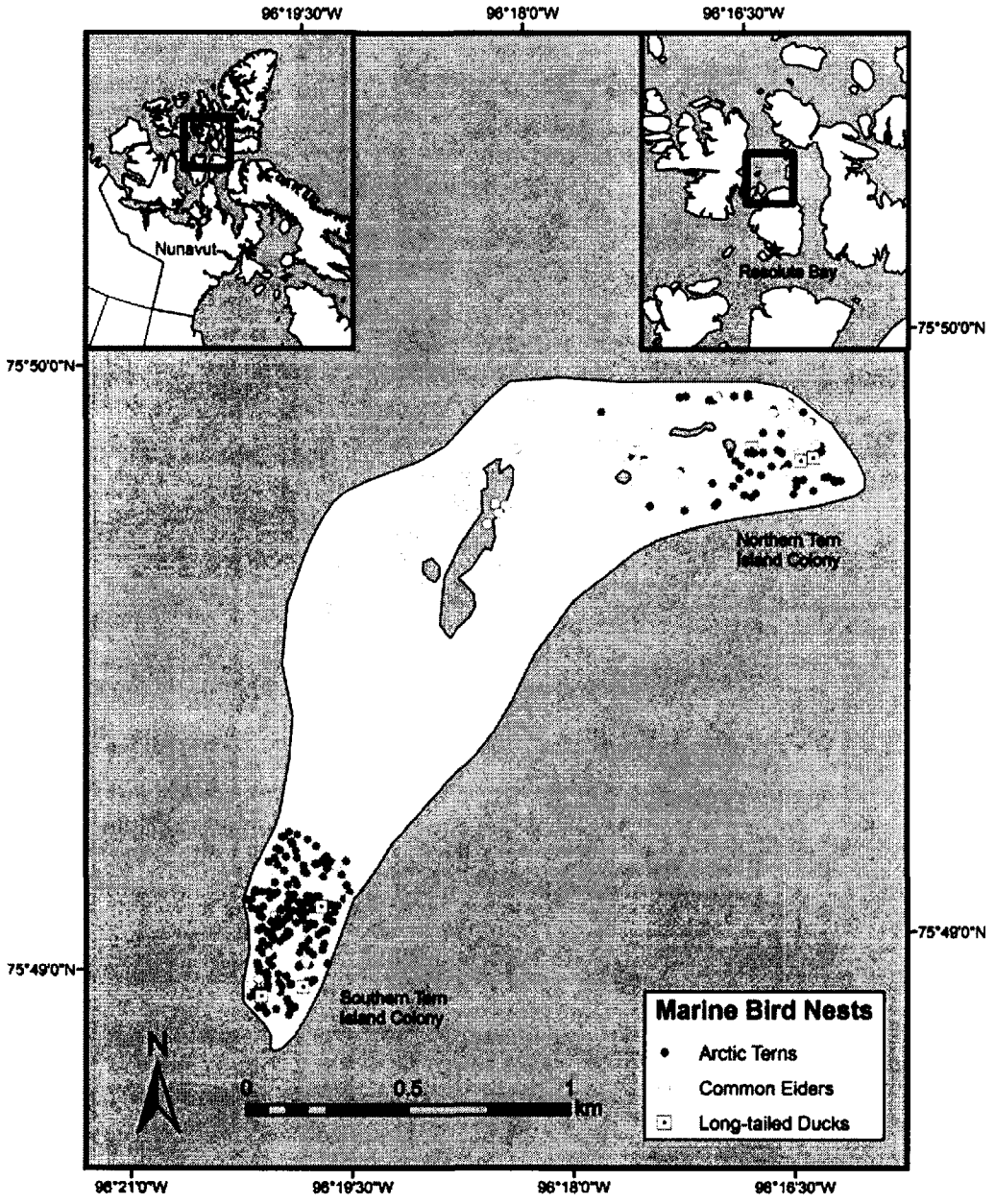

Figure 1.1. Tern Island (75°49'N, 96°18'W, ~1.4 km^2), located approximately 140 km north of the community of Resolute Bay, Nunavut.

Arctic Terns nest in colonies throughout northern regions but they have also been found nesting as far south as the United States (Evans and McNicholl 1972; Hatch 2002). Typically, Arctic Terns have a clutch of two eggs with three-egg clutches being rare (Chapdelaine et al. 1985; Robinson et al. 2001). Clutch size; however, may also be affected by food availability as well as local weather conditions (Evans and McNicholl 1972; Suddaby and Ratcliffe 1997). Arctic Terns feed on a variety of aquatic organisms such as fish, crustaceans and amphipods that are typically captured at the water surface in marine and/or freshwater environments, although insects may on occasion be an important food (Abraham and Ankney 1984; Wesławski et al. 1994; Hatch 2002). Unlike Common Eiders and Long-tailed Ducks, male Arctic Terns present food to female Arctic Terns during courtship (Monaghan et al. 1989). Furthermore, the eggs and chicks of Arctic Terns are attended by both parents throughout the breeding season (Robinson et al. 2001). In the High Arctic, eggs are usually laid in July and the full clutch is usually completed within four days of initiation (Hatch 2002).

Common Eiders are typically found in Arctic and subarctic marine environments (Goudie et al. 2000). The main prey of Common Eiders are benthic invertebrates that live in intertidal and subtidal zones (Weslawski et al. 1994; Goudie et al. 2000). Common Eiders nest in colonies, typically on small islands, and their clutch size varies from three to five eggs (Goudie et al. 2000; Robertson et al. 2001), although six egg clutches also occur (Parker and Holm 1990). Prior to egg-laying, female Common Eiders feed heavily (Parker and

Holm 1990) and accumulate fat and protein reserves (Guillemette and Ouellet 2005). Only the female attends the nest throughout the incubation period (Bolduc and Guillemette 2003). Common Eiders typically lay one egg per day initially; however, the period between the last two eggs in four-egg and five-egg clutches may be more than 24 hours (Watson et al. 1993). Weather conditions and latitudinal differences appear to affect the start of egg-laying, with egg-laying occurring as early as mid-May or late June (Goudie et al. 2000; Chaulk et al. 2005). At the start of egg-laying, females sometimes leave the nest after they have laid their first egg (Hanssen et al. 2002) to feed and drink but they generally stay on their nest after they have laid their second egg until hatch (Criscuolo et al. 2002; Hanssen et al. 2002; Bolduc and Guillemette 2003).

Long-tailed Ducks breed in Arctic and taiga regions throughout the circumpolar Arctic (Robertson and Savard 2002). They are capable of feeding on marine and freshwater food sources by either diving for their food or feeding at the water surface. Furthermore, Long-tailed Ducks also feed on various insects on their breeding grounds. Some of their main prey include fishes, various benthic organisms such as molluscs, as well as other crustaceans and amphipods in the water column. It is thought that the Long-tailed Duck is the deepest diver amongst the sea ducks (Robertson and Savard 2002). Long-tailed Ducks can lay 5 to 14 eggs per clutch (Kellett et al. 2005). Egg initiation may depend on weather and local conditions (McLaren and Alliston 1985), but egg initiation generally is between mid-May to late-June throughout the Arctic

(Robertson and Savard 2002; Kellett et al. 2005). During the start of egg-laying, Long-tailed Ducks cover their eggs with leaves or down and leave the nest for short recesses (Robertson and Savard 2002), but the frequency of these recesses may vary depending on the local abundance of food (Kellett et al. 2005).

Egg formation and egg-laying require considerable energy and nutrients (Parker and Holm 1990; Alisauskas and Ankney 1992). The Common Eider is the largest duck in North America (Goudie et al. 2000) and traditionally it has been thought to primarily rely on endogenous reserves during egg synthesis and laying (Meijer and Drent 1999). However, based on SI analysis of Common Eider eggs, Sénéchal et al. (2011) suggested that Common Eiders also relied on exogenous resources for egg production, particularly for protein. Long-tailed Ducks are smaller than Common Eiders and may rely more on exogenous resources during egg formation since it has been recorded that Long-tailed Ducks lose only 7% of their body mass during incubation, which is comparatively lower than other, similar-sized waterfowl species (Kellett et al. 2005). Arctic Terns are also small marine birds and they have been known to produce one-egg clutches during periods of low food availability (Suddaby and Ratcliffe 1997), which suggests that Arctic Terns also rely on exogenous resources during egg synthesis and laying.

In Chapter 2, I investigate intra-specific differences in early and late-laid eggs from each species. I predict that there will be SI and FA differences

between early and late-laid eggs in Common Eiders and Long-tailed Ducks since both species lay large clutches and because they likely rely on both endogenous and exogenous resources for egg production. I also predict that Arctic Terns will show little change in SI and FA profiles between early and late-laid eggs because of their primary reliance on exogenous resources for egg synthesis with a diet primarily focused on fish.

In Chapter 3, I investigate inter-specific differences in SI and FA patterns in eggs from the three species. Given that marine bird eggs reflect the diet of the laying marine bird, a comparison of eggs among marine bird species that nest in the Arctic should provide information on their respective feeding resources. Marine bird egg SIs and FAs could potentially reveal how marine bird species avoid competition for food resources during the breeding period by feeding on different foods at different trophic levels and in different locations (i.e. inshore/benthic or offshore pelagic). Because Common Eiders are principally molluscivores that arrive with large endogenous reserves and lay a large clutch early in the breeding season, I predicted that their SI and FA signatures would differ significantly from those of piscivorous Arctic Terns, which are thought to rely more on exogenous reserves for reproduction. I predicted that Long-tailed Duck signatures would differ from both of these species because they are known to utilize both saltwater and freshwater food resources; however, I also predicted that they would exhibit more overlap with Common Eiders than Arctic Terns since Arctic Terns are primarily piscivores.

CHAPTER TWO

Stable Isotope and Fatty Acid Patterns in Early and Late-Laid Eggs of Arctic Terns, Common Eiders and Long-tailed Ducks Breeding on a High Arctic Island

Introduction

Marine birds are useful as environmental monitors (Cairns 1987; Furness and Camphuysen 1997) and, as such, have been used to examine issues such as levels of contaminants in the marine environment (Braune et al. 2002; Cifuentes et al. 2003; Braune 2007) or to better understand the structure of marine food webs (Surai et al. 2001; Ramírez et al. 2009; Oppel et al. 2010). Many monitoring programs rely on the collection of eggs and traditionally these eggs have been randomly collected regardless of laying sequence within a clutch. This reflects the logistical difficulties of tracking laying sequence but such an approach does not allow for an assessment of potential intra-clutch differences in egg parameters. Such differences may be important in influencing the inferences made from monitoring data. For example, Akearok et al. (2010) found that early-laid eggs of Arctic Terns and Common Eiders had significantly greater mercury (Hg) levels than late-laid eggs. Therefore, a bias in egg collection methods, e.g. collection of eggs from incomplete clutches early in the breeding season, could overestimate Hg availability relative to other years when eggs were collected more randomly. Intra-clutch egg differences have been detected for a number of endpoints including egg size (Christians 2002; Lifjeld et al. 2005), contaminant levels (Becker 1992; Morera et al. 1997; Akearok et al. 2010), carotenoids (Newbrey et al. 2008), hormones (Groothuis and Schwabl 2002; Love et al. 2009; Hahn 2011, Kozlowski and Ricklefs 2011), stable

isotopes (Ramírez et al. 2011) and fatty acids (Royle et al. 1999). Morrissey et al. (2010) examined SIs in plasma and red-blood cells in two passerine birds during the pre-breeding and egg laying season and found that a diet shift occurred during the egg laying period. Potential diet shifts during the egg laying period could be reflected in the SI values and FA composition of bird eggs.

Intra-clutch differences in SI values have been detected in a marine bird, the Yellow-legged Gull (*Larus michahellis*) in which last-laid eggs (i.e. third egg) had significantly greater $\delta^{15}N$ values in albumen than early-laid eggs (Ramírez et al. 2011). A controlled experiment by Hobson (1995) showed that Japanese Quail egg albumen $\delta^{13}C$ values reflected a change in diet after 3 to 5 days whereas yolk reflected a change in diet after 8 days.

Intra-clutch differences in FA levels have also been detected in the Lesser Black-backed Gull (*Larus fuscus*) in which last-laid eggs (i.e., third egg) were shown to have significantly lower amounts of arachidonic acid (Royle et al. 1999). Diet changes reflected in FAs have also been demonstrated in marine bird plasma (Käkelä et al. 2005) and adipose tissue (Wang et al. 2010).

The objective of this study was to investigate possible differences in stable isotope and fatty acid profiles in early and late-laid eggs of three sympatrically nesting High Arctic marine birds: Arctic Terns, Common Eiders and Long-tailed Ducks and evaluate the extent of these differences relative to those observed across species. Differences in SI and FA patterns in early versus late-

laid eggs could reflect shifts in diet during the egg formation and laying period. Alternatively, if endogenous reserves are used for egg formation, then changes in SI and FA patterns between early and late-laid eggs could reflect differences in the relative contributions of exogenous versus endogenous resources during their formation. Here, I define exogenous resources as those acquired locally after the birds return to Tern Island to breed.

The Common Eider has traditionally been thought to rely primarily on endogenous reserves for egg synthesis (Parker and Holm 1990; Meijer and Drent 1999), although recent research by Sénéchal et al. (2011) suggested that Common Eiders also rely on exogenous resources for egg synthesis, particularly with respect to protein. With regard to Arctic Terns, Hobson et al. (1997) examined egg SIs of Arctic Terns breeding in a subarctic region and estimated that 86% of the resources used for egg production originated from exogenous resources. Quantitative estimates for Long-tailed Ducks do not exist, but Kellett et al. (2005) suggested that they rely primarily on exogenous resources for egg synthesis. All three species lay eggs at a minimum of one-day intervals (Goudie et al. 2000; Hatch 2002; Robertson and Savard 2002) so changes in diet could be detected in their clutches using SI and FA analysis, particularly for Common Eiders that lay three to five eggs (Goudie et al. 2000) and Long-tailed Ducks that lay five to fourteen eggs (Robertson and Savard 2002). For Common Eiders, this translates into a period of approximately two weeks between formation of the first

egg and ovulation of the last (Sénéchal et al. 2011). Presumably, Long-tailed Duck egg formation would take a slightly longer period. Arctic Terns would be much shorter given their smaller clutch size (modal size = 2; Hatch 2002) but the interval between egg laying can range from 1-4 days so it is possible that dietary shifts during egg formation could be reflected in the SI and FA composition of early versus late-laid eggs. Discovering early versus late-laid egg differences in SI and FA values could point to important resources that are used during the critical breeding period as well as provide preliminary insights into the use of endogenous and/or exogenous resources for egg synthesis. Based on the different breeding strategies discussed (Hobson et al. 1997; Bolduc and Guillemette 2003; Kellett et al. 2005; Mosbech et al. 2006) and the clutch size differences among the three marine birds (Goudie et al. 2000; Hatch 2002; Robertson and Savard 2002) as well as the various physical and biochemical intra-clutch differences that have been detected (Becker 1992; Morera et al. 1997; Christians 2002; Groothuis and Schwabl 2002; Lifjeld et al. 2005; Akearok et al. 2010; Newbrey et al. 2008; Love et al. 2009; Hahn 2011, Kozlowski and Ricklefs 2011; Ramírez et al. 2011), I predict that there will be significant early versus late-laid egg FA and SI differences for the Common Eider and Long-Tailed Duck. Since Long-tailed Ducks are known to feed on a wider variety of prey in both freshwater and saltwater environments (Robertson and Savard 2002), I expect that this potential dietary variability combined with the longer

period required for clutch completion may result in increased intra-clutch variability in egg SI and FA composition. Common Eiders are considered primarily molluscivores (Goudie et al. 2000) but they have been known to feed on a variety of prey (Wesławski et al. 1994) and given that they lay up to five eggs per clutch (Goudie et al. 2000) and since they accumulate large reserves prior to egg laying (Parker and Holm 1990; Guillemette and Ouellet 2005; Mosbech et al. 2006), I also expect to find Common Eider SI and FA early versus late-laid egg differences. Arctic Terns typically lay two eggs, 1-4 days apart (Hatch 2002), so egg formation only requires a relatively short period. Hence, although it is possible that dietary change during egg formation could be reflected in the chemical composition of the eggs, I predict that I will not observe significant SI and FA differences between early and late-laid eggs for this species.

Methods

Egg Collections

Arctic Tern, Common Eider and Long-tailed Duck eggs were collected at Tern Island, north of the community of Resolute Bay, Nunavut (75°49'N, 96°18'W; Fig. 1.1). Field crews arrived at Tern Island in June 2008, and soon after arrival, observations were made to record the first signs of laying for each species. Arctic Terns nested in two major colonies, the highest density and number occurred on the southern end of the island and a smaller, lower density

colony was located at the north-eastern end of the island so it was comparatively easy to determine when Arctic Terns began laying their eggs. Common Eiders nested most densely at the centre of the island near ponds but they were also scattered throughout the island. It was relatively easy to determine when Common Eiders began laying although they were more difficult than Arctic Terns to locate if they did not flush from their nests during nest checks. Nests of Long-tailed Ducks were much more difficult to locate than either Arctic Terns or Common Eiders since Long-tailed Ducks blended in with their surroundings once they began nesting and because they would conceal their eggs with down when they left the nest. Most Long-tailed Duck nests were located on the southern end of the island amidst the Arctic Terns, but a couple of Long-tailed Ducks nested in the northeast end of the island.

Arctic Terns typically lay two eggs (Hatch 2002), so we used nests where one egg was found, marked the egg using a felt marker, and then returned daily until two eggs were present, at which time we collected the entire clutch (n=34 eggs from 17 nests). Common Eiders lay larger clutches (Goudie et al. 2000) and nests with one egg were located and the egg was marked with a felt marker. During subsequent visits, late-laid eggs were marked the same way provided the egg laying sequence could be followed (Fig. 2.1). A number of Common Eider nests; however, contained two to three eggs when first located. To document laying sequence for these nests, clutches that had two or three eggs at first

sighting were labelled as early-laid eggs and later laid eggs (i.e., fourth and/or fifth laid eggs) were labelled as late-laid eggs. Complete Common Eider clutches were also found but they were not included in my research because of the lack of information regarding laying sequence. Two Common Eider clutches where an early egg was collected were predated hence those nests are not included here. In the end, 28 Common Eider eggs from 14 nests where the early and late-laid eggs were known were used in this analysis. Long-tailed Duck nests were more difficult to locate than Common Eiders or Arctic Terns, since they were relatively scarce and were more scattered throughout the island. However, we used the same approach as for the Common Eiders differentiating early and late-laid eggs. For this species, I was able to collect early and late-laid eggs from 4 nests.

Figure 2.1. A five-egg Common Eider clutch on Tern Island marked one through five documenting the laying order of the eggs.

Egg Storage and Transport

Whole eggs collected on Tern Island were packed into padded pelican cases and stored inside a tent (less than 2 weeks) until shipment to Resolute Bay was possible. They were then shipped to Ottawa via Iqaluit. Care was taken to ensure that they were kept in a refrigerator when in transit from Resolute Bay to the National Wildlife Research Centre (NWRC) in Ottawa.

Tissue Preparation Method

Upon arrival at NWRC, the marine bird eggs were dissected and processed according to NWRC method SOP-TP-PROC-04G (NWRC 2011). Eggs were rinsed in purified water to remove debris and then air dried on a hard plastic egg tray. Once dry, the egg was weighed in grams (to one decimal place). The egg was then cut open with a clean sterilized scalpel along the midline and the contents poured into a tared and chemically clean glass jar and then stored at -80°C.

Homogenization Method

In preparation for homogenization, eggs were defrosted overnight in a refrigerator at 4°C. Prior to homogenization, the biological safety cabinet interior was chemically sterilized and treated with ultra-violet radiation for 10 seconds with the air filtration system running throughout the entire homogenization

procedure. Once defrosted, the egg was moved into the sterilized biological safety cabinet with the air filtration system on. Egg contents were transferred into a sterilized Teflon beaker and homogenized using a Polytron mixer until thoroughly mixed (10 to 20 seconds). Once homogenized, two 1 g aliquots of the homogenized egg were transferred into two cryovials and labelled. One vial was used for FA and SI analysis and the other vial was archived. One g aliquots were stored at -80°C until all eggs were homogenized. The remaining portion of the homogenized eggs was transferred into labelled vials and jars and stored at -40°C.

Freeze-Drying Method

Freeze-drying of the homogenized eggs was done according to Wakeford (2003). Using a clean, metal microspatula, a 20-30 mg subsample was extracted from the 1 g aliquot of homogenized egg and placed into an acid-washed, pre-labelled 15 mL screw top test tube, which was then covered with Parafilm. Using a clean dissecting needle, 4 holes were pierced in the Parafilm to allow air movement during the freeze-drying process. The samples were then stored upright in a test tube rack in a -30°C freezer until several samples were completed and they were then placed in the freeze-dryer (Labconco FreeZone 6 Litre Freeze Dry System - Model 77530). The freeze-dryer was operated at

-40°C and the vacuum allowed to reach at least 50 x 10^{-3} mBarr. Samples were left to dry for at least 48 hours.

Fatty Acid Analysis: Lipid Extraction, Methylation, and Analysis

Lipid Extraction

Lipid extraction methods followed the procedures in Hebert et al. (2006). Egg lipids were extracted from a 20-30 mg subsample of freeze-dried sample. Subsamples were transferred into small disposable centrifuge tubes which were then placed on ice. Along with the egg samples, one blank and one standard reference material sample were included in each batch of samples undergoing the lipid extraction procedure. Two mL of 2:1 $CHCl_3$:MeOH (chloroform:methanol) and 50 µL of 1 mg/mL of cholestane internal standard were added to each tube, including the blank and standard reference material.

All of the subsamples were homogenized using a Teflon pestle (Heidolph Manufacturer) for 30-45 seconds. To ensure no cross-contamination of samples during homogenization, the pestle was rinsed between samples with 2:1 $CHCl_3$:MeOH. After all the samples were homogenized, the disposable tubes were plugged with silicon stoppers and then centrifuged at 3,200 rpm for 10 minutes at 4°C. The disposable tubes were then carefully removed from the centrifuge so as not to mix the overlying supernatant with the underlying particulate layer. The overlying supernatant was then transferred into a 12 mL

screw-cap tube using a Pasteur pipette. After all the supernatant was removed from each disposable tube, another 2 mL of 2:1 $CHCl_3$:MeOH was added to the disposable tube containing the particulates, they were then vortexed for 10 seconds. The above procedure of homogenization, centrifugation, transfer of supernatant, and vortexing was repeated two more times. At the end of the process, particulates in the disposable tubes were retained and set aside to air dry for 48 hours or more since the lipid-free particulates were used for stable isotope analysis (see Methods: Nitrogen and Carbon Stable Isotope Analysis).

The 12 mL screw-cap tubes containing the supernatant were placed in a nitrogen-evaporator (N-evap) (Organomation, Clear Brook, VA) until dry. Once dry, the 12 mL screw-cap tubes were filled with 2 mL of 2:1 $CHCl_3$:MeOH and vortexed for 10 seconds and then 200 µL were then transferred from the 12 mL screw-cap tubes into pre-weighed and labelled 100 to 250 µL gas chromatograph (GC) vials using a pre-wetted pipette tip.

Methylation

Once 200 µL were transferred into the GC vials, the 12 mL screw-cap tubes were placed in the N-evap again until the content was dry. After the N-evap was completed, 1.5 mL of toluene was added to each 12 mL screw-cap tube and then vortexed for 10 seconds without capping. Once toluene was added and vortexed, 2 mL of H_2SO_4/MeOH methylating reagent were added to

each tube. The 12 mL screw-cap tubes were then flushed with N_2 for 5 to 10 seconds, capped, and then finally vortexed for another 10 seconds. Once all the screw-cap tubes were capped and vortexed, they were incubated overnight for at least 16 hours at 50°C in a water bath.

After 16 hours in the water bath, 2 mL of 2% $KHCO_3$ were added to each tube. The tubes were then gently shaken and vented to allow any CO_2 to escape. They were then vortexed for 10 seconds, inverted, vortexed for another 10 seconds and inverted again. Afterwards, the tubes were centrifuged at 1,500 rpm for 2 minutes. Once centrifugation was completed, the upper organic toluene layer was transferred to a new 12 mL screw-cap tube using a Pasteur pipette. Once this upper layer was transferred (being careful not to transfer the underlying layer), 2 mL of hexane were added to the tube containing the underlying layer. The process of shaking, vortexing, centrifuging and transferring the upper layer to the new 12 mL screw-cap tube was repeated two more times.

The 12 mL screw-cap tube containing the upper layer fractions was placed in the N-evap until dry. Two mL of hexane were added to each tube and then 200 µL were then transferred into GC vials. The remainder of the sample was transferred into another GC vial for archiving. All the GC vials were labelled and were stored at -80°C until they were analyzed using gas chromatography. Blanks and standard reference materials were treated in an identical manner.

Analysis

Fatty acid concentrations were quantified on a Hewlett-Packard 6890 GC with the following configuration: splitless injection; column: Supelco (SP-2560 column) 100 m x 0.25 mm i.d. x 0.20 µm thick film; Oven: 140°C (hold 5 min) to 240°C at 4°C/min, hold for 12 min; helium carrier gas, 1.2 mL/min; flame ionization detector at 260°C; injector at 260°C; total run time = 42 min/sample. A 37-component FAME standard (Supelco no. 47885-U) was used to identify and quantify (4-point calibration curves) FAME in samples (unknowns) i.e., by comparing their retention times to those of the FAME standard. Results were reported as µg FAME/mg dry weight tissue.

Twenty-two FAs were identified for Long-tailed Duck and Arctic Tern eggs. For Common Eiders, 21 FAs were identified since erucic acid (22:1n-9) was not present in Common Eider eggs.

Nitrogen and Carbon Stable Isotope Analysis

Stable nitrogen isotope ($^{15}N/^{14}N$) and carbon isotope ($^{13}C/^{12}C$) ratios in the early and late-laid eggs of the three marine bird species were measured at the University of Ottawa's G.G. Hatch Stable Isotope Laboratory. These analyses were conducted using standard isotope ratio mass spectrometry, as described in Hebert et al. (1999) and Braune (2007). In brief, 1 mg subsamples from individual eggs were freeze-dried and lipids were removed using 2:1

chloroform:methanol rinses. The stable nitrogen and carbon isotope analysis was completed using these lipid free subsamples. 1.0 ± 0.2 mg of freeze-dried, lipid free sample was weighed into tin capsules (6 x 4 mm) and combusted at 1,800°C in a Vario EII III elemental analyzer (Elementar, Hanau Germany) followed by gas chromatograph separation and on-line analysis by continuous flow with a Delta Plus Advantage isotope ratio mass spectrometer (Thermo Scientific, Bremen Germany) coupled to a ConFlo III. Stable nitrogen and carbon isotope results (units were per mil (‰)) were expressed in delta (δ) notation, defined as δ=((Rx−Rstd))/Rstd) * 1000, where 'R' is the ratio of the abundance of the heavy (^{15}N or ^{13}C) to the light (^{14}N or ^{12}C) isotope, (x) denotes the ratio in a sample and 'std' denotes the ratio in a standard (i.e., atmospheric AIR for nitrogen and Pee Dee Belemnite limestone for carbon). Analytical precision, based upon repeated measurements of a standard was ±0.2‰.

Statistical Analysis

Statistical analyses were conducted using STATISTICA 7 (StatSoft 2005). For the $\delta^{13}C$ analysis, I used repeated measures ANOVA (RANOVA), applying Tukey post-hoc multiple comparison tests to determine differences in early versus late-laid eggs (RANOVA assumptions of sphericity, homogeneity of variance through Levene's test and homogeneity of co-variances through Box M-test were met). However, RANOVA assumptions were not met for the $\delta^{15}N$

analysis, so I conducted a non-parametric Friedman repeated measures ANOVA followed by a Wilcoxon matched-pairs signed-rank tests (applying sequential Bonferroni methods to p-values) to examine differences in $\delta^{15}N$ values in early versus late-laid eggs.

Fatty acid data for marine bird eggs were expressed as percentiles. For the FA analysis, a principal components analysis (PCA) was carried out to test for early versus late-laid egg differences in the FA composition of the eggs. To remove the influence of inter-specific differences in FA patterns, separate PCAs were conducted for each species. Kruskal-Wallis tests on the PC scores for each species were carried out to detect differences in FA composition of early versus late laid-eggs. Principal components analysis requires more samples than variables and since there were only eight Long-tailed Duck egg samples (four early and late-laid eggs each), the Long-tailed Duck PCA only included percent composition data for egg SAFA, MUFA, n-3 and n-6 FAs.

For each species, the % difference between the composition of early and late-laid eggs for each FA was calculated using the following formula: % difference = ((larger % - smaller %)/larger %)*100. An overall average across all FAs was also calculated.

Results

Nitrogen Isotopes in Early Versus Late-Laid Eggs

Statistically significant differences in nitrogen isotope values of early and late-laid eggs were detected in two of the three marine bird species on Tern Island (Friedman repeated measures ANOVA; X^2=16; p=0.007). $\delta^{15}N$ values in early and late-laid eggs were different in Arctic Terns (WMP test; Z=2.63; p=0.009) as well as in Common Eiders (WMP test; Z=3.01; p=0.003; Table 2.1 and Fig. 2.2). Early-laid Arctic Tern eggs had greater $\delta^{15}N$ values than late-laid eggs while Common Eider early-laid eggs had lower $\delta^{15}N$ values than late-laid eggs. Mean $\delta^{15}N$ values in early-laid eggs (13.89) of Long-tailed Ducks were not statistically different than those in late-laid eggs (13.14) (WMP test; Z=0.73, p=0.47; Fig. 2.2), likely reflecting the low power of the test to detect differences given the small sample size available.

Table 2.1. $\delta^{15}N$ (‰) and $\delta^{13}C$ (‰) values of early and late-laid eggs of Arctic Terns (ARTE), Common Eiders (COEI) and Long-tailed Ducks (LTDU) at Tern Island. CV = coefficient of variation.

Species	$\delta^{15}N$ (‰) Early Egg			$\delta^{15}N$ (‰) Late Egg			$\delta^{13}C$ (‰) Early Egg			$\delta^{13}C$ (‰) Late Egg		
	Mean	Std Dev	CV	Mean	Std Dev	CV	Mean	Std Dev	CV	Mean	Std Dev	CV
ARTE	15.67	1.03	6.57	15.27	0.68	4.45	-17.45	0.63	3.61	-17.43	0.54	3.10
COEI	13.97	0.43	3.08	14.25	0.50	3.51	-16.59	0.43	2.59	-16.75	0.41	2.45
LTDU	13.89	0.66	4.75	13.14	0.83	6.32	-17.55	0.67	3.82	-17.60	1.05	5.97

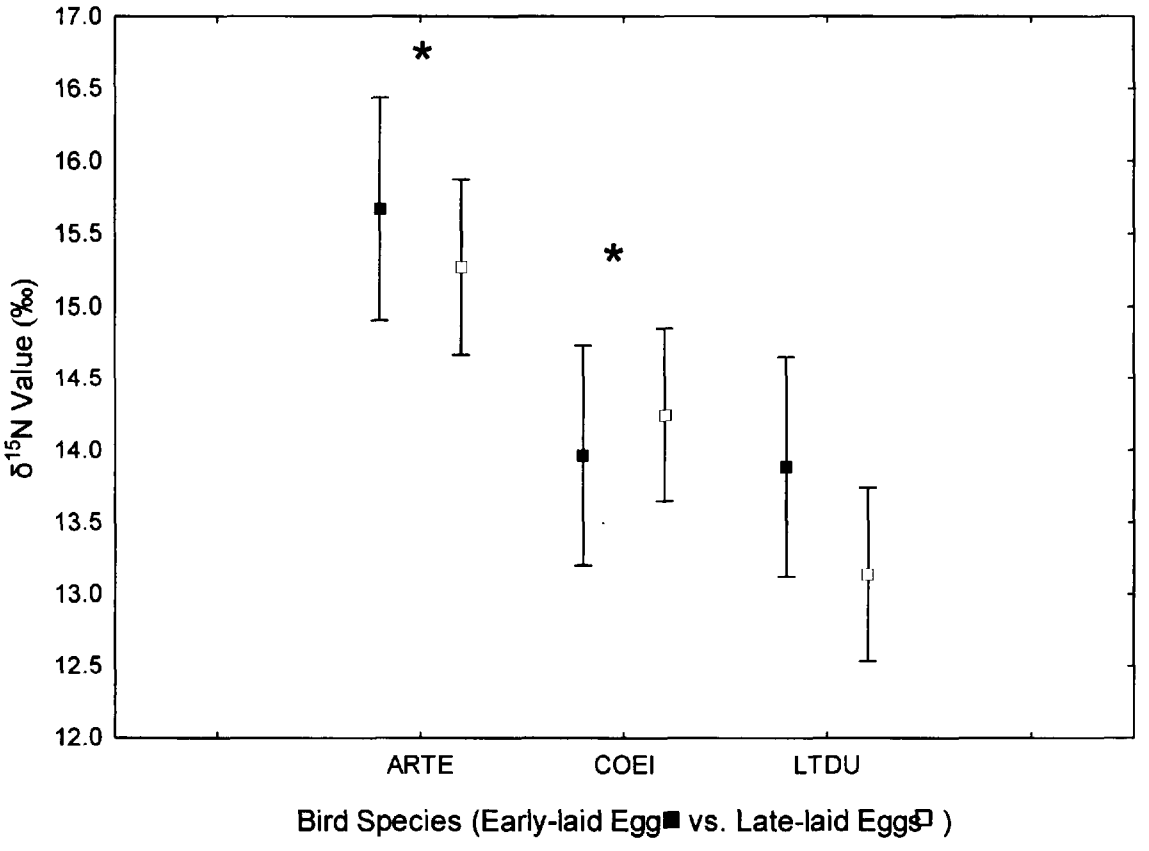

Figure 2.2. Mean (±1 std dev) $\delta^{15}N$ values for early and late-laid eggs of Arctic Terns (ARTE), Common Eiders (COEI) and Long-tailed Ducks (LTDU) collected from Tern Island. Statistically significant differences between early and late-laid eggs within species are indicated by * ($p<0.05$).

Carbon Isotopes in Early Versus Late-Laid Eggs

No significant differences were detected in mean $\delta^{13}C$ values between early and late-laid eggs for any of the three marine bird species (repeated measures ANOVA; $F_{1,35}=0.38$, $p=0.54$) (Table 2.1 and Fig. 2.3). For the early and late-laid eggs, Common Eiders had the lowest coefficient of variation, followed by Arctic Terns and Long-tailed Ducks.

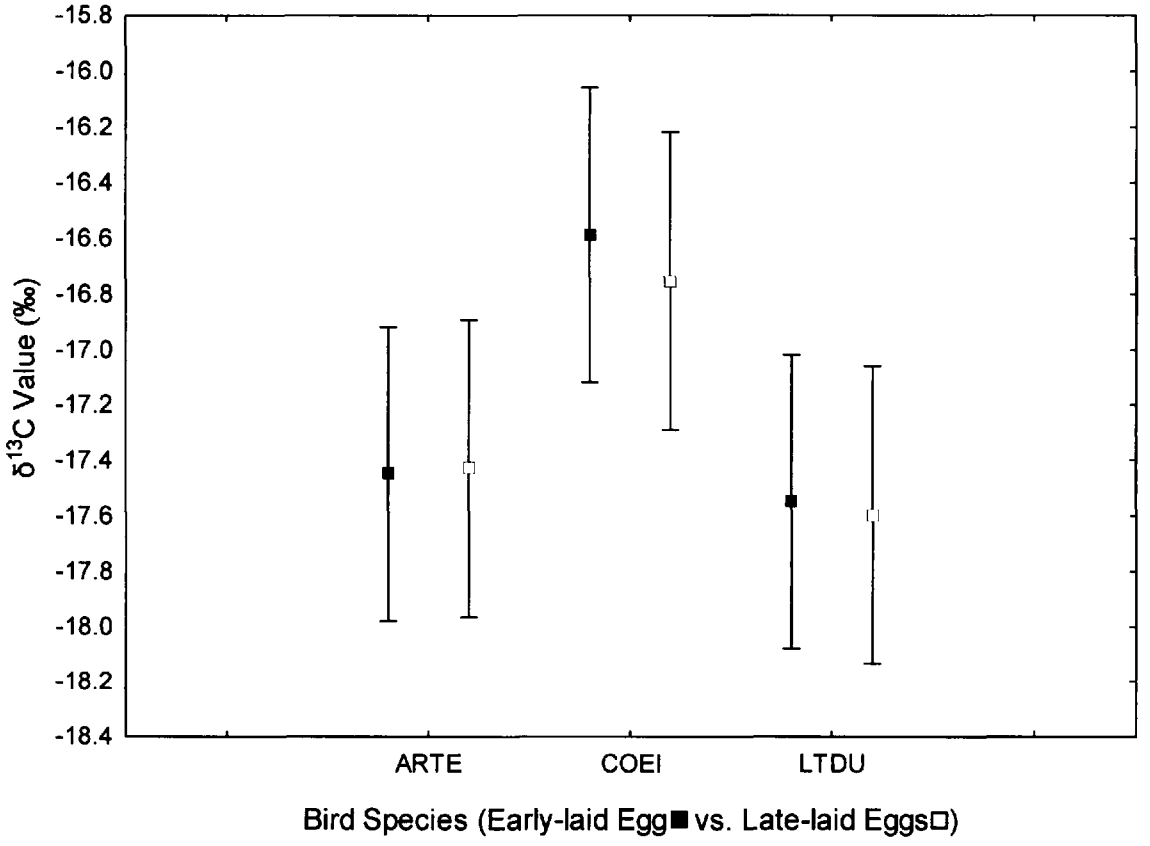

Figure 2.3. Mean (±1 std dev) $\delta^{13}C$ values for early and late-laid eggs of Arctic Terns (ARTE), Common Eiders (COEI) and Long-tailed Ducks (LTDU) collected from Tern Island.

Fatty Acids in Early Versus Late-Laid Eggs

Arctic Terns

Mean percent FA values in early and late-laid eggs are shown in Table 2.2. Saturated FAs (SAFAs, FAs with no double bonds) contributed over a third of the total FAs in both early and late-laid eggs (early-laid egg 34.4%, late-laid egg 33.5%), with palmitic acid (16:0) comprising most of the SAFAs. Both the early and late-laid egg mono-unsaturated FAs (MUFAs, FAs with one double bond) comprised over half the total FAs (early-laid egg 50.6%, late-laid egg 52.7%). Mono-unsaturated FAs were dominated by oleic acid (18:1n-9; early-laid egg 39.4%, late-laid egg 40.1%). The percent contribution of omega-6 (n-6) FAs to overall FA level was <5% (early-laid eggs 4.7%, late-laid eggs 4.4%), with arachidonic acid (20:4n-6) contributing the most (early-laid egg 3.5%, late-laid egg 3.0%). Omega-3 (n-3) FAs comprised a tenth of the total FAs (early-laid egg 10.3%, late-laid egg 9.4%) with docosahexaenoic acid (DHA, 22:6n-3) contributing over half the total n-3s (early-laid egg 5.7%, late-laid egg 5.4%) followed by eicosapentaenoic acid (EPA, 22:5n-3; early-laid-laid egg 3.1%, late-laid egg 2.7%).

Table 2.2. Percent composition of FAs in early-laid and late-laid Arctic Tern eggs.

FA Common Name	FA Structure	Early Egg Mean	Early Egg Std Dev	Late Egg Mean	Late Egg Std Dev	% Difference Between Early and Late Egg
Myristic acid	14:0	0.84	0.12	0.86	0.12	2
Pentadecanoic acid	15:0	0.15	0.13	0.16	0.07	6
Palmitic acid	16:0	24.27	0.90	24.07	0.85	1
Heptadecanoic acid	17:0	0.31	0.06	0.33	0.06	6
Stearic acid	18:0	8.76	0.53	7.91	0.65	10
Behenic acid	22:0	0.10	0.04	0.11	0.04	9
Myristoleic acid	14:1n-5	0.10	0.04	0.08	0.04	20
Palmitoleic acid	16:1n-7	8.30	2.08	9.07	1.48	8
Elaidic acid	18:1n-9t	0.27	0.10	0.27	0.10	0
Oleic acid	18:1n-9	39.41	2.90	40.12	2.38	2
Eicosenoic acid	20:1n-9	2.45	0.82	3.10	0.75	21
Erucic acid	22:1n-9	0.04	0.04	0.05	0.04	20
Nervonic acid	24:1n-9	0.02	0.02	0.03	0.02	33
Eicosadienoic acid	20:2n-6	0.07	0.02	0.08	0.01	13
Eicosatrienoic acid	20:3n-6	0.10	0.04	0.10	0.03	0
Linoleic acid	18:2n-6	0.97	0.13	1.18	0.13	18
γ-Linolenic acid	18:3n-6	0.05	0.03	0.05	0.02	0
Arachidonic acid	20:4n-6	3.50	1.06	3.02	0.83	14
α-Linolenic acid	18:3n-3	0.14	0.05	0.16	0.04	13
Eicosapentaenoic acid	20:5n-3	3.07	0.34	2.74	0.48	11
Docosapentaenoic acid	22:5n-3	1.39	0.21	1.15	0.19	17
Docosahexaenoic acid	22:6n-3	5.69	0.87	5.35	0.71	6
Saturated FA	SAFA	34.43	1.03	33.45	1.15	3
Mono-unsaturated FA	MUFA	50.58	1.84	52.71	1.65	4
Poly-unsaturated FA	PUFA	14.99	1.69	13.83	1.31	8
Omega-3 FA	n-3	10.30	1.03	9.40	0.97	9
Omega-6 FA	n-6	4.69	0.95	4.43	0.74	6
Omega-3/Omega-6 FA	n-3/n-6	2.26	0.39	2.17	0.41	4

Common Eiders

Mean percent FA values in early and late-laid eggs are shown in Table 2.3. For Common Eider early and late-laid eggs, the SAFAs also comprised approximately one third of the total FAs (early-laid eggs 35.1%, late-laid egg 35.7%) with palmitic acid (16:0) representing most of the total SAFAs (early-laid egg 26.7%, late-laid egg 27.5%). Mono-unsaturated FAs comprised over half of the FA total in early and late-laid eggs with oleic acid (18:1n-9) dominating the MUFAs in the early (40.6%) and late-laid (38.7%) eggs. The n-6 FAs comprised approximately 3% both in the early and late-laid eggs with arachidonic acid present in the greatest amount (early-laid egg 1.9%, late-laid egg 1.8%). Common Eider early and late-laid eggs contained 7.8% and 7.6% n-3 FAs with DHA and EPA present in the greatest amounts.

Table 2.3. Percent composition of FAs in early-laid and late-laid Common Eider eggs.

FA Common Name	FA Structure	Early Egg Mean	Early Egg Std Dev	Late Egg Mean	Late Egg Std Dev	% Difference Between Early and Late Egg
Myristic acid	14:0	1.08	0.11	1.11	0.14	3
Pentadecanoic acid	15:0	0.14	0.08	0.19	0.12	26
Palmitic acid	16:0	26.72	0.65	27.52	0.61	3
Heptadecanoic acid	17:0	0.43	0.08	0.50	0.07	14
Stearic acid	18:0	6.66	0.42	6.36	0.41	5
Behenic acid	22:0	0.03	0.01	0.03	0.01	0
Myristoleic acid	14:1n-5	0.09	0.02	0.10	0.03	10
Palmitoleic acid	16:1n-7	12.42	1.07	14.10	1.63	12
Elaidic acid	18:1n-9t	0.26	0.05	0.22	0.06	15
Oleic acid	18:1n-9	40.61	1.68	38.69	1.80	5
Eicosenoic acid	20:1n-9	0.58	0.10	0.52	0.09	10
Nervonic acid	24:1n-9	0.03	0.01	0.02	0.01	33
Eicosadienoic acid	20:2n-6	0.08	0.03	0.08	0.02	0
Eicosatrienoic acid	20:3n-6	0.09	0.02	0.07	0.01	22
Linoleic acid	18:2n-6	0.92	0.18	1.08	0.20	15
γ-Linolenic acid	18:3n-6	0.06	0.02	0.06	0.01	0
Arachidonic acid	20:4n-6	1.94	0.27	1.76	0.24	9
α-Linolenic acid	18:3n-3	0.32	0.06	0.36	0.11	11
Eicosapentaenoic acid	20:5n-3	3.05	0.50	2.34	0.42	23
Docosapentaenoic acid	22:5n-3	1.00	0.16	0.93	0.14	7
Docosahexaenoic acid	22:6n-3	3.48	0.34	3.97	0.29	12
Saturated FA	SAFA	35.07	0.96	35.70	0.86	2
Mono-unsaturated FA	MUFA	54.00	1.59	53.65	1.47	1
Poly-unsaturated FA	PUFA	10.94	0.85	10.65	0.80	3
Omega-3 FA	n-3	7.84	0.72	7.60	0.66	3
Omega-6 FA	n-6	3.09	0.41	3.05	0.42	1
Omega-3/Omega-6 FA	n-3/n-6	2.58	0.42	2.54	0.40	2

Long-tailed Ducks

Mean percent FA values in early and late-laid eggs are shown in Table 2.4. SAFAs made up a third of the total FAs (early-laid eggs 31.3%, late eggs 33.0%) with palmitic acid (16:0) making up three quarters of the SAFAs. Mono-unsaturated FAs constituted over half the total FAs in early (58.1%) and late-laid (52.6%) eggs with oleic acid (18:1n-9) comprising most of the MUFAs (37.7% and 32.2% of total FAs in early and late-laid eggs, respectively). Palmitoleic acid (16:1n-7), another MUFA also made up a considerable amount with the early-laid eggs containing 18.3% and late-laid eggs containing 18.6% of total FAs. In the early-laid eggs, n-6 FAs comprised 2.6% and in late-laid eggs comprised 4.8%, with linoleic acid (18:2n-6) present in the greatest proportions in late-laid egg (3.0%) versus early-laid egg (1.1%). Arachidonic acid comprised a significant portion of the n-6 FAs, with the early-laid eggs containing 1.3% and the late-laid eggs containing 1.5% of total FAs. The n-3 FAs comprised 8.1% and 9.6% of total FA levels in early and late-laid Long-tailed Duck eggs. Most important n-3 FAs were DHA and EPA. Early and late-laid Long-tailed Duck eggs contained 3.9% and 4.1% of DHA, respectively and 2.9% and 3.1% of EPA, respectively.

Table 2.4. Percent composition of FAs in early-laid and late-laid Long-tailed Duck eggs.

FA Common Name	FA Structure	Early Egg Mean	Early Egg Std Dev	Late Egg Mean	Late Egg Std Dev	% Difference Between Early and Late Egg
Myristic acid	14:0	1.21	0.18	1.27	0.24	5
Pentadecanoic acid	15:0	0.22	0.02	0.33	0.17	33
Palmitic acid	16:0	23.84	1.27	24.63	1.72	3
Heptadecanoic acid	17:0	0.31	0.02	0.55	0.41	44
Stearic acid	18:0	5.65	1.48	6.23	0.97	9
Behenic acid	22:0	0.03	0.01	0.03	0.02	0
Myristoleic acid	14:1n-5	0.12	0.02	0.14	0.03	14
Palmitoleic acid	16:1n-7	18.25	3.67	18.63	7.90	2
Elaidic acid	18:1n-9t	0.21	0.04	0.21	0.07	0
Oleic acid	18:1n-9	37.73	4.83	32.22	2.79	15
Eicosenoic acid	20:1n-9	1.67	0.65	1.28	0.71	23
Erucic acid	22:1n-9	0.06	0.03	0.05	0.04	17
Nervonic acid	24:1n-9	0.06	0.02	0.05	0.03	17
Eicosadienoic acid	20:2n-6	0.05	0.01	0.06	0.04	17
Eicosatrienoic acid	20:3n-6	0.07	0.03	0.09	0.01	22
Linoleic acid	18:2n-6	1.08	0.37	3.04	3.83	64
γ-Linolenic acid	18:3n-6	0.07	0.02	0.10	0.03	30
Arachidonic acid	20:4n-6	1.28	0.19	1.50	0.98	15
α-Linolenic acid	18:3n-3	0.28	0.10	0.99	1.03	72
Eicosapentaenoic acid	20:5n-3	2.89	0.94	3.11	1.15	7
Docosapentaenoic acid	22:5n-3	1.01	0.25	1.39	0.21	27
Docosahexaenoic acid	22:6n-3	3.90	0.85	4.09	0.14	5
Saturated FA	SAFA	31.26	1.12	33.03	2.24	5
Mono-unsaturated FA	MUFA	58.10	1.67	52.59	5.91	9
Poly-unsaturated FA	PUFA	10.64	0.80	14.38	4.64	26
Omega-3 FA	n-3	8.08	0.61	9.58	0.62	16
Omega-6 FA	n-6	2.56	0.23	4.80	4.88	47
Omega-3/Omega-6 FA	n-3/n-6	3.16	0.17	3.37	1.88	6

FA Patterns in Eggs - Principal Components Analysis (PCA)

All Species

To investigate FA patterns in eggs I conducted a PCA using data for 22 FAs for early and late-laid eggs from all 3 species (Fig. 2.4). PC1 and PC2 explained 46% of the variation in the data. It was evident that most of the variation in the FA dataset stemmed from inter-specific differences (Figure 2.4). To remove the influence of inter-specific differences on the interpretation of FA patterns in early versus late-laid eggs, separate PCAs were conducted for each species.

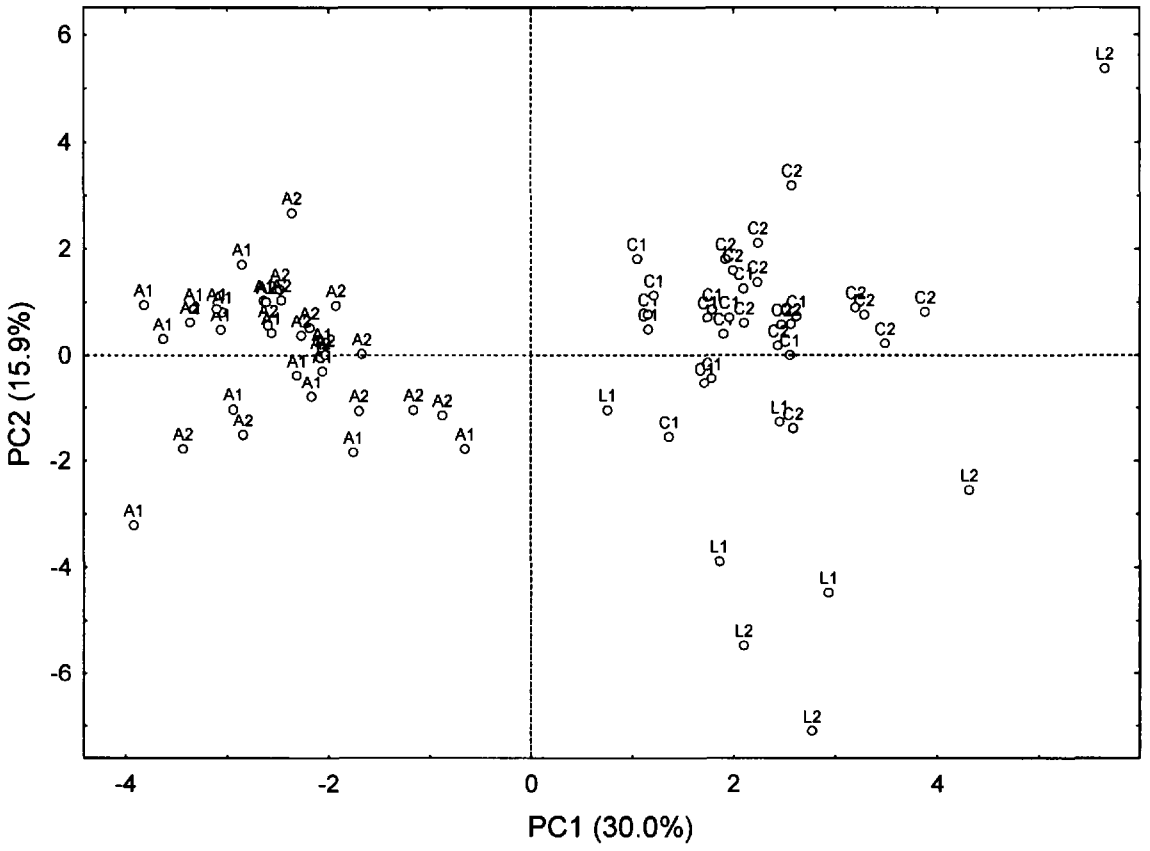

Figure 2.4. Plot of principal component scores summarizing FA composition in early (1) and late-laid (2) eggs of three bird species collected on Tern Island in 2008. Principal component 1 (PC1) and PC2 explained 46% of the variance in the data. Arctic Tern early egg (A1), late egg (A2); Common Eider early egg (C1), late egg (C2) and Long-tailed Duck early egg (L1), late egg (L2) are shown.

Arctic Tern PCA

For Arctic Terns, only scores on principal component 3 (PC3) showed a significant difference between early and late-laid eggs (KW-$H_{1,33}$=12.71, p=0.0004). PC3 accounted for 13% of the total variation in the Arctic Tern FA data. Early and late-laid eggs tended to group separately on PC3 (Fig. 2.5). FA loadings on PC3 were dominated by 5 of the 22 FAs (Table 2.5). Two of the n-3 PUFAs (DPA, 22:5n-3 and DHA, 22:6n-3) had negative loadings with the early and late-laid eggs indicating that early-laid eggs had greater %DPA and %DHA than late-laid eggs. Two omega-9 (n-9) MUFAs (eicosenoic acid, 20:1n-9 and erucic 22:1n-9 acid) had the greatest positive loadings, indicating that late-laid eggs had higher %eicosenoic and %erucic acid. Myristic acid (14:0), a SAFA, also had a positive loading along PC3 indicating greater percent contributions to total FA levels in late-laid eggs.

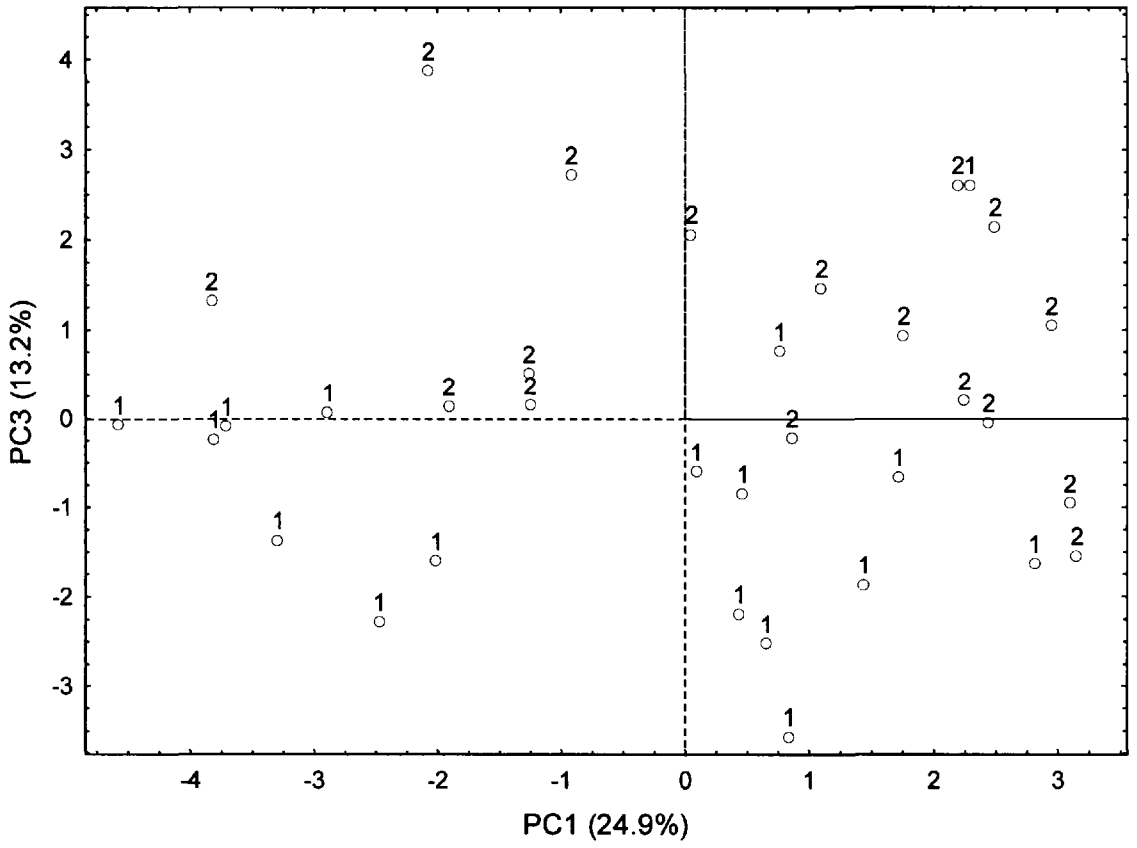

Figure 2.5. Plot of principal component scores summarizing FA composition in early (1) and late-laid (2) eggs of Arctic Terns from Tern Island. Principal component 1 (PC1) and PC3 explained 38% of the variance in the data. Differences between early and late-laid eggs were only detected on PC3. FAs with the greatest loadings on PC3 are shown in Table 2.5.

Table 2.5. FA loadings on PC1-3 for early and late-laid Arctic Tern eggs. Significant differences between early and late-laid eggs in PC scores were only detected for PC3. FAs with greater loadings are highlighted by (*).

FA Common Name	FA Structure	PC1	PC2	PC3*
Myristic acid	14:0	-0.45	-0.55	0.55*
Pentadecanoic acid	15:0	-0.33	0.01	-0.02
Palmitic acid	16:0	0.44	-0.33	-0.42
Heptadecanoic acid	17:0	-0.77	0.06	0.26
Stearic acid	18:0	-0.40	-0.54	-0.35
Behenic acid	22:0	0.74	0.33	-0.36
Myristoleic acid	14:1n-5	-0.36	-0.30	-0.23
Palmitoleic acid	16:1n-7	0.33	-0.83	0.30
Elaidic acid	18:1n-9t	-0.53	0.34	0.05
Oleic acid	18:1n-9	0.36	0.88	-0.14
Eicosenoic acid	20:1n-9	-0.15	0.15	0.87*
Erucic acid	22:1n-9	-0.01	0.14	0.56*
Nervonic acid	24:1n-9	-0.31	0.25	0.25
Eicosadienoic acid	20:2n-6	0.48	-0.08	0.12
Eicosatrienoic acid	20:3n-6	0.70	-0.54	-0.05
Linoleic acid	18:2n-6	0.68	0.10	0.43
γ-Linolenic acid	18:3n-6	0.38	-0.82	0.10
Arachidonic acid	20:4n-6	-0.90	0.15	-0.05
α-Linolenic acid	18:3n-3	-0.11	-0.03	0.37
Eicosapentaenoic acid	20:5n-3	-0.31	-0.56	0.08
Docosapentaenoic acid	22:5n-3	-0.67	-0.31	-0.50*
Docosahexaenoic acid	22:6n-3	-0.43	-0.05	-0.45*

Common Eider PCA

For the Common Eider PCA, 21 FAs were used instead of 22 since there was no erucic acid present in the Common Eider eggs. PCA scores on PC1 and PC2 separated early and late-laid Common Eider eggs along both axes (Fig. 2.6). Egg FA PC1 and PC2 scores were significantly different between early and late-laid eggs ($KW\text{-}H_{1,27}=12.52$, $p=0.0004$ for PC1 and $KW\text{-}H_{1,27}=4.08$, $p=0.04$ for PC2).

PC1 and PC2 explained 46% of the variance in the FA percent composition data (Fig. 2.6). On PC1 (Table 2.6), three of the shorter carbon chain SAFAs (15:0, 16:0, 17:0) loaded negatively in addition to two of the n-3 PUFAs (DHA – 22:6n-3 and ALA – 18:3n-3) indicating that late-laid eggs had greater percent contributions of these FAs to overall FA levels than did early-laid eggs. Two n-6 PUFAs (linoleic acid – 18:2n-6 and eicosadienoic acid – 20:2n-6) also had negative loadings. Positive loadings were observed for one n-3 PUFA (EPA – 20:5n-3), one n-6 PUFA (eicosatrienoic acid – 20:3n-6) and oleic acid (18:1n-9) indicating that percent contributions of these FAs were greater in early-laid eggs.

On PC2 (Table 2.6), nine FAs had high positive loadings. In general, these FAs contributed greater percentages to total FA levels in early-laid eggs than in late-laid eggs. Of particular interest were greater positive loadings of the

n-3 and n-6 FAs: DHA (22:6n-3), DPA (22:5n-3), EPA (20:5n-3) and ARA (20:4n-6).

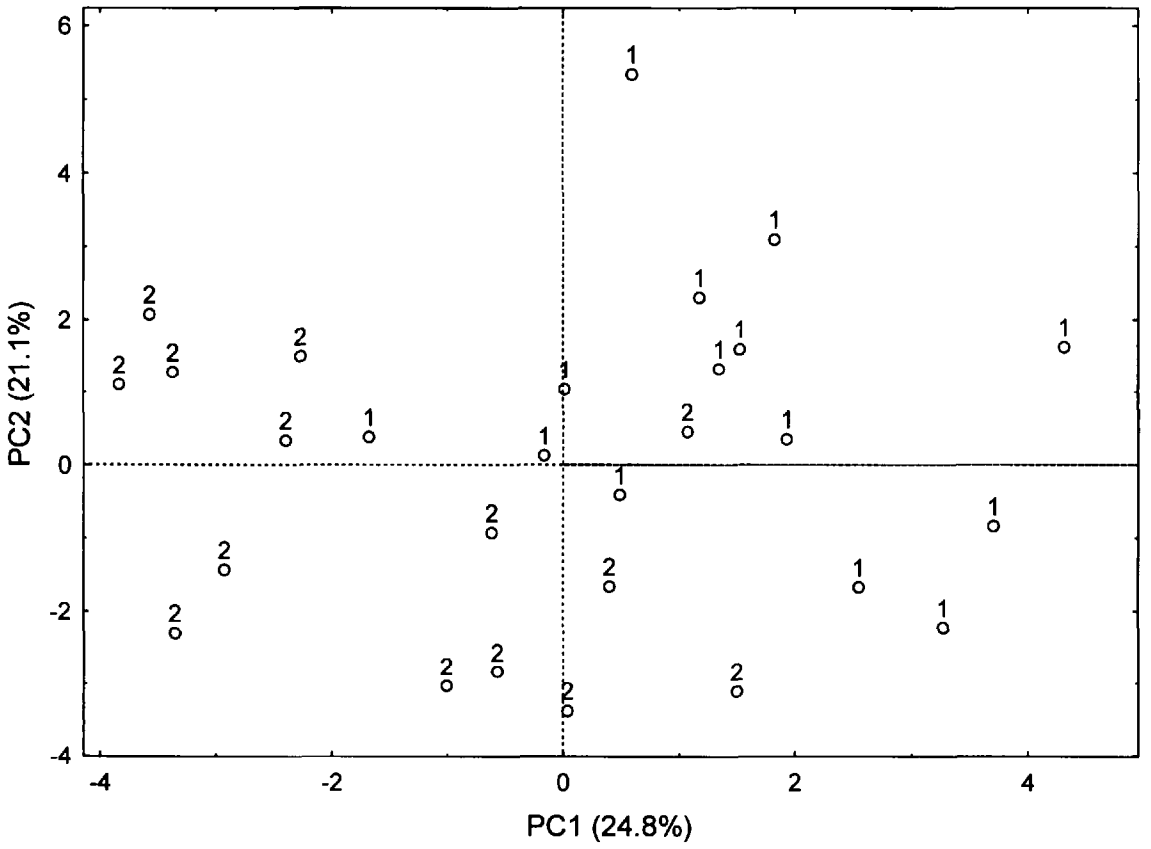

Figure 2.6. Plot of principal component scores summarizing FA composition in early (1) and late-laid (2) eggs of Common Eiders from Tern Island. Principal component 1 (PC1) and PC2 explained 46% of the variance in the data. FAs with the greatest loadings on PC1-2 are shown in Table 2.6.

Table 2.6. FA loadings on PC1-2 for early and late-laid Common Eider eggs. Significant differences between early and late-laid eggs in PC scores were detected for PC1 and PC2. FAs with greater loadings are highlighted by (*).

FA Common Name	FA Structure	PC1	PC2
Myristic acid	14:0	-0.47	0.46
Pentadecanoic acid	15:0	-0.64*	0.23
Palmitic acid	16:0	-0.67*	-0.08
Heptadecanoic acid	17:0	-0.83*	0.21
Stearic acid	18:0	0.25	0.57*
Behenic acid	22:0	0.06	0.68*
Myristoleic acid	14:1n-5	-0.30	0.19
Palmitoleic acid	16:1n-7	-0.39	-0.31
Elaidic acid	18:1n-9t	-0.01	0.72*
Oleic acid	18:1n-9	0.70*	-0.29
Eicosenoic acid	20:1n-9	-0.04	0.58*
Nervonic acid	24:1n-9	0.49	0.06
Eicosadienoic acid	20:2n-6	-0.56*	0.55*
Eicosatrienoic acid	20:3n-6	0.57*	0.61*
Linoleic acid	18:2n-6	-0.68*	-0.10
γ-Linolenic acid	18:3n-6	0.29	0.11
Arachidonic acid	20:4n-6	-0.14	0.67*
α-Linolenic acid	18:3n-3	-0.52*	0.04
Eicosapentaenoic acid	20:5n-3	0.59*	0.64*
Docosapentaenoic acid	22:5n-3	0.03	0.83*
Docosahexaenoic acid	22:6n-3	-0.75*	-0.02

Long-tailed Duck PCA

For the Long-tailed Duck eggs, the SAFA, MUFA, n-3 and n-6 FAs were used for the PCA and only scores on PC1 showed a significant difference between early and late-laid eggs ($KW\text{-}H_{1,3}=4.03$, $p=0.04$; Fig. 2.7). PC1 accounted for 65.5% of the total variation in the Long-tailed Duck FA data.

Along PC1, the SAFA, MUFA and n-6 FAs loaded strongly (Table 2.7). The SAFA and the n-6 FAs loaded negatively indicating that they were lower in the early-laid eggs. The MUFA loaded positively indicating that they were higher in the early-laid eggs.

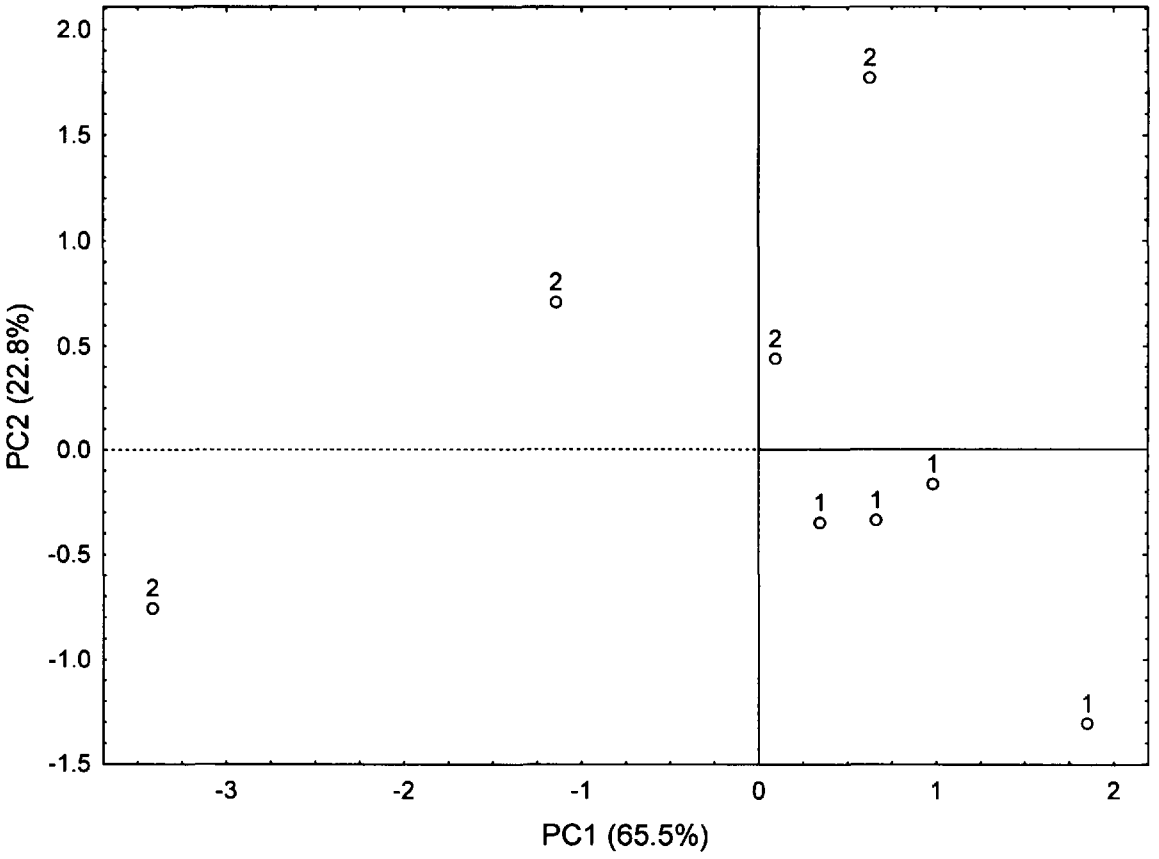

Figure 2.7. Plot of principal component scores summarizing FA composition (SAFA, MUFA, n-3 and n-6) in early (1) and late-laid (2) eggs of Long-tailed Ducks from Tern Island. Principal component 1 (PC1) and PC2 explained 98.3% of the variance in the data. FAs with the greatest loadings on PC1-2 in Table 2.7.

Table 2.7. FA loadings on PC1-2 for early and late-laid Long-tailed Duck eggs. Significant differences between early and late-laid eggs in PC scores were detected for PC1. FAs with greater loadings are highlighted by (*).

Fatty Acid	PC1*	PC2
SAFA	-0.83*	-0.05
MUFA	0.99*	0.07
n-3	-0.43	0.89
n-6	-0.86*	-0.32

Discussion

Stable Nitrogen and Carbon Isotopes

Significant differences were observed in $\delta^{15}N$ values between early and late-laid Arctic Tern eggs. Late-laid eggs had lower $\delta^{15}N$ values. These results suggest that Arctic Terns may have been feeding on different prey during the breeding period and/or that they were utilizing both exogenous and endogenous resources for egg synthesis. In captive feeding trials using Japanese Quail, a change in diet was reflected in a shift in $\delta^{13}C$ values in albumen after 3 to 5 days and after 8 days in yolk (Hobson 1995). Arctic Terns typically lay two eggs per clutch with eggs laid at 1-4 day intervals (Hatch 2002), so it is possible that differences in $\delta^{15}N$ values between early and late-laid eggs reflected shifts in diet. With an interval of 3-4 days between egg laying, there would be sufficient time for changes in exogenous resources with differing $\delta^{15}N$ values to affect egg $\delta^{15}N$ values. It is less likely that $\delta^{15}N$ differences between early and late-laid eggs reflected different proportions of exogenous versus endogenous resources being used for egg synthesis given that Hobson et al. (2000) estimated that subarctic nesting Arctic Terns relied almost exclusively on exogenous resources (86%) for egg production. Furthermore, $\delta^{15}N$ values were lower in late-laid eggs which is opposite to the expected pattern if greater endogenous reserves were used in late-laid eggs (see below for Common Eiders). It is possible that if foraging opportunities were more limited during the formation of early-laid eggs,

perhaps as a result of sea-ice cover, that more endogenous reserves would have been used for the formation of early-laid eggs resulting in greater $\delta^{15}N$ values in early-laid eggs. However, based upon Hobson et al.'s (2000) results, endogenous reserves do not appear to be important for egg formation.

Long-tailed Ducks exhibited no statistically significant differences in $\delta^{15}N$ values between early and late-laid eggs. This contrasted with Common Eiders which, like Arctic Terns, showed significant differences in $\delta^{15}N$ values between early and late-laid eggs. Unlike Arctic Terns; however, late-laid Common Eider eggs had greater $\delta^{15}N$ values than early-laid eggs. This same pattern of increasing $\delta^{15}N$ values in late-laid eggs was observed in subarctic nesting Common Eiders (Sénéchal et al. 2011) and was attributed to the possible incorporation of more endogenous resources into late-laid eggs. Greater $\delta^{15}N$ values in late-laid eggs were consistent with the pattern observed in fasting birds. Hobson et al. (1993) found that birds fasting during incubation showed a significant increase in their tissue $\delta^{15}N$ values. Common Eiders are known to fast during incubation (Goudie et al. 2000; Criscuolo et al. 2002), hence, $\delta^{15}N$ differences between early and late-laid eggs may have reflected changes in the relative use of endogenous versus exogenous resources for egg synthesis. However, Sénéchal et al. (2011) found that Common Eiders primarily relied on exogenous protein resources for egg production. Given that Common Eiders lay up to 5 eggs per clutch (Goudie et al. 2000; Hanssen et al. 2002) over a 5-day

period (Watson et al. 1993; Goudie et al. 2000) it is then possible that $\delta^{15}N$ differences could have reflected dietary change because the time required for clutch completion is consistent with isotopic turnover rates as measured in the laboratory feeding studies in Japanese Quail eggs (Hobson 1995). Clearly, more research is required to conclusively determine what factors are most important in regulating differences in $\delta^{15}N$ values between early and late-laid eggs. In general, although statistically significant differences in egg $\delta^{15}N$ values between early and late-laid eggs were detectable in Arctic Terns and Common Eiders, these differences were small, i.e. 0.3-0.4‰, relative to the magnitude of differences associated with trophic enrichment of ^{15}N, i.e. $\delta^{15}N$ increase of 3 to 5‰ at each successive trophic level.

No significant differences in $\delta^{13}C$ values were detected between early-laid and late-laid eggs for any of the three bird species. In aquatic ecology, carbon isotopes are typically used to deduce the origin of food resources, i.e., pelagic vs. offshore, freshwater vs. saltwater (France and Peters 1997; Michener and Kaufman 2007). The lack of difference in $\delta^{13}C$ values between early and late-laid eggs suggests that all three bird species continued to use their same general foraging areas during the laying period. Based upon previous studies, Common Eiders are primarily nearshore benthic feeders (Goudie et al. 2000) whereas Arctic Terns are pelagic feeders that forage for prey at the water surface (Hatch 2002). Long-tailed Ducks are thought to have a more varied diet than Common

Eiders or Arctic Terns. This was reflected in the somewhat larger coefficient of variation for $\delta^{13}C$ values in early (CV = 3.8) and, particularly, late-laid eggs (CV = 6.0) of Long-tailed Ducks than in Arctic Terns or Common Eiders (Table 2.1). Long-tailed Ducks are known to feed in both freshwater and saltwater environments by diving for prey as well as by feeding at the water surface (Robertson and Savard 2002). The potential for greater variability in the Long-tailed Duck diet might have been expected to lead to differences in early and late-laid egg $\delta^{13}C$ values if they fed in different zones during the breeding period. However, the lack of statistically significant differences suggests that they also continued to feed in a similar area throughout the egg formation/laying period. However, small sample sizes for Long-tailed Ducks likely contributed significantly to the lack of statistically significant differences in carbon and nitrogen isotope values between early and late-laid eggs.

Fatty Acids

Principal components analysis of percent FA composition data for all three species indicated that the majority of the variance in the FA data was the result of inter-specific differences. Although detectable, differences between early and late-laid eggs within species were relatively small for all three marine bird species. For example, for Arctic Terns the mean percent difference between the percent composition of early and late-laid eggs across all 22 FAs was 10.42%

(range 0-33%, Table 2.2), the Common Eider FA mean percent difference across 21 FAs was 11.25% (range 0-33%, Table 2.3). The Long-tailed Duck FA mean percent difference across 22 FAs was 20.03% (range 0-72%, Table 2.4), the greater range is likely due to the small sample size (n=4). This suggests that the FA composition of eggs may be regulated to some degree perhaps to ensure adequate supplies to the developing embryo (see Royle et al. 1999). However, individual species PCA results highlighted changes in egg FA composition between early and late-laid eggs.

For Arctic Terns, three FAs (DPA, DHA and eicosenoic acid) were of particular interest in elucidating possible shifts in diet composition that could have contributed to the changes in egg chemical composition. Two of these FAs were the n-3 FAs, docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). These FAs contributed more to total FA levels in early-laid eggs than late-laid eggs. DPA is typically an intermediate FA between eicosapentaenoic acid (EPA, 20:5n-3) and DHA (Kaur et al. 2011) and all three n-3 FAs are typically found in high amounts in fish (Whelan and Rust 2006), especially DHA (Surai et al. 2001). Vertebrates are not capable of synthesizing n-3 FAs since they lack the enzymes needed to produce them so their n-3 stores have to come from their diet (Neuringer et al. 1988; Surai et al. 2001; Williams and Buck 2010). Lower percent contributions of DPA and DHA to total FA levels in late-laid eggs suggest that female Arctic Terns may have fed less on fish

during the formation of late-laid eggs. This interpretation was consistent with the decline in egg $\delta^{15}N$ values in late-laid eggs if female Arctic Terns were shifting to a lower trophic level food resource. The other FA of particular interest was eicosenoic acid (20:1n-9). Eicosenoic acid is typically found in high amounts in Arctic calanoid species (Scott et al. 2002; Kattner et al. 2007) and eicosenoic acid has frequently been used as an FA biomarker for calanoid consumption, either directly, or indirectly through the consumption of organisms feeding on calanoids (Wesławski et al. 1994; Dahl et al. 2003; Käkelä et al. 2007; Kattner and Hagen 2009; Loseto et al. 2009; Wilson et al. 2010) like amphipods (Graeve et al. 1997; Auel 2002; Wold et al. 2011). At Tern Island, I frequently observed Arctic Terns feeding offshore near the island. The Arctic Terns were diving along the ocean surface for extended periods of time, possibly feeding on invertebrates such as calanoid species or organisms that were feeding on calanoids. It is possible that these invertebrate food resources became more important as the period of egg formation progressed resulting in greater percent contributions of eicosenoic acid in late-laid eggs as well as lower egg $\delta^{15}N$ values.

The Common Eider FA PCA indicated that there were FA differences between the early and late-laid eggs and of particular interest were the four PUFAs: DHA (22:6n-3), DPA (22:5n-3), ARA (20:4n-6), and EPA (20:5n-3). Three are considered essential FAs (DHA, ARA and EPA; Speake and Deans 2004; Ahlgren et al. 2009; Gladyshev et al. 2009) since most vertebrates are not

capable of synthesizing the n-3 and n-6 PUFAs so they have to be acquired through the diet (Neuringer et al. 1988; Williams and Buck 2010). DPA (22:5n-3) typically is a precursor to EPA and DHA (Kaur et al. 2011) and all three n-3 FAs (EPA, DPA and DHA) are typically found in high amounts in fish (Whelan and Rust 2006) although they are sometimes broadly used as a marine biomarker (Neuringer et al. 1988; Hanson et al. 2010; Larsen et al. 2011). ARA has been found to increase in marine food webs (Kainz et al. 2004) and has been found in higher amounts in higher trophic level consumers (i.e., fish) (Kuusipalo and Käkelä 2000; Koussoroplis et al. 2011). Common Eiders are primarily known as molluscivores (Goudie et al. 2000) but they have been known to consume fish as well (Wesławski et al. 1994). The higher amount of ARA, EPA and DPA in the early-laid eggs may indicate that the Common Eiders were consuming fish during the formation of the early-laid egg but DHA, which is also rich in fishes (Whelan and Rust 2006; Rubio-Rodríguez et al. 2010) was also higher in the late-laid eggs. Furthermore, the Common Eider late-laid egg had higher $\delta^{15}N$ values, which is typically associated with consumption of higher trophic level prey like fish (Hobson et al. 1994; Thompson et al. 1999). Higher late-laid egg $\delta^{15}N$ values in Yellow-legged Gulls were attributed to potential utilization of endogenous resources (Ramírez et al. 2011). As well, the tissues of fasting birds have been shown to have increased $\delta^{15}N$ values (Hobson et al. 1993). Common Eiders are known to fast during incubation (Goudie et al. 2000). Obviously, the

factors regulating SI and FA differences in early versus late-laid Common Eider eggs are not clear but shifts in diet along with alterations in the use of endogenous and/or exogenous resources for egg formation may be acting together to influence egg compositional differences.

The Long-tailed Duck FA PCA also revealed differences between the early and late-laid eggs. The SAFA and n-6 FAs contributed more to the late-laid Long-tailed Duck eggs whereas the MUFAs contributed more to the early-laid eggs. Lab experiments on fish showed that MUFAs and PUFAs (n-3 and n-6 FAs) were largely linked with diet, although the relationship was stronger for the PUFAs (Elsdon 2010). Long-tailed Ducks have been known to lay up to 14 eggs per clutch and are known to consume a diverse array of prey at both terrestrial and marine ecosystems (Robertson and Savard 2002) and they are thought to rely primarily on exogenous resources for egg production (Kellett et al. 2005), so the FA differences could potentially reflect diet shifts during the breeding period. The SAFAs were higher in the late-laid egg but most SAFAs can be produced endogenously (Arts et al. 2001; Dalsgaard and St. John 2004; Käkelä et al. 2007; Iverson 2009) or are precursors to other FAs (Dalsgaard and St. John 2004), thus speculating on possible dietary sources of SAFAs is not possible without further study. Specific MUFAs like eicosenoic acid (20:1n-9) have been linked to pelagic food sources (Wold et al. 2011), which may indicate that the Long-tailed Ducks were feeding in the pelagic zone during the early breeding period.

However, greater proportions of MUFA in early-laid eggs also represent five other FAs that have not been as well established as eicosenoic acid as a biomarker, thus making inferences about feeding patterns difficult. The n-6 egg differences are more promising in pointing to diet shifts since n-6 FAs typically have to be obtained through the diet since most vertebrates lack the enzymes needed to produce them (Williams and Buck 2010). Arachidonic acid (20:4n-6) is considered an essential n-6 FA (Ahlgren et al. 2009; Gladyshev et al. 2009) and it has been found in higher levels in higher trophic level consumers (Kuusipalo and Käkelä 2000; Koussoroplis et al. 2011), so the higher n-6 FA in the late-laid egg could potentially indicate that the Long-tailed Ducks were consuming higher trophic level prey. However, further research is needed to conclusively determine if Long-tailed Ducks are indeed switching to higher trophic level prey during the breeding period given the small sample size that was available for this study.

The stable isotope and fatty acid results suggest that diet shifts may occur during the breeding season but it is still unclear as to what proportions of endogenous and/or exogenous resources are utilized for egg production. Nonetheless, all three marine birds appear to be capable of consuming different prey during the breeding period. Such plasticity may be beneficial in the short term when unpredictable sea-ice cover may make access to specific prey difficult. Potential long term changes in the Arctic such as increased shipping,

increasing levels of contaminants, increased offshore oil and gas interests, increased fishing (Huntington 2009) and sea-ice reduction (Polyak et al. 2010) could further affect food resources for Arctic-nesting birds potentially affecting their populations. Marine bird reproduction has already been shown to be affected by sea-ice, or lack thereof, in the Arctic (Gaston et al. 2005) but how these factors will work together to affect marine birds is difficult to predict. Nonetheless, the early and late-laid egg SI and FA data gathered in this study is the first biochemical information obtained at this important High Arctic marine bird breeding colony and thus could be useful for tracking potential changes in the Arctic marine ecosystem.

CHAPTER THREE

Inter-specific Examination of Fatty Acid and Stable Isotope Profiles in Eggs of Three Sympatrically Nesting Marine Birds Breeding on a High Arctic Island

Introduction

Inter-specific differences in the feeding patterns of Arctic Terns, Common Eiders and Long-tailed Ducks have been documented, both through direct, e.g. observational, gut contents (Goudie et al. 2000; Bustnes and Systad 2001; Hatch 2002; Robertson and Savard 2002) and indirect methods, e.g. stable isotopes (SI), fatty acids (FA) (Hobson 1993; Thompson et al. 1999; Hobson et al. 2000; Dahl et al. 2003). Stable isotopes of nitrogen and carbon (Hobson et al. 1994; Hebert et al. 1999; Thompson et al. 1999; Forero and Hobson 2003; Inger and Bearhop 2008) and fatty acids (Käkelä et al. 2007; Karnovsky et al. 2008; Price 2010; Wold et al. 2011) have been used to show inter-specific differences amongst various marine birds by measuring these dietary tracers in various marine bird tissues (Williams and Buck 2010), including eggs (Surai et al. 2001; Hebert et al. 2006, 2009; Oppel et al. 2010).

In marine bird ecology, stable nitrogen isotopes (^{15}N and ^{14}N) are used to infer trophic relationships since the tissues of marine birds that consume higher trophic level organisms are typically more enriched in the heavier ^{15}N-isotope than tissues of marine birds that consume lower trophic level prey (Hobson et al. 1994; Thompson et al. 1999). Carbon isotopes (^{13}C and ^{12}C) are primarily used to identify potential food sources (France and Peters 1997; Michener and Kaufman 2007). Benthic prey typically are enriched in the heavier ^{13}C-isotope than organisms in offshore or freshwater ecosystems (Hobson et al. 1994;

France 1995). Hence, predators feeding in offshore, pelagic locations generally have more negative $\delta^{13}C$ values than predators feeding on benthic prey.

Fatty acid analysis is a relatively new method for determining the feeding ecology of marine organisms (Barrett et al. 2007). Following consumption, prey FA signatures remain relatively intact in consumer tissues (Iverson et al. 2004), hence, by examining FA profiles in predator tissues it is possible to infer the composition of their diets (Iverson et al. 2004; Pierce and McWilliams 2005; Williams and Buck 2010).

The objective of this study was to investigate possible differences in the stable isotope and fatty acid profiles of three sympatrically nesting High Arctic marine birds: Arctic Terns, Common Eiders and Long-tailed Ducks. Stable isotopes and fatty acids have been used to show inter-specific differences (Dahl et al. 2003; Hobson 1993; Wold et al. 2011) amongst various marine bird species but few studies have utilized them together. Previously, Arctic-breeding Common Eiders have been shown to have lower $\delta^{15}N$ values in their tissues compared to marine birds that feed on higher trophic level prey (Hobson 1993; Akearok et al. 2010). Furthermore, marine birds, like Common Eiders, that feed in inshore benthic zones tend to have less negative $\delta^{13}C$ values than marine birds that feed in offshore pelagic areas (Hobson et al. 1994; Dahl et al. 2003). In contrast, Arctic Terns are known piscivores (Hatch 2002) and marine birds that feed on higher trophic level prey like fish tend to have greater $\delta^{15}N$ values in their

tissues (Hobson 1993; Hebert et al. 1999; Thompson et al. 1999). Also, organisms that typically feed in offshore pelagic areas, like Arctic Terns, tend to have more negative $\delta^{13}C$ values relative to inshore benthic feeders (Dahl et al. 2003). Long-tailed Ducks are capable of exploiting a wide variety of food resources possibly overlapping with the diets of Arctic Terns and Common Eiders. Braune et al. (2005) found highly regional differences in both nitrogen and carbon isotopes in Long-tailed Duck tissues.

Fatty acids have also been used to distinguish marine birds that feed on pelagic versus benthic prey (Dahl et al. 2003). Two pelagic marine birds (Black-legged Kittiwake *Rissa tridactyla* and Northern Fulmar *Fulmarus glacialis*) were separated from a benthic feeding species (Common Eider) based upon the FA composition of their tissues. Of particular note were the higher levels of eicosenoic acid (20:1n-9) in the pelagic feeders (Dahl et al. 2003). Arctic Terns are also pelagic feeders as well as known piscivores (Hatch 2002) and fish tend to contain high levels of n-3 FAs (Whelan and Rust 2006; Huynh and Kitts 2009; Řezanka and Sigler 2009). Long-tailed Ducks feed in both freshwater and saltwater environments and can dive for their food or capture prey at the water's surface (Robertson and Savard 2002) so their diets could overlap with those of Common Eiders and Arctic Terns.

Given that Arctic Terns are known piscivores that typically feed in offshore pelagic areas (Hatch 2002), I expect that their egg nitrogen isotopes will reflect

consumption of higher trophic level prey through greater $\delta^{15}N$ values than the Common Eider and Long-tailed Duck eggs. I also expect that since Arctic Terns typically feed in offshore pelagic areas, their $\delta^{13}C$ values will be more negative relative to the Common Eiders and Long-tailed Ducks. Common Eiders are considered to be primarily molluscivores (Goudie et al. 2000), I therefore expect that their egg nitrogen isotope values will reflect feeding on lower trophic level prey through lower egg $\delta^{15}N$ values. I also expect that Common Eider eggs will show less negative $\delta^{13}C$ values in their eggs since ^{13}C is typically enriched in the tissues of organisms that feed in inshore benthic areas (Hobson et al. 1994; France 1995). Long-tailed Ducks feed on a wide variety of prey in more diverse ecosystems (Robertson and Savard 2002), I therefore expect that both their egg nitrogen and carbon isotopes will show greatest variability amongst the three marine bird species but that those values will be intermediate between those in the Arctic Tern and Common Eider eggs.

Since FAs have been used to demonstrate inter-specific differences in feeding patterns of marine birds (Dahl et al. 2003; Wold et al. 2011), I expect that given the different feeding strategies among Arctic Terns (Hatch 2002), Common Eiders (Goudie et al. 2000), and Long-tailed Ducks (Robertson and Savard 2002) that it will be possible to distinguish these species based upon egg FA patterns. This will depend on whether FA profiles in eggs remain stable after collection. In remote field sites, such as Tern Island, it is not possible to store samples at

cryogenic temperatures, i.e. -85^{0}C, or immediately transport samples to locations where such facilities exist. Hence, a concern with the collection of tissue samples in remote environments for FA research is the possible degradation of FAs, particularly through oxidation. If that occurs, then the usefulness of such samples for determination of inter-specific differences in FA profiles will be compromised and FA profiles in eggs would be expected to show somewhat random patterns with little in the way of consistent inter-specific patterns.

However, if egg FA patterns are consistent within and across species, then this will indicate that such samples are useful for examining inter-specific differences in the diets of different species. In this case, I expect that Arctic Tern egg FA profiles should reflect that they are primarily fish consumers and since fish typically contain high levels of n-3 FAs (Whelan and Rust 2006; Rubio-Rodríguez et al. 2010) this should result in higher proportions of n-3 FAs in their eggs compared to Common Eider and Long-tailed Duck eggs. In addition, based on the research by Dahl et al. (2003), I expect that Arctic Tern eggs may contain greater proportions of FA associated with pelagic food webs, i.e. eicosenoic acid (20:1n-9), than do either of the other two species. FA profiles in Common Eider eggs should have lower proportions of this FA based upon the preponderance of benthic food in their diets. I expect that Long-tailed Duck egg FA profiles may show greater variability than both Arctic Tern and Common Eider profiles but that

Long-tailed Duck profiles will fall between those of the other two marine bird species.

Canadian Arctic marine ecosystems are currently undergoing rapid, anthropogenically caused changes (Huntington 2009; Polyak 2010). These changes could affect the structure of Arctic marine food webs thereby altering the diets of Arctic marine nesting birds. Gaston et al. (2003) showed that Thick-billed Murres nesting in a subarctic region are increasingly consuming fish species that, in the past, were typically found in more southerly locations. Such redistributions of prey may be related to climate change. My study will generate the first stable isotope and fatty acid information for these three marine bird species at Tern Island, an important breeding colony. The SI and FA data will provide a baseline against which future patterns can be compared thus providing the means to track marine food web change.

Methods

Egg collection, storage and transport, tissue preparation, homogenization, freeze drying, lipid extraction, FA methylation, and FA analysis are identical to what is described in Chapter 2. Similarly, methods used for stable nitrogen and carbon isotope preparation and analysis are the same as those outlined in Chapter 2.

Statistical Analysis

Prior to statistical analyses, mean SI and %FA values were calculated for each species by averaging SI and FA data for early and late-laid eggs. These mean values were used to examine inter-specific differences in SI and FA composition. Statistical analyses were conducted using STATISTICA 7 (StatSoft 2005). For the egg stable isotope data, I used a Kruskal-Wallis (KW) ANOVA to test for inter-specific differences in mean egg $\delta^{15}N$ and $\delta^{13}C$ values among Arctic Terns (n=17), Common Eiders (n=14) and Long-tailed Ducks (n=4), followed by a multiple comparison test (MCT) (Siegel and Castellan 1988).

Principal components analysis (PCA) was used to explore inter-specific differences in egg FA patterns, i.e. percent contribution of individual FA to total FA levels. KW ANOVAs were then conducted to investigate possible inter-specific differences in principal component scores on axes 1-2.

To test whether the composition of FAs in eggs was related to egg $\delta^{15}N$ and $\delta^{13}C$ values, I used Pearson correlation analyses. FAs that exhibited high loadings along PC axes 1-2 were used in the correlation analyses as well as a Pearson correlation analysis between n-3, n-6, and the ratio of n-3/n-6 FAs to egg $\delta^{15}N$ and $\delta^{13}C$ values. A correlation analysis was also carried out between $\delta^{15}N$ and $\delta^{13}C$ values. Sequential Bonferroni correction was then applied to adjust p-values to account for multiple statistical tests (n=21).

Results

Inter-specific Differences in Egg Stable Isotope Values

$\delta^{15}N$

$\delta^{15}N$ egg values showed that the Arctic Tern eggs had the highest mean values, followed by the Common Eider and the Long-tailed Duck eggs (Table 3.1). The Arctic Tern eggs also had the highest standard deviation and coefficient of variation (CV) (Table 3.1).

Mean $\delta^{15}N$ values in eggs differed significantly among the species (Table 3.1; Fig. 3.1) (KW-$H_{2\text{-}34}$=21.16; $p<0.0001$) and a multiple comparison test (MCT) showed that Arctic Tern eggs had significantly greater $\delta^{15}N$ values than Common Eider (MCT; p=0.001; Fig. 3.1) or Long-tailed Duck eggs (MCT; p=0.0004). There was no significant difference between the $\delta^{15}N$ values in Common Eider and Long-tailed Duck eggs (MCT; p=0.42; Fig. 3.1).

$\delta^{13}C$

The Common Eider eggs had less negative $\delta^{13}C$ values than the Arctic Tern and Long-tailed Duck eggs (Table 3.1). The Long-tailed Ducks had the greatest coefficient of variation, followed by the Arctic Tern and Common Eider eggs (Table 3.1).

There were significant inter-specific differences in egg $\delta^{13}C$ values (KW-$H_{2,34}$=13.45; p=0.001) (Table 3.1, Fig. 3.2). Common Eider eggs had less

negative $\delta^{13}C$ values than Arctic Tern (MCT; p=0.002; Fig. 3.2) or Long-tailed Duck eggs (MCT; p=0.04). No significant difference was detected between Arctic Tern and Long-tailed Duck eggs (MCT, p=1.00).

Table 3.1. Mean $\delta^{15}N$ values (‰) and $\delta^{13}C$ values (‰) in eggs of Arctic Terns (ARTE), Common Eiders (COEI) and Long-tailed Ducks (LTDU) at Tern Island.

Species	$\delta^{15}N$ (‰)			$\delta^{13}C$ (‰)		
	Mean	Std Dev	Coefficient of Variation	Mean	Std Dev	Coefficient of Variation
ARTE	15.47	0.84	5.43	-17.44	0.55	3.15
COEI	14.11	0.45	3.19	-16.67	0.35	2.10
LTDU	13.51	0.26	1.92	-17.57	0.74	4.21

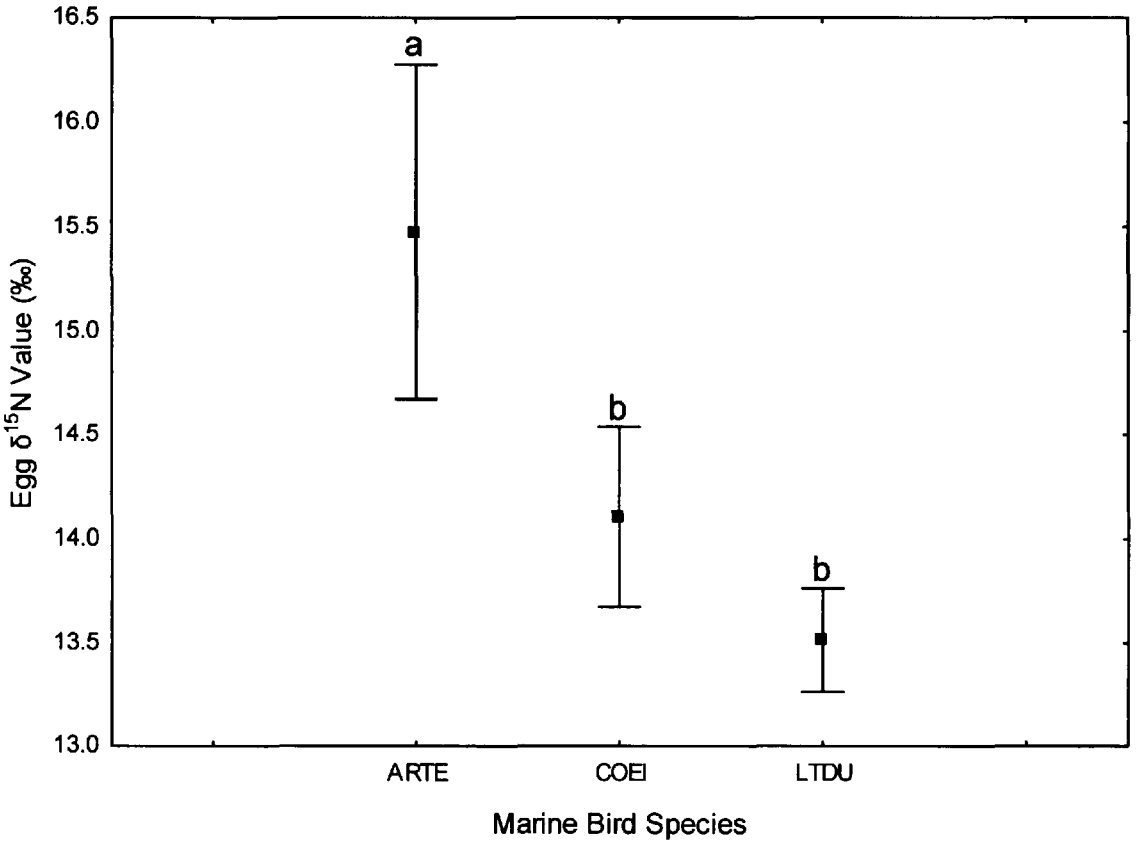

Figure 3.1. Mean (± 1 std dev) $\delta^{15}N$ values (‰) in eggs from three marine bird species nesting at Tern Island (ARTE – Arctic Tern; COEI – Common Eider; LTDU – Long-tailed Duck). Means with different letters are statistically different ($p<0.05$).

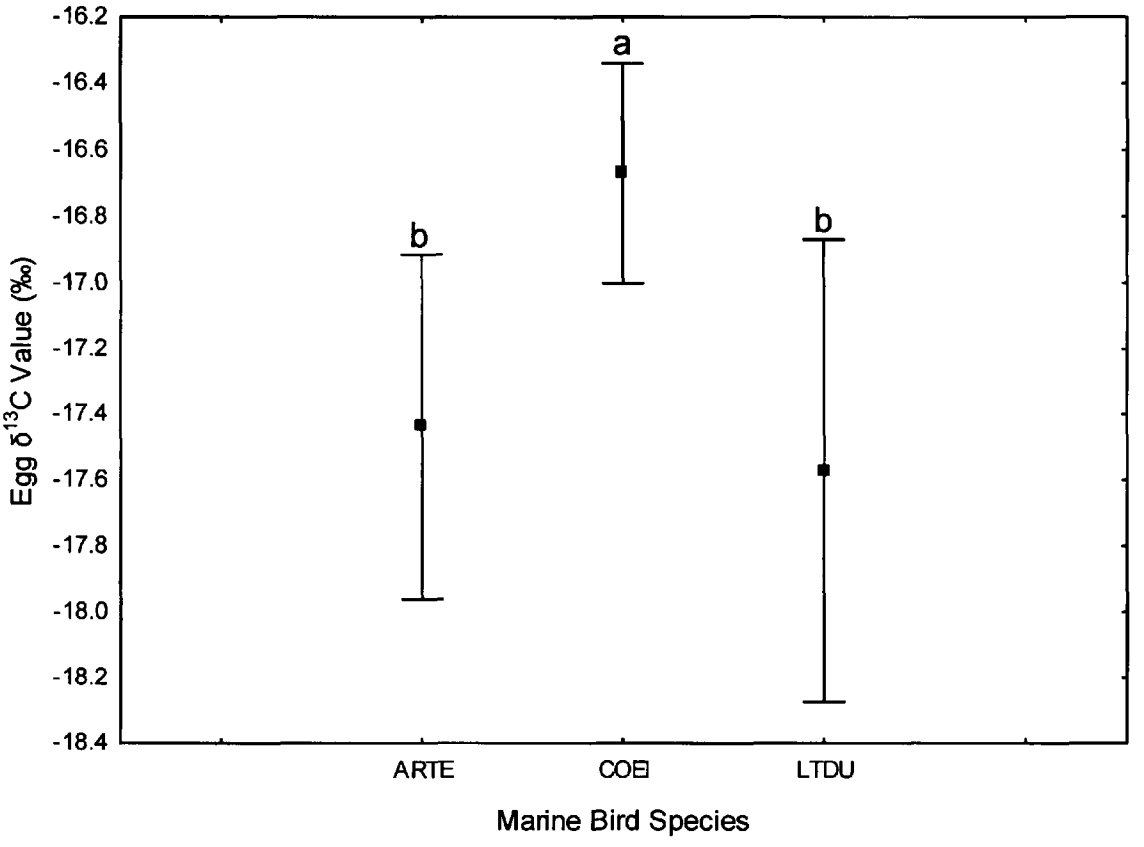

Figure 3.2. Mean (± 1 std dev) $\delta^{13}C$ values (‰) in eggs from three marine bird species nesting at Tern Island (ARTE – Arctic Tern; COEI – Common Eider; LTDU – Long-tailed Duck). Means with different letters are statistically different ($p<0.05$).

Inter-specific Differences in Egg FA Profiles

Arctic Tern Fatty Acid Composition

The mean FA profile of Arctic Tern eggs contained over a third of SAFAs (33.9%), with palmitic acid (16:0) comprising 24.2% of the total SAFAs, followed by stearic acid (18:0, 8.3%; Table 3.2). The MUFAs accounted for 51.7% of the total FAs with oleic acid (18:1n-9) being found in the greatest proportions (39.8%), followed by palmitoleic acid (8.7%). The PUFAs comprised 14.4% of the total FAs with docosahexaenoic acid (22:6n-3) accounting for 5.5% of the total PUFAs, followed by eicosapentaenoic acid (20:5n-3, 2.9%). The n-3 and n-6 PUFAs totalled 9.9% and 4.6% of the total FAs, respectively.

Common Eider Fatty Acid Composition

For Common Eider eggs, total FAs were comprised of over a third of SAFAs (35.4%; Table 3.2), with palmitic acid constituting the most (27.1%). The MUFAs contributed over half of the total FAs (53.8%) with oleic acid being most important (39.7%). The PUFAs comprised 10.8% of total FAs with DHA accounting for 3.7% followed by eicosapentaenoic acid (EPA, 20:5n-3) at 2.7%. The n-3 and n-6 FAs comprised 7.7% with 3.1%, respectively.

Long-tailed Duck Fatty Acid Composition

Like the Arctic Tern and Common Eider eggs, Long-tailed Duck SAFAs comprised nearly a third of the total FAs (32.1%) with palmitic acid being found in the greatest proportions (24.2%) (Table 3.2) followed by stearic acid (5.9%). The MUFAs totalled over half the total FAs (55.3%) with oleic acid being most important (35.0%). The PUFAs constituted 12.5% of total FAs with DHA and EPA being most important at 4.0% and 3.0%, respectively. The n-3 FAs made up 8.8% and the n-6 FAs comprised 3.7% of total FAs.

Table 3.2. Mean (± 1 std dev) percent composition data for fatty acids in eggs of three marine bird species from Tern Island (ARTE = Arctic Tern, COEI = Common Eider, LTDU = Long-tailed Duck).

FA Common Name	FA Structure	ARTE		COEI		LTDU	
		Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
Myristic acid	14:0	0.85	0.10	1.10	0.11	1.24	0.21
Pentadecanoic acid	15:0	0.16	0.08	0.16	0.07	0.27	0.09
Palmitic acid	16:0	24.17	0.78	27.12	0.48	24.24	1.25
Heptadecanoic acid	17:0	0.32	0.06	0.46	0.07	0.43	0.20
Stearic acid	18:0	8.34	0.55	6.51	0.33	5.94	1.20
Behenic acid	22:0	0.11	0.04	0.03	0.01	0.03	0.01
Myristoleic acid	14:1n-5	0.09	0.03	0.10	0.02	0.13	0.02
Palmitoleic acid	16:1n-7	8.68	1.72	13.26	0.96	18.44	5.57
Elaidic acid	18:1n-9t	0.27	0.07	0.24	0.04	0.21	0.04
Oleic acid	18:1n-9	39.76	2.52	39.65	1.54	34.98	3.74
Eicosenoic acid	20:1n-9	2.78	0.71	0.55	0.08	1.47	0.56
Erucic acid	22:1n-9	0.04	0.03	0.00	0.00	0.06	0.03
Nervonic acid	24:1n-9	0.03	0.02	0.02	0.01	0.06	0.02
Eicosadienoic acid	20:2n-6	0.08	0.01	0.08	0.02	0.06	0.02
Eicosatrienoic acid	20:3n-6	0.10	0.03	0.08	0.01	0.08	0.02
Linoleic acid	18:2n-6	1.07	0.11	1.00	0.17	2.06	1.84
γ-Linolenic acid	18:3n-6	0.05	0.02	0.06	0.01	0.09	0.01
Arachidonic acid	20:4n-6	3.26	0.93	1.85	0.22	1.39	0.52
α-Linolenic acid	18:3n-3	0.15	0.03	0.34	0.07	0.64	0.47
Eicosapentaenoic acid	20:5n-3	2.90	0.38	2.69	0.42	3.00	0.88
Docosapentaenoic acid	22:5n-3	1.27	0.18	0.97	0.14	1.20	0.22
Docosahexaenoic acid	22:6n-3	5.52	0.75	3.72	0.30	4.00	0.48
Saturated FAs	SAFA	33.94	0.92	35.38	0.70	32.14	1.30
Mono-unsaturated FAs	MUFA	51.65	1.60	53.82	1.31	55.34	2.29
Poly-unsaturated FAs	PUFA	14.41	1.44	10.79	0.75	12.51	1.96
Omega-3 FAs	n-3	9.85	0.92	7.72	0.65	8.83	0.54
Omega-6 FAs	n-6	4.56	0.82	3.07	0.37	3.68	2.36
Omega-3/Omega 6 FAs	n-3/n-6	2.21	0.36	2.55	0.39	3.02	1.31

Principal Components Analysis (PCA)

A PCA of percent FA data in eggs showed a clear separation among Arctic Terns, Common Eiders and Long-tailed Ducks (Fig. 3.3). PC1 explained 34.0% of the variation in the egg FA data and Arctic Tern eggs were clearly separated from both Common Eider and Long-tailed Duck eggs. PC2 explained 18.3% of the variation in the FA data and Common Eider eggs were separated to some degree from Long-tailed Duck eggs. A KW-ANOVA revealed significant inter-specific differences in sample scores for both PC1 ($KW\text{-}H_{2,34}=26.49$; $p<0.0001$) and PC2 ($KW\text{-}H_{2,34}=13.99$; $p=0.0009$) but not for PC3 ($KW\text{-}H_{2,34}=3.17$; $p=0.20$).

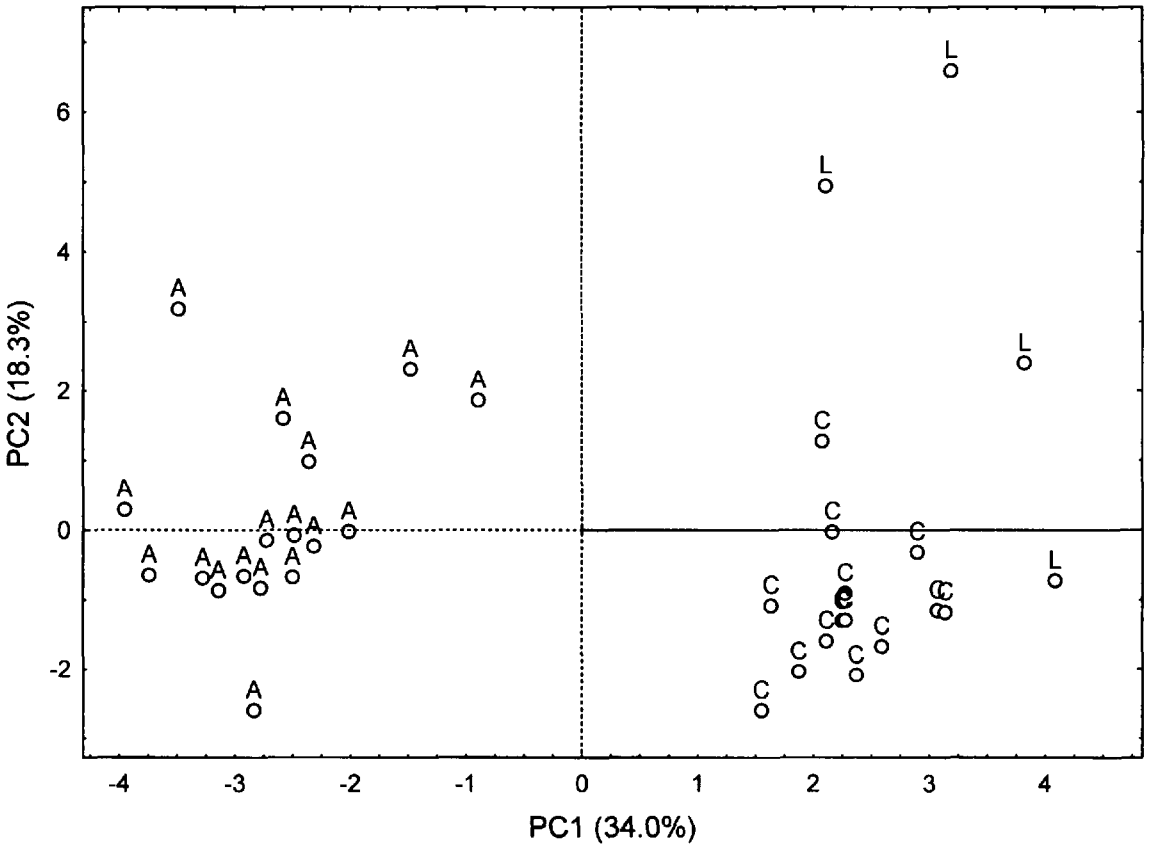

Figure 3.3. Principal component analysis of percent fatty acid composition data in eggs of Arctic Terns (A; n=17), Common Eiders (C; n=14) and Long-tailed Ducks (L; n=4).

The factor loading scores along PC1 (Table 3.3) showed five SAFAs with high factor loadings. Of those five SAFAs, two SAFAs had negative loadings (18:0 - stearic acid; 22:0 – behenic acid) suggesting that they contributed more to FA levels in Arctic Tern eggs than in Long-tailed Ducks or Common Eiders. Three of the SAFAs had positive loadings (14:0 – myristic acid, 16:0 – palmitic acid; 17:0 – heptadecanoic acid) indicating that they were more important in Long-tailed Duck and Common Eider eggs than in Arctic Tern eggs. Two of the MUFAs also had high factor loadings. Palmitoleic acid (16:1n-7) loaded positively along PC1, which suggested that it was more important in the duck eggs than in eggs of Arctic Terns. Eicosenoic acid (20:1n-9) loaded negatively, which indicated it contributed more to egg FA profiles in Arctic Terns versus Common Eiders or Long-tailed Ducks. Two n-6 PUFAs also loaded strongly on PC1 with γ-linolenic acid (18:3n-6) loading positively, highlighting its lower contribution to FA levels in Arctic Tern eggs, while arachidonic acid (ARA, 20:4n-6) loaded negatively indicating greater contributions to egg FA levels in Arctic Terns versus Common Eiders or Long-tailed Ducks. Three of the four n-3 PUFAs had high factor loadings. Only α-linolenic acid (ALA, 18:3n-3) loaded positively while the others (DPA, docosapentaenoic acid, 22:5n-3; DHA, docosahexaenoic acid, 22:6n-3) had negative loadings indicating that the latter two FAs were more important in eggs of Arctic Terns than in Long-tailed Ducks and Common Eiders.

Along PC2, six FAs had high factor loading scores (Table 3.3). Three MUFAs (palmitoleic acid, 16:1n-7; nervonic acid, 24:1n-9; erucic acid, 22:1n-9) had positive loadings as did two of the three n-3 PUFAs (eicosapentaenoic acid, EPA, 20:5n-3; docosapentaenoic acid, DPA, 22:5n-3). These results reflect the lower contribution of these FAs to total FA levels in Common Eiders versus the other species.

The dominant negatively loading FA was a MUFA (oleic acid, 18:1n-9), indicating that it was less important in the Long-tailed Duck eggs than in the Common Eider and Arctic Tern eggs.

Table 3.3. FA loadings on PC1 (34.0% of data variance) and PC2 (18.3% of data variance) from the PCA of percent FA data for Arctic Tern, Common Eider and Long-tailed Duck eggs at Tern Island. Asterisks (*) highlight FAs with strong factor loadings.

FA Common Name	FA Structure	PC1	PC2
Myristic acid	14:0	0.79*	0.39
Pentadecanoic acid	15:0	0.26	0.16
Palmitic acid	16:0	0.69*	-0.49
Heptadecanoic acid	17:0	0.63*	-0.34
Stearic acid	18:0	-0.86*	0.02
Behenic acid	22:0	-0.83*	-0.09
Myristoleic acid	14:1n-5	0.46	0.45
Palmitoleic acid	16:1n-7	0.78*	0.52*
Elaidic acid	18:1n-9t	-0.38	-0.21
Oleic acid	18:1n-9	-0.32	-0.82*
Eicosenoic acid	20:1n-9	-0.83*	0.36
Erucic acid	22:1n-9	-0.40	0.63*
Nervonic acid	24:1n-9	0.09	0.65*
Eicosadienoic acid	20:2n-6	-0.04	-0.49
Eicosatrienoic acid	20:3n-6	-0.28	0.23
Linoleic acid	18:2n-6	0.24	-0.05
γ-Linolenic acid	18:3n-6	0.61*	0.47
Arachidonic acid	20:4n-6	-0.76*	-0.07
α-Linolenic acid	18:3n-3	0.68*	-0.06
Eicosapentaenoic acid	20:5n-3	-0.16	0.68*
Docosapentaenoic acid	22:5n-3	-0.55*	0.57*
Docosahexaenoic acid	22:6n-3	-0.82*	0.15

Correlation Analysis Between Stable Isotopes and Fatty Acids

Across the eggs of three marine bird species sampled at Tern Island, I found significant correlations between the percent contribution of certain FAs to total FA levels in eggs and the trophic level at which the female was feeding before she laid the egg, as indexed by nitrogen isotopes (Table 3.4). Correlation analysis of those FAs contributing significantly to inter-specific differences in FA profiles (as inferred from the PCA analysis described above) indicated that after Bonferroni correction 10 FAs were correlated with $\delta^{15}N$ values (Table 3.4). Negative correlations were observed for shorter-chain SAFAs (14:0, 16:0), one MUFA (16:1n-7), one n-6 FA (18:3n-6), and one n-3 PUFA (18:3n-3) (Table 3.4). Positive correlations were observed for 5 other FAs including two longer-chain SAFAs (18:0, 22:0), one MUFA (20:1n-9), one n-6 FA (20:4n-6), and one n-3 PUFA (22:6n-3) (Table 3.4). Arctic Tern eggs contained greater proportions of FAs showing positive correlations with $\delta^{15}N$, that is, total FA levels in Arctic Tern eggs were comprised of greater proportions of 18:0, 22:0, 20:1n-9 (Fig. 3.4), 20:4n-6 (Fig. 3.5), and 22:6n-3 (Fig. 3.6) then were eggs of the other two species.

Table 3.4. Results of correlation analyses between $\delta^{15}N$ or $\delta^{13}C$ and percent FA composition of eggs of Arctic Terns, Common Eiders and Long-tailed Ducks.

FA Common Name	FA Structure	$\delta^{15}N$	$\delta^{13}C$
Myristic acid	14:0	r = -0.61	r = 0.20
		p < 0.0001**	p = 0.26
Palmitic acid	16:0	r = -0.55	r = 0.71
		p = 0.001**	p < 0.0001**
Stearic acid	18:0	r = 0.63	r = -0.36
		p < 0.0001**	p = 0.04*
Behenic acid	22:0	r = 0.49	r = -0.31
		p = 0.003**	p = 0.08
Palmitoleic acid	16:1n-7	r = -0.75	r = 0.33
		p < 0.0001**	p = 0.05
Eicosenoic acid	20:1n-9	r = 0.67	r = -0.67
		p = 0.0001**	p < 0.0001**
γ-Linolenic acid	18:3n-6	r = -0.75	r = 0.34
		p < 0.0001**	p = 0.05*
Arachidonic acid	20:4n-6	r = 0.85	r = -0.51
		p < 0.0001**	p = 0.002**
α-Linolenic acid	18:3n-3	r = -0.51	r = -0.06
		p = 0.002**	p = 0.75
Eicosapentaenoic acid	20:5n-3	r = -0.02	r = -0.10
		p = 0.91	p = 0.56
Docosapentaenoic acid	22:5n-3	r = 0.44	r = -0.46
		p = 0.007*	p = 0.005*
Docosahexaenoic acid	22:6n-3	r = 0.66	r = -0.43
		p = 0.0001**	p = 0.009*
Omega-3	n-3	r = 0.53	r = -0.49
		p = 0.001**	p = 0.003*
Omega-6	n-6	r = 0.60	r = -0.63
		p = 0.0002**	p < 0.0001**
Omega-3/Omega-6	n-3/n-6	r = -0.52	r = 0.33
		p = 0.001**	p = 0.05*

**- significant correlation after Bonferroni correction.

*- non-significant correlation after Bonferroni correction.

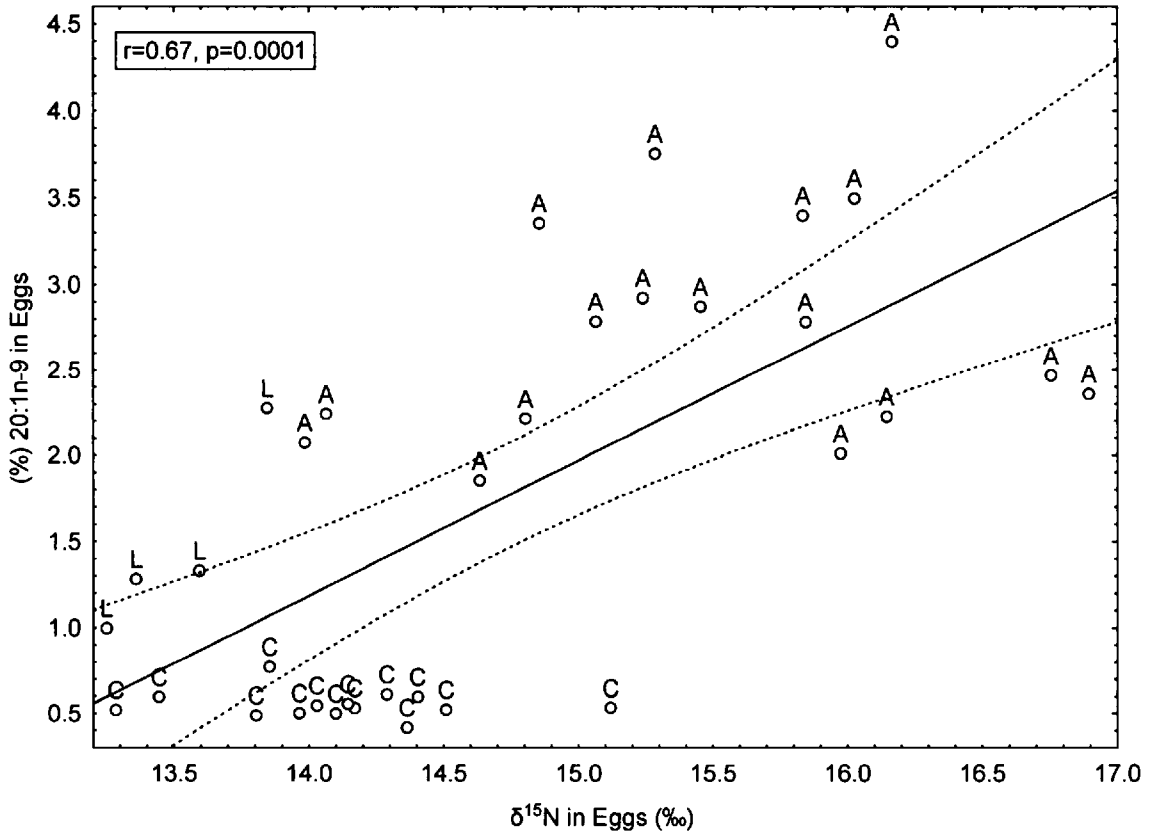

Figure 3.4. Relationship between $\delta^{15}N$ (‰) and eicosenoic acid (20:1n-9) in eggs of Arctic Terns (A), Common Eiders (C) and Long-tailed Ducks (L). Confidence bands represent 95% confidence interval.

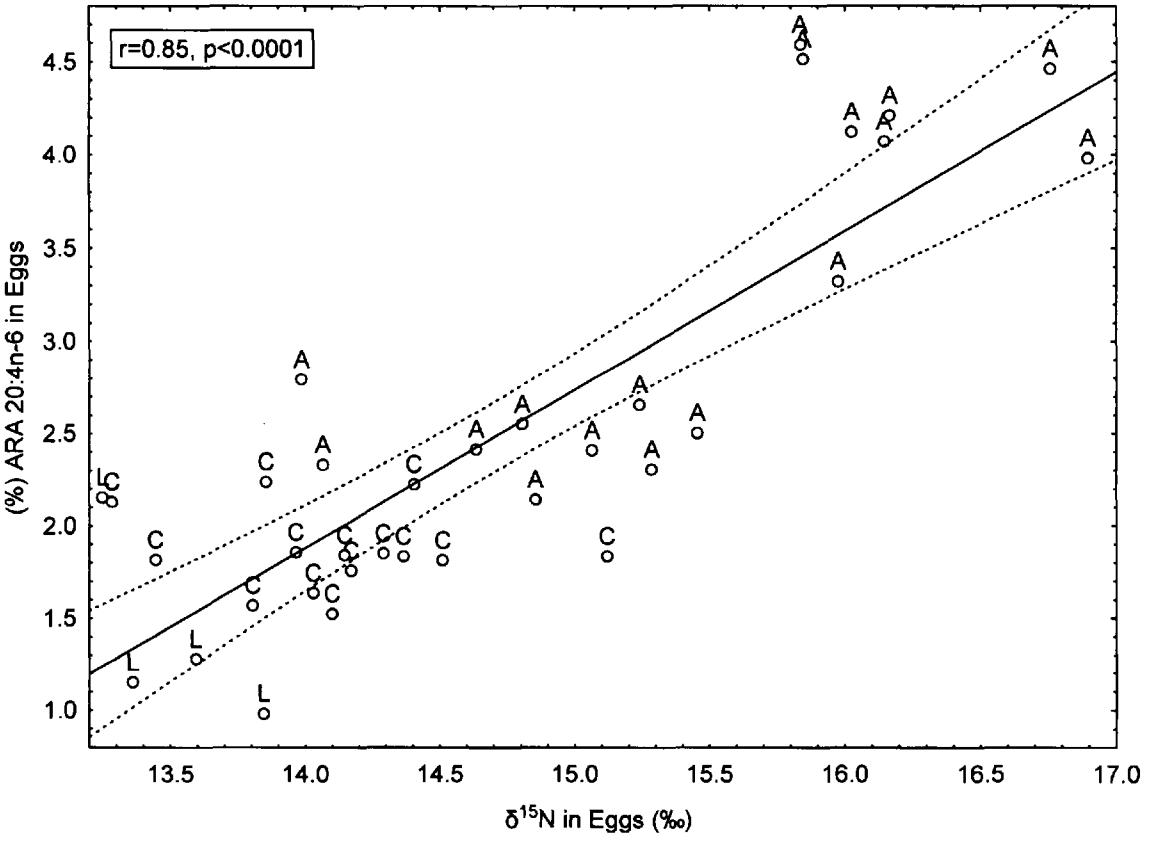

Figure 3.5. Relationship between $\delta^{15}N$ (‰) and arachidonic acid (ARA; 20:4n-6) in eggs of Arctic Terns (A), Common Eiders (C) and Long-tailed Ducks (L). Confidence bands represent 95% confidence interval.

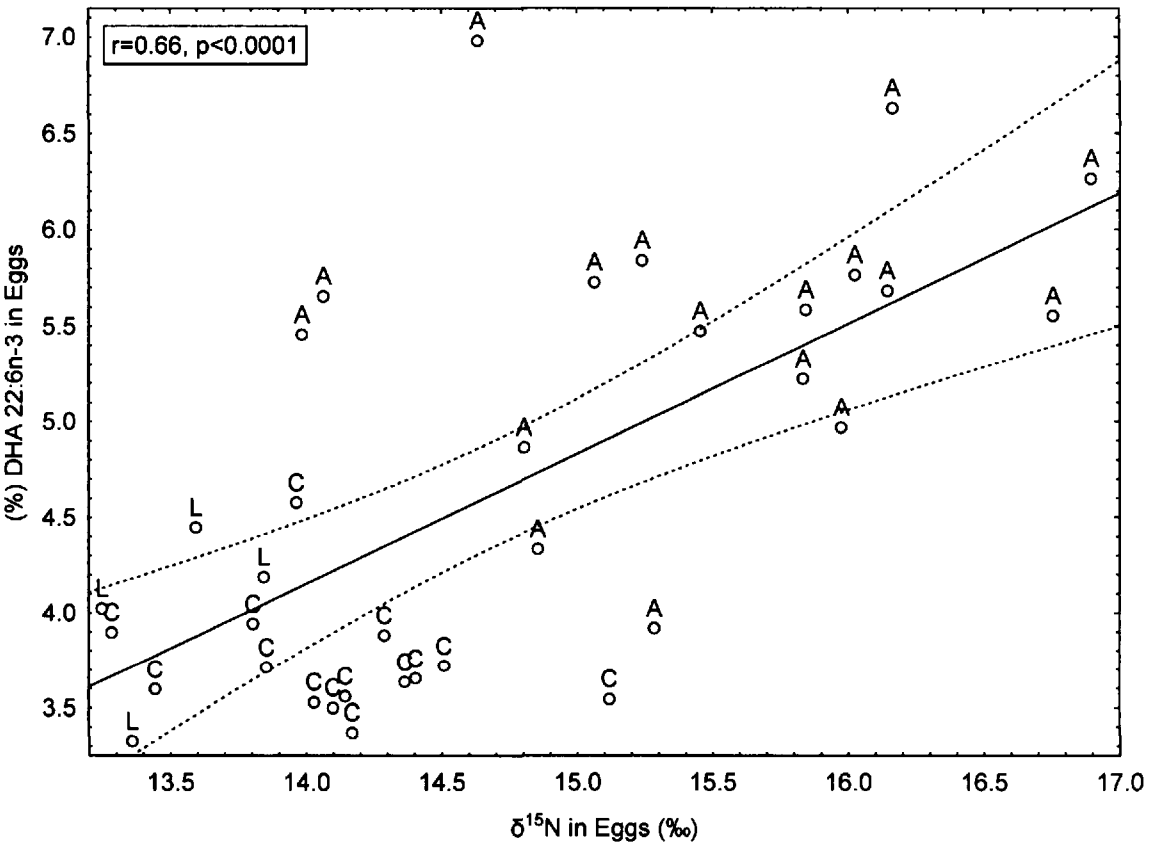

Figure 3.6. Relationship between $\delta^{15}N$ (‰) and docosahexaenoic acid (DHA, 22:6n-3) in eggs of Arctic Terns (A), Common Eiders (C) and Long-tailed Ducks (L). Confidence bands represent 95% confidence interval.

Compared with $\delta^{15}N$, fewer significant correlations were observed between egg FA composition data and $\delta^{13}C$ values (Table 3.4). Egg $\delta^{13}C$ values were positively correlated with percent contribution to total FA levels of 16:0 and negatively correlated with percent contributions of 20:1n-9 and 20:4n-6 (Table 3.4). Across all species, proportions of 20:1n-9 and 20:4n-6 in eggs declined as egg $\delta^{13}C$ values became less negative (Fig. 3.7, Fig. 3.8). Although neither DPA (22:5n-3) nor DHA (22:6n-3) exhibited significant correlations after Bonferroni correction, both had negative correlation coefficients with $\delta^{13}C$ values (Table 3.4).

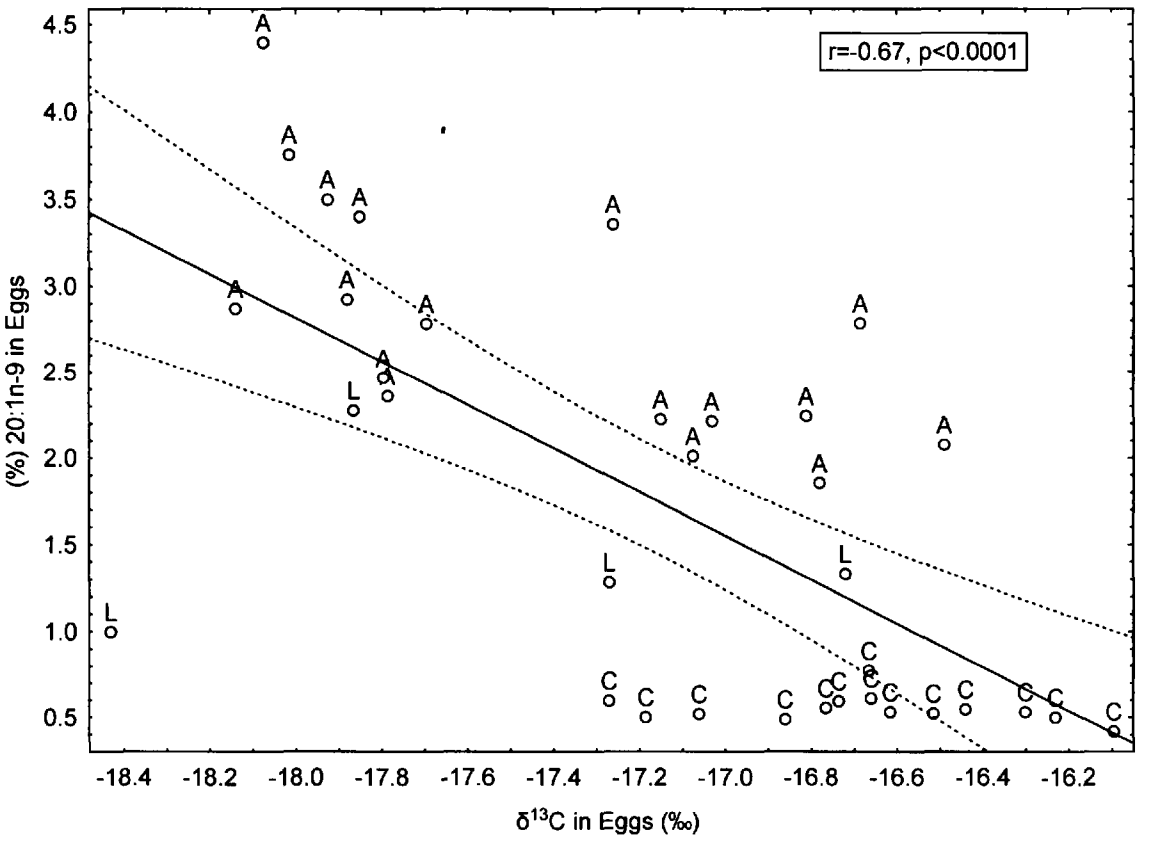

Figure 3.7. Relationship between $\delta^{13}C$ (‰) and eicosenoic acid (20:1n-9) in eggs of Arctic Terns (A), Common Eiders (C) and Long-tailed Ducks (L). Confidence bands represent 95% confidence interval.

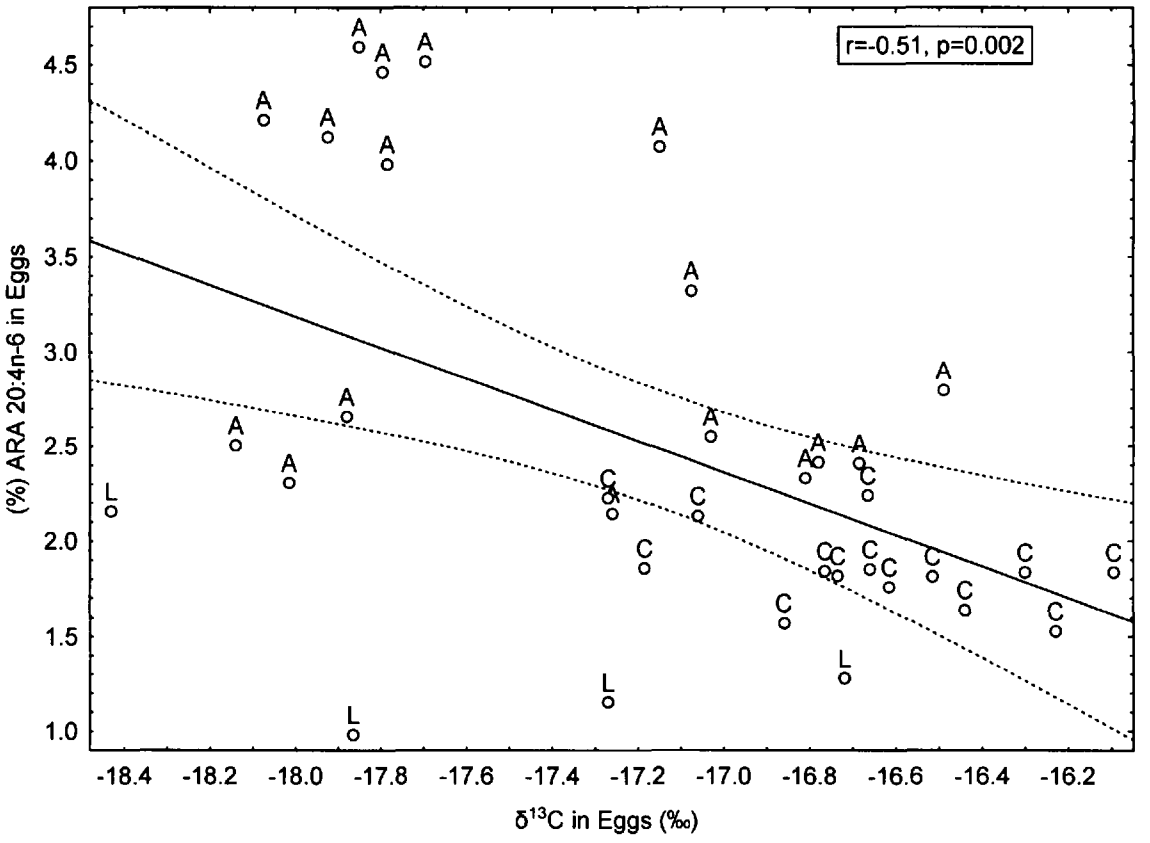

Figure 3.8. Relationship between $\delta^{13}C$ (‰) and arachidonic acid (20:4n-6) in eggs of Arctic Terns (A), Common Eiders (C) and Long-tailed Ducks (L). Confidence bands represent 95% confidence interval.

Omega-3 FAs are typically high in fishes (Rubio-Rodríguez et al. 2010; Zheng et al. 2009) and n-3 FAs are considered to be essential FAs for most vertebrates since they are generally not capable of synthesizing them (Williams and Buck 2010), I therefore conducted a correlation analysis to test whether egg stable isotope values were related to percent contribution of n-3 FAs to total FA levels. A positive correlation was detected between % n-3 FAs and $\delta^{15}N$ ($r=0.53$, $p=0.001$; Table 3.4) with Arctic Tern eggs containing greater proportions of n-3 FAs and having greater $\delta^{15}N$ values. The n-6 PUFAs are also considered essential FAs (Ahlgren et al. 2009) and a positive correlation was observed between their percent contribution to total FA levels in eggs and egg $\delta^{15}N$ values ($r=0.60$, $p=0.0002$; Table 3.4) with the Arctic Terns again showing greater contributions of n-6 FAs to total FA levels. Percent contributions of n-3 and n-6 PUFAs were negatively correlated with $\delta^{13}C$ values (Table 3.4). In both correlations, Arctic Terns had more negative $\delta^{13}C$ values and greater percent contributions of the n-3 and n-6 PUFAs. n-3/n-6 ratios are frequently used to assess an organisms' n-3/n-6 requirement for optimal physiological processes (Ahlgren et al. 2009; Parrish 2009). Egg $\delta^{15}N$ was negatively correlated with this ratio but the relationship with egg $\delta^{13}C$ values was non-significant (Table 3.4).

Egg carbon and nitrogen isotope values were negatively correlated ($r=-0.44$, $p=0.008$). Arctic Tern eggs had greater $\delta^{15}N$ values and more negative $\delta^{13}C$ values than the other species (Fig. 3.9).

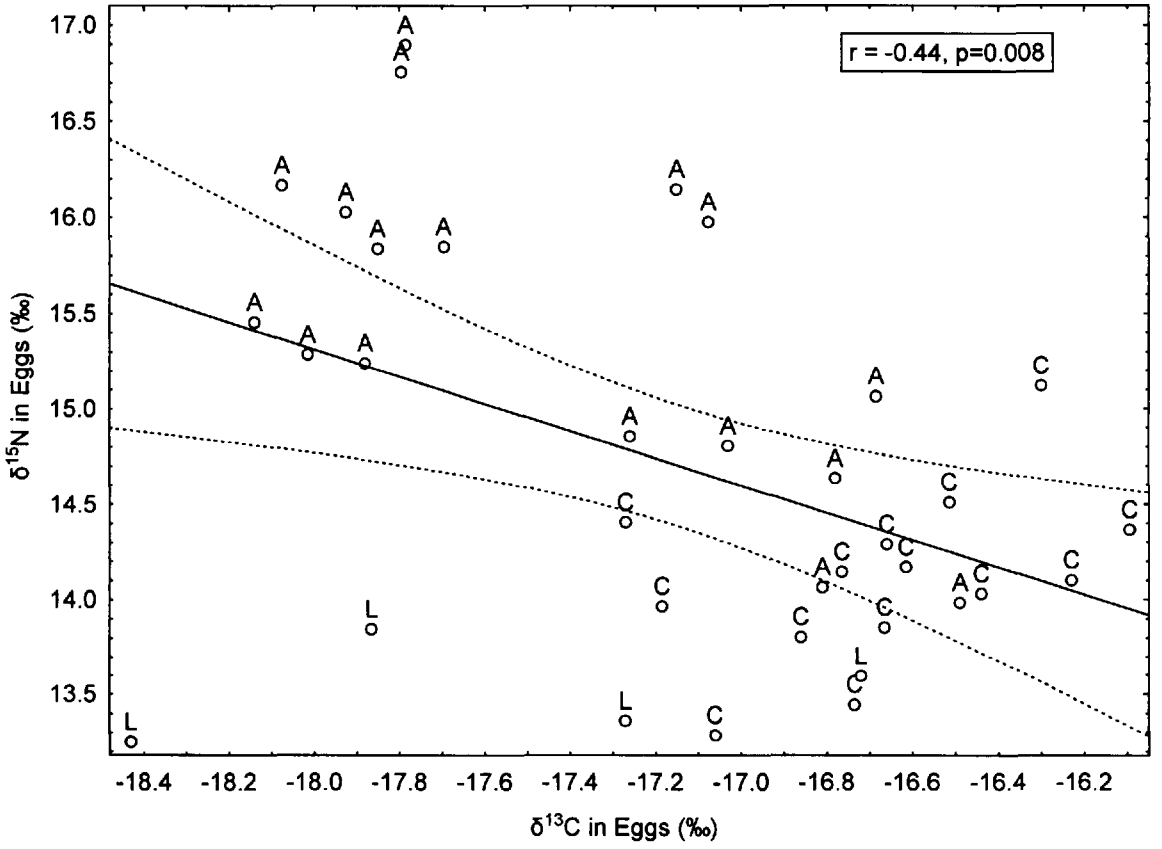

Figure 3.9. Relationship between $\delta^{13}C$ and $\delta^{15}N$ values in eggs of Arctic Terns (A), Common Eiders (C) and Long-tailed Ducks (L). Confidence bands represent 95% confidence interval.

Discussion

Inter-specific Differences in Egg Stable Isotope Values

$\delta^{15}N$ values in the three marine bird species showed expected patterns. Organisms feeding on higher trophic level prey have greater $\delta^{15}N$ values (Hobson 1993; Hebert et al. 1999; Thompson et al. 1999). Here, Arctic Terns had greater $\delta^{15}N$ values than the other two species reflecting their high trophic level diet consisting of fish (Hatch 2002). The lack of a statistically significant difference in egg $\delta^{15}N$ values between Common Eiders and Long-tailed Ducks suggests that they were feeding on prey at a similar lower trophic level compared to Arctic Terns.

The less negative $\delta^{13}C$ values in Common Eider eggs was also expected given the preponderance of benthic food in their diet. Benthic feeders tend to have less negative $\delta^{13}C$ values relative to offshore pelagic feeders (Hobson et al. 1994; Dahl et al. 2003). However, the lack of difference in $\delta^{13}C$ values between Arctic Terns and Long-tailed Ducks was somewhat surprising given previously identified dietary information for the two species (Robertson and Savard 2002). Here, the similarity between these two species may have reflected the fact that they were both feeding on pelagic organisms during the breeding period. However, the lack of statistical difference between Long-tailed Duck and Arctic Tern eggs in $\delta^{13}C$ values and Long-tailed Duck and Common Eider eggs in $\delta^{15}N$

values may have also been a reflection of the small sample size for Long-tailed Ducks.

An unexpected result was that egg $\delta^{15}N$ values showed greater variation in Arctic Terns than the other two marine birds (Table 3.1). Given the varied diets of Long-tailed Ducks I expected that they would show the greatest variation in stable isotope values; however, Arctic Terns feed on crustaceans, insects and invertebrates (Hatch 2002) which could vary substantially in their $\delta^{15}N$ values. If Arctic Terns were feeding on a variety of food items then this could have accounted for their greater variability in $\delta^{15}N$ values. Furthermore, different Arctic fish species may also vary in their $\delta^{15}N$ values (Jæger et al. 2009), so it may be that Arctic Terns were consuming different fish species that varied inter-specifically in their $\delta^{15}N$ values. The smaller $\delta^{15}N$ coefficient of variation in Long-tailed Duck eggs suggests that females of that species were consuming prey with a narrower range of $\delta^{15}N$ values than female Arctic Terns. In contrast, species-specific $\delta^{13}C$ coefficients of variation were greatest in Long-tailed Duck eggs (Table 3.1), suggesting that females of that species were feeding in more diverse habitats than Arctic Terns or Common Eiders. Long-tailed Ducks have been known to exploit a diverse array of prey in both freshwater and saltwater environments and to capture prey throughout the water column (Robertson and Savard 2002). However, the choice of prey available to the Long-tailed Ducks

during the breeding period at Tern Island may have been limited to lower trophic level prey in all their foraging habitats.

Inter-specific Differences in Egg FA Profiles and Relationships with Egg SI

Consistent patterns within and among species indicated that eggs collected in remote environments retained their FA profiles and provided a good medium for evaluating inter-specific differences in FA patterns. Principal components analysis highlighted several FAs that were useful in distinguishing eggs from the three marine bird species. This suggested that these species were exploiting different food resources throughout the breeding period. In particular, Arctic Tern eggs showed clear separation from the Common Eider and Long-tailed Duck eggs.

Five of the six SAFAs distinguished the Arctic Tern eggs from the Common Eider and Long-tailed Duck eggs. The sources of most SAFAs tend to be difficult to determine (Bianchi and Canuel 2011) and quite often can be synthesized endogenously (Arts et al. 2001; Dalsgaard and St. John 2004; Käkelä et al. 2007; Iverson 2009) or act as precursors to other FAs (Dalsgaard and St. John 2004). However, some SAFAs have been linked with specific food sources (Bianchi and Canuel 2011). For example, heptadecanoic acid (17:0) has been linked to detrivorous food webs (Kuusipalo and Käkelä 2000; Käkelä et al. 2005) and proportions of that FA were greater in eggs of Common Eiders and

Long-tailed Duck eggs compared to Arctic Terns. The greater proportions of 17:0 in Common Eiders is in keeping with their benthic feeding habits (Goudie et al. 2000) and Long-tailed Ducks may also utilize benthic food as they dive for food (Robertson and Savard 2002). Stearic acid (18:0), another SAFA, was found in greater proportions in Arctic Tern eggs versus Common Eider and Long-tailed Duck eggs. Stearic acid is one of the most common FAs in most organisms but it may be a useful marker of fish consumption in that it is structurally-linked to docosahexaenoic acid (DHA, 22:6n-3) in phospholipid bilayers in fishes (Farkas et al. 2000). DHA is found in high amounts in fish (Whelan and Rust 2006; Rubio-Rodríguez et al. 2010). Hence, fish consumption could lead to elevated levels of stearic acid, in addition to DHA, in predators. Proportions of both DHA and stearic acid were greater in Arctic Tern eggs versus the two marine bird species supporting observations that Arctic Terns are piscivores (Hatch 2002). Furthermore, DHA and stearic acid were positively correlated with egg $\delta^{15}N$ values lending further support to the idea that consumption of higher trophic level prey, namely fish, leads to greater proportions of these FAs in predators.

MUFAs may also be useful in tracking the use of food resources by predators. For example, proportions of palmitoleic acid (16:1n-7) were greater in Long-tailed Duck eggs compared to Common Eider and Arctic Tern eggs. Wold et al. (2011) found that palmitoleic acid, along with eicosapentaenoic acid

(20:5n-3), was found in high levels in benthic fish. Long-tailed Ducks are one of the deepest divers and they appear to opportunistically feed on a wide diversity of prey (Robertson and Savard 2002), so they may have had access to benthic fishes during the breeding period at Tern Island.

Oleic acid (18:1n-9), a short-chain MUFA, constituted a third of total FAs for all three marine bird eggs. It was lower in Long-tailed Duck eggs and dietary sources of oleic acid, like most MUFAs, are typically difficult to determine. Most MUFAs act as precursors to other FAs (Parrish 2009). However, it is possible that some MUFAs may be useful indicators of food consumption. For example, erucic acid (22:1n-9) and nervonic acid (24:1n-9) are generally found in high concentrations in plants (Impallomeni et al. 2011). Long-tailed Ducks are known to feed on prey from terrestrial ecosystems as well as terrestrial vegetation (Robertson and Savard 2002). Hence, greater proportions of these FAs in Long-tailed Duck eggs may have reflected their use, to some degree, of terrestrial food sources. Inferring the potential feeding habits of Long-tailed Ducks based on the current SI and FA data from Tern Island; however, must be treated with caution due to the small sample size.

Another MUFA showing greater promise as a tracer of food web interactions was eicosenoic acid (20:1n-9). It showed large differences in percent contributions to total FA levels in eggs from the three bird species. Greater proportions of it were found in eggs of Arctic Terns versus the other two

marine bird species. Eicosenoic acid is typically found in Arctic calanoid species (Scott et al. 2002; Kattner et al. 2007) and has been used as an FA biomarker for organisms that feed on calanoids or organisms that are part of calanoid-based food webs (Wesławski et al. 1994; Dahl et al. 2003; Käkelä et al. 2007; Karnovsky et al. 2008; Kattner and Hagen 2009; Loseto et al. 2009; Wilson et al. 2010). Amphipods contain high amounts of eicosenoic acid as a result of their consumption of calanoid species (Graeve et al. 1997; Auel 2002; Wold et al. 2011) and Arctic Terns are known to consume amphipods at the water's surface (Hatch 2002). Arctic fish also consume crustaceans like amphipods (Dolgov 2009; Orlova et al. 2009; Wojciech et al. 2011) so the greater contribution of eicosenoic acid to FA levels in Arctic Tern eggs may have reflected direct consumption of invertebrates or consumption of fish that had consumed calanoids. Percent egg eicosenoic acid data were positively correlated with egg $\delta^{15}N$ values and negatively correlated with egg $\delta^{13}C$ values. Wold et al. (2011) found that Arctic marine birds that were part of calanoid-based food webs had greater levels of eicosenoic acid along with more negative $\delta^{13}C$ values. The positive correlation between $\delta^{15}N$ and eicosenoic acid and the negative correlation between $\delta^{13}C$ and eicosenoic acid suggested that the Arctic Terns were feeding on higher trophic level prey, i.e. fish, linked to a calanoid food chain, most likely in pelagic waters as reflected by more negative $\delta^{13}C$ values. In Common Eider eggs; however, egg eicosenoic acid content remained relatively

constant while both nitrogen and carbon showed some variation, indicating that Common Eiders are not relying on calanoid-based food webs. Long-tailed Duck eggs contained eicosenoic acid but to a lesser degree than for Arctic Terns indicating that this species relied less on calanoid-based food webs.

Among the n-3 FAs, docosapentaenoic acid (DPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) were important in differentiating egg FA composition among species. DHA and EPA are considered essential FAs (Gladyshev et al. 2009; Parrish 2009) since most vertebrates lack the enzymes needed to produce n-3 FAs so they have to be obtained through the diet (Williams and Buck 2010). DPA is typically characterized as an intermediate form between EPA and DHA but it may also perform some important biochemical functions (Kaur et al. 2011). All three n-3 FAs (DPA, DHA and EPA) are typically found in high amounts in fish (Sargent et al. 1999; Whelan and Rust 2006; Kaur et al. 2011). Proportions of both DHA and DPA were greater in Arctic Tern eggs compared to Long-tailed Duck and Common Eider eggs. DHA was positively correlated with $\delta^{15}N$ after Bonferroni correction. The greater percent contributions of DHA and DPA to total FA levels in Arctic Tern eggs and the positive correlation between egg $\delta^{15}N$ values and DHA reflects the known piscivorous diet of Arctic Terns (Hatch 2002). The lesser importance of DHA and DPA to overall FA levels in Common Eider and Long-tailed Duck eggs, in addition to their lower $\delta^{15}N$ values, provides evidence of diet

separation between the Arctic Terns and the other two species. Proportions of EPA were greater in the Long-tailed Duck eggs versus the Arctic Tern and Common Eider eggs. Wold et al. (2011) found that EPA, in conjunction with palmitoleic acid (which was also higher in the Long-tailed Duck eggs), was characteristic of benthic fish, which may indicate that the Long-tailed Ducks were consuming more benthic fish compared to the Common Eiders and Arctic Terns.

As expected, there was a positive correlation between the n-3 FAs and $\delta^{15}N$ ($r=0.53$, $p=0.001$; Table 3.4) and a negative correlation with $\delta^{13}C$ ($r=-0.49$, $p=0.003$; Table 3.4). This reflected the higher trophic position of Arctic Terns, likely reflecting their greater reliance on n-3 rich fish (Sargent 1999; Whelan and Rust 2006; Rubio-Rodríguez 2010) than the Common Eiders and Long-tailed Ducks. Less negative egg $\delta^{13}C$ values and lower proportions of egg n-3 FA in Common Eider eggs were consistent with their benthic feeding habits (Goudie et al. 2000).

The n-6 FAs, e.g. arachidonic acid (ARA, 20:4n-6) and γ-linolenic acid (GLA, 18:3n-6), were also useful in distinguishing eggs from the three species. In particular, proportions of ARA were greater in Arctic Tern eggs than in Common Eider or Long-tailed Duck eggs. In planktonic food webs, ARA has been known to accumulate from sestonic organisms to macro-zooplankton (Kainz et al. 2004) and has been found at higher levels in higher trophic level consumers (i.e., fish) (Kuusipalo and Käkelä 2000; Koussoroplis et al. 2011).

Here, a significant positive correlation was observed between egg $\delta^{15}N$ values and egg ARA (Table 3.4, Fig. 3.5) reflecting an increase in the percent contribution of ARA to egg FA levels with increasing trophic position. In addition, egg $\delta^{13}C$ showed a negative correlation with percent ARA levels in eggs indicating that species utilizing pelagic food webs, perhaps based upon higher trophic level prey, e.g. fish, were acquiring more ARA. Proportions of GLA were greater in the Long-tailed Duck and Common Eider eggs than in the Arctic Tern eggs. GLA can act as a precursor to other PUFAs like ARA (Arts et al. 2001; Guil-Guerrero et al. 2010; Williams and Buck 2010) and it can also perform some of the same functions as ARA (Guil-Guerrero et al. 2010). Its utility as a dietary tracer requires further research.

Not surprisingly, there was a positive correlation between n-6 FAs and $\delta^{15}N$ ($r=0.60$, $p=0.0002$; Table 3.4) and a negative correlation with $\delta^{13}C$ ($r=-0.63$, $p<0.0001$; Table 3.4). This is consistent with higher trophic level prey that are consumed by Arctic Terns (Hatch 2002) over the Common Eiders and Long-tailed Ducks (Goudie et al. 2000; Robertson and Savard 2002). The negative relationship between n-6 FAs and $\delta^{13}C$ is also in agreement with the pelagic feeding patterns of Arctic Terns (Hatch 2002) and the benthic feeding habits of Common Eiders (Goudie et al. 2000).

The negative relationship between $\delta^{15}N$ and $\delta^{13}C$ ($r=-0.44$, $p=0.008$; Fig. 3.9) was also not surprising since it supports the observations of the higher

trophic level prey that are consumed by the Arctic Terns (Hatch 2002) over the Long-tailed Ducks (Robertson and Savard 2002) and Common Eiders (Goudie et al. 2000). It also supports the observations that the Common Eiders typically consume prey at inshore benthic habitats (Goudie et al. 2000). The Long-tailed Duck $\delta^{13}C$ egg values varied whereas the $\delta^{15}N$ egg values remained relatively constant, which may indicate that the Long-tailed Ducks fed on lower trophic prey over a wider variety of habitats (i.e., terrestrial, marine, offshore, inshore) than the Arctic Terns and Common Eiders.

Inter-specific differences in fatty acid and stable isotope composition have been shown in previous studies (Dahl et al. 2003; Ramírez et al. 2009; Wold et al. 2011) and the Tern Island marine bird eggs also showed such differences. All three marine birds are known to feed on different prey (Hatch 2002; Goudie et al. 2000; Robertson and Savard 2000) and the inter-specific egg FA and SI differences observed here likely reflect their different feeding strategies. My results demonstrate the value of utilizing SI and FA in combination when using biochemical tracers to examine the feeding ecology of Arctic marine birds.

In the Canadian Arctic, anthropogenically induced changes are rapidly occurring (Huntington 2009; Polyak 2010), and these changes could affect food supplies for birds in High Arctic marine ecosystems. My egg SI and FA data from Tern Island are the first baseline biochemical information gathered for High Arctic marine birds at this important breeding site. These baseline data could be

important in identifying future change in diet composition. For example, in Arctic Terns, shifts to less negative $\delta^{13}C$ values in eggs combined with reductions in the proportions of specific FAs in eggs, e.g. eicosenoic acid, could be indicative of reductions in the availability of pelagic prey. Periodic monitoring of biochemical markers in eggs could provide a tool to identify such change thereby gaining an effective early warning indicator that could be used to inform management decisions.

CHAPTER FOUR

Summary and Conclusions

Summary and Conclusions

While it is possible to analyze and display the data in other ways, my study has laid the groundwork for possible future biochemical tracer studies in High Arctic marine birds. Egg stable isotope and fatty acid results indicated that there were differences in the chemical composition of early and late-laid eggs in three species of marine birds. These differences may have been related to shifts in diet during the period of egg formation, particularly for Arctic Terns and Long-tailed Ducks since they likely rely more on exogenous resources for egg synthesis (Hobson et al. 2000; Kellett et al. 2005). In early-laid Arctic Tern eggs, greater $\delta^{15}N$ values along with greater proportions of n-3 FAs suggested that fish may have constituted more of the diet during early egg formation. Lower $\delta^{15}N$ values in late-laid Arctic Tern eggs coupled with higher contributions of eicosenoic acid (20:1n-9), a MUFA frequently associated with pelagic calanoid food webs (Wesławski et al. 1994; Dahl et al. 2003; Käkelä et al. 2007; Kattner and Hagen 2009; Loseto et al. 2009; Wilson et al. 2010; Wold et al. 2011), suggested that Arctic Terns may have included more invertebrates in their diets later in the egg formation period. Long-tailed Duck egg FA results also suggested that diet shifts may have occurred during the breeding period but future work with a larger sample size is needed to confirm this. Common Eider stable isotope and fatty acid results indicated that diet shifts may have occurred during the period of egg formation but other factors (i.e. changing use of

endogenous versus exogenous resources for egg formation, nutritional state of the females) may have played a role in regulating differences in the SI and FA composition of early versus late-laid eggs.

More research is clearly required to conclusively determine the factors that are most important in regulating differences in $\delta^{15}N$ values and FA composition between early and late-laid eggs. In general; however, differences in egg biochemical composition between early and late-laid eggs were relatively small compared to the magnitude of temporal changes observed in Arctic marine bird eggs (Braune 2007) and elsewhere (Hebert et al. 1999, 2008). For example, Braune (2007 and pers. comm.) documented temporal (1975-2008/09) changes in egg $\delta^{15}N$ of five species of Arctic marine birds. Through time, $\delta^{15}N$ values in Glaucous Gulls (*Larus hyperboreus*) ranged over 4.1‰, Black-legged Kittiwakes (*Rissa tridactyla*) 3.3‰, and Black Guillemots (*Cepphus grille*) 2.3‰. Lower variation was observed in two other species: Northern Fulmar 1.3‰ and Thick-billed Murre 1.1‰. Only in these latter two species does inter-egg variability in $\delta^{15}N$ values (0.3-0.4‰) approach the changes that have been observed through time. Unfortunately, no Arctic data are available to assess temporal trends in egg FA composition so a similar comparison with differences between early and late-laid eggs for FA is not possible. However, temporal alterations in the FA composition of Herring Gull eggs from the Laurentian Great Lakes (Hebert et al. 2008) showed much larger changes than the differences observed here between

early and late-laid eggs. This is important because marine bird egg monitoring programs are based upon the interpretation of data generated from the analysis of randomly collected eggs. Based upon my results, analysis of biochemical markers in such eggs should provide useful information for evaluating inter-specific differences in diet and for assessing temporal changes in diet composition that may be linked to ecosystem change.

The Tern Island marine bird eggs exhibited consistent inter-specific differences in FA patterns indicating that protocols used for the collection, storage, and analysis of High Arctic marine bird eggs were appropriate for the generation of reliable FA data that were useful in evaluating the ecological niches of the study species. Inter-specific differences in the egg fatty acid and stable isotope data likely reflected differences in diet among Arctic Terns, Common Eiders and Long-tailed Ducks. Stable isotopes of carbon (more negative egg $\delta^{13}C$ values) and greater proportions of eicosenoic acid in eggs (linked to pelagic calanoid-based food webs; Wesławski et al. 1994; Dahl et al. 2003; Käkelä et al. 2007; Karnovsky et al. 2008; Kattner and Hagen 2009; Loseto et al. 2009; Wilson et al. 2010) supported the pelagic feeding patterns of Arctic Terns. Consumption of higher trophic level prey, i.e. fish, by Arctic Terns was reflected in both egg nitrogen isotopes (greater egg $\delta^{15}N$ values) and egg FA patterns (greater proportions of n-3 FAs which are rich in fish; Whelan and Rust 2006; Rubio-Rodríguez 2010). Similarly, the benthic feeding habits of Common Eiders were

confirmed using carbon isotopes (less negative egg $\delta^{13}C$ values) and through greater contributions of heptadecanoic acid (17:0) to Common Eider eggs. This SAFA is frequently associated with detrivorous food webs (Kuusipalo and Käkelä 2000; Käkelä et al. 2005). Greater proportions of eicosapentaenoic acid and palmitoleic acid in Long-tailed Duck eggs may have also reflected consumption of benthic prey, since both FAs have been found in greater amounts in Arctic benthic organisms such as benthic fish (Wold et al. 2011). The dependence of Common Eiders and Long-tailed Ducks on lower trophic level prey, e.g. molluscs, was highlighted by lower egg $\delta^{15}N$ values. However, the higher coefficient of variation for Long-tailed Duck egg $\delta^{13}C$ values may have reflected that species utilization of a wider variety of feeding habitats. Long-tailed Ducks are thought to eat a diverse array of prey in saltwater, freshwater, and terrestrial ecosystems (Robertson and Savard 2002).

Small sample sizes for Long-tailed Ducks make definitive conclusions for that species difficult. At Tern Island, Long-tailed Duck nests were more difficult to locate than Arctic Tern and Common Eider nests and future research to obtain larger sample sizes for Long-tailed Duck eggs at Tern Island would likely require more time spent in blinds to locate Long-tailed Duck nests. Unlike Arctic Terns and Common Eiders which usually flushed from their nests when I was doing nest checks, Long-tailed Ducks would stay on their nests and were much more difficult to locate since they were well camouflaged. I did; however, have more

success during observations from blinds (unrelated research on Arctic Terns for the Canadian Wildlife Service) since I would sometimes notice female Long-tailed Ducks fly off, perhaps to feed, and then return to nest. Future studies using this method early in the marine bird breeding season would likely increase the chances of obtaining a larger sample size.

Fatty acid research by Iverson et al. (2004) and Wang et al. (2010) has utilized the quantitative FA approach to quantifiably determine diet composition in predators. In the future, if time and funding permits, sampling of prey items at Tern Island may make quantifying the specific diets of Arctic Terns, Common Eiders and Long-tailed Ducks possible thus improving our ability to track potential changes in Arctic marine ecosystems. In addition, utilizing a wider array of FAs specific to certain types of organisms may also facilitate the tracking of such changes. For example, poly-methylene-interrupted fatty acids (PMI-FA) are typically found in high amounts in marine and freshwater bivalves (Kawashima and Ohnishi 2004; Thiemann et al. 2007, Mezek et al. 2011) and they appear to hold promise as a biomarker of bivalve-based food webs. Measuring PMI-FAs in eggs of the species studied here could increase the likelihood of identifying their prey in future studies. For example, I would expect that PMI-FAs would be found in greater proportions in Common Eider eggs. Other fatty acids, such as odd-numbered, very-long chain FAs (Řezanka and Sigler 2009), also hold promise as

dietary biomarkers. Utilization of such FAs may further aid in discerning the diets of marine birds.

Marine ecosystems in the Canadian Arctic are currently undergoing rapid anthropogenically-induced changes (Huntington 2009) and it is important to recognize the impacts of these changes on the marine environment. Analysis of biochemical markers in recently collected eggs to those analyzed in the past (or concurrently, if archived samples are available) represents an opportunity to place current conditions into an historical context. Using this approach, dietary changes were identified in Herring Gulls on the Laurentian Great Lakes (Hebert et al. 2008). In that study, temporal changes in Herring Gull egg SI and FA values were related. Declines in Herring Gull trophic position (as inferred from $\delta^{15}N$ values) were correlated with changes in egg $\delta^{13}C$ values and egg FA composition. In turn, these dietary markers were linked to declines in the abundance of pelagic prey fish resulting in the birds incorporating more terrestrial food into their diets. Integration of stable isotope and fatty acid data to understand ecosystem change holds promise for monitoring in Arctic marine ecosystems as well. Egg stable isotope and fatty acid data collected at Tern Island can be used as a baseline against which to compare the results of future egg analyses. Arctic Tern eggs, for instance, were characterized by relatively high proportions of n-3 FAs and high $\delta^{15}N$ values. These biochemical markers may be good candidates for monitoring ecosystem change as reductions in egg

n-3 FA (which are typically high in fish; Whelan and Rust 2006; Zheng et al. 2009; Rubio-Rodríguez 2010) or egg $\delta^{15}N$ values (which reflect consumption of higher trophic level prey; Hobson 1993; Thompson et al. 1999) could point to potential declines in fish abundance or the trophic position of prey consumed by Arctic Terns. Other potential markers for monitoring marine ecosystem change are eicosenoic acid, an FA frequently associated with calanoid-based food webs (Wesławski et al. 1994; Dahl et al. 2003; Käkelä et al. 2007; Karnovsky et al. 2008; Kattner and Hagen 2009; Loseto et al. 2009; Wilson et al. 2010), and egg $\delta^{13}C$ values. Alterations in egg composition of these endpoints could reflect changes in the degree to which pelagic food webs are being utilized by marine birds.

The amount of sea-ice in the Arctic has been declining (Polyak et al. 2010) and Budge et al. (2008) found a relationship between sea-ice algae and eicosapentaenoic acid, an n-3 FA that is considered an essential FA (Gladyshev et al. 2009; Parrish 2009). Sea-ice decline (Polyak et al. 2010) may therefore result in lower quantities of eicosapentaenoic acid being incorporated into marine food webs thereby reducing the availability of this essential FA for marine bird egg synthesis. How this may affect marine bird reproduction is currently impossible to predict. However, monitoring potential changes in egg eicosapentaenoic acid content could be an important endpoint to track through time.

Research in the Arctic is logistically difficult and expensive and using biochemical tools such as stable isotopes and fatty acids is challenging since the laboratory work and analysis of these biochemical tracers must be done in southern Canada. The results of this study; however, demonstrate that despite the difficulties and potential setbacks (i.e. weather, logistics, etc) associated with research in the Arctic, that stable isotopes and fatty acids can be used for Arctic marine bird research. With the changes that are occurring in Arctic marine ecosystems (Huntington 2009; Polyak 2010) it is important to improve our ability to recognize ecosystem change. Incorporation of stable isotope and fatty acid markers into marine bird monitoring programs may provide valuable tools in Arctic marine ecosystem monitoring.

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