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ENERGY STORAGE DYNAMICS IN BREEDING ARCTIC SEABIRDS

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Dedicated to

Al and Suzanne Maas

-with all my thanks

"I have always been fascinated by the gifts of the magi;

Frankensense, Gold, and

a Murre!"

Lister Sinclair

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Abstract

This thesis describes investigations into the energy store (defined as the size of lipid stores) dynamics in breeding Arctic seabirds. The first chapter compares three species feeding at similar trophic levels but with different life history, physiological and foraging strategies, and breeding strategies. They managed their stores differently during poor environmental conditions. Though lipids were the primary tissue driving changes in body composition in Thick-billed Murres and Northern Fulmars, lean tissues were most dynamic in Black-legged Kittiwakes. Chapter two presents a method for evaluating the size of lipid stores non-lethally in Thick-billed Murres and Northern Fulmars using plasma fatty acid composition and concentration. Traditional (morphometric) tools had less predictive ability (especially for Thick-billed Murres) than plasma fatty acids. In Chapter three, I show that the plasma fatty acid signatures of Thick-billed Murres breeding at low and high Arctic colonies differ; likely an effect of differing diets. In addition, the concentration of plasma fatty acids increases during the breeding season, and is highest during chick-rearing. It is proposed that this may reflect a greater need for metabolic fuels as chick-rearing is likely the period of greatest energy expenditure. In Chapter four, the results of an egg replacement study are presented. Thick-billed Murres were made to lay a replacement egg following the removal of the first laid egg. The fatty acid signatures of the female and male plasma and of the first and replacement eggs were identified. Fatty acid signatures of eggs were highly conserved and those fatty acids found in the highest concentration in eggs were depleted in females after laying the replacement egg. In addition, from comparisons of the fatty acid signatures of eggs and potential prey species, I suggest that local sources of fatty acids could be utilised for the production of the replacement egg. In Chapter five, the way in which breeding adults manage an increase in the cost of diving is examined. In two experiments, conducted during two breeding seasons, the chick growth and plasma fatty acid concentrations of handicapped versus control adults were compared. Generally, adults passed on the increase in cost of diving to their chicks as chick mass gain was significantly less in some of the treatment groups. In addition, a feeding watch study suggests that this was because handicapped adults fed their chicks less frequently than control groups. This thesis represents the most physiologically intensive studies that have been conducted to date on wild breeding auks and is presented in the context of the potential effects of environmental change.

Contents

List of Figures

Figure 3.3: Components 1 vs. 2 from a Principal Component Analysis of plasma neutral lipid signatures from Thick-billed Murres sampled during late chick-rearing on Prince Leopold (open squares) and Coats (solid squares) Islands.

Figure 3.4: Components 2 vs. 3 from a Principal Component Analysis of plasma neutral lipid signatures from Thick-billed Murres sampled during late chick-rearing on Prince Leopold (open squares) and Coats (solid squares) Islands.

Figure 4.1: Mass of adult females (light bars) and males (dark bars) just after laying the first and replacement eggs 2004.

Figure 4.2: Fatty acid composition (% ± SE) for individual fatty acids for first versus replacement eggs. Fatty acids 16:0 and 18:1 are identified. A trendline representing the equation x=y is presented for comparison of values.

Figure 4.3: Components 1 vs. 2 for first (dark circles) and replacement eggs (open circles) from a principal component analysis. All fatty acids with component loadings of less than 0.5 for both components are excluded from the figure.

Figure 4.4: Components 2 vs. 3 for first (dark circles) and replacement eggs (open circles) from a principal component analysis. All fatty acids with component loadings of less than 0.5 for both components are excluded from the figure.

Figure 4.5: Total plasma lipid concentrations (mg/mL ± SE) of male (light bars) and female (dark bars) Brünnich's guillemots just after laying the first and replacement eggs at Coats Island in 2004.

Figure 4.6: Male plasma fatty acid composition (% of each fatty acid) after laying of the first and replacement egg (trendline in bold, r^2= 0.98). A line with equation x=y is also presented (fine line).

Figure II: A possible relationship between the timing of ice breakup (and consequently, prey availability) and reproductive success on Coats Island and Prince Leopold Island.

List of Tables

INTRODUCTION

Environmental changes in polar climates

Changes in the Arctic surface air temperature are associated with changes in precipitation, snow cover, area and depth of permafrost, vegetation distribution and sea ice cover (Moritz et al. 2002). While contention is still present over the precise allocation of responsibility and effects, it is difficult to deny that the climate is changing and that these changes are influencing the environment and ecology (Trivelpiece and Fraser 1996, Emslie *et al.* 1998; review Saether et al. 2004). The reason that few direct-evidence studies exist (see Moritz *et al.* 2002) lies with a lack of long-term data (Smith *et al.* 1999). Instead, investigators have utilised less direct measures, for example, using a combination of historical observations, modern instrumental records, ice core and marine sediment data, and seabird distributions to support the notion of rapidly warming polar climates (Smith *et al.* 1999). The effects of climate change are likely to be observed first at the Earth's poles and changes are predicted to be amplified compared with changes in more temperate environments (Morison et al. 2000). In the Arctic, water from the Atlantic Ocean is exerting a strong influence on the Arctic Ocean (e.g. Eastern water type is currently 20% greater in area than previously observed), there have been changes in surface ice drifts, and atmospheric circulation has shifted (Morison et al. 2000).

Seabirds as indicators

Identifying the relevant bioindicators is necessary for investigations into the effects of environmental change. A wide variety of species, representing all kingdoms, have been studied for this purpose. Birds, as the most visible and most widely distributed animals of all the ecosystems in which they are found, tend to be easily accessible (Montevecchi

1993) and, in the case of migration, travel great distances between different climates. Seabirds are also desirable bioindicators because they are at the top of the food chain, are particularly widespread, sample prey over large areas from a point source (colony), are very sensitive to changes in the distribution of prey (e.g. Davoren and Montevecchi 2003), and data are less expensive to collect than those from research cruises (Cairns 1987). In addition, evidence suggests that seabird populations experience large-scale changes in abundance due to environmental change and anthropogenic influence (Birkhead and Furness 1984; Wooler et al. 1992; Boersma 1998; Davoren and Montevecchi 2003). Though most literature evaluating the use of seabirds as bioindicators refers specifically to their value as indicators of marine ecosystem health - in particular, fish population abundance, and those of economic value above all - it is important to remember that other factors, which may or may not affect food distribution (i.e. air temperatures, wind speeds, frequency of storms, etc.), will also impact avian populations (e.g. Wooller et al. 1992; Miller et al. 1994; Cotegreave 1995; Eeva et al. 2002). Arctic seabirds may be especially useful as bioindicators because of growing concern for the impacts of environmental change on polar climates in particular.

Biological processes in Arctic and Antarctic marine ecosystems are closely tied to annual cycles of sea-ice break up and freeze up. Seabirds forage at ice edges where there is a higher concentration of prey species (Stirling 1997) than in open water. Current global warming trends are likely to advance the break up of sea-ice and reduce the amount of floating ice present at sea in the summer. Early ice break up may create unfavourable conditions for Thick-billed Murres (Gaston and Hipfner 2000, Coats Island, Nunavut) because it is at the ice edges that this species feeds to replenish energy stores. Conversely, earlier ice breakup in more northern regions (e.g. Prince Leopold Island) may at first, be beneficial for breeding seabirds and we may soon observe a slow northward displacement of seabird colonies (Gaston et al. 2005a). At present, little has been published on the effects of recent climatic changes on Eastern Canadian High Arctic marine ecosystems (but see Aanes et al. 2002; Morison et al. 2000) or their seabirds (but see Gaston et al. 2005a, 2005b). Research conducted on Prince Leopold Island and Coats Island presents an excellent opportunity to elucidate seabird reproductive strategy (see section Study Species) and success in relation to varying climatic conditions. Furthermore, manipulative experimentation on the southern colony may allow identification of the magnitude of influence that certain factors (e.g. egg production and prey availability) have with respect to reproductive success.

Reproductive investment

Throughout the life time of an individual specific decisions must be made concerning the division of effort between different activities (e.g. when to eat, rest, molt, forage, defend territory and, of particular interest to us, reproduce; Stearns 1992). It is generally assumed that organisms would reproduce continuously unless otherwise constrained. That continuous reproduction is not prevalent in nature suggests that some combination of factors confines reproduction to distinct periods. Decisions related to when to engage in an activity influence life-time reproductive success and are dependent upon the 'state' of the individual. The 'state dependent' approach to parenthood examines the physiological and environmental impacts on life history (Wendeln and Becker 1999), as opposed to the 'age related pattern' approach, which makes specific predictions regarding the outcomes of an individual's choices with reference only to the age of that individual (review: Wooller et al. 1992). Declining emphasis on the importance of age alone comes from the observation of variation in 'state' (size of energy stores, for example) among individuals of the same cohort that result in a range of responses to similar environmental conditions and may affect life-time reproductive success among individuals. In addition, and with reference specifically to long lived organisms, age cannot be the sole factor in the decision to reproduce if reproduction can be, individually, delayed (Wendeln and Becker 1999).

To support the 'state dependent' approach further, it is important to observe organisms controlling their physiological state (or body condition, defined here as the size of energy stores) in response to, among other things, environment, status (activity), and life history strategy. We can imagine a continuum of potential strategies, from one in which the organism carries the largest volume of energy stores that their body can support or that they can obtain from the environment (e.g. individuals carrying small energy stores are the result of poor environmental conditions or inexperience), to, at the other extreme, a situation in which energy stores are managed entirely by the individual and optimized with respect to current activities (e.g. stores during chick-rearing represent an optimum tradeoff between wing-loading and parental survival). In reality, most organisms must exist for most of their lives in some intermediate state: a compromise between what is available and what is optimal.

Observations of programmed anorexia during, or in anticipation of, an activity (i.e. chick-rearing) have been documented in a variety of avian species (Freed 1981; Croll *et al.* 1991, Gaston and Perrin 1993). Conversely, 'fattening' in anticipation of an energetically demanding activity (i.e. migration, McWilliams et al. 2004; Pierce and McWilliams 2005) or long period of resource unavailability (i.e. hibernation) supports the theory that animals rarely carry their maximum energy load (Gosler 1996). Gosler (1996) demonstrated that energy (lipid) stores in Great Tits (*Parus major*) were higher during years with poor environmental conditions and among individuals of lower social dominance. This observation is not consistent with the idea that lipid stores are passively determined by the environmental circumstances and suggests that, at least of this species, birds do not carry the maximum that the environment will permit but rather the minimum that the environment (and physiology) will allow. Likewise, Gaston and Hipfner (2006) showed that older, experienced Thick-billed Murres lose more mass than younger birds during chick-rearing, demonstrating that energy stores can be individually managed depending upon the degree to which they are willing to invest in current reproductive effort. It also suggests that age may be an important component of 'state'. Alternative explanations may exist, including that there is a difference in diet of older and younger birds, coupled with an annual decline in food availability for older birds. This, however, seems rather unlikely and the previous explanation has been supported by pervious literature on energy stores dynamics in other species (see Gosler 1996).

In birds and mammals, the size of lipid stores provides a very close estimate of the total size of energy stores. Though energy stores as defined by Blem (1990) include both carbohydrates and proteins, the size of carbohydrate stores are always very small (<5% of total) and proteins are not usually oxidized unless under extreme conditions (i.e. prolonged starvation or long-distance migration; approximately 20% of metabolic rate; but see Gaunt et al. 1990). Consequently, my research focused upon lipid stores and mobilisation, which I used as an index of body condition and energy expenditure.

Effects of food availability

Biological processes are commonly divided into those required for personal maintenance and survival and those required for reproduction, though some activities cannot be clearly assigned to one or the other of these processes (i.e. adult foraging during chick-rearing). All biological processes require an input of energy for execution and lipids are generally the primary source of the energy because lipid molecules yield more ATP than any other biological fuel (Allen 1976). Species within the class Aves have the highest rates of energy use and storage, most of which is lipid in the form of triglycerides, or neutral lipids (Blem 1976, Christie 2006). Though they can be produced within the body (Allen 1976), lipids are also derived from the food which an individual consumes. Essential fatty acids are those found within the diet which cannot be produced within the body, but are required for biological functioning.

The availability of food affects reproductive behaviour and success. Measuring different aspects of reproductive behaviour and performance for a seabird population is, potentially, an excellent technique for monitoring the effects of changes in food availability, and therefore, the effects of environmental change (Wooller et al. 1992).

Links have been made, for example, between variations in food quality at two breeding colonies and reproductive success (e.g. Atlantic Puffins *Fratercula arctica*: Harris 1983 in Birkhead and Furness 1984; Pigeon Guillemots *Cepphus columba*: Litzow et al. 2002; Humbolt Penguins *Spheniscus humboldti*: Hennicke and Culik 2005), between annual variations in food availability and reproductive success (e.g. McIntyre and Adams 1999) or both (e.g. Black-legged Kittiwakes *Rissa tridactyla*: Suryan et al. 2000). However, using measurements of reproductive success alone is not desirable if an early-detection system is preferred or if conditions have not caused dramatic differences in prey distribution (Cairns 1987). Birds are potentially able to buffer the effects of variable food supplies by altering their foraging effort (Burger and Piatt 1990) and therefore could limit the precision of reproductive success as a monitoring tool. Compensation, or buffering, however, requires an increase in parental investment, costing time and energy. Hence, using an index of body condition, or the rate of energy utilisation, is likely to be a sensitive early-detection tool, comparable among different breeding colonies and years. The size of lipid stores, used as an index of adult body condition can therefore provide information regarding the health of both the local food supply (Cairns 1992) and of the individual (Drent and Daan 1980; McNab 2002).

Changes in the availability of prey or the composition of available prey species, due to either changes in prey distribution or changes in ice conditions which limit accessibility, may have consequences for the survival and reproductive success of other species dependent upon that source of energy (Figure I). Responses to changing prey availability during reproduction are thought to be hierarchical (Cairns 1987). As prey availability declines, the first aspect to be affected is time budgets of adults. For example, adults may spend less time preening and resting and more time foraging (Burger and Piatt 1990; Common Murres *Uria aalge*). As prey availability continues to decline, the next activity to be affected is reproduction. For example, adults may have lighter chicks compared with reproductive seasons where prey is more readily available (Dawson and Bortolotti 2000; Baillie and Jones 2004), or may delay breeding until conditions improve (Barboza and Jorde 2002; Davoren and Montevecchi 2003). When there are drastic declines in the availability of prey adults will cease reproduction and individual survival will be affected (Vader *et al.* 1990, Barrett and Krasnov 1996).

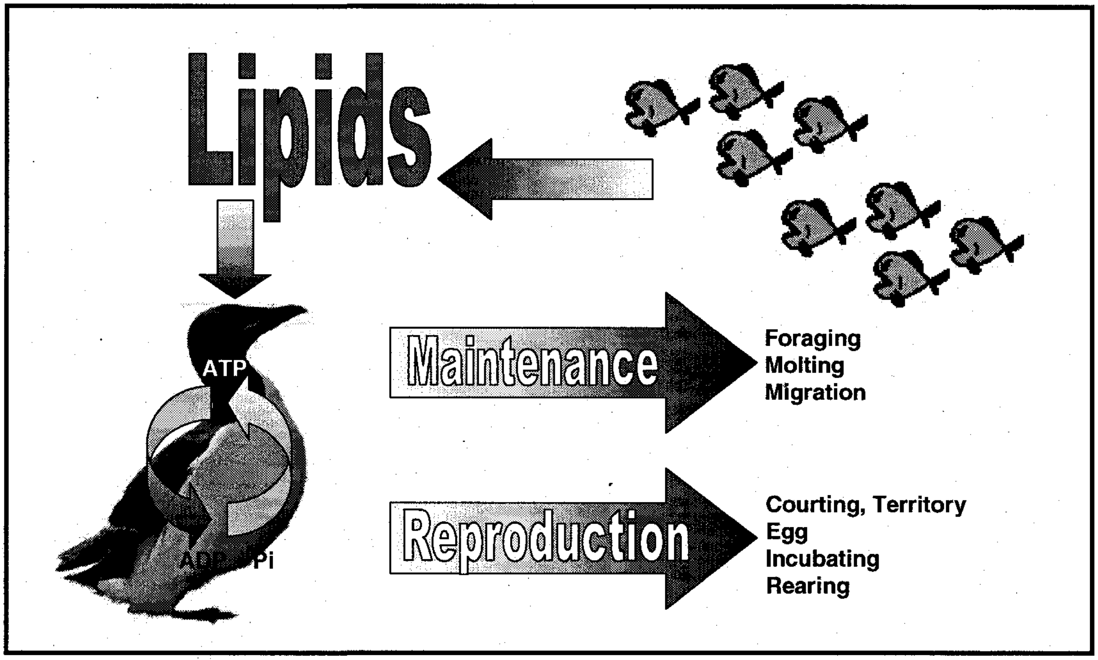

Figure I: Cycle of influence of prey availability on biological processes (with a few examples of specific activities). The availability of prey influences an individual's ability to acquire the necessary lipid for self-maintenance and reproduction. Consequently, a decline in prey availability will influence the allocation of energy to the two axes of biological activity.

Limitations related to foraging strategy or flight parameters may also direct the response to changes in prey availability. For example, Carscadden et al. (2002) found that during a decline in surface water prey species, Black-legged Kittiwakes' reproductive success declined because this species is unable to dive more deeply for prey. However, Common Murres (*Uria aalge*), and Atlantic Puffins (*Fratercula artica*) did not show breeding failures because they have more flexibility in diving range and dove deeper to compensate. In a similar study on the same decline in prey species availability, Bryant et al. (1999) found that both Common and Thick-billed (*Uria lomvia*) Murres were able to compensate completely for the change by seeking another prey species. There were no declines in reproductive success measures as a result.

Limitations may also be imposed by foraging range. Seabirds which have low wing-loading and store large amounts of food within the stomach, can afford to have longer foraging distances and times. Consequently, these species may be able to buffer changes in prey availability by seeking new foraging grounds over their wider range. Species which cannot forage at greater distances (eg. Due to limitations in wingload or incubation shift lengths), would have less of a buffer within which to search for new sources of prey. Similarly, the same applies to species that forage by pursuit diving. Surface feeders are unable to buffer changes in prey availability by foraging at different depths. Pursuit divers have access to a wider range of available species as a function of a greater vertical foraging range, though the cost associated with exploiting these larger ranges must also be considered.

Studying the effects of changes in food availability requires a significant amount of baseline knowledge, including, most importantly, the way in which individuals manage their energy stores and the importance that the size of these stores plays in successful reproduction. Biological factors such as wing loading, foraging range, and foraging strategy of each species will influence the directions of responses to changes. Only once this information is acquired can we begin to assess the effects of changing food availability as a consequence of events like environmental change.

Dramatic changes in the Arctic climate may cause a change in the human usage of this environment. Therefore, we must also consider the impacts upon those ecosystems whose conditions are not directly a consequence of changing environmental conditions. Recent expressions of national and international interest in Canada's Arctic combined with an increase in prospecting, oil exploration, and shipping are of great concern. Though migratory birds are generally protected under migratory bird legislation, we know that seabirds are particularly sensitive to large-scale changes in prey availability and local disturbances (Birkhead and Furness 1984; Gaston and Hipfner 2000). Increased shipping due to mining operations and the search for new routes connecting the Atlantic and Pacific Oceans will likely cause oil spills, environmental contamination, and shoreline degradation.

RATIONALE FOR THE THESIS RESEARCH

Body condition Indices

The accumulation of lipid is considered the main method of energy storage in most animals and the analysis of variation in the size of energy stores is a useful way to assess body condition (Johnson *et al.* 1985). Lipid is the most variable component of body composition, including in birds (Speakman 2001). For example, birds accumulate lipid stores prior to periods of reproduction (Drent and Daan 1980; Bromley and Jarvis 1993; Alisauskas and Ankney 1992) and declines in body condition are associated with the desertion of an egg or chick (i.e. Davis 1982). A negative correlation between mortality rates and body mass, independent of body size, has been observed in Herring Gulls (Coulson *et al.* 1983) and it was also observed that, mortality, over the course of a year was highest when the body mass of gulls is lowest (end of the breeding season). Lipid is also considered to be the most limiting nutrient throughout avian lifecycles (Johnson *et al.* 1985), limiting, for example, the duration of incubation and brooding shifts (McNab 2002). The storage of lipid is particularly important for birds. Both carbohydrates and protein storage, requiring large volumes of water (1 gram of fat stores more than 8 times the energy of hydrated glycogen; Weis-Fogh 1952), are cumbersome and maladapted for flight (Stryer 1995).

Determining the size of lipid stores can be accomplished in three ways: lethally, invasively, and non-invasively. Lethal methods are highly accurate because, generally, they include the chemical extraction of lipids from either the entire organism or from tissue subsamples. The non-lethal methods (invasive and non-invasive), and their resultant condition indices, must be shown to be accurate by comparing the results with those obtained employing the lethal method on the same individual. Condition indices derived without validation are difficult to interpret. However, condition indices can only be related to fitness if subsequent reproduction and survival can be measured (Figure I), in which case terminal sampling is impossible. An index based on a marker in the plasma avoids the requirement of killing birds (Chapter 2).

Once an accurate method for the non-lethal assessment of body condition, or energy mobilisation, has been developed, the strategies by which birds adapt to increases in the cost of reproduction can be examined.

Cost management, reproduction, and adult decisions

Previous studies on artificial increases in the cost of reproduction suggest that there is a difference in cost management between long and short-lived species (review; Mauck and Grubb 1995). In addition, the authors also suggest that there is a relationship between the response and the clutch size of the species. They suggest that because a single egg clutch in long-lived species contributes less to their lifetime reproductive success than multiple egg clutches do in short-lived species, parents of long-lived species are less likely to compromise their survival by increasing investment in reproduction than are short-lived species (Newton 1989; Wooller et al. 1992; Mauk and Grubb 1995). Short-lived species are therefore predicted to place a higher value (in terms of life time reproductive success) on current reproductive efforts because the chance of reproducing the following year is lower than for long-lived species. I describe two experiments where this prediction can be tested: increases in the cost of producing an egg (Chapter 5), and increases in the cost of foraging (Chapter 6). If the prediction by Mauk and Grubb (1995) holds true, then it is predicted that in both these experiments adults should maintain their condition at an expense to their current reproductive success. Since lipid is the primary source of energy for birds (Blem 1990), I anticipate that birds will manage the size of their stores in a way which maximises their reproductive success, given neutral environmental conditions or which maximises individual survival, at a cost to current reproductive success, should these conditions deteriorate.

Though the precise methods of lipid store mobilisation and utilisation have yet to be completely identified, it is known that they are mobilised through the plasma and delivered to muscle tissue for oxidation (McWilliams et al. 2004). Consequently, as the cost of reproduction increases between egg incubation and chick-rearing (Gaston 1985; Paredes et al. 2005), I anticipate that the concentration of plasma lipids will increase. Should parents respond to a further increase in the cost of reproduction by working harder, I also anticipate higher plasma lipid concentrations. However, if adults are preserving their own condition, and passing on the cost to current offspring, I anticipate that there will be no change in plasma lipid concentrations.

The study species

Thick-billed Murres are pursuit-diving seabirds that breed on cliffs in the Eastern Canadian Arctic during the summer, migrating northward from wintering areas in the NW Atlantic. They lay a single, large egg that can be replaced once or twice, following a 12-14 day period, if the first egg is lost due to predation or dislodgment. Males and females take 12 hour (Coats Island), 24 hour (Prince Leopold Island) (Gaston and Hipfner 2000) shifts incubating the egg or guarding the chick while the other forages for itself and/or the chick. Murres have a very high wingload and are highly adapted for foraging by pursuit diving, attaining average maximum depths of 105 m (this study; Chapter 5; Table I). At Coats Island, males incubate during the day while females incubate at night. Incubation lasts 32-33 days. Chick provisioning begins within a day of hatching and lasts 21 days on average. Once the chick has reached approximately one quarter adult mass, the chick departs the colony with the male, embarking on the longest swimming migration of any birds with the exception of penguins. The female remains at the breeding site for up to 14 days, after which point, she embarks on a flying migration to the common wintering grounds. (Gaston and Hipfner 2000)

Though there is a clear divergence of behaviour after the chick has fledged and begins to migrate, it has been generally assumed that males and females share equally in the responsibilities of chick-rearing while on the colony (Gaston and Hipfner 2000). However, a recently published paper (Paredes et al. 2006) showed that this may not always be the case.

Northern Fulmars also lay single egg clutches but if lost, these eggs are not replaced. The eggs are incubated for 48 days before hatching. The incubation shifts of Northern Fulmars are considerably longer than for Thick-billed Murres, lasting up to 15 days, though they are generally shorter (4-7 days; Hatch and Nettleship 1998). Chicks are first fed within 24 hours of hatching and are reared at the nesting site by parents for about 14 days. After this period, chicks are left unattended and parents return with food every 1-3 days. Fledging usually occurs after 50 days post-hatch, though some variability in the timing has been noted for Prince Leopold Island (Hatch and Nettleship 1998). Fledglings are generally heavier than adults at departure and leave by flying.

Northern Fulmars have a broad diet consisting of primarily invertebrates, including squid, and Arctic cod (*Boreogadus saida*) and utilise several methods of foraging including surface seizing, pursuit diving (up to 3 m in depth), surface diving, and piracy (Hobson and Welch 1991). The species has a lower wingload than Thick-billed Murres, and long foraging ranges (Table I).

Black-legged Kittiwakes can lay up to 3 eggs per clutch, with eggs laid at 2 -3 day intervals. Chicks generally hatch 25 days after clutch completion. Males and females take turns incubating for 5-6 hours each. However, sometimes the nest is unattended, especially when food availability is low (Baird and Gould 1983). Chick feeding begins immediately after hatching and both parents deliver food to the nest. Fledging occurs between 34 and 58 days post-hatch, though some chicks take short flights before departing permanently.

Black-legged Kittiwakes are also surface feeders and capture pelagic schooling fish, such as sandlance (*Ammodytes hexapterus*) by plunge diving (Jodice et al. 2002) at short distances from the breeding colony. They have the lowest wingloading of the species considered here (Table I).

Table I: Relevant biological differences between the study species Thick-billed Murre (TBMU), Northern Fulmar (NOFU), and Black-legged Kittiwake (BLKI).

Species	Chicks per nest	Incubation Shift Length	Foraging Range (km)	Max. Diving Depth (m)	Wing Load[a] N/cm^2	Foraging Mode	Sources
TBMU	1	12-24 hr	<100	105	15.7	Pursuit Diver	Gaston et al. 2003
NOFU	1	4-7 d	120-466	3	5.9	Surface	Weimerskirch et al. 2001; Hobson and Welsh 1991
BLKI	1-3	5-6 h	20-60	Neg.[b]	3.5	Surface	Suryan et al. 2000

[a] Defined as weight per unit area of the wing: 9.8 (mass)/wing area (Gaston and Elliot 2005)
[b] Negligible

Study sites

The research for this thesis was conducted on two Canadian Arctic seabird colonies. Prince Leopold Island is located in Barrow Sound and is Canada's 4th largest seabird community in the Arctic. It is the only colony where large numbers of four different species (Thick-billed Murres, Black-legged Kittwakes, Northern Fulmars, Black Guillemots) breed and it has been visited by researchers since the 1970s. The limestone cliffs on which seabirds nest at Prince Leopold Island are 245-265 meters in height. Coats Island is located at the north end of Hudson Bay in Canada's Low Arctic region. Two small cliffs, approximately 75 meters high, support Thick-billed Murres colonies: one of these colonies has been studied since the 1980s. In the first three Chapters, I describe research that was conducted on three seabird species (Thick-billed Murres, Northern Fulmars, Black-legged Kittiwakes) on Prince Leopold Island from data collected during the 2002 breeding season. In Chapter 4, I describe research conducted on Thick-billed Murres sampled on Prince Leopold Island in 2002 and Coats Island in 2003. Chapters 5 and 6 describe research conducted on Thick-billed Murres breeding on Coats Island during the 2003 and 2004 breeding seasons.

Objectives

In Chapter 1, I tested whether breeding seabirds adjust the size of their energy stores in relation to reproductive activity. I predicted that any observed changes would reflect the different breeding strategies of each species and the local environmental conditions. In Chapter 2, I developed a non-lethal body condition index (a predictor of the size of energy stores) in Thick-billed Murres and Northern Fulmars. I predicted that the use of plasma fatty acids for the development of a condition index would improve the predictive ability of the model over traditional measurements. In Chapter 3, I tested the assumption that chick-rearing is the most energetically demanding reproductive period for adult

Thick-billed Murres breeding on Prince Leopold Island and Coats Island during different years (2002 and 2003, respectively). In Chapter 4, I increased the cost of egg production in female Thick-billed Murres. I tested the prediction that adults preserve their own condition at the expense of the current reproductive effort and that exogenous energy stores are utilised in the production of a replacement egg. In Chapter 5, I increased the cost of chick-rearing in male and female Thick-billed Murres. I tested the prediction that adults preserve their own condition at the expense of current reproductive effort and searched for possible mechanisms by which this cost is transferred.

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CHAPTER 2

PLASMA FATTY ACID PROFILE IMPROVES PREDICTION OF BODY FAT: COMPARISON OF LETHAL AND NON-LETHAL BODY CONDITION INDICES FOR NORTHERN FULMARS AND THICK-BILLED MURRES

ABSTRACT

Changes in the reproductive success of birds are often used to monitor the status of marine ecosystems in relation to food availability and environmental change. However, internal energy stores can buffer variable food supply, and changes in the size of lipid stores can provide a more sensitive, early-detection indicator of environmental shifts. Direct measurements of body condition are most accurate but lethal, and traditional non-lethal indices are rarely validated. The goal of this study was to develop improved, non-lethal indices of total fat stores based on plasma lipids. Total fat stores and the fatty acid composition of plasma lipids (neutral lipids, free fatty acids and phospholipids) were determined for 42 Thick-billed Murres (*Uria lomvia*) and 25 Northern Fulmars (*Fulmaris glacialis*). Using a backward stepwise multiple regression, I found that multivariate indices based upon fatty acid concentrations and one or two external measures were better predictors (R^2_a=0.72 for murres and 0.87 for fulmars) of body lipid than traditional indices based exclusively on body mass and external measures (R^2_a=0.36 for murres and 0.72 for fulmars). A non-lethal body condition index potentially matching the accuracy of traditional lethal indices may now be available to investigate a fundamental aspect of avian physiology: variation in the energy stores of wild birds.

INTRODUCTION

Lipid is the main form of energy storage in animals (Griminger 1986), making variation in lipid stores a good monitoring tool for assessing body condition (Johnson et al. 1985). The use of lipid as a source of energy is particularly crucial for birds because alternative metabolic fuels require large volumes of water for storage (one gram of fat stores more than 8 times the energy of hydrated glycogen; Weis-Fogh 1952), and are therefore cumbersome and maladaptive for flight (Stryer 1995). In Chapter 1, I show that lipid stores change in Thick-billed Murres and Northern Fulmars depending upon reproductive activity, and a decline in body condition below a threshold can be associated with egg or chick desertion in seabirds (Drent and Daan 1980; Davis 1982). Fat stores are considered one of the limiting factors throughout avian lifecycles (Johnson et al. 1985), limiting, for example, the duration of incubation and brooding shifts (McNab 2002). Though energy stores also include other metabolic fuels, carbohydrate stores are always very small (<5% of total) and a significant contribution from proteins is limited to extreme conditions of prolonged starvation or long-distance migration (but see Gaunt et al. 1990). Hence, in this study, I develop a new tool to measure fat stores, and use this parameter as an index of total energy stores, or body condition.

Generally, there are two approaches to assessing energy stores: 1) lethal methods (dissection and carcass analysis), and 2) non-lethal methods divided in 2 categories: a) external methods (e.g. mass, body measurements), and b) internal methods (e.g. blood analyses).

Lethal methods

Because the direct measurement of whole-organism energy stores is costly and time consuming, the use of tissue subsamples has been exploited as a more convenient alternative. Previous indirect measures include: skin mass (Bailey 1979, Chappell and Titman 1983), gizzard lipid mass (Kierth and Guthery 1983), wing lipid mass (Norman and Kirkpatrick 1981, in Blem 1990), and abdominal lipid mass (Thomas et al. 1983). Lethal approaches exclude the possibility of a longitudinal study and can compromise the population status of endangered species (Johnson et al. 1985, Blem 1990, Speakman 2001). In addition, differences in body condition can only be related to fitness if subsequent reproduction and survival can be measured.

Non-lethal methods

External. For practical and ethical reasons, total body mass combined with some other measure of body size has been used routinely to estimate the body condition of wild animals (e.g. Chastel et al. 1995, three petrel species; Cattet et al. 2002, three ursid species; Dinsmore and Collazo 2003, Sanderlings *Calidris alba*; Erikstad et al. 1997, Atlantic Puffins *Fratercula arctica*; Golet and Irons 1999, Kitaysky et al. 1999a, Black-legged Kittiwake *Rissa tridactyla*; Jakob et al. 1996, three arachnid species). However, body mass alone is a poor indicator of total lipid stores (Mascher and Marcstrom 1976, Golet and Irons 1999) and incorporating other measures of body size is based on scaling relationships that are not isometric (Speakman 2001). For birds, indices based on body mass corrected for size are inaccurate for these and other reasons: food in the gut; changes in water content; differences in protein content with individual size (Iverson and

Vohs 1982, Blem 1990). They can usually only provide a rough indication of changes in lipid content with some, species-specific exceptions. For example, breast cavity lipid is routinely assessed by external inspection in passerines, but this method is not available for species with opaque skin or very dense feathers such as seabirds.

In species for which energy stores fluctuate dramatically, mass, whether or not corrected for size, may be an accurate predictor of energy stores. However, size-corrected mass indices are unlikely to be accurate predictors for species in which energy stores fluctuate over a narrow range, and this range probably depends on the particular activities of individual species. For example, individual Thick-billed Murres *Uria lomvia* incubate eggs for no more than three days at a time (Gaston and Hipfner 2000), while Northern Fulmars *Fulmaris glacialis*, have been observed incubating for more than 15 days (Hatch and Nettleship 1998, AJG unpubl.). In addition, Thick-billed Murres experience the highest wing-loading of any bird while Northern Fulmars have a relatively low wing-load and are therefore more likely to carry relatively more lipid than murres. Because the range in the size of energy stores of Northern Fulmars will likely exceed those of Thick-billed Murres, I anticipate that size-corrected body mass will be a more accurate predictor of energy stores for Northern Fulmars than for Thick-billed Murres.

Internal. Internal techniques typically involve blood sampling, and to a lesser extent, tissue sampling. Many plasma-derived parameters (metabolites, hormones etc.) may be correlated with the size of energy stores, and using indices based on such parameters avoids the need to kill animals [e.g. mice *Mus musculus* using leptin (Hwa 1997), Herring

Gull *Larus argentatus* using glucose, triacylglycerides, uric acid, non-esterified fatty acids, or corticosterone (Jeffrey 1985), Black-legged Kittiwake using corticosterone (Kitaysky et al. 1999b)]. Though not commonly used by ecologists, some success has been achieved with these techniques and interest is growing in spite of the relative complexity of sampling and analysis. Here, I reasoned that indices derived from circulating lipids might yield better estimates of fat stores than indices based on other compounds.

The lethal approach is the most accurate method for evaluating the size of lipid stores and it will be used in this study to evaluate the accuracy of non-lethal approaches (external and internal). I chose to use Thick-billed Murres and Northern Fulmars as test species because of their very different incubation strategies (incubation shifts of 12-48 h vs. 2-14 d, respectively, AJG unpubl.), foraging ranges, wing-loading, feeding strategy, which likely reflect differences in energy store fluctuations. The goal of this study was to develop a novel body condition index combining the accuracy of traditional lethal methods with the convenience of non-lethal ones. I anticipated that the concentrations of key plasma lipids, either alone, or in combination, could be used as accurate predictors of the size of lipid stores.

MATERIALS AND METHODS

During June-August 2002, I collected 42 Thick-billed Murres and 26 Northern Fulmars from breeding colonies at Prince Leopold Island (74’02°N, 90’00°W), Nunavut, Canada. Most birds were identified as breeders (incubating or brooding when collected), though a 4 appeared to be non-breeders (they were not sitting on eggs or chicks). Birds were taken after egg laying, at the end of incubation (murres only), and after hatching; at times when the variation in body condition was expected to be greatest. At time of capture, each bird was weighed and 10 mL of blood were collected from the brachial vein in vacutainer tubes using syringes (23 gauge needles). Blood samples were placed immediately on ice (for up to 6 hours). The bird was then killed using a guillotine and placed in a sealed plastic bag. Upon returning to the field camp, blood samples were centrifuged and the plasma was transferred to cryovials. Morphometric measures (wing, tarsus, and culmen lengths) were taken from all birds. I measured tarsus and culmen length using calipers (±0.05mm), and wing length (longest primary) was measured using a ruler (±0.5cm). Carcasses and plasma were placed in frozen storage in a propane-operated freezer (approx. -20°C) until they could be shipped to the laboratory in Ottawa, Canada, in a dry nitrogen shipper (plasma) or on wet ice in a well sealed cooler (carcasses).

Ethical concerns associated with the killing of wild migratory birds were considered intensely. The benefit of developing a non-lethal method for the assessment of the size of fat stores necessitates the killing of individuals to accomplish this goal. All birds were used in conjunction with two other research projects. All required permits were obtained and include a permit for scientific take or disturbance of migratory birds (NUN-SCI-01-03 (CWS); Wildlife Research Permit WL000174, Government of Nunavut), National Wildlife Research Centre Animal Care Committee Permit (02M00G02, NWRCACC operated under the guidelines of the Canadian Council on Animal Care) and an Animal Care Committee Permit from the University of Ottawa (BL-172, ACVS).

Upon arrival in Ottawa, carcasses were thawed partially and feathers were removed (all but head and wing feathers). The abdomen and breast feathers of Thick-billed Murres were shaved because they were very difficult to pluck and because plucking these feathers would have extracted fat and muscle from beneath the skin. After plucking and weighing the feathers, the birds were refrozen until they were completely thawed for dissection.

Total body lipid

Total lipid was measured by adding the contributions of lipid from various tissues. Lipid extractions were conducted on five tissues (or combined tissues); breast muscle, liver, skin, combined visceral organs (except liver), and combined carcass (including bones). The skin, organs and carcass were homogenized prior to collection of the sub-samples. Breast muscle and liver samples were collected from the whole, unground organ. Each tissue was homogenized in Folch reagent (chloroform: methanol, 2:1 v/v). After filtration (Whatman 1) to remove solid material, KCl (0.25% dissolved in distilled water) was added to remove any remaining non-lipid components. Samples were placed in a water bath at 70°C for 5 min to produce a two phase solution. After removal of the aqueous phase, the organic phase was evaporated to yield only lipids, which were weighed (+/- 0.001g).

Based upon the mass of the lipid in each subsample, total lipid mass was determined for each whole tissue. Total lipid mass for the individual bird (minus the legs, wings, head) was the sum of these five values. Percent body lipid was determined by dividing total lipid mass by live body mass measured at capture.

Plasma Lipid Extraction and Analysis

Plasma was added to Folch reagent (2:1 v/v). An internal standard (Heptadecanoic acid, 17:0, 30 mg/100 mL hexane) was added for gas chromatography (GC) analysis of the non-esterified fatty acids (NEFA). The resulting mixture was centrifuged at 3000 RPM for 10 min. The supernatant was filtered and KCl (0.25% in distilled water) was added to eliminate water-soluble compounds. This mixture was centrifuged at 3000 RPM for 10 min to facilitate the separation of an aqueous phase. This phase was eliminated with a water-powered vacuum pump and the remaining solvent was N_2 evaporated at 70 °C.

Separation of Neutral Lipid, Non-esterified Fatty Acid and Phospholipid Fractions.

Total plasma lipids can be divided in 3 classes: neutral lipids (NL), non-esterified fatty acids (NEFA) and phospholipids (PL). These classes were separated to analyze their fatty acid composition. Supelclean solid-phase extraction tubes (LC NH_2, 100 mg: Sigma) were conditioned with hexane. The lipids were resuspended in chloroform and transferred into the columns. The NL fraction was eluted by flowing chloroform:isopropanol (2:1 v/v) through the columns. An internal standard (17:0, 30mg/100mL hexane) was added to the neutral lipid fraction. The solvent was evaporated under N_2 at 70 °C.

The NEFA fraction was eluted by flowing isopropyl ether:acetic acid (98:2 v/v) through the columns. The solvent was evaporated under N_2 at 70 °C.

The PL fraction was eluted by flowing methanol through the columns. An internal standard (17:0, 30mg/100mL hexane) was added to the neutral lipid fraction. The solvent was evaporated under N_2 at 70 °C.

Methylation of NEFA fraction for Gas Chromatography Analysis. Methylation is required to increase the volatility of the lipid samples for GC analysis. NEFA were resuspended in methanol, dimethoxypropane and 6N HCl. The mixture was incubated at room temperature for 20 min and evaporated under N_2 at 70 °C. The methylated lipids were resuspended in isooctane and transferred into GC autosampler tubes.

Acid Transesterification of Neutral Lipid and Phospholipid fractions for Gas Chromatography Analysis. Lipids were resuspended in an acetyl chloride solution (7.2 mL acetyl chloride in 100 mL methanol). Samples were incubated at 90 °C for 2 h after which the acetyl chloride in methanol was evaporated under N_2 at 70 °C. Fats were resuspended in methanol and evaporated under N_2 at 70 °C. The lipids were resuspended in isooctane and transferred to GC autosampler tubes.

All samples were then analysed using GC (HP 5890 series II with HP 7673 autosampler and flame-ionization detector). The retention times of the fatty acids were compared with those from known standards.

Statistical analyses

Backward stepwise multiple regression analysis (*F-stat* entry criterion = 4.00) was used to determine which variables were the best predictors of total body lipid for each species. However, the results presented in Tables 1-4 show a cumulative model to clarify the contribution of each variable to the accuracy of the prediction. Data presented in Tables 1 and 2 are means ±S.E. All statistical analyses were conducted using SYSTAT version 9 software and the level of significance was set at 0.05.

RESULTS

Mean body lipid was 6.4% (±2.5) of body mass for Thick-billed Murres and 12.0% (±4.1) for Northern Fulmars ($F_{1,66} = 51.9$, $p<0.001$, n=68; Figure 2.1). The difference in percent body lipid between these species probably reflects their large difference in the duration of incubation shift.

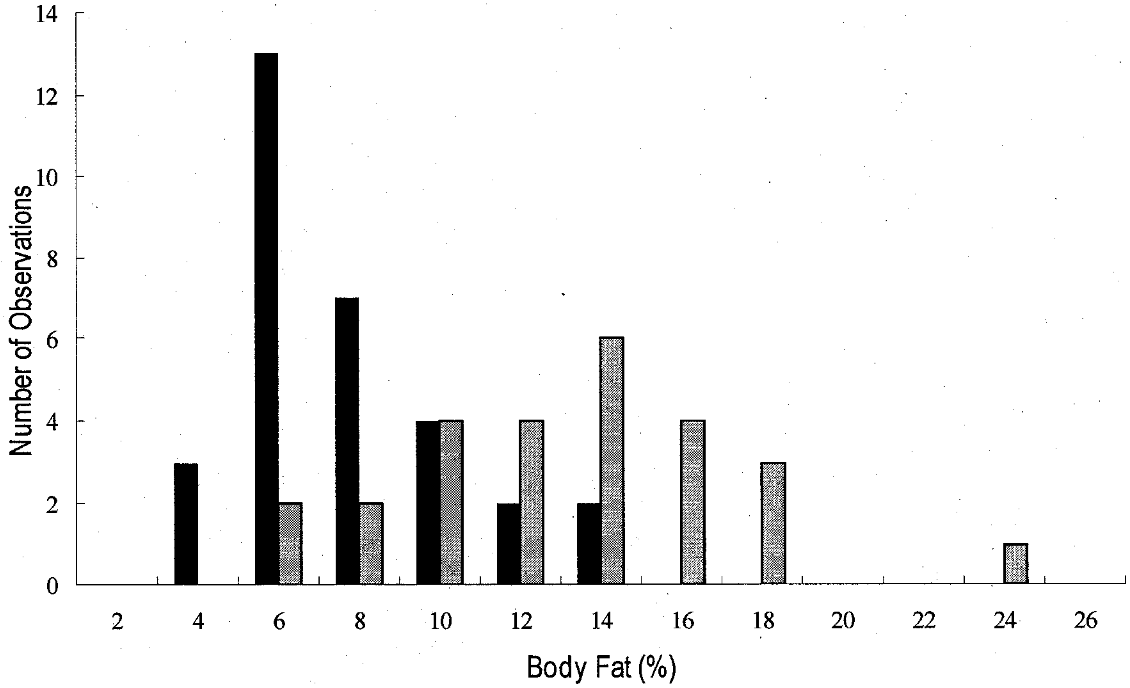

Figure 2.1: Frequency distribution of percent body lipid for Thick-billed Murres (N=42, black bars) and Northern Fulmars (N=26, grey bars).

The most lipid was found in skin, which contained 53% total lipid in Thick-billed Murres and 47% in Northern Fulmars (Table 2.1). For both species, skin lipid mass was the most accurate predictor of total body lipid (Thick-billed Murres: $R^2= 0.92$; Northern Fulmars: $R^2=0.90$) and had the highest percent lipid composition of all tissues (Thick-billed

Murres: average = 29%, Northern Fulmars: average = 46%) (Table 2.1) Liver lipid mass (Northern Fulmars) and breast muscle lipid mass (Thick-billed Murres) were not correlated with percent body lipid.

Table 2.1: Lethal predictors of total lipid (mean ± SE, R_a^2) in Thick-billed Murres and Northern Fulmars.

	Thick-billed Murres N=42		Northern Fulmars N=26	
Predictor Variable(s)	Mean lipid mass	R_a^2 of Total body lipid[b]	Mean lipid mass	R_a^2 of Total body lipid
Breast muscle lipid mass	2.4 (0.9)	0.00	2.6 (0.8)	**0.64***
Visceral organ lipid mass	2.8 (1.2)	**0.35***	6.2 (4.2)	**0.68***
Liver lipid mass	1.8 (0.6)	**0.49***	0.8 (0.2)	0.04
Subcutaneous lipid mass[a]	23.6 (11.9)	**0.94***	40.3 (18.1)	**0.90***
Carcass lipid mass	19.8 (6.7)	**0.67***	35.3 (12.4)	**0.57***

* $p < 0.001$, significant results are bolded.

[a] Thick-billed Murre: Total lipid=15.09+1.311(Subcutaneous lipid mass)
Northern Fulmar: Total lipid=25.92+1.43(Subcutaneous lipid mass)

[b] Total Body Lipid = (Visceral organ lipid mass)+(Liver lipid mass)+(Subcutaneous lipid mass)+(Carcass lipid mass, including breast muscle)

External measurements, including mass, were poor predictors of total body lipid in Thick-billed Murres (R^2 values ranged from 0.27 to 0.36, Table 2.2). In accordance with the prediction based upon the range in the size of lipid stores, R^2 values (or adjusted R^2

values) for models using morphometric and mass measures were higher for Northern Fulmars (maximum $R^2 = 0.72$) than for Thick-billed Murres (maximum $R^2 = 0.36$).

Table 2.2: Non-lethal external predictors of total body lipid in Thick-billed Murres and Northern Fulmars.

Predictor Variable(s)	Thick-billed Murres (N=42) R_a^2	Northern Fulmars (N=26) R_a^2
Mass	**0.27***	**0.52****
Mass, Culmen	**0.36***[a]	**0.56***
Mass, Culmen, Wing	**0.35***	**0.57***
Mass, Culmen, Wing, Tarsus	**0.35***	**0.67***
Mass, Culmen, Tarsus, Tube Depth	NA	**0.72***[b]

*$p < 0.05$, **$p < 0.001$

[a] For Thick-billed Murres: Total lipid=-275.79+0.191(Mass)-4.95(Culmen)

[b] For Northern Fulmars: Total lipid=138.14+0.35(Mass)-8.50(Culmen)-4.81(Tarsus)+14.03(Tube depth)

Plasma lipids, combined with one (Thick-billed Murres) or two (Northern Fulmars) morphometric or mass measures, were the most accurate, non-lethal predictors of total body lipid in both species (Tables 2.3 and 2.4). For Thick-billed Murres, the best prediction using a single fatty acid was obtained from NL 20:5 (R^2=0.32); the R^2 was 0.66 when 7 fatty acids were used and this value reached a maximum of 0.72 when a combination of lipids and external measurements was used (culmen + 7 fatty acids). For Northern Fulmars, the best prediction using a single fatty acid was obtained from PL 20:1 (R^2=0.43); the R^2 was 0.68 when 6 fatty acids were used and this value reached a maximum of 0.87 when a combination of plasma lipids and external measurements was used (culmen, mass and 6 fatty acids). NL 20:5 appeared in most combinations of variables for both species (all in Thick-billed Murres and all but two in Northern Fulmars) while PL 20:5 appeared in the best three for Thick-billed Murres and the best predictor for Northern Fulmars.

Table 2.3: Non-lethal internal predictors of total body lipid in Thick-billed Murres.

Predictor Variables	R_a^2 (N=42)
NL[a] 20:5	**0.32***
NL 20:5, NEFA[b] 20:5	**0.39***
NL 20:5, NEFA 20:5, Culmen	**0.46***
NL 18:0, 18:1, 20:5, 22:6, Culmen	**0.58***
NL 18:0, 18:1, 20:5, 22:6, NEFA 16:1, 18:1, PL[c] 20:5	**0.66***
NL 18:0, 18:1, 20:5, 22:6, NEFA 16:1, 18:1, PL 20:5, Culmen[d]	**0.72***

* $p < 0.001$

[a] Neutral Lipid

[b] Non-esterified Fatty Acid

[c] Phospholipid

[d] Total Lipid = -96.90+63.15(NL18:0) - 83.27(NL18:1) + 207.65(NL20:5) 150.48(NL22:6)-225.54(NEFA16:1)+135.20(NEFA18:1)-5.85(PL20:5) + 4.46(Culmen)
Percent Body Lipid= -10.62+6.23(NL 18:0)-9.51(NL 18:1)+22.41(NL 20:5)-17.02(NL 22:6)-23.75(NEFA 16:1)+14.64(NEFA 18:1)-0.76(PL 20:5)+0.51(Culmen), R^2=0.75.

Table 2.4: Non-lethal internal predictors of total body lipid in Northern Fulmars.

Predictor Variables	R_a^2 (N=26)
PL[a] 20:1	**0.43***
PL 20:1, Mass	**0.67***
NL[b] 20:4, 20:5, NEFA[c] 22:6, PL 16:1, 22:0, 22:6	**0.68***
NL 20:5, PL 20:1, Mass, Culmen	**0.71***
NL 20:5, NEFA 16:1, 18:1, PL 18:2, 20:1, 20:5, 22:6, Mass, Culmen[d]	**0.87***

* $p < 0.001$

[a] Phospholipid

[b] Neutral Lipid

[c] Non-esterified Fatty Acid

[d] Total lipid=102.51-42.54(NL 20:5)-622.96(NEFA 16:1)+151.88(NEFA 18:1)+147.35(PL 18:2)-20.72(PL 20:1)+48.70(PL 20:5)-35.85(PL 22:6)+0.43(Mass)-10.63(Culmen).

Percent Body Lipid=20.96-7.26(NL 20:5)-84.82(NEFA 16:1)+17.63(NEFA 18:1)+22.28(PL 18:2)-1.65(PL 20:1)+6.99(PL 20:5)-4.99(PL 22:6)+0.04(Mass)-1.34(Culmen), R^2=0.87.

DISCUSSION

The results show that a combination of specific plasma fatty acids and external measurements can be used to predict the size of energy stores more accurately than traditional techniques. The technique proposed here does not require the killing of birds and allows researchers to monitor the same individuals over time.

Marine birds are being used increasingly to assess changes in marine ecosystems (Springer et al. 1986, Cairns 1987, 1992, Montevecchi 1993a, b). Measuring different aspects of their reproductive behaviour and performance could be an excellent strategy for monitoring the effects of changes in food availability, and hence the effects of environmental change (e.g. changes in prey stocks, climate change). However, birds may buffer the effects of variable food supplies by altering their foraging effort (Burger and Piatt 1990). Compensation, or buffering, requires an increase in parental investment, costing time and energy. Hence, using an index of body condition can provide a sensitive early-detection tool, comparable among breeding colonies and years. Body condition influences both reproductive success and individual survival (Davis 1982, Loman 1984, Monaghan et al. 1989, Wendeln and Becker 1999, Hanssen et al. 2002, Ost et al. 2003), and can therefore provide information regarding the state of local food supply (Cairns 1992), of the individual (Davis 1982, Drent and Daan 1980, McNab 2002) and, in a single measure, can quantify key factors influencing fitness (Speakman 2001).

Problems exist with body condition indices based upon external measures because depending upon how the index is calculated, widely different conclusions are reached. Moreover, indices are often ambiguous because they are used as indicators of very different things (i.e. fitness, health, status) (Dabbert et al. 1997, Gosler and Harper 2000, Speakman 2001). In addition, this and other studies (Bailey 1979, Redhead Duck *Aytha americana*; Iverson and Vohs 1982, Sandhill Crane *Grus canadensis*; Conway et al. 1994, Wood Thrush *Hylocichla mustelina*; Golet and Irons 1999, Black-legged Kittiwake *Rissa tridactyla*; Quillfeldt and Peter 2000, Wilson's Storm-Petrel *Oceanites oceanicus*) demonstrated that external measures alone, though simple to execute, are not adequate predictors of lipid stores. Studies demonstrating no change in body condition may not have been sensitive enough to detect any due to the imprecise nature of the tool. In this study however, external measures did increase the predictive power, suggesting that lipid mass is partly explained by size.

Previous measures using blood markers to predict body lipid, body composition, or mass loss have shown some success. Alonzo-Alvarez et al. (2002) found that plasma cholesterol concentrations accounted for 75% of the deviance in percent total body mass loss in Caspian Gull *Larus cachinnans*. Dabbert et al. (1997) found that combining plasma triglyceride concentration with total body mass in mallards increased the R^2 by 0.12, but only for males. Quillfeldt et al. (2004) found that plasma triglycerides were positively correlated with body mass in adult Wilson's Storm-Petrel *Oceanites oceanicus* (R^2=0.13, P=0.015). Guglielmo et al. (2002) suggested that, though rates of fattening are positively correlated with plasma concentrations of triglycerides (neutral lipids), phospholipid concentrations should also be considered in combination because they are likely to improve predictive accuracy.

Problems associated with not validating body condition indices are obvious, but non-validated indices are frequently used. In addition, Spengler *et al.* (1995) observed that the relationships among condition indices and lipid mass vary among species. This study demonstrates interspecific variation in accuracy that can be achieved using external measures alone as an indication of lipid stores. In species that experience high fluctuations in the lipid stores, body mass, corrected by external measures, may be sufficient depending upon the precision of the measure required. Studies that have found a good correlation between external measures and body lipid have been conducted on species with lipid stores that fluctuate over a wide range (e.g. Skagen *et al.* 1993, sandpipers; Chappel and Titman 1983, Greater Scaup *Aythya marila* and Lesser Scaup *A. affinis*). A comparative study assessing the size of lipid stores in 4 species showed that mass was the least accurate predictor in the species with the lowest variation in total lipid mass (Red-winged Blackbird *Agelaius phoeniceus*, Spengler *et al.* 1995).

The majority of energy used to power flight and diving is derived from the oxidation of lipids stored in adipose tissue (McWilliams et al. 2004). Support for this comes from observations that the fat loads in adipose tissue of migrant avian species can comprise up to 50% of body mass and that adipose tissue can be severely depleted by migration. Though the specific functions of individual fatty acids are still largely unknown, the types of fatty acids found in the circulation have specific functions. Neutral lipids, generally found in the form of triglycerides (three fatty acid chains esterified to a glycogen backbone) have been identified as the primary energy storage molecule and are mobilised from energy stores through the circulation in the form of lipoproteins (packages of lipids bound within a phospholipid membrane). Non-esterified fatty acids, bound to albumin, can be transported from the energy stores, though the circulation to the muscle for oxidation (review McWilliams et al. 2004) though my study shows that they are relatively unimportant (as a function of concentration) in the three seabirds studied. Rather, it seems likely that liproproteins (composed primarily of neutral lipids and phospholipids) are an important transport mechanism of fatty acids from adipose tissues and the liver through the circulation to muscle tissue for oxidation (Jenni-Eiermann and Jenni 1992). Phospholipids are structural fatty acids, found primarily in cell membranes (Stryer 1995).

Because plasma and dietary fatty acids are related (Budge *et al.* 2002), it is possible that the usefulness of this new indices will be reduced for interannual or inter-population comparisons when dietary shifts occur. However, in environments where diet is constant, this tool should allow serial, non-lethal sampling of the same individuals and will therefore allow researchers to collect data on changes in the size of energy stores in response to specific activities or season. The results show that plasma fatty acid profiles add significantly to our ability to predict the size of lipid stores without killing birds. This method is particularly useful in species such as Thick-billed Murres, where variation in the size of lipid stores is relatively small and hence hard to determine from mass, even when corrected for size.

To my knowledge, this is the first study in which plasma fatty acid signatures have been proposed as a tool to investigate changes in the size of energy stores. This new approach has allowed development of a more accurate, non-lethal body condition index than previously used in ecological studies. At first consideration, the technical knowledge required to carry out the analyses described here may appear overwhelming. However, this relative complexity is a small price to pay in view of the advantage provided by the accuracy of this new tool. A non-lethal body condition index matching the accuracy of traditional lethal indices is now available to investigate a fundamental aspect of ecological systems: the variation in the energy stores of wild animals.

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CHAPTER 3

CHANGES IN PLASMA FATTY ACID CONCENTRATIONS DURING THE REPRODUCTIVE SEASON:

A COMPARISON OF THICK-BILLED MURRES BREEDING AT HIGH AND LOW ARCTIC COLONIES

ABSTRACT

The chick-rearing period is thought to be the most energetically demanding for Thick-billed Murres. Plasma lipid concentrations increase with exercise in a variety of avian species (McWilliams et al. 2004). Consequently, if chick-rearing requires an increase in the mobilisation of energy stores to fuel higher demands, I predict that plasma lipid concentrations will be higher in birds rearing chicks than those incubating eggs, reflecting a depletion in the size of their lipid stores. This prediction is tested by comparing the plasma concentrations of total lipids and neutral lipids during the breeding season on Prince Leopold Island in 2002, and Coats Island in 2003. Total plasma lipid concentrations increased throughout the season on Coats Island and there was a trend towards increasing concentrations on Prince Leopold Island. Neutral lipid concentration also increased on Coats Island but there was no significant increase on Prince Leopold Island. In addition, it was found that the fatty signatures were different in birds for each colony, suggesting that their diets are different. These results are discussed in the context of challenging environmental conditions on Prince Leopold Island in 2002, and dietary differences between the High and Low Arctic colonies.

INTRODUCTION

There is significant uncertainty regarding the way in which lipid stores are managed by birds. Migrating birds fuel their high-intensity exercise by mobilising fatty acids from energy stores (adipose tissue) through the circulatory system, and exercise causes an increase in the mobilisation of lipids from energy stores in a variety of species (McWilliams et al. 2004). However, the way in which these fatty acids are packaged and which fatty acids are most important remains a mystery. There is evidence suggesting that lipids are packaged and transported as lipoproteins and, consequently associated with an increase in plasma neutral lipid concentrations (Jenni-Eiermann and Jenni 1992) because they are the primary component of low density lipoproteins. Most studies on changes in plasma lipids and lipid utilisation metabolites have been conducted on birds during migration or periods of intensive fattening (i.e. in preparation for migration). Very little information exists on lipid mobilisation during reproduction, though it is generally assumed that this is a very costly activity (i.e. Golet and Irons 1999; Vezina and Williams 2002).

Thick-billed Murres migrate long distances between their wintering grounds off the coast of Newfoundland, to various colonies in the Eastern Arctic. Consequently, Thick-billed Murres expend significant amounts of energy and time traveling to favourable breeding grounds. Though migration for reproduction may decrease the total energy required to reproduce, compared with what may be required if migration did not occur, reproduction is still considered to be energetically costly. Of all reproductive activities (i.e. egg production, incubation, chick-rearing), chick-rearing is considered to be the most energetically costly activity for adults (Gaston 1985; Parades et al. 2005). Consequently, I predict that lipid plasma concentrations should be higher during chick-rearing than during incubation. In addition, because neutral lipids are the primary energy molecule, and lipids may be transported to muscle as low density lipoprotein (McWilliams et al. 2004), I predict that a similar trend will also be found for neutral lipids.

I investigated the changes in plasma lipid concentrations of Thick-billed Murres during reproduction at two colonies, Prince Leopold Island, and Coats Island, Nunavut. Prince Leopold Island is in the High Arctic, and Coats Island is in the Low Arctic. It is thought that the breeding conditions are more favourable at Coats Island because of warmer temperatures and earlier ice breakup. However, current warming trends suggest that conditions may be declining on Coats Island while they may improve on Prince Leopold Island in the future (Gaston et al. 2005). In addition, I use the data to test whether there are differences in the lipid signatures between Thick-billed Murres breeding on Prince Leopold Island and on Coats Island. Previous research (i.e. Budge et al. 2002) suggests that there are inter-specific and geographic (within species) differences in the lipid signatures. This analysis will help determine the viability of using the condition index based upon plasma lipids (Chapter 2) on individuals sampled at different colonies and could be useful for identifying inter-colony and ocean regime changes by monitoring the fatty acid signatures of individuals throughout the breeding season an between colonies.

MATERIALS AND METHODS

Total lipid and neutral lipid of plasma samples from Prince Leopold Island 2002 and weekly plasma samples from Coats Island 2003 were used for this analysis (Table 3.1). All lipid extractions were conducted according to methods in Chapter 2. Occasionally, there was a 'no detection' signal from the GC results. In this case, this sample was eliminated from the analysis. All plasma lipid analyses were conducted according to the methods in Chapter 2.

Table 3.1: Sample sizes used for plasma total lipid analysis of Thick-billed Murres from Prince Leopold Island (P.L.I.; 2002) and Coats Island (C.I.; 2003).

Colony	Early Incubation		Late Incubation		Early Chick-rearing		Late Chick-rearing	
	Males	Females	Males	Females	Males	Females	Males	Females
P.L.I.	5	8	7	11	NA	NA	4	4
C.I.	18	12	11	4	27	22	5	4

Table 3.2: Sample sizes used for plasma neutral lipid analysis of Thick-billed Murres from Prince Leopold Island (P.L.I.; 2002) and Coats Island (C.I.; 2003).

Colony	Early Incubation		Late Incubation		Early Chick-rearing		Late Chick-rearing	
	Males	Females	Males	Females	Males	Females	Males	Females
P.L.I.	5	8	7	11	NA	NA	4	4
C.I.	22	16	19	5	29	23	5	4

Based upon the reproductive activity in which birds were engaged at the time of sampling, data were organised into four sampling periods: early incubation (<16 days after mean laying date), late incubation (≥16 days after mean laying date), early chick-rearing (<11 days after mean hatching date), and late chick-rearing (≥11 after mean hatching date).

Statistical Analysis

Changes in total lipid and neutral lipid concentrations were tested using a General Linear Model with period, sex, and colony as independent variables.

To determine if there were differences in the plasma lipid signatures of Thick-billed Murres sampled during the same period on Prince Leopold Island and Coats Island, I used MANOVA on all 18 variables, Principal Component Analysis and Discriminant Function Analysis (SYSTAT version 8.0). In cases where the number of variables exceeds the number of replicates, it is generally best to use a data reducing method, such as the Principal Component Analysis. These data have sufficient replicates to avoid data reduction, however, this technique is used in a later chapter and for consistency, it is preferable to note both results. Data from samples collected during the late chick-rearing period were used to compare potential dietary difference between Prince Leopold Island and Coats Island because differences in diets were likely greatest between the colonies at the end of the season. All statistical results with p values of less than 0.05 were considered significant.

RESULTS

Plasma Fatty Acids

There was a significant effect of sampling period on the total concentration of plasma lipids (Period: $F_{3,156}=4.53$, $p=0.004$, Colony: $F_{1,137}=0.35$, $p=0.55$, Sex: $F_{1,137}=1.85$, $p=0.18$; Figure 4.1) as lipid concentrations increased during reproduction. There was a significant effect of sampling period and colony on the total neutral lipid concentration (Period: $F_{3,157}=5.02$, $p=0.002$, Colony: $F_{1}=4.14$, $p=0.043$, Sex: $F_{1,157}=0.83$, $p=0.37$; Figure 4.2).

When the data were separated by colony, neutral lipid concentrations did not increase significantly on Prince Leopold Island during the reproductive season ($F_{2,37}=0.12$, $p=0.89$) and total plasma lipid increased though the effect was just short of significant ($F_{2,37}=2.92$, $p=0.06$). Both effects remained significant for those samples collected on Coats Island.

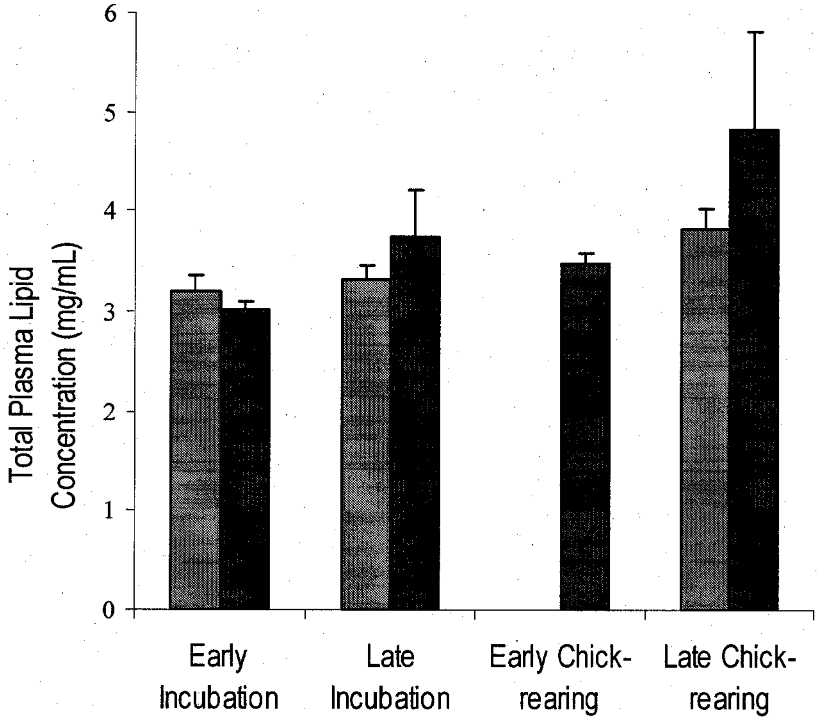

Figure 3.1: Total plasma lipid concentrations (mean mg/mL ± SE) during different reproductive activities for Thick-billed Murres breeding on Prince Leopold Island (light bars) and Coats Island (dark bars) in 2002 and 2003.

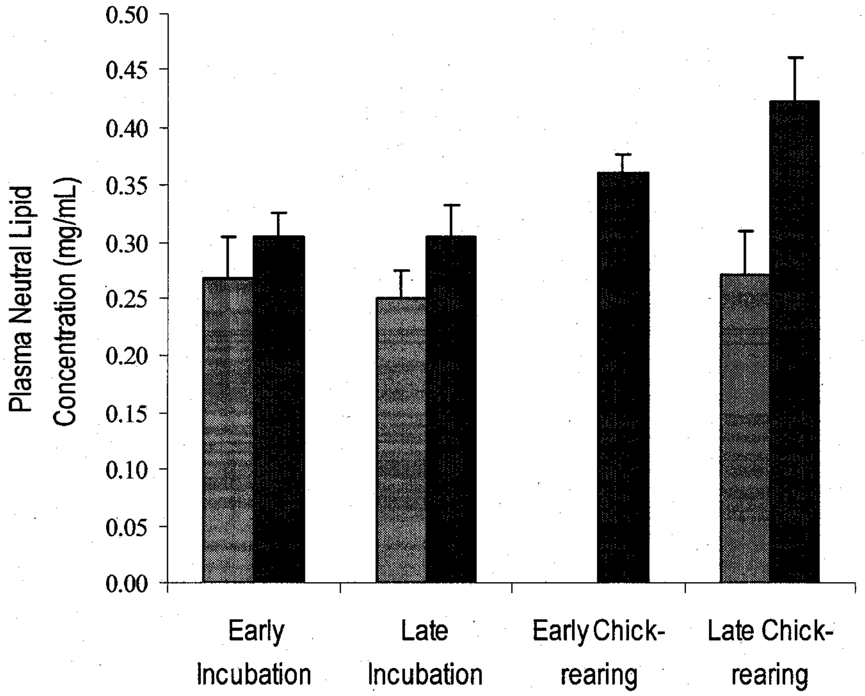

Figure 3.2: Total plasma neutral lipid concentrations (mean mg/mL ± SE) during different reproductive activities for Thick-billed Murres breeding on Prince Leopold Island (light bars) and Coats Island (dark bars) in 2002 and 2003.

Principal Component Analysis

The principal component analysis revealed that components 1, 2, and 3 accounted for 34, 13 and 11% of the variance in neutral lipid fatty acid concentrations, respectively. Most of the separation between the neutral lipid fatty acid signatures at the two colonies occurred along the component 2 axis though there was some separation along the first component axis (Figure 3.3). Fatty acids 22:1, and 20:4 loaded the highest in contributing towards the separation along the second component, though 16:0, 16:1, 18:2, and 22:5 also contributed to the separation of neutral lipid fatty acid signatures on Prince Leopold Island and Coats Island (Figure 3.4).

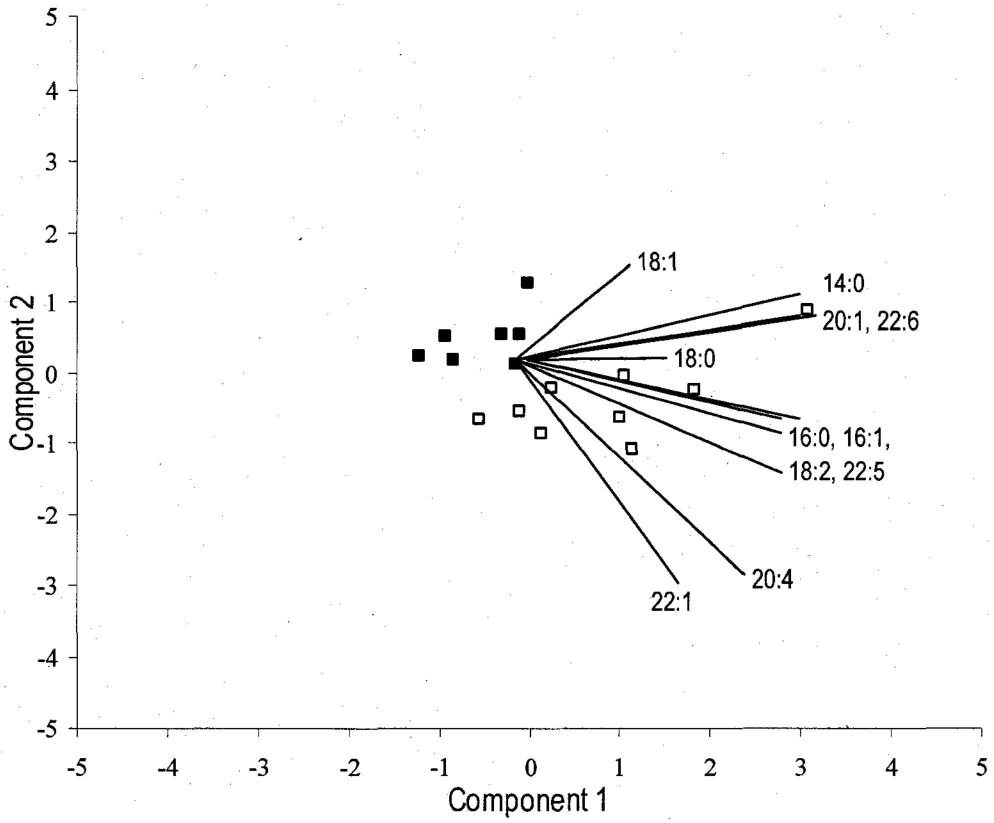

Figure 3.3: Components 1 vs. 2 from a Principal Component Analysis of plasma neutral lipid signatures from Thick-billed Murres sampled during late chick-rearing on Prince Leopold (open squares) and Coats (solid squares) Islands.

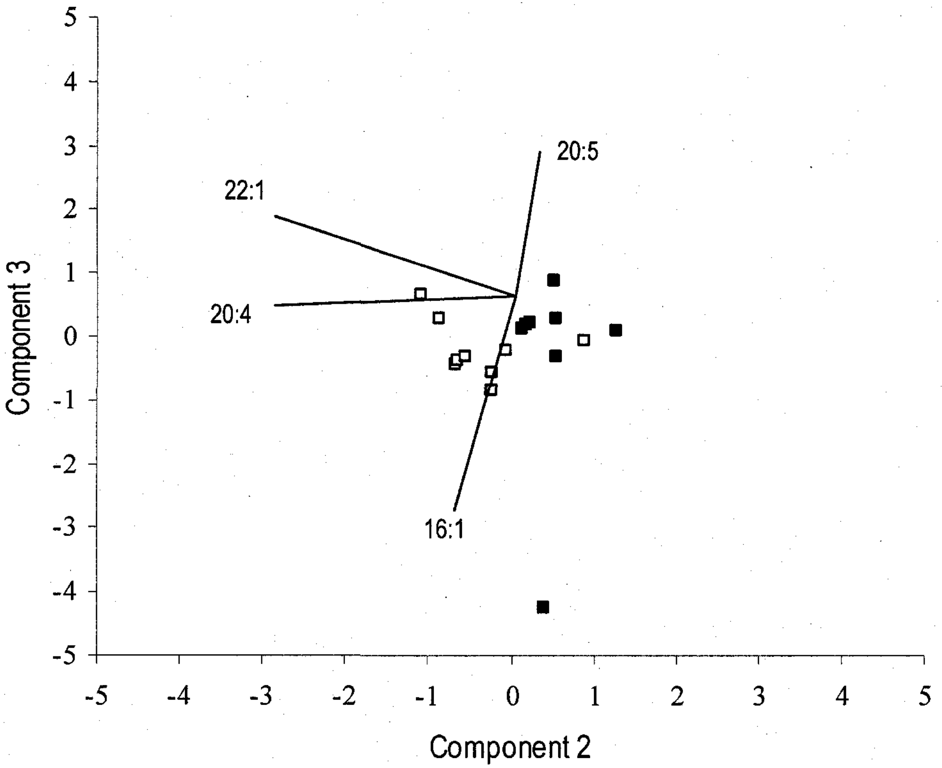

Figure 3.4: Components 2 vs. 3 from a Principal Component Analysis of plasma neutral lipid signatures from Thick-billed Murres sampled during late chick-rearing on Prince Leopold (open squares) and Coats (solid squares) Islands.

A Discriminant Function Analysis showed that there was a significant difference in the distributions of the plasma neutral lipid signatures between Thick-billed Murres breeding on Prince Leopold Island and Coats Island ($F_{18,71}$= 27.63, p< 0.001). A similar result was obtained using the first three factors of a Principal Component Analysis on the combined fatty acid concentration variables ($F_{1,3}$=5.47, p=0.002). There was no significant different between the fatty acid signatures of Thick-billed Murres breeding on Prince Leopold Island at different stages of reproduction ($F_{36,40}$=1.55, p=0.09), however, the signatures did change in birds breeding on Coats Island throughout the season

($F_{54,300}$=2.82, $p<0.001$). Similar results were obtained using the first three factors of the Principal Component Analysis (PLI: $F_{6,70}$=2.09, p=0.09; Coats Island: $F_{9,277}$=4.46, $p<0.001$).

DISCUSSION

I show that on Coats Island, plasma concentrations of neutral lipids and total lipid concentrations increase throughout the season and were highest during chick-rearing. On Prince Leopold Island, total plasma lipid concentrations increased slightly but there was no change in the neutral lipid concentration. At Coats Island, the largest change occurred between early and late chick-rearing, a period when breeding Thick-billed Murres typically lose mass (Gaston et al. 2005b), though data from my study (Chapter 1) show only a trend toward a declining mass that is not significant.

As described in Chapter 1, the environmental conditions on Prince Leopold Island in 2002 were particularly challenging and the reproductive success from this year was likely very low. In this situation, it is unlikely that adults would have managed their energy stores as though the environmental conditions were normal. However, the trends found from adults sampled on Coats Island follow my predictions as both neutral lipids and total lipids were elevated during chick-rearing.

The Principal Component and Discriminant Function Analyses showed that the neutral lipid fatty acid signatures of adults breeding on Prince Leopold Island and Coats Island are different and that a variety of fatty acids are responsible for these differences. Gaston (1983) showed that the diversity of prey species delivered to chicks on Prince Leopold Island was the lowest of all the colonies examined, and that the diversity on Coats Island was the highest. This further supports the prediction that the condition index developed on birds from Prince Leopold Island (Chapter 2) could not be applied to birds from other colonies where the diets are likely to be different. In addition, further interpretation is complicated because it is thought that the breeding conditions were particularly challenging on Prince Leopold Island in 2002. Consequently, this study should have been conducted from samples collected in the same year and when the conditions are thought to be approximately equal between colonies.

The analysis also demonstrates however, that fatty acid signatures on Coats Island changed throughout the season while those on Prince Leopold Island did not. If fatty acid signatures vary with diet (see Budge et al. 2002), then these data suggest that there was a change in the diet of birds breeding on Coats Island throughout the breeding period. This observation is supported by feeding watch data from both of the colonies (see Gaston 1983; Gaston et al. 2005a). Though it is not currently feasible to assess the diets of adult birds, it is possible to determine what their chicks are feeding by conducting feeding watches on study plots. Birds breeding on Prince Leopold Island feed their chicks primarily on Arctic Cod (*Boreogadus saida*), while those on Coats Island show a temporal (and annual) change in species throughout chick-rearing (Davidson 2005; Gaston et al. 2005a). Collecting accurate chick-feeding data is labour intensive and also difficult when birds are observed from a distance. Examining changes in prey composition between and within colonies may be facilitated by analysing the fatty acid profiles of breeding adults and their chicks.

These data support the prediction by Gaston (1985) and Paredes et al. (2005) that chick-rearing is the reproductive activity of highest energy demand, and provides a method for monitoring the rate of lipid use in Thick-billed Murres. This type of analysis may allow future researchers to identify inter-colony and ocean regime changes.

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CHAPTER 4

FATTY ACID SIGNATURES OF THE PLASMA OF FEMALE THICK-BILLED MURRES SUGGEST RELIANCE ON LOCAL PREY FOR REPLACEMENT EGG PRODUCTION

ABSTRACT

The laying of smaller replacement eggs by adult Thick-billed Murres has been described as a time saving adaptation because chicks generally grow faster once hatched than inside the egg. However, chicks hatched from smaller eggs have, potentially, lower survival. Consequently, a smaller replacement egg represents a cost to the offspring. I predicted that females will show physiological signs of preserving their own condition at this cost to their offspring. I test this prediction by measuring adult mass changes and plasma lipid concentration changes in males and females breeding on Coats Island, Nunavut. Between laying the first and replacement egg, plasma fatty acid concentrations declined in females and increased in males, suggesting that females mobilise less lipid to preserve their condition after laying the replacement egg. In females, plasma lipid concentrations of the dominant fatty acids found in the eggs (16:0 and 18:1) declined between first and replacement egg laying and plasma concentrations of 20:1 increased in males and females. I made comparisons of the fatty acid signatures of first and replacement egg to potentially determine if the lipid source for their production is different. Principal Component and Discriminant Function Analyses showed that the fatty acid signatures of replacement eggs were closer to the signatures of the local prey than first eggs. I suggest that it is likely that females rely upon local sources of energy for the production of the replacement egg, or that the available food sources change between the time of producing the first and replacement eggs.

INTRODUCTION

In Thick-billed Murres, there is generally a seasonal decline in egg volume, as earlier laying birds tend to lay larger eggs (Birkhead and Nettleship 1982; Hipfner et al. 2003). Where replacement eggs are laid, once a first egg has been lost by predation or parental mishandling, these eggs are generally smaller as well (Hipfner et al. 2003). Birkhead and Nettleship (1982) found these smaller eggs result in lighter (but not structurally smaller) chicks at hatching and proposed that the smaller replacement, or late-laid, egg is a time saving strategy that favours rapid chick growth after hatching. Consequently, the local prey availability may influence decisions related to whether to lay smaller eggs more quickly or delay laying in favour of a larger egg. For example, in one year, the egg size of Thick-billed Murres increased with date throughout the laying period (Gaston and Nettleship 1981) and was linked with evidence of unusually abundant prey availability. This suggests that prey availability, in addition to female quality, can influence egg size. The effects of the availability of prey (lipids and nutrients) during egg production may be particularly challenging, because most high latitude birds time reproduction so that chick-rearing coincides with maximum prey availability (Williams 2005). Consequently, limiting fatty acids to the composition of eggs may be more difficult to find for the production of replacement eggs.

Many questions relating to the strategy of the female in the production of the replacement egg are still unanswered. This is largely because studies addressing the cost of reproduction focus upon the chick-rearing stage (Williams 2005). Those which do address egg development, have concentrated on precocial species. In Thick-billed Murres (*Uria lomvia*), it is not known whether females lose body mass (or lipid stores) and whether there is a difference in the lipid (fatty acid) composition of eggs that may reflect changes in the lipid source for the production of the replacement egg. Though total composition of eggs has been tested on other species (i.e. Arnold et al.1991; Hipfner et al. 2003), we do not know if the composition of fatty acids within the eggs is conserved between the first and replacement egg. In some species, there is no decline (or change) in female body mass during, or after, egg laying (Williams 1996). In some precocial species, it is known that they rely upon endogenous stores and that the size of these stores limits egg production (Gauthier et al. 2003).

Studies investigating the composition of eggs have largely focused on domestic chickens. Those studies which have been conducted on wild species or other domestic avian species (i.e. Hipfner et al. 2003; Brand et al. 2003) have not included detailed fatty acid composition analysis. Instead, they focus on the larger divisions of composition, including total lipid, total protein, contaminants, yolk size, albumen volume, and egg shell mass. For example, in a study on four alcid species, including Thick-billed Murres, Birkhead and Nettleship (1984) found that the absolute yolk size increased with egg size but the relative contribution of the yolk remained the same except for Atlantic Puffins (*Fratercula arctica*), in which it was comparatively smaller. Hipfner et al. (2003) found no difference in the biochemical composition of Thick-billed Murre replacement eggs when compared to first laid eggs, however, the yolk to albumin ratio was smaller in late laying and replacement eggs. The authors suggest that changes in the composition of eggs are largely a function of size and timing of laying.

Determining whether birds rely upon endogenous or exogenous stores is an important component of assessing the cost of egg production. If endogenous stores are used in the production of a replacement egg there could be consequences to individual survival or future reproductive success should environmental conditions change when those stores are accumulated or if individuals are forced to replace eggs. If exogenous stores (local prey) are used, changes in food availability at the breeding colony may affect an individual's ability to produce the replacement egg. Certain biochemical components may be essential for the proper development of chicks which may be available locally in the concentrations necessary to successfully rear replacement broods. However, changes in the distribution and availability of certain prey types may remove essential components, such as fatty acids, thus reducing the reproductive success of replacement egg laying birds.

To determine whether exogenous or endogenous stores are utilised, complementary analyses are desirable. A comparison of fatty acid signatures from the eggs with those of prey items may allow us to determine if the signatures from the replacement eggs are more closely related to the signatures of the prey items. Stable isotope analysis can help identify the trophic level at which individuals were feeding when a tissue (i.e. egg) was produced (i.e. Hobson et al. 1997). Consequently, if there is no difference in the signatures (fatty acids and isotopes) of the first and replacement egg, we could support the notion that endogenous stores are being used for the production of both eggs.

However, if there is a difference in the fatty acid and stable isotope signatures, it would suggest that the materials for the production of the replacement egg are derived from an alternate and local source. There is evidence that stable isotope signatures between first and replacement eggs are different (K. Hobson pers. comm.). The stable isotope analysis could not be completed in time for the writing of this thesis. Consequently, the data presented reflect only the results of fatty acid analyses.

In addition to obtaining fatty acid signatures for the first and replacement eggs, a comparison of the plasma fatty acid signatures in the plasma of males and females during the laying period will help us identify if any changes in the egg fatty acid composition are reflected in the plasma. Should these changes be present in the plasma, a stronger case for exogenous use of lipids would exist, especially if we see changes in both males and females. In addition, I will use the total fatty acid composition within the plasma to determine if there is a difference in the mobilisation of lipids between males and females after laying the first and replacement egg.

The study of fatty acid signatures is relatively recent and many gaps in the literature require filling, including identifying the functions of specific fatty acids. However, their use as biomarkers in prey / predator relationship studies has been increasing (i.e. Kirsch et al. 1998, Hooker et al. 2001). We know that there is a conservative transfer of fatty acids in neutral lipids (Lee et al. 1971), but that they can be converted to other fatty acids in the liver and that fatty acid signatures are significantly different with species and body size (Budge et al. 2002; Christie 2006). Despite this, Kirsch et al. (1998) show that whole body fatty acid composition in fish can be used to identify diet of predators at upper trophic levels.

I measured the dimensions and masses of first and replacement eggs, though it is already known that replacement eggs are smaller than first laid eggs (Hipfner et al. 1997). I compared egg and plasma fatty acid concentrations and compositions of Thick-billed Murres during the reproductive season of 2004 on Coats Island, Nunavut. I predicted that total lipid concentrations will not differ between first and replacement eggs. I anticipated that there may be a difference in the fatty acid signature that may be associated with changes in diet by the female. Based upon theories of life-history strategy, and the observations that egg size is reduced in replacement eggs, I predicted that females preserve their own individual body condition at an expense to the offspring, in the form of a smaller egg. I predicted that there is a difference in the plasma concentrations of males and females, likely reflecting differences in energy demand with different reproductive activities (males defend nest site, females produce eggs), and the potential depletion of key fatty acids for the production of an egg by the female. I compared the changes observed in the concentrations of specific fatty acids with those found in the prey items collected from the colony ledges in 2003. I use these results to determine whether exogenous or endogenous stores are utilised for the production of the replacement egg.

MATERIALS AND METHODS

Experimental design and sample collection

The experiment was conducted during the chick rearing period of 2004 on Coats Island (62°57'N, 82°00'W), Nunavut, in the Canadian Eastern Low Arctic. I arrived at the colony before the date of mean egg lay and identified the plot (S) that would be used for this experiment. On the day of egg laying for each breeding pair, the incubating adult was caught and the egg was removed. It was replaced with a painted hard boiled chicken egg and I waited after the release of the captured adult to ensure that it accepted incubation of the chicken egg. All adults accepted the chicken egg as their own, though a couple of individuals took a few extra minutes to begin incubating. After the first changeover with its mate, I also caught and sampled the mate. Upon sampling both members of the pair and the removal of the first egg, the chicken egg was removed. The same sampling procedure was used after the laying of the replacement egg, approximately 14 days later. 11 first and replacement eggs were collected from the same breeding sites. 8 complete sets of adult plasma (plasma samples from both adults of the pair at first and replacement egg laying) were collected and utilised for the analysis.

The adults were weighed with a pesola weigh scale (±1 g) and a blood sample was taken. Approximately 3 mL of blood was taken using a syringe and 27 gauge butterfly needle. The blood was transferred immediately to a 3 mL vaccutainer lined with heparin. The birds were released within 5 min of capture. Upon arriving at the camp, blood samples were centrifuged for 5 min and the plasma was transferred to cryovials. Samples were then frozen in a propane freezer (-20°C) and stored there until being shipped to Ottawa in a dry nitrogen shipper.

Prior to the period of the second sampling the generator broke down and was no longer available for powering the centrifuge. Plasma was obtained by hand centrifuging for up to 2.5 h, or until there was sufficient plasma.

Each egg was weighed and measured (length and width ±0.5 mm). Eggs were then cracked and the contents of each were scrambled thoroughly with an egg beater until 2 min after the egg appeared homogenous. Approximately 6mL of each egg were then transferred to cryovials. Egg samples were frozen in a propane freezer (-20°C) and stored until being shipped to Ottawa in a dry nitrogen shipper.

The sex of adult Thick-billed Murres was identified using DNA markers according to methods described by Crump et al. (2006, manuscript submitted for publication).

Prey samples were collected opportunistically during the 2003 field season. On many occasions, fresh prey items were found on the cliff ledges. Details of prey species and sample sizes are in Table 4.1. Each item was collected, weighed, measured (fork length) and then frozen in a plastic bag at -20°C until shipping on wet ice in a cooler to Ottawa. Mean masses of different species were similar to those estimated from larger samples collected at Coats Island over may years (A. Gaston and K. Elliott, unpubl.).

Table 4.1: Prey collected on cliffs during the 2003 field season on Coats Island, Nunavut. Fork length and mass values for prey items with N>1 are mean values (±S.D.).

Prey Item	N	Fork length (mm)	Mass (g)
Capelin	6	118.8 (7.7)	9.2 (2.3)
Arctic Cod	3	140.7 (12)	19.5 (5.1)
Daubbed Shanny	3	126.0 (11.5)	5.3 (1.2)
Sandlance	4	132.3 (17.6)	6.3 (3)
Sculpin	5	124.2 (23.6)	17.1 (8.6)
Amphipoda	-	NA	1.3[a]
Fish Doctor	1	172	4.8
Cephalopoda	1	66	3.2
Decapoda	1	90	5.3

[a] Denotes mass of sample used in fatty acid analysis. Sample contained many individuals too small to analyse individually.

Egg fatty acid analysis

0.05 g of egg sample was transferred into 6mL of Folch reagent (2:1 v/v). The resulting mixture was centrifuged at 3000 RPM for 10 min. The supernatant was filtered and KCl (0.25% in distilled water) was added to eliminate water-soluble compounds. This mixture was centrifuged at 3000 RPM for 10 min to facilitate the separation of an aqueous phase. This phase was eliminated with a water-powered vacuum pump and the 2 mL of methanol were added to each tube. Samples were frozen at -20°C with N_2 to reduce oxidation rate.

Prey fatty acid analysis

Lipid extraction for prey items followed the protocol for egg fatty acid analysis except that the lipids from the whole prey item were extracted and then a subsample of the fat was removed for analysis. This was done because the whole prey items are not homogenous and direct subsampling of a single tissue would have introduced insurmountable error given that some species were not fish. Consequently, only percent composition was analysed.

Plasma fatty acid analysis

Plasma was added to Folch reagent (2:1 v/v). An internal standard (Heptadecanoic acid, 17:0, 30 mg/100 mL hexane) was added for gas chromatography (GC) analysis of the non-esterified fatty acids (NEFA). The resulting mixture was centrifuged at 3000 RPM for 10 min. The supernatant was filtered and KCl (0.25% in distilled water) was added to eliminate water-soluble compounds. This mixture was centrifuged at 3000 RPM for 10 min to facilitate the separation of an aqueous phase. This phase was eliminated with a water-powered vacuum pump and the remaining solvent was evaporated N_2 at 70 °C.

Separation of Lipid Fractions

Total lipids can be divided in 3 classes: neutral lipids (NL), non-esterified fatty acids (NEFA) and phospholipids (PL). These classes were separated to analyze their fatty acid composition. Supelclean solid-phase extraction tubes (LC NH_2, 100 mg: Sigma) were conditioned with hexane. The fats were resuspended in choloroform and transferred into the columns. The NL fraction was eluted by flowing chloroform:isopropanol (2:1 v/v) through the columns. The NEFA fraction was eluted by flowing isopropyl ether:acetic acid (98:2 v/v) through the columns. The PL fraction was eluted by flowing methanol through the columns. An internal standard (17:0, 30mg/100mL hexane) was added to each lipid fraction. The solvent from all samples was evaporated under N_2 at 70 ˚C and the lipids were resuspended in Folch reagent and frozen at -20˚C with N_2 until Methylation.

Methylation of NEFA fraction for Gas Chromatography Analysis

Methylation is required to increase the volatility of the lipid samples for GC analysis. NEFA were resuspended in methanol (100ul), dimethoxypropane (1mL) and 6N HCl (50ul). The mixture was vortexed and incubated at room temperature for 20 min and evaporated under N_2 at 70 ˚C. The methylated fats were resuspended in isooctane and transferred into GC autosampler tubes.

Acid Transesterification of Neutral Lipid and Phospholipid fractions for Gas Chromatography Analysis

Fats were resuspended in an acetyl chloride solution (7.2 mL acetyl chloride in 100 mL methanol). The samples were incubated at 90 ˚C for 2 h after which the acetyl chloride in methanol was evaporated under N_2 at 70 ˚C. The fats were resuspended in methanol and evaporated under N_2 at 70 ˚C. The fats were resuspended in isooctane and transferred to GC autosampler tubes.

All samples were then analysed using Gas Chromatography (HP 5890 series II with HP 7673 autosampler and flame-ionization detector; see McClelland, Hochachka and Weber 1999). The retention times of the fatty acids were compared with those from known standards and the concentration of each fatty acid was determined by comparing the size of the peak of the internal standard with that of all the other fatty acids. The analysis of two plasma samples were incomplete as no NL (male after first lay) or PL (male after replacement lay) were detected. These samples were excluded from the statistical analysis.

Statistical Analyses

Fatty acid concentrations in plasma, eggs, and prey were analysed using MANOVA with all fatty acid concentrations as dependents within the same analysis. Individual analyses of fatty acids were done in a univariate posthoc test of MANOVA and Bonferroni adjustments were made for multiple comparisons. Fatty acid signatures of eggs and female plasma were compared using either only a Discriminant Function Analysis, or a Principal Component Analysis (PCA) with eigenvalues of greater than 1.0 followed by Discriminant Function Analysis on the first three factors to identify if the there were significant differences in the fatty acid signatures. The results from both of these approaches are presented because a Discriminant Function Analysis generally should not be conducted when there are fewer sample replicates than variables. Consequently, data reduction techniques, such as a PCA may be favourable in this case. For comparison of fatty acid signatures of eggs and plasma, and of eggs and prey, arcsine transformed percent contributions of each of the fatty acids were used. All statistical analyses were conducted using SYSTAT version 8 software and the level of significance was set at 0.05 when not indicated otherwise.

RESULTS

Adult Mass changes

In 2004, all but two males (of n=8) lost mass while six females (of n=8) gained mass between first and replacement egg laying (average mass change males: -19.2 g ± 8.2; females: -8.6 g ± 12). Males did not lose significantly more mass than females ($F_{1,15}$=0.53, p=0.24), nor were there differences in total mass between the sexes ($F_{1,15}$=1.35, p=0.13; Figure 4.1).

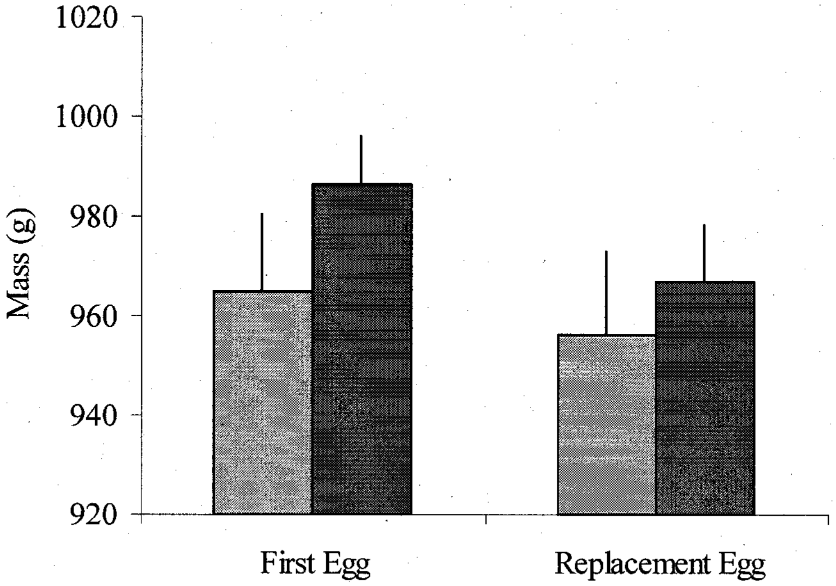

Figure 4.1: Mass of adult females (light bars) and males (dark bars) just after laying the first and replacement eggs 2004.

Egg Measurements

First laid eggs were generally heavier, wider and longer than replacement eggs though the differences were not significant (Table 4.2). When egg dimensions were used to calculate volume, replacement eggs were generally smaller than first eggs but this difference was also not significant. Studies with larger sample sizes have shown that differences in egg dimensions between first and replacement eggs are significant and by a similar amount (e.g. 6%, Hipfner et al. 2003).

Table 4.2: Measurements of first and replacement eggs (n=22) in 2004. Values presented are means (± SE). Results of ANOVAs for differences between means are also presented.

	Mass (g)	Length (mm)	Width (mm)	Volume[a] (mm^3)	Lipid Concentration (mg/mL)
First Eggs	109.4 (2.3)	80.5 (0.9)	50.6 (0.5)	206211.4 (4395.8)	42.16 (3.24)
Replacement Eggs	103.5 (2.6)	79.6 (0.7)	49.6 (0.6)	196084.5 (5260.2)	39.44 (2.46)
% Difference	5.3	1.1	2.0	4.9	7.1
$F_{1,20}$	5.36	1.42	2.99	4.2	Table 4.3
p[b]	0.03	0.25	0.10	0.05	Table 4.3

[a] Volume = (height x $width^2$)

[b] With the Bonferroni correction for multiple comparisons, the level of significance is set at p=0.013.

Egg Fatty Acids

Total egg lipids accounted for 4.2 and 3.9 % of total egg content in first and replacement eggs, respectively (excluding the shell) though this difference was not significant. As replacement eggs were generally smaller in size and volume, no change in the concentration of lipids from a scrambled egg suggests that the relative size of the yolk does not increase in the replacement egg, but remains constant (but see Hipfner et al. 2003). Phospholipids accounted for 50% of the total fatty acid concentration, while NL and NEFA accounted for approximately 49 and 1 % respectively. Fatty acids 16:0 and 18:1 were highest in concentration in both the first and replacement eggs compared with all others. Fatty acids 20:1 and 22:1 (though not after Bonferroni correction) increased in concentration from the first egg to the replacement egg (Table 4.3). For the remaining fatty acids, there was no significant change in concentration between the eggs. This suggests that the fatty acid composition is highly conserved and that 16:0 and 18:1 are essential fatty acids of the egg for successful development of chicks (Figure 4.2).

Table 4.3: Fatty acid concentrations (mg/mL ± SE) of first and replacement eggs of Thick-billed Murres breeding in 2004 (n=22).

Fatty Acid	Mean Concentration (mg/g) Egg 1 (SE)	Egg 2 (SE)	F df=1,20	P
14:0	0.19 (0.03)	0.17 (0.02)	0.10	0.76[a]
16:0	10.09 (0.83)	9.08 (0.71)	0.61	0.44
16:1	0.20 (0.05)	0.37 (0.12)	2.00	0.17
18:0	2.34 (0.12)	2.17 (0.06)	1.13	0.30
18:1	21.34 (1.72)	19.92 (1.49)	0.24	0.63
18:2	0.22 (0.04)	0.21 (0.03)	0.03	0.86
20:1	0.49 (0.06)	1.02 (0.08)	26.24	<0.001
20:4	1.81 (0.80)	1.02 (0.09)	0.96	0.34
22:1	0.07 (0.02)	0.12 (0.01)	5.74	0.03
20:5	2.29 (0.11)	2.22 (0.09)	0.45	0.51
22:5	0.51 (0.04)	0.42 (0.04)	2.50	0.13
22:6	2.54 (0.15)	2.64 (0.09)	0.23	0.64
Total egg fat	42.16 (3.24)	39.44 (2.46)	0.46	0.51

[a] With the Bonferroni correction for multiple comparisons, the level of significance is set at p=0.004

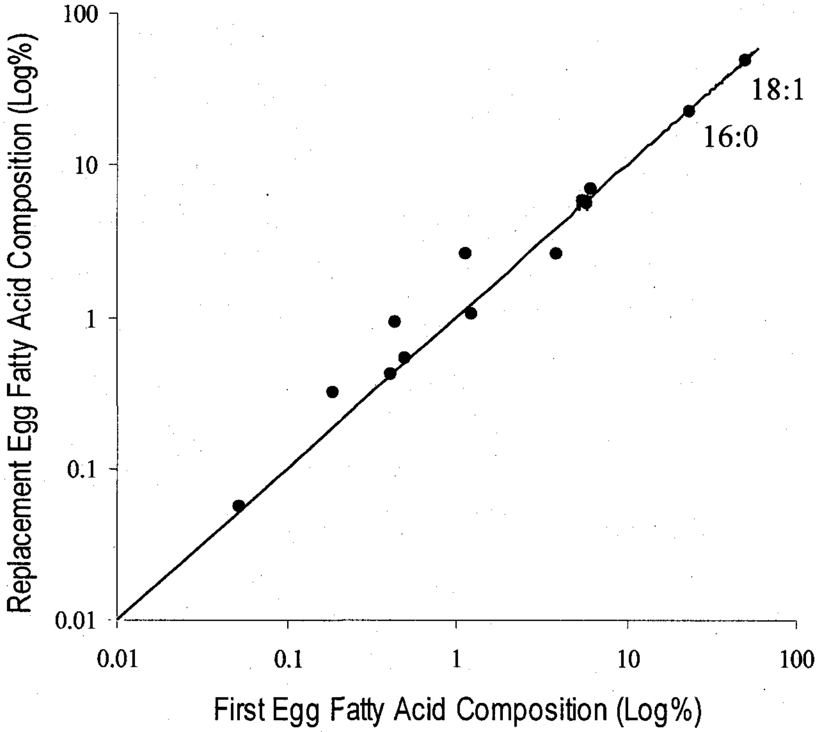

Figure 4.2: Fatty acid composition (% ± SE) for individual fatty acids for first versus replacement eggs. Fatty acids 16:0 and 18:1 are identified. A trendline representing the equation x=y is presented for comparison of values.

For comparing the fatty acid signatures of the first and replacement eggs, univariate analyses, like those presented above, do not account for interactions in variability and therefore, principal component analysis (for variable reduction) and discriminant function analysis are required (Grahl-Nielsen 1999). Results from the principal component analysis of fatty acid concentrations (mg/mL) are presented in both Figures 4.3 and 4.4. The first three factors accounted for 68% of the variation in the data. From these figures, we see that the fatty acid signatures of first and replacement eggs are separated along the Component 2 axis. The two fatty acids with the highest loading in this component are 20:1 and 22:1 and are the only ones which increase significantly in concentration in the eggs (Table 4.3). Consequently, the fatty acid signatures of first and replacement eggs are differentiated by changes in the concentration of 20:1 and 22:1. Though conducting a Discriminant Function Analysis on data with a large number of variables can decrease the power of the analysis and is unadvisable in this case, there is no significant difference in the fatty acid signatures between first and replacement eggs (F_{15}=1.54, 0.31). However, a Discriminant Function Analysis of the first, second, and third factors of the Principal Component Analysis showed that there is a significant difference in the loadings of these factors between first and replacement eggs (F_3= 4.36, p = 0.03) and that they are significantly spread.

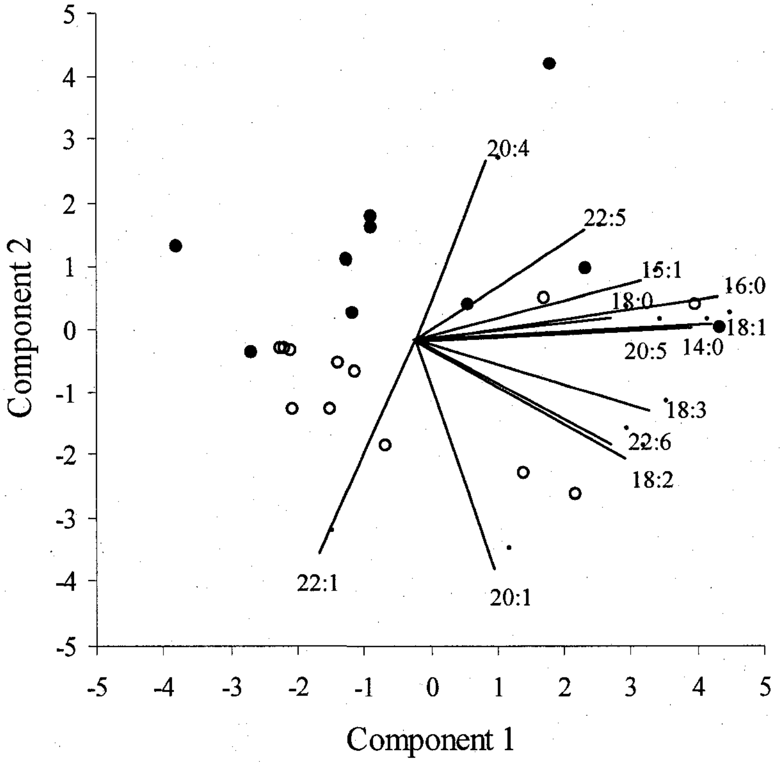

Figure 4.3: Components 1 vs. 2 for first (dark circles) and replacement eggs (open circles) from a principal component analysis. All fatty acids with component loadings of less than 0.5 for both components are excluded from the figure.

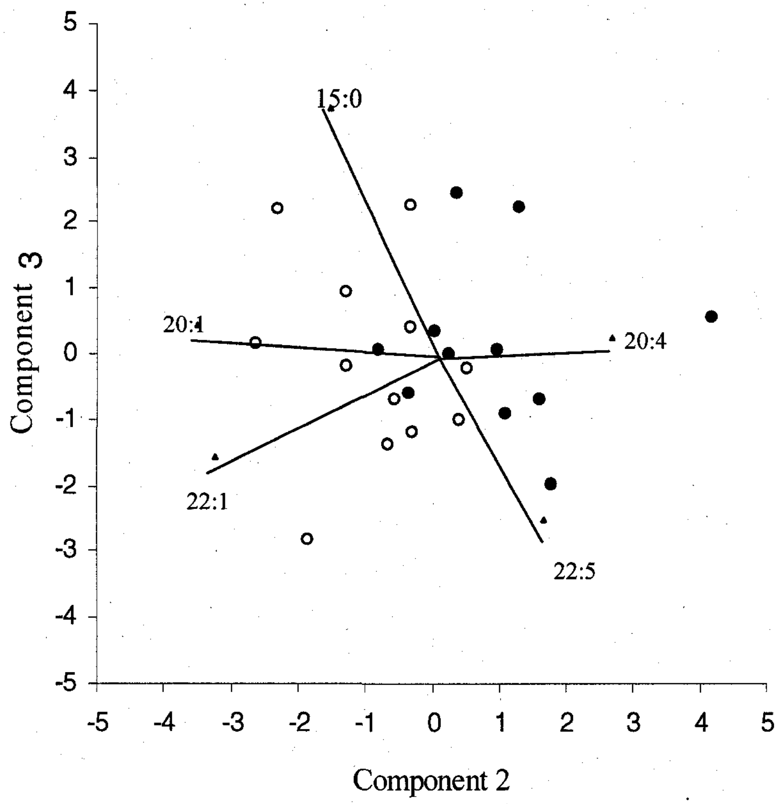

Figure 4.4: Components 2 vs. 3 for first (dark circles) and replacement eggs (open circles) from a principal component analysis. All fatty acids with component loadings of less than 0.5 for both components are excluded from the figure.

Plasma Fatty Acids

There was a significant interaction between sex and treatment (after first or replacement egg laying) on total plasma fatty acid concentrations ($F_{1,26}$=6.25, p=0.02). The total plasma lipid concentration was significantly higher in females than males after laying the first egg ($F_{1,13}$=10.3, p=0.007) and suggests that females were mobilising more lipids than males at this time. In females, the concentration of plasma lipids decreased between first and replacement egg laying. In males, plasma lipid concentrations increased. After laying the replacement egg, there was no significant difference in the plasma concentrations of lipids between males and females ($F_{1,13}$=0.04, p=0.84) (Figure 4.5).

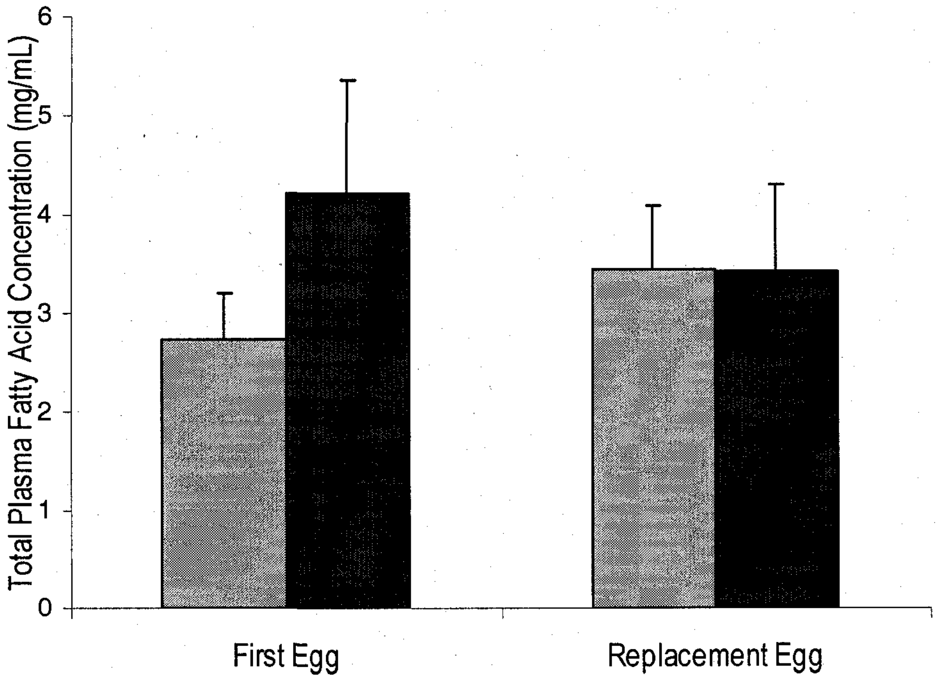

Figure 4.5: Total plasma lipid concentrations (mg/mL ± SE) of male (light bars) and female (dark bars) Brünnich's guillemots just after laying the first and replacement eggs at Coats Island in 2004.

In males, the fatty acid composition was similar after the laying of the first and replacement eggs (Figure 4.6). However, the concentrations of 16:0 and 18:1 increased significantly (16:0: $F_{1,12}$=8.29, p=0.01, 18:1: $F_{1,12}$=15.05, p=0.002). In females, those fatty acids found in the highest concentration in the eggs decreased in concentration significantly between first and replacement egg laying (16:0: $F_{1,14}$=10.94, p=0.01, and 18:1: $F_{1,14}$=4.89, p=0.04, Figure 4.7).

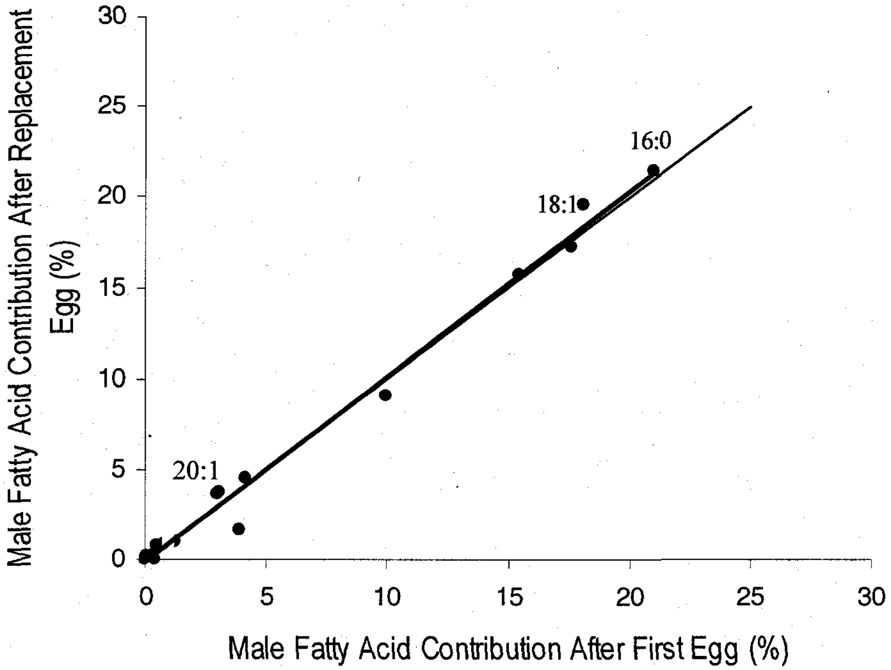

Figure 4.6: Male plasma fatty acid composition (% of each fatty acid) after laying of the first and replacement egg (trendline in bold, $r^2 = 0.98$). A line with equation x=y is also presented (fine line).

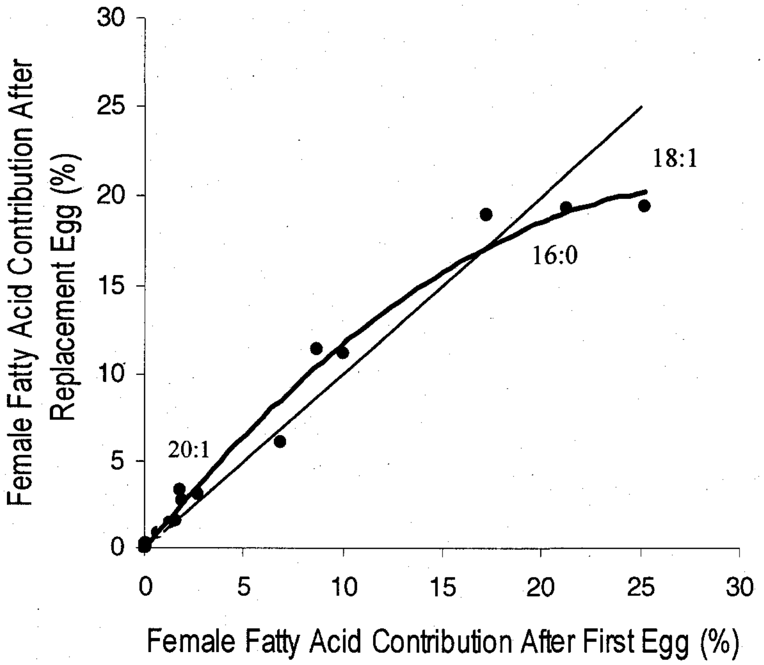

Figure 4.7: Female plasma fatty acid composition (% of each fatty acid) after laying of the first and replacement egg (trendline in bold, r^2= 0.99). A line with equation x=y is also presented (fine line).

Comparisons of changes in the percent contribution of individual fatty acids to egg lipid composition and female plasma composition after laying the first egg and after laying the replacement egg show clearly that 16:0 and 18:1 are found in higher proportion in eggs and that these are depleted in females after laying the replacement egg (Figure 4.8).

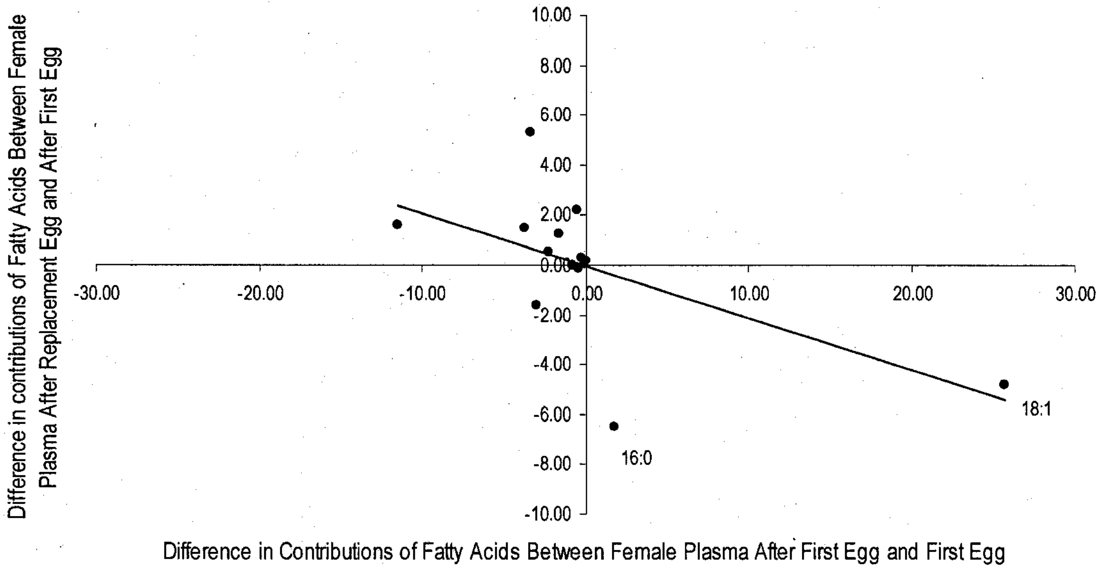

Figure 4.8: Comparison of changes in percent lipid composition between female plasma (after laying the first egg) and the first egg, and differences in percent lipid composition of female plasma after laying the first egg and the replacement egg. A trendline is provided.

The percent contribution of each fatty acid to the total lipid composition show some differences in males versus females after laying the first and replacement eggs. Females showed higher percent contributions of both 16:0 and 18:1, which were then depleted in females after laying the second egg. The contribution of 16:0 declined to below that of males, which 18:1, even when depleted in females after laying the replacement egg, was still higher than males. In addition, males had higher contributions of the fatty acid 20:5 than females (after first egg: $F_{1,13}$=6.65, p=0.02; after replacement egg: $F_{1,13}$=7.60, p=0.02). Though there are no clear predictions about why this would be found, it is potentially related to the higher concentrations of total plasma lipids and their use for fueling flight, or perhaps due to a higher consumption of amphipods, which have the highest percent contribution of 20:5 compared to the remaining prey items.

Based upon the changes in fatty acid composition of first and replacement eggs, and the Principal Component Analysis of those data, I compared the changes in the concentration of fatty acids 20:1 and 22:1 in the plasma of the adults. Changes in the concentration of fatty acids in the first and replacement eggs are reflected in one of the two fatty acids in the adult plasma. Male plasma concentrations of 20:1 increase significantly ($F_{1,12}=6.83$, $p=0.02$) while those in females did not ($F_{1,14}=1.26$, $p=0.28$).

A Discriminant Function Analysis using the raw fatty acid concentrations showed that there is no significant difference in the fatty acid signatures between after laying the first and after laying the replacement egg ($F_{14}=0.35$, $p=0.9$). A Discriminant Function Analysis using the first three PCA factors shows that there is a significant difference in the fatty acid signatures of females after laying the first egg and after laying the replacement egg ($F_{3}=4.09$, $p=0.03$).

Prey Fatty Acids

There was a significant effect of species on the (arcsine) percent total 20:1 in the entire body ($F_{8,15}=3.21$, $p=0.036$). Of the species analysed, capelin (11.2 ± 1.3), sandlance (10.1 ± 1.7), and squid (12.9) had the highest percent 20:1 (Table 4.5). A comparison of the percent composition of all other measured fatty acids showed that there were no significant differences with species ($F_{8,15}<2.44$, $p>0.05$). Prey species also had a high concentration of 16:0, 18:1, 20:5 and 22:6 (Table 4.4).

Table 4.4: Percent fatty acid composition (±SE) of prey items collected off the ledges at the Coats Island Thick-billed Murre colony in 2003.

	Percent Fatty Acids Composition								
Species	Amphipod (n=1.3 g)	Capelin (n=5)	Cod (n=3)	Fish Dr. (n=1)	Sandlance (n=4)	Sculpin (n=5)	Shanny (n=3)	Shrimp (n=1)	Squid (n=1)
14:0	2.3	1.6	1.3	0.9	1.7	1.2	1.1	1.3	1.4
		(0.4)	(0.4)		(0.2)	(0.3)	(0.2)		
15:0	0.3	0.1	0.1	0	0.1	0.1	0.2	0.4	0.4
		(0.1)	(0.1)		(0.1)	(0.0)	(0.1)		
15:1	1.2	1.4	0	0	0	0.5	0	0	0
		(1.4)				(0.5)			
16:0	15.0	12.5	15.0	14.0	14.1	12.1	12.9	13.0	13.8
		(1.2)	(1.0)		(0.5)	(2.2)	(0.4)		
16:1	5.7	6.7	10.1	7.4	7.3	9.0	10.6	7.3	7.1
		(1.3)	(1.7)		(0.7)	(1.4)	(1.1)		
18:0	6.3	4.7	5.2	6.6	6.1	5.1	4.4	6.8	2.5
		(0.3)	(0.8)		(0.8)	(0.8)	(0.3)		
18:1	16.3	11.9	17.6	18.0	10.8	26.5	18.2	18.9	19.4
		(0.5)	(1.4)		(1.2)	(8.8)	(1.4)		
18:2	2.8	1.6	1.4	1.9	1.4	1.8	1.7	1.1	1.2
		(0.2)	(0.4)		(0.3)	(0.1)	(0.2)		
20:1	3.5	10.7	7.4	2.8	9.6	4.8	6.1	3.4	12.9
		(2.1)	(0.7)		(0.7)	(1.2)	(1.4)		
22:0	0	0.1	0.5	0.2	0.1	0.1	0.2	0.2	0.5
		(0.0)	(0.1)		(0.0)	(0.1)	(0.1)		
20:4	2.2	1.8	0.5	1.4	0.3	0.6	2.1	2.5	0.9
		(1.4)	(0.2)		(0.2)	(0.3)	(0.6)		
22:1	2.3	9.7	8.2	7.1	12.3	4.1	8.2	14.7	3.0
		(2.5)	(3.0)		(3.5)	(2.1)	(3.1)		
20:5	22.7	14.2	15.2	20.7	14.2	14.8	17.2	19.6	15.1
		(1.4)	(1.5)		(1.7)	(2.5)	(0.4)		
22:5	5.2	1.5	3.2	3.1	3.8	2.1	4.2	3.2	0
		(0.8)	(2.1)		(1.8)	(1.1)	(1.1)		
22:6	13.8	21.3	14.1	15.6	17.7	17.0	11.0	7.5	21.5
		(2.6)	(2.2)		(2.1)	(3.6)	(3.5)		

Principal component analysis on percent fatty acid contributions of first and replacement eggs and prey showed that the fatty acid signatures of replacement eggs are closer to the fatty acid signatures of prey than first laid eggs. The first and second components accounted for 54 and 10% of the variation respectively. There was still quite a separation between eggs and prey as they are very different tissues (Figure 4.9).

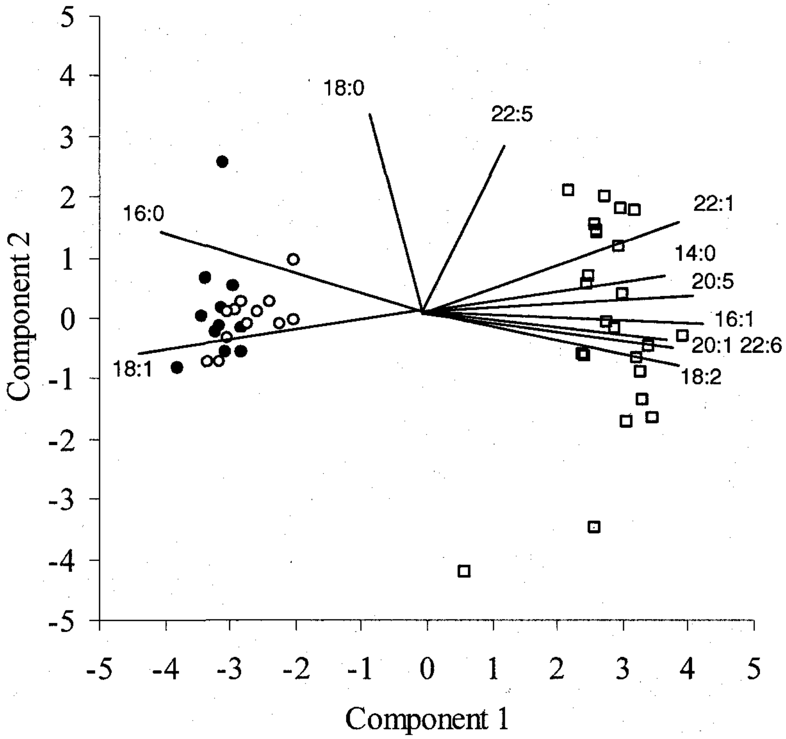

Figure 4.9: Components 1 vs. 2 for first (dark circles) and replacement (open circles) eggs and prey (open squares) from a principal component analysis.

DISCUSSION

I show that females do not lose as much mass as males and that mass changes are not significant between laying the first and replacement eggs. Plasma lipid concentrations in females just after laying the first egg were higher than just after laying the replacement egg. This suggests that females were mobilising fewer lipids after laying the replacement egg than they did after laying the first. No decline in mass, and a reduction in plasma lipid circulation suggests that females were preserving their own condition at a cost to the chick when producing the smaller replacement egg. Because the overall size of the egg is smaller, and the relative contribution of the yolk is the same or smaller (Hipfner et al. 2003; indirectly, this study), less lipid is laid down in the replacement egg as a function of size. Hipfner and Gaston (1999) found that chicks hatched from larger eggs show increased wing length growth and that these chicks fledged before those from smaller eggs. Therefore, there is a likely relationship between egg size and chick survival, as wing chord length is positively correlated with chick survival (U. Steiner, pers. comm.).

Some confusion still remains regarding whether female plasma lipid concentrations around the time of laying reflect lipid mobilisation for egg development or for the individual's energy expenditure. However, in another seabird it was found that there is a 4-5 day lag between the deposition of lipids for the egg and the time of laying (Astheimer 1986). This suggests that the concentration of plasma fatty acids of the females after laying reflects energy mobilisation for individual activities, excluding egg production. In contrast to the females, male plasma fatty acid concentration increased significantly.

Fatty acid concentrations have been shown to increase in exercising birds (review: McWilliams et al. 2004). Therefore, these results suggest that males were engaged in more exercise after the female laid the replacement than after the first egg and than females just after laying the replacement egg.

The plasma fatty acid concentration changes in females reflect the fatty acid composition within the eggs, as only those fatty acids found in high concentration within the eggs were found to decline in female plasma. This suggests that sources of 16:0 and 18:1 are limiting in the production of the replacement egg. In males, all those fatty acids which changed in concentration increased, contributing to the overall increase in plasma fatty acids after the laying of the replacement egg. It was found that one of the two fatty acids which increased in concentration in the replacement eggs also increased in the plasma of males (20:1). From the principal component and discriminant function analyses, the fatty acid signatures of first and second eggs are separated by concentrations of 20:1 and 22:1 and the signatures of replacement eggs are closer to the prey than first eggs. Exogenous sources of energy for egg production have been shown to be more important than thought previously in other avian species (Bromley and Jarvis 1993; Perrins 1996; Hobson et al. 1997) and it is likely that this applies to Thick-billed Murres.

Of all possible prey items previously analysed, squid had a higher percent concentration of 20:1 than fish (Lea et al. 2002) but fish species also higher in percent 20:1 were capelin and sandlance (this study). In Norway, male and female capelin showed seasonal increases in percent 20:1 and 22:1 (Henderson et al. 1984) and percent composition ranged between 1.0 and 11.8 % for these two fatty acids. From the results presented in this study, the percent concentration of 20:1 and 22:1 for capelin fall within the range presented above (10.7 and 9.7 % respectively). All the prey items analysed for lipid composition show a higher percent composition of 20:1 than was found in the eggs and could have contributed to the increase of 20:1 found in the eggs. 20:1 increases from 1.2 to 2.5 % and 22:1 increases from 0.2 to 0.3 %. These values are very small because of the high concentrations of other fatty acids within the tissue. If those fatty acids which are highest in concentration are removed from the calculation of percent contribution, 20:1 increases from 4.6 to 9.8 %, and 22:1 increases from 0.7 to 1.2 %. In the plasma, the concentration of 20:1 increased significantly, from 2.3 to 3.5 %, or 13 to 20 % if the dominant fatty acids are removed.

In adult plasma, fatty acids 16:0, 18:0, 18:1, 20:5, and 22:6 account for 83% of all lipids (21, 17, 22, 13, and 10 % respectively), and in eggs, fatty acids 16:0 and 18:1 account for 75 % of all lipids (24 and 51 % respectively). These fatty acids are highly conserved because their concentration and percent contribution do not change. In the prey, percent contributions of 16:0, 18:0, and 18:1 (except for sculpin), were lower than in the plasma and percent contributions of 16:0 and 18:1 were also lower than in eggs. Thick-billed Murres are therefore either 1) preferentially selecting these fatty acids from their energy stores or 2) creating them *de novo* to generate the appropriate concentrations. Consequently, I suggest that these fatty acids are essential for either survival (in adult plasma) or chick development (in eggs) and that those fatty acids found in lower concentrations can be used as biomarkers to identify changes in diet.

Thick-billed Murres at Coats Island bring back a variety of prey items to their chicks and are also considered to be generalist feeders (Davidson 2005). The only information regarding the diet of adults comes from stomach analysis from dead adults. Based upon these studies, it has been found that the diets of Thick-billed Murres vary geographically and temporally (Gaston and Hipfner 2000). In Chapter 3, I showed that the fatty acid signatures of birds breeding on Prince Leopold Island and Coats Island were different and I suggest that this was likely due to differing diets. In addition, other studies have shown variation in fatty acid signatures among individuals to be due to geographic effects (Budge et al. 2002), and dietary effects (Kirsch 1998; Iverson et al. 2002; Pierce and McWilliams 2005), and that there is an interaction between these two (Budge et al. 2002). Because it was found that there was a change in the fatty acid composition of plasma between first and replacement egg laying and the fatty acid signatures of first and replacement eggs differed, I suggest that females are likely using the local food supply for the production of the replacement egg, though it is possible that females are relying upon an alternative endogenous lipid store with a different fatty acid signature. However, since fatty acid signatures appear to move in the direction of prey, and other studies have shown that though endogenous and exogenous stores are utilised for egg production, exogenous sources represent a larger contribution (Hobson et al. 1997).

The eggs in this study were scrambled to allow for multiple analyses by different researchers. For the purposes of lipid analysis alone it would have been preferable to separate the tissues and conduct a lipid analysis of the yolk separately, including measuring the mass of the yolks for first and replacement eggs. However, in a study on Common Murres, it was found that the albumin contained no traces of lipid (Birkhead and Nettleship 1984) and, consequently, my measures of total egg lipid (and not composition) are not as readily comparable with other studies. Birkhead and Nettleship (1984) found that in Common Murres, egg yolks contained 34% lipid. If tissue masses are corrected for dry analysis, their values show that of the total egg mass, Common Murres had 5.6% lipid, compared with the result of 4.2 and 3.9 % on Thick-billed Murre eggs. Differences in measures could be the result of differences in lipid extraction protocols (Dobush 1985), or of variation between breeding colonies (e.g. female body size, Michel et al. 2003; egg volume; Birkhead and Nettleship 1981).

Based upon these data, it was not possible to determine precisely the sources of lipids (either exogenous or endogenous) for the production of eggs. However, it is shown that the fatty acid compositions are different in both first and replacement eggs and adult plasma. Therefore, two possibilities exist: 1) Females rely upon lipid sources at the breeding colony for the production of both the first and replacement eggs but the composition of prey species changes during the time of the deposition of lipids, 2) Females rely upon lipid sources at the wintering grounds by storing that lipid during migration for the production of the first egg and the lipid sources at the breeding colony for the production of the second egg. Though the time for the development of the first egg in Thick-billed Murre is unknown, it was found to be approximately 14 days in Cassin's Auklets (*Ptychoramphus aleuticus*; Astheimer 1986); a related species that also lays replacement eggs within 14 days of losing the first. Given that Thick-billed Murres generally arrive on the breeding colony several weeks before median egg laying (Gaston and Hipfner 2000), and that migration is a very costly activity and storing lipid for use after migration presents little adaptive advantage, it seems likely that females do rely upon food sources at the breeding colony for the development of both eggs.

Further research is required to investigate the plasma fatty acid concentrations and compositions at shorter intervals between first and replacement egg laying and, if possible, prior to laying. In addition, prey and plasma could be collected from wintering grounds and compared with those collected at multiple breeding colonies.

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CHAPTER 5

EFFECTS OF INCREASING THE COST OF CHICK-REARING IN THICK-BILLED MURRES

ABSTRACT

To investigate the management of stress during reproduction by free-ranging Thick-billed Murres, I experimentally increased the cost of diving for individuals rearing chicks. Chick mass, feeding rates, diving depths, and plasma neutral lipid concentrations were measured in handicapped males and females and compared with controls. The experiments were conducted in 2003 using singly handicapped and control groups, and in 2004 using singly handicapped, doubly handicapped, and control groups. I conducted a study of the effects of handicaps on diving profiles of Thick-billed Murres using diving data recorders deployed for 24 h. During the handicapping study, all sampling was conducted on days 1, 6 and 12 of the experiments. Handicapped males and females had lighter chicks or showed significant decline in mass gain than controls in the first experiment and doubly handicapped females had lighter chicks than controls in the second. Manipulated males, but not females, had significantly higher neutral lipid concentrations in their plasma on day 6 of the experiment but they returned to baseline levels by day 12. Females with double handicaps fed their chicks fewer times than controls. Lower masses in chicks may influence their ability to survive their long post-departure migration. The increase in plasma lipids may be a symptom of increased energy mobilisation. I concluded that variable responses to handicapping between years and sexes suggested that individuals managed increases in costs differently and was perhaps due to unmeasured, local annual variation.

INTRODUCTION

Biological processes are generally divided into those required for self-maintenance and those required for reproduction. Any increase in current reproductive effort may be detrimental to self-preservation and to future reproductive success (Fisher 1975; Clark and Ydenberg 1990). This trade-off has been discussed in the context of parent-offspring conflict, since it is in the offspring's interest to increase the investment of the parent in the current reproductive effort, and in the parents' interest to invest cautiously to maximize the chances of future reproductive success (e.g. Trivers 1974). To investigate how individuals manage the trade-off between reproduction and factors affecting subsequent survival, we can manipulate the cost of reproduction and observe the way in which this cost is managed; which activities are compromised, and whether responses vary depending on sex or local environmental differences between years.

All of the biological processes considered require energy, derived in most cases from diet. Therefore, changes in the availability of food will influence an individual's ability to perform these processes and will influence the decisions that are made. In marine birds it is generally thought that there is a continuum of behavioural changes that occurs with declining prey availability that result in various measurable outcomes (review by Cairns 1987); poor to moderate prey availability affects the rate of chick weight gain and, potentially breeding success, though the integration time varies (months for breeding success, weeks for chick weight gain), and it is this section of the continuum that is of interest here. Minor declines influence activity budgeting, while major declines affect individual survival. Determining the threshold at which parents pass on costs to offspring or cease to invest in current reproduction is an important component of predicting the effects of environmental change and the development of responsible population management practices.

Frequently, questions relating to the effects of declining prey availability are addressed only serendipitously, when changes in prey availability, or changes in environmental conditions occur beyond the control of the researcher (e.g. El Niño effects, Barber and Chavez 1983; oil spills, Maccarone and Brzorad 2000; commercial fishing, Nettleship 1991; Bingham 2002). Alternatively, differences observed among years have been attributed, *post hoc*, to a decline in environmental conditions (e.g. Baillie and Jones 2004). Though this type of study can reveal important results, especially when baseline data are available, experiments which manipulate the cost of foraging are desirable because they can be conducted on a predictable temporal scale and allow for the use of control groups. However, these manipulations must simulate a biologically relevant change. The reduction of wing span, thus increasing wing loading, is a common practice in these types of experiments (e.g. Mauck and Grubbs 1995; Velando and Alonzo-Alvarez 2003). For many species, it is likely that a decline in food availability will require an increase in time of flight. For diving seabirds, a change in the availability of food is likely to require an increase in effort during the diving component of prey acquisition, rather than only in flight. Manipulating the cost of diving can address questions relating to how diving seabirds may respond to rising ocean temperatures. Ocean community structures have been reorganised as a result of climate change at higher latitudes (i.e. Anderson and Piatt 1999) and consequently, there have been changes in the diet of some seabird populations (Gaston et al. 2003). In the Eastern Canadian

Arctic, it appears as though arctic cod (*Boreogadus saida*) are being replaced by capelin. Though capelin have been shown to be higher in caloric content by mass (Bradstreet and Brown 1985), they are also generally smaller in size and weigh less (Davidson 2005), and therefore represent less nutritional value per food delivery, given that adult Thick-billed Murres deliver one fish at a time. Arctic cod are distributed deeper in the water column and, therefore, the possibility of changing prey species is only available to diving species, such as murres, though they may require more energy to capture than those species found higher in the water column.

Previous studies on artificial increases in the cost of reproduction suggest that there is a difference in cost management between long and short-lived species (Newton 1989; review: Mauck and Grubb 1995). In addition, Mauck and Grubb (1995) suggest that there is also an interaction between parental investment and clutch size. Ainley and Boekelheide (1990) show that seabirds with smaller clutch sizes showed lower coefficients of variation in breeding success and that lower clutch sizes fledged more chicks per egg than larger clutches. However, if the contribution to life time reproductive success of a single egg clutch in long-lived species is less than multiple egg clutches in short-lived species (Mauk and Grubb 1995), parents of long-lived species are less likely to compromise their survival than short-lived species, whose multiple egg clutches, if reared successfully, represent a higher contribution to life time reproductive success per clutch (Wooler et al. 1992). If these predictions are true, then I anticipate that increasing the cost of parental investment - conducted on a long-lived seabird with a single egg clutch - will result in adults passing on the cost to their chicks.

Several methods for measuring parental investment in reproduction have been utlitised, though behavioural monitoring is most common. For example, Duriez et al. (2000) used data collected on foraging trip duration as an indication of energetic investment in chick-rearing. They showed that chick-rearing parents alter their foraging trip duration based upon their individual body condition. Mauk and Grubb (1995) used data collected on chick-feeding and found that parents adjusted their provisioning rates to the energetic cost of foraging. Taking an interesting approach to explaining interspecific variation in parent investment, Hipfner et al. (2001) found that nest site safety was also an important determinant of parental investment; individuals with safer nesting sites invested more energy into reproduction than those with more exposed nesting sites.

Measuring changes in body components to quantify parental investment has also been explored. For example, Velando and Alonzo-Alvarez (2003) concluded that changes in adult body mass may determine the level of reproductive investment that each individual was willing to make. Similarly, Hillstrom (1995) showed that there is a positive correlation between fledging success and body mass changes in female Pied Flycatcher (*Ficedula hypoleuca*).

More recently, measurements of physiological changes, in lieu of measurements of changes in body mass, for the quantification of energy expenditure have been explored and I suggest that, based upon studies linking the changes in plasma lipid concentrations with energy store depletion (review: McWilliams et al. 2004) that this may be used as an indicator of parental investment. Alonzo-Alvarez et al. (2002) found that the plasma concentration of cholesterol was the best indicator of body mass changes in Yellow-legged Gulls (*Larus cachinnans).* It has been shown that plasma lipid concentrations can be used as an index of lipid mobilisation and, consequently, of lipid use (review by McWilliams et al. 2004). Jenni-Eiermann and Eiermann (1992) found that concentrations of plasma neutral lipids and very low density lipoproteins (composed mostly of neutral lipids; Stryer 1995) increased during migratory flight. Since the maintenance of adequate body stores (most importantly, lipids) is an essential component of individual survival (Blem 1990), we can predict that parents will manage their energy stores at a compromise between the costs of parental investment and individual maintenance. Therefore, if I predict adults pass on the cost of increases in the cost of reproduction to their offspring, there should be no change in the plasma neural lipid concentration when the cost of reproduction is increased.

In this study, I artificially increased the cost of diving in wild Thick-billed Murres, a species known to dive to approximately 100 m when foraging, and observed the management of this cost. I was especially interested in 1) whether adults transferred the costs entirely to chicks and 2) whether the tactical adjustments, if any, differed between the sexes.

I approached these goals from a variety of perspectives. I determined whether there was a change in the diving behaviour of adults with handicaps using data loggers to measure the diving depths and times of individuals. To measure investment in reproduction, I measured chick mass gain and wing growth and adult mass at three periods during the experiment. I measured total plasma neutral lipid concentrations as indicators of energy stores mobilisation, and consequently, energy utilisation. I conducted feeding watches on handicapped and control pairs to determine whether the handicaps influenced feeding rates and total prey mass delivery.

MATERIALS AND METHODS

All manipulations and observations were conducted during the chick rearing periods of 2003 and 2004 on Coats Island, Nunavut in the Canadian Eastern Low Arctic.

Diving Experiment

During the period of late egg incubation in the season of 2004 (July 19-22), the effects of single handicaps on the diving profiles of Thick-billed Murres were investigated. Sixteen adult birds of unknown sex and age were randomly assigned either the treatment of floater and depth gauge or, as a control, only a depth gauge. Half of the birds were caught during the day and the other half at night to attempt an even split between males and females. After capture, the birds were handled as briefly as possible to help ensure that minimal disturbance occurred (to help ensure the recovery of the depth gauges and handicaps). Consequently, no data were collected on these birds prior to deploying the gauges. LOTEK 1100LTD TDRs (Lotek Marine Technology, St. John's, Newfoundland, Canada) were secured with duct tape to plastic bands that were attached to the legs of Murres. The "Lotek" time and depth gauges were cylindrical (mass = 4.5 g) and attached to a leg band with duct tape. The gauges measured temperature and pressure (±2 m; K. Elliott pers. comm.). After approximately 24 hours, the birds were recaptured and all gauges and floaters removed. At this point, I measured the mass of the adults with a pesola spring balance and took a blood sample from the brachial vein for sex determination (see below). Upon returning to the camp, the data from the depth gauges were downloaded to a laptop computer and analysed using the program TAGTALK

(Conservation Devices Inc., Belmont, Massachusetts). Of the 16 devices deployed, I successfully recovered data from 12.

Handicapping experimental design and sample collection

A month prior to manipulating the birds, observations were made daily to determine the date of hatching at each site. On Day 1 (27/07) of the experiment in 2003, 16 adults were caught for the experimental group and 16 individuals were caught for the control group. Handicaps were applied to half of the individuals at random (Figure 6.1). In order to maximize the chances of collecting an even sample of males and females, eight individuals for each group were caught during the day and the remaining 8 were caught at night (Table 6.1). Previous observations have shown that males brood chicks for approximately 12 h during the day while females brood chicks for 12 h during the night (Kober and Gaston 2003).

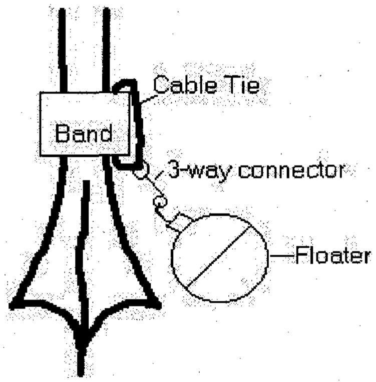

Figure 5.1: Diagram of apparatus (1.2 cm diameter) attached to the leg band to increase the cost of diving.

All handicaps were purchased at a local fishing supply store. Before deployment, the seams in the handicaps were covered with epoxy to ensure that it did not fill with water. On Day 1 (31/07) of the experiment in 2004, 15 adult individuals were caught for the two handicap treatment groups, 15 individuals for the one handicap treatment group, and 14 individuals for the control group. The treatments were randomly applied and as before, with half of the individuals caught during the day and the other half caught at night (Table 5.1).

Table 5.1: Sample sizes of adults and chicks used in the 2003 and 2004 experiments.

		Two Handicaps			One Handicap			Control		
Year	Day	Females	Males	Chicks	Females	Males	Chicks	Females	Males	Chicks
2003	1	--	--	--	10	6	16	7	8	16
	6	--	--	--	4	6	10	3	6	10
	12	--	--	--	7	6	15	4	5	10
2004	1	7	8	15	6	9	15	7	7	14
	6	7	8	15	6	9	15	6	6	13
	12	7	7	14	6	8	14	6	6	12

On each of the sampling days (days 1, 6 and 12) the chicks at each site of the experiment were caught by hand and weighed with a pesola scale. Wing chord was measured with a small ruler to the nearest mm. On day 1, the chicks were banded with a metal band and, in 2004, a small dot of coloured nailpolish was added to their culmen for easier identification. Each chick was released within 3 min of capture and replaced upon the ledge where they were collected. After all chicks were measured, the adult samples were collected. Each bird was captured with a noose pole and placed inside a canvas bag. The adults were weighed with a pesola weigh scale and a blood sample was taken (in 2004 for sex identification only), though it was not possible to catch all birds on days 6 or 12 (Tables 5.1). Approximately 3 mL of blood was taken using a syringe and 27 gauge butterfly needle. The blood was transferred immediately to a 3 mL vaccutainer lined with heparin. The birds were released within 5 min of capture. Upon arriving at the camp, blood samples were centrifuged for 5 min and the plasma was transferred to cryovials. Samples were then frozen in a propane freezer (-20° C) and stored there until they were shipped to Ottawa in a dry nitrogen shipper.

Plasma fatty acid analysis (2003)

Plasma was added to Folch reagent (2:1 v/v). An internal standard (Heptadecanoic acid, 17:0, 30 mg/100 mL hexane) was added for gas chromatography (GC) analysis of the non-esterified fatty acids (NEFA). The resulting mixture was centrifuged at 3000 RPM for 10 min. The supernatant was filtered and KCl (0.25% in distilled water) was added to eliminate water-soluble compounds. This mixture was centrifuged at 3000 RPM for 10 min to facilitate the separation of an aqueous phase. This phase was eliminated with a water-powered vacuum pump and the remaining solvent was evaporated N_2 at 70 °C.

Separation of Neutral Lipid. Total plasma lipids can be divided in 3 classes: neutral lipids (NL), non-esterified fatty acids (NEFA) and phospholipids (PL). These classes were separated to analyze their fatty acid composition. Supelclean solid-phase extraction tubes (LC NH_2, 100 mg: Sigma) were conditioned with hexane. The fats were resuspended in chloroform and transferred into the columns. The NL fraction was eluted by flowing chloroform:isopropanol (2:1 v/v) through the columns. An internal standard (17:0, 30mg/100mL hexane) was added to the neutral fat fraction. The solvent was evaporated under N_2 at 70 ˚C.

Acid Transesterification of Neutral Lipid Fraction for Gas Chromatography Analysis. Fats were resuspended in an acetyl chloride solution (7.2 mL acetyl chloride in 100 mL methanol). The samples were incubated at 90 ˚C for 2 h after which the acetyl chloride in methanol was evaporated under N_2 at 70 ˚C. The fats were resuspended in methanol and evaporated under N_2 at 70 ˚C. The fats were resuspended in isooctane and transferred to GC autosampler tubes.

All samples were then analysed using GC (HP 5890 series II with HP 7673 autosampler and flame-ionization detector). The retention times of the fatty acids were compared with those from known standards.

Sexing analysis (2003 and 2004)

The sex of adult Thick-billed Murres were identified using DNA markers according to methods described by Crump et al. (2006, manuscript submitted for publication).

Feeding Watches (2004)

During the 2004 experiment, feeding watches were conducted during times when data from previous years showed an increase in the frequency of feeds that coincide with the changeover time between the partners. On days 2-5 and 8-10, feeding watches were conducted in the early morning (approx. 04.30-09.30) and in the evening (16.00-20.00).

The numbers of hours of feeding watches were not always equal per day, nor were they necessarily evenly split between those watches where more females were feeding or more males. All feeds of birds used in the experiment were recorded and included the time of delivery, the species delivered, and the approximate size (measured by comparing with the length of the culmen). In addition, all deliveries made by the partner of the individual used in the experiment were recorded, though observational priority was given to those individuals used in the experiment; if two birds were feeding at the same time, and one was not part of the experiment, priority with respect to determining the species and size of prey item fed to chicks was given to the experimental bird.

The estimated sizes and species of the prey were used to convert all measures of size to mass based upon regressions of species specific prey size and mass data collected since 1998 (K. Elliot and A.J. Gaston unpublished). For those species analysed for lipid content (Chapter 5) in 2003, all mass data were converted to lipid mass. In cases where samples sizes were greater than one for the lipid analysis, the lipid conversions were done using the equation of a regression of mass versus total body lipid. Where lipid analysis data for a species were conducted on only one individual, lipid conversions were done based upon the percent lipid content. Species observed during the 2004 feeding watches where there was no previous lipid analysis were omitted from the data set (two cases of very rare prey item where no sample has ever been collected). Amphipod lipid content was estimated based upon an average mass of 0.5 g per individual with a delivery of 3 individuals per feed.

Statistical Analyses

Adult masses, plasma neutral lipid concentrations, and feeding watch data were analysed using ANOVAs. Chick masses and wing lengths were analysed using ANCOVA with chick age as the covariate to account for any differences in the age distribution of chick among treatments, although, within experiments, there was no significant difference in ages among the treatment groups. Due to the high among-individual variation in diving profiles, a mixed model ANOVA with Satterthwaite approximate F-test was used to identify any significant differences between the diving profiles of birds with and without floaters. High variability in the diving profiles of individuals within a pair and among pairs has been observed for other species of diving birds (Adelie Penguins *Pygoscelis adeliae*; Takahashi et al. 2003). I calculated the total time for each dive as the difference between the time of submersion below 1m (which resulted in a dive deeper than 5 m) and the time of that the depth of the dive returned to less than 1 m. Maximum depth was defined as the deepest depth recorded by the data logger of a dive which exceeded 5 meters. No individuals were excluded from the analysis following these criteria.

All statistical analyses were conducted using SYSTAT version 9 software and the level of significance was set at 0.05. All tests were one-tailed where there were strong *a priori* predictions about the direction of an effect. Two-tailed tests were used for the diving experiment, for comparisons between sexes, and for testing changes in plasma neutral lipid concentrations (two-tailed analyses indicated).

RESULTS

Diving Experiment

373 dives by 5 males (2 controls, 3 handicapped) and 498 dives by 7 females (3 controls, 4 handicapped) were analysed. Though the average time per dive was lower in handicapped birds (Table 5.2), it was not found to be significant. Birds among the control group dove significantly deeper than those birds with handicaps. There was no significant effect of sex for either dive times or depths (two tailed: $F_{1,10}<2.59$, $p>0.05$).

Table 5.2: Average maximum diving depths and dive times (±SE) for male and female Thick-billed Murres in control and singly handicapped experimental groups.

	Male		Female			
	Control	Handicapped	Control	Handicapped	$F_{1,10}$	p^a
Average time (s)	42.6 (1.9)	31.8 (1.0)	61.2 (2.1)	38.7 (1.7)	3.67	0.11
Average maximum depth (m)	81.0 (2.6)	68.2 (1.9)	105.7 (2.7)	79.1 (2.3)	6.05	0.046

[a] p values are for two-tailed analyses.

Handicapping Experiments

Adult Mass. In the 2003 experiment, handicapped adults did not weigh less after the experiment than controls ($p=0.12$, $F_{5,65}=1.384$). In 2004, doubly handicapped adults were significantly lighter than singly handicapped or control groups with no significant effect of sex and no interaction between sex and treatment (Treatment: $F_{2,34}=2.93$, $p=0.034$). However, further analysis with separate sexes showed that adult males with two floaters were significantly lighter (886.4g±11.9) than singly handicapped individuals

(926.9g±11.2) or controls (936g±14.1) on day 12 of the experiment ($F_{2,17}$=4.54, p=0.026) while there was no significant difference among females, though mean mass was lower (doubly handicapped: 882.9g ± 25.3, singly handicapped: 938.3g ± 27.3, controls: 927.5g ± 28.0, $F_{2,16}$=1.274, p=0.15). Though there seems to be a decline in mass among the females based upon the comparison of the mean mass with that of the males in the doubly handicapped group, the standard errors are much higher in the female group and the statistical analysis is not significant. In 2003, with all treatments combined, males were significantly heavier than females ($F_{1,69}$=4.019, p=0.025). In 2004, there was no difference in mass between the sexes ($F_{1,41}$=0.42, p=0.26).

Chick Mass 2003. Chick masses at the onset of the experiment in 2003, when controlled for age using an ANCOVA, did not differ significantly among treatments (2003: $F_{2,30}$=0.06, p=0.81). No comparisons of chick mass between and within groups were significant except for the mass of chicks with handicapped males on the 12th day in 2003 ($F_{1,8}$=23.04, p=0.001; Figure 5.2a). The slopes of the regression lines for chick mass on age for female handicapped parents in 2003 was significantly different from that for chick mass of controls on day 12 ($t_{3,7}$= 134.22, p<0.001; Figure 5.2b), suggesting that there was a decline in rate of mass gain though the absolute difference in mass was not significant ($F_{1,10}$=0.89, p=0.18).

Chick Mass 2004. The control chicks in 2004 were an average of 10 g lighter than the singly or doubly handicapped groups, although the differences were not significant at the beginning of the experiment. When mass of chicks reared by pairs where the male was handicapped were analysed separately, the chicks in the control group were significantly lighter than those in the single handicap group ($F_{2,15}$=6.31, p=0.004). No comparisons of chick mass between and within groups were significant except that chicks with doubly handicapped mothers were lighter than the other treatments on the 12th day ($F_{2,16}$=5.06, p=0.02 Figure 5.3). Because the mass of the chicks with control fathers in 2004 was significantly lighter than singly-handicapped, I looked at the mass gain of those chicks throughout the experiment. Chicks with singly-handicapped fathers gained less mass than controls ($F_{1,12}$=3.81, p=0.038). Chicks with doubly-handicapped mothers gained less mass than singly-handicapped mothers and controls ($F_{2,16}$=2.8, p=0.045).

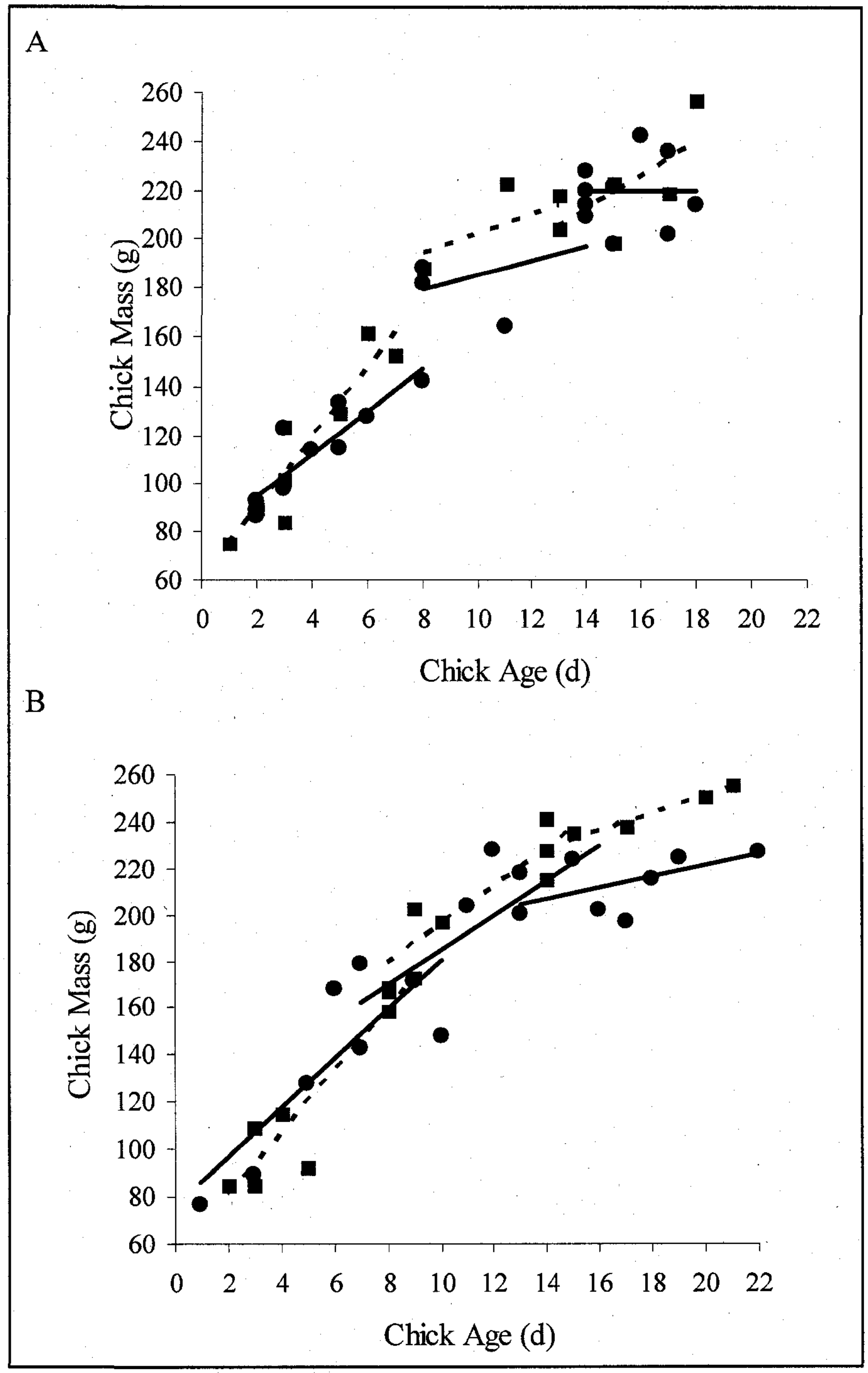

Figure 5.2: Mass of chicks with handicapped (circles and solid trendlines) and control (squares and dashed trendlines) female parents (A) and male parents (B) in the 2003 experiment.

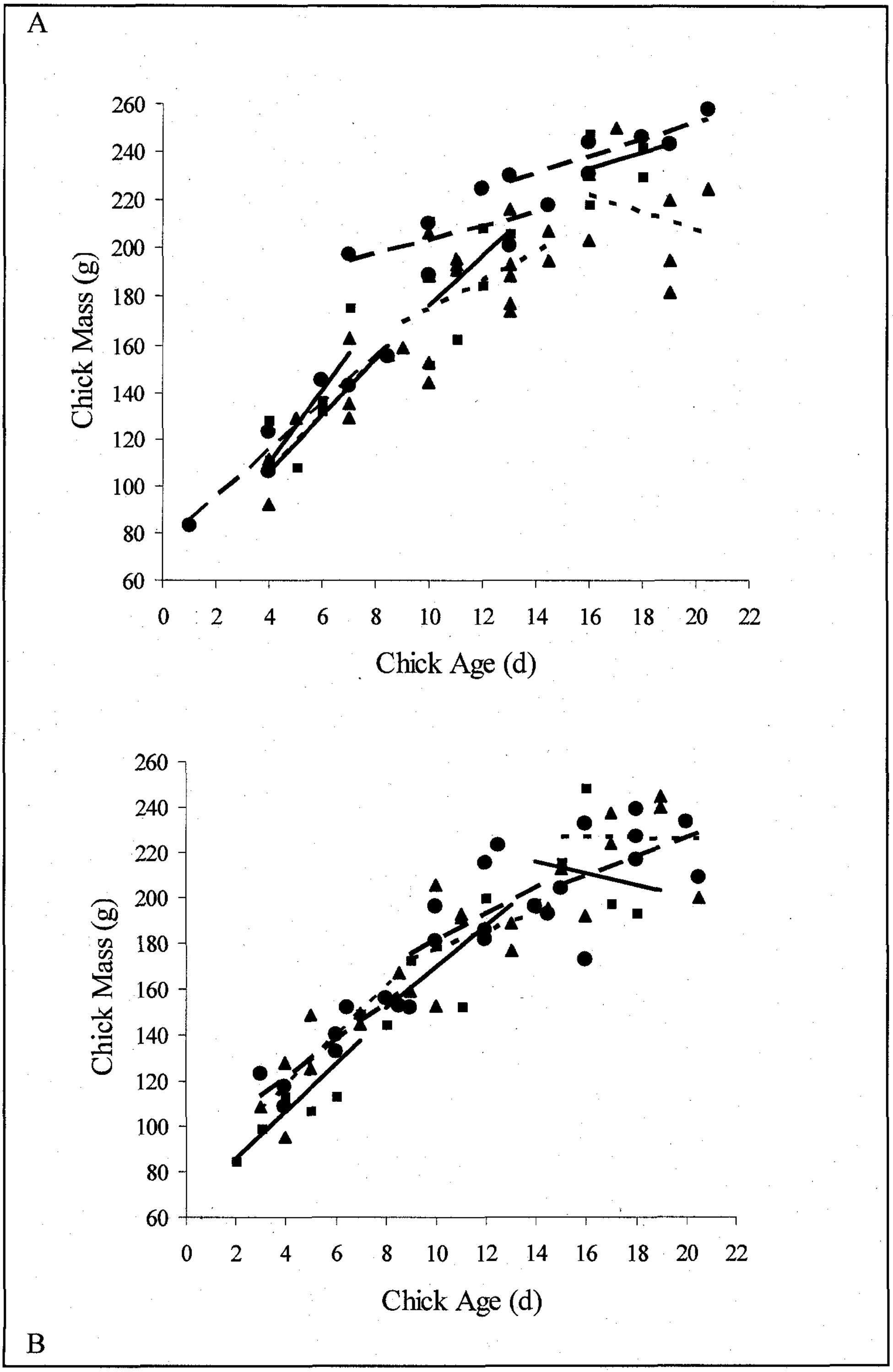

Figure 5.3: Mass of chicks with doubly handicapped (triangles with dotted trendlines), singly handicapped (circles and dashed trendlines) and control (squares and solid trendlines) female parents (A) and male parents (B) in the 2004 experiment.

Chick Wing Length. Chick wing development appears to be conserved, despite the lower mass, or mass gain, of the chicks with handicapped parents, as chick wing length was not different in this group when compared with those with control parents on any of the sampling days (Table 5.3).

Table 5.3: Wing length of chicks on day 12 (mm ± SE) of the handicapping experiment with singly handicapped, doubly handicapped, and control parents in 2003 and 2004.

	Single Handicap	Double Handicap	Control	F_2	p
2003	71.5 (1.9)	--	74.0 (2.8)	0.54	0.3
2004	69.0 (0.2)	66.5 (0.2)	65.8 (0.2)	2.2	0.06

Adult Plasma Fatty Acids (2003). Total fatty acid concentration was significantly higher on day six in handicapped males ($F_{2,12}$=6.88, p=0.01). When the lipid fractions were analysed separately, plasma neutral lipid concentration peaked on day 6 in handicapped males ($F_{2,14}$=19.125, p<0.001; Figure 5.4) when compared with the concentrations from the same individuals on days 1 and 12 and with male and female controls on day 6 ($F_{2,15}$=10.15, p=0.001). A further analysis of the concentrations of specific neutral lipid fatty acids showed that the increase in total neutral lipid concentration on day 6 of the male adults with floaters was due to a significant increase in the concentration of neutral lipid 15:1 ($F_{5,31}$=2.73, p=0.02), 20:1 ($F_{5,31}$=2.91, p=0.015), 20:5 ($F_{5,31}$=4.48, p=0.002), and 22:6 ($F_{5,31}$=6.3, p=0.0001). In females, plasma neutral lipid concentrations were unchanged in the same individuals throughout the experiment, however, on day 6 neutral lipid concentrations were significantly lower in birds with floaters compared with controls ($F_{1,5}$=16.72, p=0.01; Figure 5.4). These levels, however, were not different from those samples collected on day 1 of the same group ($F_{1,9}$=0.889, p=0.19), suggesting that handicapped females do not show increasing plasma neutral lipid concentrations as is the trend in the control sample. The increase in plasma neutral lipid concentrations was not significant ($F_{2,11}$= 1.29, p=0.31) in this experiment with small sample sizes (but see Chapter 3).

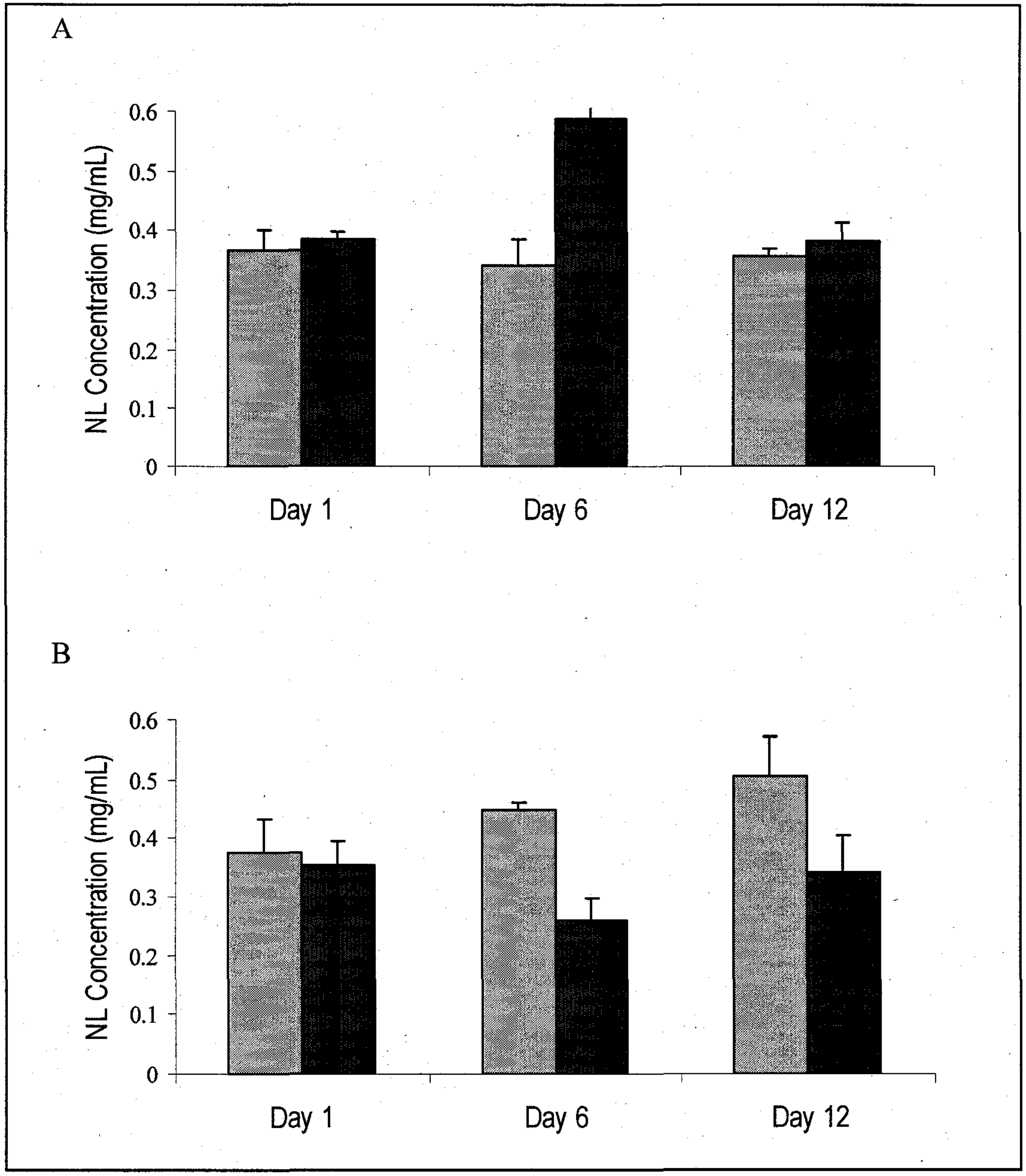

Figure 5.4: Plasma neutral lipid concentration (±SE) in male (A) and female (B) Thick-billed Murres in the control group (light bars) or with a single handicap (dark bars).

Feeding Watches (2004). Feeding data were log transformed because the distribution of the data was not normal. There was no difference between the feeding rates of the males and females among the control group (two-tailed: $F_{1,23}$=3.54, p=0.073), though the feeding watches were not conducted over 24 h and, consequently, observation times could have favoured one sex. Though I could not control for this variable, other studies on this species have shown that there is no difference in feeding rates between the sexes (Gaston and Nettleship 1981; but see Paredes et al. 2006).

When all data were analysed together in a multiple ANOVA, there was a significant effect of treatment on the log-transformed feeding frequencies of adults (two-tailed: $F_{11,62}$=1.98, p=0.047; Figure 5.5).

When the data were separated by the sex of experimental individuals, doubly handicapped females made the fewest prey deliveries to their chicks compared with females in all other groups ($F_{5,30}$=2.56, p=0.024). When compared with females only in the control groups, this difference was also significant (5.00±1.08; $F_{2,14}$=3.92, p=0.01). There is some evidence of compensation by the male partners of the doubly handicapped females because the increase in feeding rate of these males was significant when compared with control males ($F_{1,9}$=3.58 p=0.045). There was no significant effect on the number of food deliveries in singly handicapped groups when compared with control groups within the sexes ($F_{7,42}$=0.73, p=0.32). There was no effect of treatment or sex on the average size of prey delivered to chicks (Treatment: $F_{5,63}$=0.51, p=0.77, Sex: $F_{1,63}$= 0.0001, p=0.99).

Capelin delivered by parents to chicks during the experiment were significantly lighter than cod (Mean calculated mass: capelin = 1.39 g ± 0.08, cod = 7.2 g ± 0.2; $F_{1,657}=765.8$, $p<0.0001$). Consequently, if we apply the caloric values for each species determined by Bradstreet and Brown (1985), who show that capelin is the most energy rich fish in their study, we find that adult Thick-billed Murres are delivering, on average, 10.4 kJ of energy per capelin and 42.5 kJ per arctic cod. Comparisons of average fat content delivered per fish based upon the regressions from the lipid analysis also show that the absolute amount of fat delivered per fish is higher in cod ($F_{1,658}=1515.6$, $p<0.0001$).

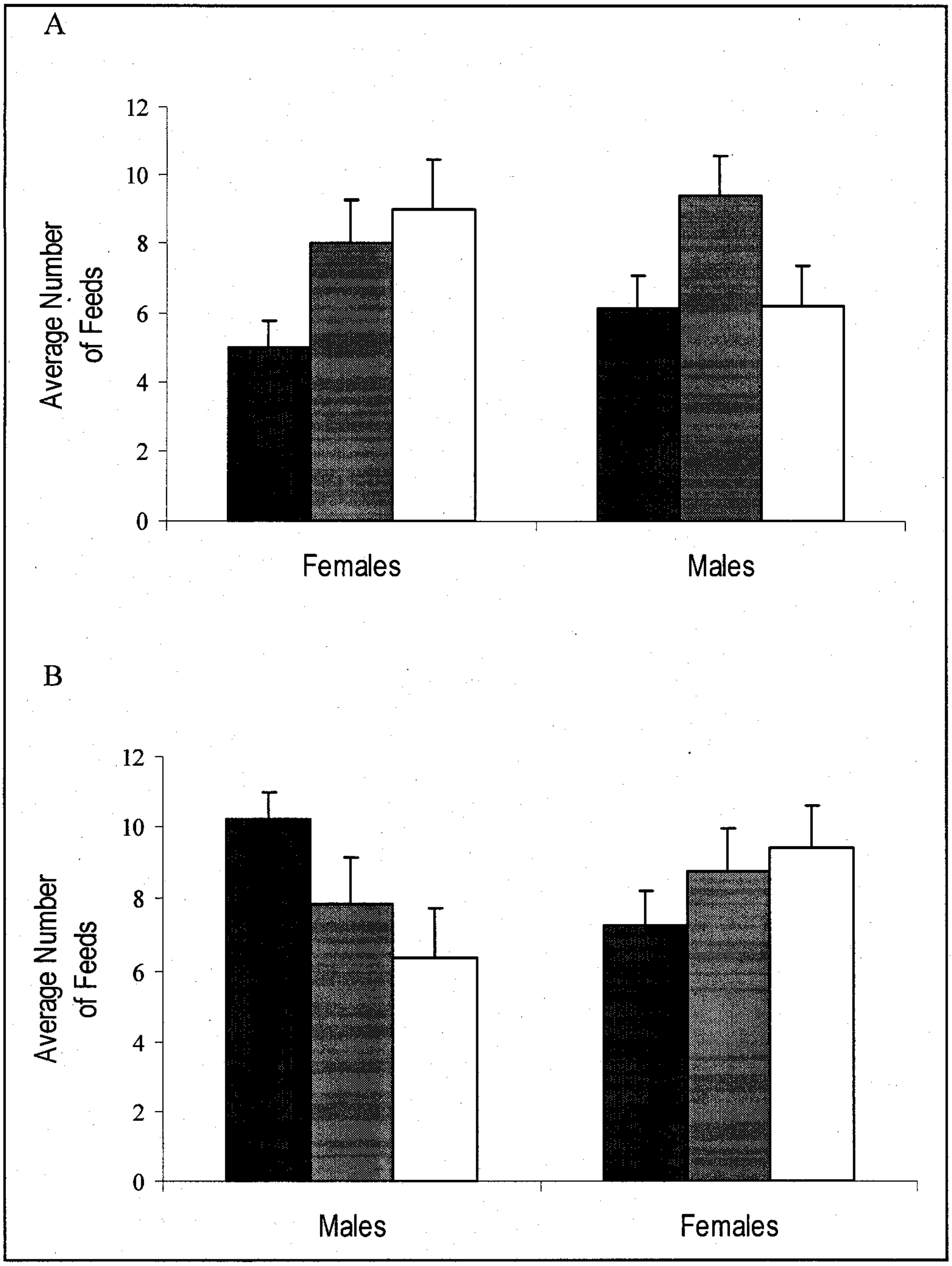

Figure 5.5: Average number of food deliveries to chicks by A) adults used in the experiment and B) their partners (±SE). Treatment groups were doubly handicapped (darkest bars), singly handicapped (intermediate bars), and control (lightest bars).

Average prey mass was calculated by estimating the total mass of all prey items delivered to the chick during all feeding watches, divided by the number of prey items delivered for each site. In a two-way ANOVA, I found that the decreased mass fed to chicks by handicapped adults compared with controls, was not significant (doubly handicapped: 2.16 g ± 0.42; singly handicapped 3.17 g ± 0.40, combined controls and partners: 3.01 g ± 0.21, $F_{2,70}$=1.95, p=0.07). However, doubly handicapped adults fed less mass to their chicks when compared with controls (Table 5.4). The lower average mass delivered to chicks by doubly handicapped females was not significant when compared with control females (doubly handicapped: 2.17 g ± 0.66; controls: 2.96 g ±0.3, $F_{1,28}$=1.21, p=0.14). Doubly handicapped males did not deliver significantly less mass to their chicks than controls (doubly handicapped: 0.9 g ± 0.25; controls: 1.1 g ± 0.15, $F_{1,29}$=0.37, p=0.27).

Table 5.4: Average prey mass (g ± SE) delivered to chicks by singly handicapped, doubly handicapped, and control parents in 2004.

	Single Handicap	Control	Double Handicap
Average prey mass	3.17 (0.4)	3.01 (0.4)	2.16 (0.4)
F	0.06		4.11
p	0.41		0.02

When the lipid equations were used to transform prey mass to total lipid by species (See Appendix 1 for conversions), doubly handicapped adults delivered less fat to their chicks than singly handicapped or control adults and the partners of the treated adults ($F_{2,70}$=3.6, p=0.03). Comparisons by sex revealed that doubly handicapped females delivered less fat than control females ($F_{1,29}$=4.59, p=0.04). Doubly handicapped males did not feed significantly less fat to their chicks than control males ($F_{1,13}$=0.197, p=0.17).

DISCUSSION

I show that the increase in the cost of reproduction by handicapping can be passed on by parents to their offspring. In those cases where this occurs, presumably the increase in the cost of self maintenance results in a reduction in parental effort, because chicks with handicapped parents gained less mass, or were lighter than those with control parents. These results also show that the increase in the cost of the handicaps was not so great as to force parents to abandon reproduction entirely (Drent and Daan 1980). Though I did not see a change in mass among the parents in 2003, it was found that during the chick-rearing period males showed elevated plasma neutral lipid concentrations, an indication of increased mobilization of lipid stores, which then returned to control levels as the chicks decline in mass gain. Mass loss among the doubly handicapped males in 2004 suggests that the double handicap was more costly than the single handicap and that males were further affected to the point of reducing their energy stores. Differences in the observed response to handicapping between the years suggests that Thick-billed Murres can respond to increasing challenges in a variety of ways and that the local conditions, which often vary among years, may have differed in 2003 and 2004 (Table 5.5).

Table 5.5: Summary of all effects for singly and doubly handicapped adults on Coats Island, Nunavut in 2003 and 2004.

	Females			Males		
	Single 2003	Single 2004	Double 2004	Single 2003	Single 2004	Double 2004
Diving depth	NA[a]	- effect	NA	NA	- effect	NA
Diving time	NA	No effect	NA	NA	No effect	NA
Adult mass	No effect	No effect	- effect	No effect	No effect	- effect
Chick mass	- effect	No effect	- effect	- effect	- effect	No effect
Chick wing growth	No effect	No effect	No effect	No effect	No effect	No effect
Adult plasma lipids	No effect	NA	NA	+ effect	NA	NA
Feeding rate	NA	No effect	- effect	NA	No effect	No effect[b]
Prey mass	NA	No effect	- effect[c]	NA	No effect	- effect[c]
Prey lipid mass	NA	No effect	- effect	NA	No effect	No effect

[a] Not measured.

[b] Though male partners of doubly handicapped females did compensate for lower feeding rate of their mate.

[c] Data from sexes combined.

Effects on Adults

The traditional method for evaluating the effects of increased cost to reproduction on adults (mass) did not reveal any significant differences between the handicapped and control groups in 2003. Mass has shown to be an inaccurate method for the evaluation of the size of energy stores and, because mass incorporates not only lipids but also protein and water, it is less likely to be as dynamic and as energetically relevant as measures of lipids. Though the size of lipid stores has shown to affect activities such as reproduction and migration (Robin et al. 1998, 2001, Groscolas et al. 2000, Velando and Alonzo-

Alverez 2003), the detection of declining lipid stores using mass might not occur because lipids represent a smaller component of body mass. A similar handicapping study on Thick-billed Murres breeding off the coast of Labrador by Paredes et al. (2005) found significant declines in adult body mass when they were fitted with a time-depth recorder (TDR) mounted on their backs. In addition, they found that males lost mass more quickly than females. Data from this study show that doubly handicapped males lost mass while females did not. Given that there appears to be a difference in the cost of carrying the handicaps versus TDRs due to differences in shape and location of placement, I did not expect a matching result, however, these data support the direction of effect found in Paredes et al. (2005) as males lost mass before females.

Though the mechanisms for the mobilization of lipids are still not entirely clear for birds, and alternatives to the mammalian models have been proposed (i.e. Jenni-Eiermann and Jenni 2002), it is known that lipids are the primary source of energy for birds during migration (high intensity exercise; Blem 1976, 1990). The pathway for the mobilization to the muscles for oxidation of lipids is through the circulation; increased plasma lipid concentrations occur during increased energy expenditure in birds and other species (pigeon; Vallyathan and George 1969, mummichog (small fish); Jensen and Taylor 2002, birds; review by McWilliams et al. 2004). Since neutral lipids are the primary lipids utilised for energy production, they can be used as an indicator of energy expenditure (i.e. Jenni-Eiermann and Eiermann 1992).

The chick rearing period is the time thought to be the most energetically demanding for Thick-billed Murres (Gaston 1985; Paredes et al. 2005). This is supported by the observation that plasma lipid concentration increased during chick-rearing (Chapter 4). During the 2003 experiment, there was an increase in plasma neutral lipid concentrations in male adult Thick-billed Murres with handicaps that then declined to day 1 levels by the end of the trial. Male adults appeared to be compensating for the cost of the floater by increasing the mobilization of lipids up until at least day 6, whereby within the following six days, the mobilization of lipids returned to day 1 concentrations. Between days 6 and 12, the cost appeared to be passed on to the offspring which was manifested by lower mass in those chicks. From the 2004 experiment, the mechanism by which this cost is passed appears to be by a reduction in feeding rates and, consequently, a reduction in lipids delivered to chicks. These results from the diving experiment suggest that some compensation occurs and that the handicaps present a challenge with respect to achieving normal diving depths.

Given that I obtained less of a difference in chick mass in singly handicapped females, it suggests that pairs with singly handicapped females were able to maintain the mass gain of their chick longer than pairs with singly handicapped males but failed to do so in 2004 with doubly handicapped females while handicapped (singly and doubly) males were able to overcome the cost. In another study on Thick-billed Murres, it was found that males spent more time brooding chicks than females, and that females made more frequent prey deliveries to chicks than males (Paredes et al. 2006). The authors suggest that 1) males may be at a disadvantage with respect to chick provisioning because of a large energetic investment at the beginning of the season defending the breeding site and 2) females may be unable to rear the chick at sea because of their high investment in rearing on the colony. They do not consider directly any cost of egg production though they suggest that the cost of egg production by birds that lay single, large eggs may be greater than for those that lay multiple small eggs. Studies conducted on Prince Leopold Island and Coats Island have not revealed any differences between the sexes regarding egg incubation or chick-rearing (Gaston and Hipfner 2000). In addition, Paredes et al. (2005) found that there were no differences in feeding rate of males and females provisioning chicks during the first two weeks of chick-rearing. This suggests that reproductive strategies and investment partitioning between the sexes may vary among years or with differing local conditions.

In 2003, female handicapped Thick-billed Murres did not increase the utilisation of their lipid stores during the experiment. It is likely that the adaptation to the handicap was expressed by a change in foraging behaviour that did not compromise the females' condition. Differences in the average diving depth of handicapped individuals were observed, though I did not detect a difference with sex. Doubly handicapped females were not able to provision their chicks at the same rate as controls and, consequently were unable to deliver the same amount of energy (lipid) to their chicks as the control and partner group. That there was no apparent physiological effect on females (i.e. neither a decline in mass nor increased mobilization of lipids) was surprising, though it is possible that the female's mate compensated and the experiment did not include observations of mate in this year. However, in the 2004 feeding watch study, the provisioning rates of males with doubly handicapped female partners did increase, though not significantly.

Moody et al. (2005) have shown that, in Common Murres, adults work harder than predicted because the likelihood of divorce increases with a decreased effort during chick-rearing in the previous reproductive season. This prediction is supported by Paredes et al. (2005) who observed an increase in divorce rate when parental effort was reduced the previous year. Consequently, the reproductive pair may experience a reproductive success of zero if they fail to compensate for a decline in reproductive effort (Paredes et al. 2005) or increased environmental challenges, such as declining prey availability. Confirmation of a higher incidence of divorce was not possible in this study because observations of breeding were not made in the year following the experiment. However, it was generally noted that very few birds from the previous study were caught from that breeding plot the following year.

A model of parental care by MacNamara et al. (1999) predicts that, should compensation occur by the unhandicapped partner, it will not account for the entire decline in parental effort by the handicapped mate. The feeding watch data suggest that there may be compensation by the mate when provisioning rates decrease by the handicapped partner. However, there is still a reduction in mass gain among the chicks with singly (2003) or doubly (2004 – females) handicapped parents, suggesting that though there may have been a slight increase in provisioning rates of the unhandicapped partner, it was not enough to prevent a cost transfer to the chicks. Paredes et al. (2005) found that though compensation by unhandicapped partners was evident by higher feeding rates, the increase in rates were still below the feeding rates of control groups. Chick growth parameters were not measured in their study and so they were unable to determine the extent to which there was a transfer of cost to the chicks.

The effects of the handicaps on diving profiles further demonstrate clearly that there is an increase in the cost of the handicaps. Individuals with handicaps either chose not to or could not dive as deeply as controls. Depth is an important component of diving as many fish are only found at greater depth or on the ocean floor. Handicapped birds may have experienced reduced access to these species and increased energetic cost of catching any prey in the water column. This could limit their ability to provision chicks while maintaining their own condition. Also, birds that fly further from the colony do not dive as deeply (K. Elliott, pers. comm.). Therefore, it is possible that handicapped birds could have offset the handicap by flying further.

The strategy for coping with the handicaps apparently involves adjusting several activities associated with reproduction (i.e. increasing lipid mobilization, reducing feeding rate, diving less deeply). Variation in results between years could reflect different environmental conditions and was beyond my control. Should we continue to execute these types of real-situation manipulations, we will be able to use the differences in response between years as an indicator of not only the magnitude of environmental changes but also the quality. For example, if we observed that females have reduced their chick-feeding rates, then perhaps there is a change in the local environment influencing the distribution of prey items found only where females forage. Or, if both males and females have elevated plasma lipid concentrations than in previous years, then perhaps both are working harder, compromising their body condition to overcome an environmental challenge equally experienced by both sexes.

After the chick leaves the breeding site, male and female Thick-billed Murres participate in different activities (Gaston and Hipfner 2000). Males embark on a long-distance swimming migration with the chick and continue to provision it, while females remain on the colony for many days before starting a flying migration. The swimming migration is, presumably, of high energetic cost because the male is required to provide for the nutritional requirements for both itself and the chick (Harris and Birkhead 1985). The female, on the other hand, should experience very little energetic stress prior to migration. The reasons for this difference between the sexes is unknown but, with further analysis, the data collected on control groups and the way in which handicapping is managed by males and females may provide answers to some of these questions.

Effects on Chicks

These data support the prediction by Mauck and Grubb (1995) that in long-lived species, the cost of an increase in parental investment is passed on to the chicks. In two cases, the handicap(s) seemed to have an effect on chick mass. In 2003, chicks with singly handicapped males and in 2004, chicks with doubly handicapped females, were lighter than controls.

Differences between the sexes have been observed in other handicapping studies (i.e. Velando and Alonzo-Alvarez 2003; Blue-footed Boobies). If I had conducted the 2003 experiment for a longer period, we might have seen a significant difference among the chicks with handicapped adult females, and not only a significant difference in the rate of mass gain. However, given that some chicks were 20 days old by day 12 of the experiment, and fledging usually occurs after 21 days, it would not have been possible to extend this experiment. That there was no effect on the mass of chicks with handicapped male parents in the 2004 experiment, and only on chicks with doubly handicapped female parents, suggesting that the local conditions were such that the handicaps affected only those individuals foraging during the day, and who therefore dive more deeply.

Paredes et al. (2006) showed that females deliver more prey to chicks during rearing, while males forage further from the colony. This difference in reproductive activity between the sexes has not been found for birds breeding on Coats Island or Prince Leopold Island. Another study by Paredes et al. (2005) showed that there was no difference in feeding rate among males and females within the first two weeks of chick-rearing.

I show that the decline in mass gain of chicks with handicapped parents is likely a result of decreased feeding rates. Takahashi et al. (2003) observed a similar trend on Adelie Penguins *Pygoscelis adeliae*; the frequency of prey delivery to chicks was positively correlated with chick growth rates. In many bird species, there is a relationship between nestling weight and juvenile survival (review in Magrath 1991; Hedgren 1981, Barrett and Rickardsen 1992; Keedwell 2003) and between feeding rate and juvenile survival, fledging mass and fledging period (review Barrett and Rikardsen 1992). In addition, dramatic changes in the composition of prey species delivered to chicks has been shown to affect only their mass (Atlantic Puffins *Fratercula arctica*: Baillie and Jones 2004; Common Murres *Uria aalge*: Davoren and Montevecchi 2003). Davoren and Montevecchi (2003) also show that annual declining availability of gravid capelin (linked to an overall decline in the energy delivered to chicks) was correlated with a decline in chick mass by 2 grams per year, and a significant decline in chick body condition. Ricklefs et al. (1984) show that variable conditions of prey availability likely explain the differences in mass gain of chicks at different colonies in the Galapagos Islands (Blue-footed Boobies *Sula nebouxii*).

In this study, chicks appeared to be conserving their wing growth at the expense of compromising mass gain. Preservation of wing growth is an important component of successful fledging (Hipfner and Gaston 1999) and a study by Wilhelm and Storey (2004) has demonstrated that wing growth is increased in years of poor conditions in order to, potentially, facilitate an early fledging. Here I show that wing growth preservation for fledging is more important than mass, despite a tradeoff in survival during migration which may impact their survival. Nestling mass is an important determinant of population recruitment, though studies which address this issue are few and limited to a small number of species (Magrath 1991).

It has been suggested that lipid storage by chicks is not an adaptation to overcoming periods of food shortage (or fasting; Lack 1968) but, rather, a byproduct of consuming prey with high lipid and relatively poor nutrient content (Ricklefs et al. 1984). However, it is also possible that energy accumulation could occur in preparation for energy expenditure, as we see in adults of many species (e.g. in preparation for migration), though I know of nowhere where this has been suggested previously. Thick-billed Murre chicks embark on an extended swimming migration only three weeks after hatching. Consequently, the accumulation of adequate energy stores is likely to be necessary for survival during migration, even if they are provisioned by the adult male during this time.

In addition, Thick-billed Murre chicks do not lose mass prior to fledging from the nesting site. Therefore, lower fledging masses are likely to influence chick survival during migration.

In cases where there appear to have been dramatic declines in prey availability, studies have shown significant effects on seabird populations (Cairns 1987; Diamond and Devlin 2003). Vader *et al.* (1990) concluded that a dramatic drop in Guillemot numbers in 1986 and 1987 was likely due to a collapse in their traditional prey. A decline in Thick-billed Murre population in the 1970s was associated with a decline in polar cod stocks (Barrett and Krasnov 1996). But seabird populations (not reproductive success) have also shown to be poor monitors of changes in fish stocks (due to low fecundity and delayed maturity; Barrett and Krasnov 1996) and suggest that using measurements of seabird population dynamics will only reveal significant effects when there is a dramatic decline in prey availability (Cairns 1987).

In order to ensure that populations are managed sustainably and early warning detectors to evaluate the health of the marine Arctic ecosystem are established, sensitive tools are required. Diamond and Devlin (2003) suggest that changes in prey availability will likely affect foraging effort, timing of breeding, breeding success, and adult survival. Of these the authors identify that breeding success, defined to include condition and growth of chicks, will be affected within weeks/months, and within the foraging range for that colony. Baillie and Jones (2004) found that of all measures of Atlantic Puffins, chick growth (defined here as mass gain) was the most sensitive measure of changes in prey abundance.

Either large scale climatic changes or local disturbances are associated with changes in prey availability (Anderson and Piatt 1999, Golet et al. 2002). In the Arctic, changes due to both are likely compounded; melting will allow for intensive shipping which is associated with local disturbance. Thick-billed Murres are known to be impacted deeply by local disturbances such as oil spills (Gaston and Hipfner 2000) and now I show that changes in prey availability may influence the growth of chicks and, potentially, the reproductive success of breeding Thick-billed Murres.

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APPENDIX 1

Conversions from fork length to body mass:

Cod: body mass = $8 \times 10^{-6} \times (\text{fork length})^{2.9569}$ (r^2=0.85)

Capelin: body mass = $8 \times 10^{-8} \times (\text{fork length})^{3.8461}$ (r^2=0.84)

Sculpin: body mass = $5 \times 10^{-5} \times (\text{forklength})^{2.5704}$ (r^2=0.88)

Sandlance: body mass = $4 \times 10^{-6} \times (\text{forklength})^{2.8944}$ (r^2=0.85)

Fish Dr.: body mass = $5 \times 10^{-6} \times (\text{fork length})^{2.8573}$ (r^2=0.89)

Blennies: body mass = $2 \times 10^{-4} \times (\text{fork length})^{2.0732}$ (r^2= 0.83)

Shrimp: body mass = $12 \times 10^{-4} \times (\text{length})^{1.8779}$ (r^2=0.81)

Squid: body mass = $39 \times 10^{-4} \times (\text{length})^{1.6192}$ (r^2=0.81)

Amphipods: body mass = 0.5 g

Conversions from body mass to lipid mass:

Cod: lipid mass = $0.1071(\text{body mass})^{0.701}$ (r^2=0.08)

Capelin: lipid mass = $0.0377(\text{body mass})^{1.2711}$ (r^2= 0.74)

Sculpin: lipid mass = $0.057(\text{body mass})^{0.9883}$ (r^2=0.81)

Sandlance: lipid mass = $0.0668(\text{body mass})^{0.9648}$ (r^2=0.85)

Fish Dr.: lipid mass = (body mass) x 0.086

Blennies: lipid mass = $0.9303(\text{body mass})^{-0.5785}$ (r^2=0.71)

Shrimp: lipid mass = (body mass) x 0.131

Squid: lipid mass = (body mass) x 0.079

Amphipods: lipid mass = (0.5 x 3) x 0.044

DISCUSSION

In Chapter 1, I examined the changes in body composition in Northern Fulmars, Thick-billed Murres and Black-legged Kittiwakes. It was shown that in a very poor year for reproduction these species manage their stores differently during reproduction. In Northern Fulmars and Thick-billed Murres, body lipid changes were observed between egg incubation and chick-rearing. However, in Black-legged Kittiwakes, lean mass changed while lipid mass remained constant during reproduction. For Thick-billed Murres, I used changes in the variance and coefficients of variation to suggest the identification of a minimum body lipid threshold for chick-rearing, presumably below which reproductive effort would cease for that year.

In Chapter 2, I found that plasma fatty acids can be used as a better predictor of total body fat in Thick-billed Murres and Northern Fulmars than other indices based solely on external measurements. Unfortunately, it was not possible to test the reliability of the lipid condition index, and I am not convinced that it would be an effective use of researchers' time. From literature and the results presented in this thesis, I believe that the fatty acid composition is likely a reflection of dietary fatty acid composition, while the plasma concentrations are indicators of energy expenditure (see below for more discussion).

Chapter 3 describes the changes in plasma lipid concentrations throughout reproduction on both Prince Leopold Island and Coats Island. The environmental conditions observed on Prince Leopold Island in 2002 suggest that birds were facing especially challenging conditions during this year. On Coats Island, I found the total plasma lipid and neutral lipid concentrations increased during chick-rearing. This suggested that adults were working harder while foraging for their chicks compared to efforts during egg incubation.

The experiment described in Chapter 4, conducted on first and replacement eggs, supports the prediction by Mauk and Grubb (1995) that reproducing female adults of long-lived, single egg clutches species pass increases in the cost of reproduction to their offspring while maintaining their own condition. It was found that females do not mobilise more plasma lipids after laying a replacement egg, and suggests that they reduce their energy expenditure accordingly. When compared to the fatty acid composition of first and replacement eggs, changes in specific fatty acid concentrations were reflected by changes in similar fatty acids within the adult plasma. The fatty acids of highest concentration in both the first and replacement eggs (16:0 and 18:1) declined in concentration between the laying of the first and replacement eggs in female plasma. I also compared these changes to the fatty acid composition of some of the prey species collected at the colony. Those fatty acids that increased from first to replacement egg were those most concentrated in sandlance, squid, and capelin; however all prey species showed higher concentrations than was found in the eggs. The results suggest that females preserve their own future reproductive success at a cost to their chicks (because replacement eggs are generally smaller), and that they do not increase the mobilization of lipids within their own bodies. However, the depletion of certain fatty acids in the female between the first and replacement laying suggests that there may be some cost to relaying in terms of the availability of specific fatty acids critical to egg production. The fatty acid signatures also suggested that females are relying upon local food supplies for the production of the replacement egg, or that the availability of specific prey items changes during the breeding period. Further study is required using stable isotope analysis. If stable isotope analyses concur with the conclusion based upon the fatty acid signatures, then the local region around Coats Island is an important source of lipids and nutrients for egg production. Consequently, any changes in the local environment may affect Thick-billed Murres' ability to reproduce successfully. Very little research has been conducted on the importance of prey availability during egg production (Williams 2005). To my knowledge, this study is the first to investigate changes in fatty acid signatures of eggs and adult plasma during egg laying.

In Chapter 5, I show that adults pass on to their offspring a proportion of the cost associated with an increase in the cost of foraging. Chicks with handicapped parents generally grew more slowly than controls and there were no permanent changes in the lipid mobilisation of parents, though plasma neutral lipid concentrations did increase on the sixth day in singly handicapped males in 2003. There were differences in the response to handicapping between years that are not readily explicable, suggesting that conditions in the local environment could have presented different challenges to breeding pairs in the two years of the study. Comparisons of our results with those from the study by Paredes et al. (2006) is difficult because males and females incubate during opposite times of day (Gannet Islands; males incubate at night, Coats Island, females incubate at night). With the reversal in incubation shifts and the different constraints upon birds foraging either at night or during the day, direct comparisons of male and female adults is not possible between the two studies. These types of experiments, however, conducted with larger sample sizes, over a number of years, may provide researchers with information regarding the availability of prey among years and the way in which breeding Thick-billed Murres respond.

The functions of fatty acids

The function of lipids as a source of energy has been known for quite some time (i.e. George and Vallyathan 1963). However, it has long been thought that specific fatty acids also have specific physiological functions. While some studies have shown definitively that certain fatty acids turn over faster with a certain activity (McWilliams et al. 2002; Pierce et al. 2004), those studies focusing on fatty acid analysis have generally shown that certain fatty acids are found in higher concentrations than others (Blem 1990) or that they are good identifiers of species, individual size, and geographic region (Smith et al. 1997; Kirsch et al. 1998; Iverson et al. 2002; Lea et al. 2002). Consequently, it is not easy to explain the changes in composition of fatty acids within this research. From these data, there are certain trends that compliment what little literature does exist, though I was rarely able to find literature which contradicted the findings (but see McWilliams et al. 2002; literature review). The change in the composition of 20:1 and 22:1 in eggs, linked with the high concentrations of these fatty acids found in the marine food web (Christie 2006), supports the suggestion that these two fatty acids can be used as indicators of diet in species feeding at higher trophic levels. Animal fats tend to have high concentrations of 16:0 and 18:1, while plants show more variability in which fatty acids are dominant among species, though 18:1 tends to be found in high concentrations. The higher (compared with adult plasma percent contributions) and conserved concentrations of 16:0 and 18:1 in eggs suggest that these fatty acids are essential to the development of chicks. High contributions of 16:0, 18:0, 18:1, 20:5, and 22:6 in adult plasma suggest that these fatty acids are most utilised for fueling metabolism and exercise. Up to 90% fat stores of birds just prior to migration are composed of 16:0, 18:1, and 18:2 (Bairlein 2002, Pierce and McWilliams 2005). McWilliams et al. (2004) state that this seemingly common fatty acid composition among many species exists in spite of diverse food habits. The results from Chapter 3 demonstrate that though there may be many similarities in the fatty acids composition among individuals breeding at two different colonies, the signatures were still distinct using the principle components (Grahl-Nielsen 1999). Pierce and McWilliams (2005) found that the difference in fatty acid composition of lipid stores among Red-eyed Vireos (*Vireo olivaceous*) was primarily the result of diet fatty acid composition. Though I was not able to collect prey species on Prince Leopold Island, it is likely that the difference in fatty acid signatures between the colonies is due to differences in diet.

Saturated (without double bonds in carbon chain) fatty acids contain higher energy content than monounsaturated or polyunsaturated fatty acids (Blem 1990). Polyunsaturated fatty acids are, however, more readily mobilised (Raclot and Groscolas 1995) and could be preferentially mobilised during periods of endurance exercise. Significant changes in the plasma concentrations of 20:5 and 22:6 (and two other monounsaturated fatty acids) in singly handicapped males suggests that these fatty acids are mobilised to cope with increases in energy demands. This observation is supported by Davenport et al. (2004) who show that dietary supplementation of 20:5 and 22:6

increases the endurance of an exercising mammal. Fatty acid 22:6 has been shown to increase in concentration within the cell membrane lipids from dietary sources (Awad 1986) and are known to facilitate the movement of other fatty acids through the membrane from oxidation (Stillwell and Wassal 2003). More generally, subcutaneous lipid stores tend to be higher in unsaturated fatty acids than intra-organ lipid stores (Christie 2006). Further analysis on the fatty acid composition of the lipids from the different tissues described in Chapter 1 may help to elucidate some of the more specific roles of individual fatty acids.

Plasma fatty acid concentration seems to be an indicator of energy expenditure. We see from the handicapping experiments that, in some cases, the handicapped individual (male) showed an increase in plasma lipid concentration. We also see an increase in the plasma lipid concentrations from incubation to chick-rearing (the reproductive activity said to be the most costly in birds) in Thick-billed Murres. This suggests that the increased energy expenditures, fueled by lipids, can be detected by measuring plasma fatty acid concentrations. Though few studies have investigated the effects of exercise on plasma fatty acid concentration, those which do have shown increases in lipoprotein concentration (composed mostly of neutral lipids), glycerol, neutral lipids, and free-fatty acids (i.e. Vallyathan and George 1969; Jenni Eiermann and Jenni 1992; Guglielmo et al. 2002 (Though Guglielmo et al. (2002) suggest that elevated plasma fatty acid concentrations in migrants is a result of fattening, and not exercise, and Jenni-Eiermann et al. (2002) suggest that there may be differences in observed plasma lipid concentrations between passerines and non-passerines or because researchers handled their study subjects differently between studies. However, studies showing increases in plasma fatty acid concentrations were conducted on fasted birds, thus eliminating the potential effects of the distribution of dietary fatty acids for storage (Jenni-Eiermann et al. 2002). Since total body fat is significantly lower during chick-rearing than during incubation, and because plasma fatty acid concentrations seem to fluctuate depending upon the activity in which individuals are engaged, I propose we have observed the mobilisation of energy stores to fuel the increased energy expenditure. However, this is not necessarily at a cost to future reproductive success because, in the case of pursuit diving seabirds rearing chicks, it may be adaptive to have a lower wing loading during periods of intense exercise. Pre-programmed mass loss prior to chick-rearing triggered by hatching has been observed in Thick-billed Murres (Gaston and Perin 1993). Further study on the transition period between incubation and chick-rearing may reveal some important information about the physiological differences between adaptive mass loss to reduce wing-loading and increased energy expenditure to provision chicks.

Limitations to conducting eco-physiology research

There are several methodological and scientific challenges to conducting research on physiological changes from birds in their natural environment. The tissue preparation protocols are often limiting and special equipment is needed. The transportation and modification of this equipment to reach isolated research stations and operate using the available energy sources, and the cold temperatures and exposure, can sometimes compromise the storage requirements for analysis. In captive studies, tissue samples are collected from captive individuals and are stored immediately in the required conditions until analysis. However, samples collected and transported from the Arctic are not always subjected to the ideal conditions. For example, plasma separation should be done immediately after the blood sample is collected. Samples collected for this research were separated up to 2 h after collection. They were then placed in a propane-operated freezer and often took several hours to freeze. The plasma was placed in a 1.0 mL cryovials and filled to maximum capacity to minimise contact with oxygen. Similarly, the bird carcasses were not processed immediately and were shipped whole to the laboratory in Ottawa. Though there was no evidence that samples were damaged or had thawed upon arrival, it is always possible that some changes occurred in the tissues prior to analysis. To minimise the effects of any compromise in storage and handling, all samples were treated in the same manner prior to analysis.

Field studies limit the researcher's ability to control influential variables other than those explicitly studied. For example, the poor weather conditions in 2002 on Prince Leopold Island were beyond our control, and therefore shifted the interpretation of the results. However, it is the unmeasured variables that are of greater concern. If we are asking questions related to changes in breeding behaviour in relation to differences in climate or as a result of environmental changes, larger sample sizes will help to limit the effects of, for example, age, sex, and past reproductive success. However, it is often difficult to collect a large number of samples given the physical conditions typical of cliff-breeding seabird colonies. The large number of Thick-billed Murres of known age and sex on Coats Island will allow us to investigate further if there are any differences in energy store utilisation among birds of different age or sex.

Though very challenging to conduct in a natural setting, eco-physiology studies are necessary to verify whether the observations made during captive studies occur in real systems. However, the manipulation must simulate a real scenario and this is not easily validated. For example, in order to study the effects of declining prey availability on chick growth, I should have directly altered the distribution of prey within the foraging range of Thick-billed Murres breeding on Coats Island. These types of large scale environmental manipulations have occurred for studies conducted on the patch size effects of species diversity in tropical rainforests. However, since this type of environmental modification was not possible, handicaps were used to simulate a condition which may occur with a real decline in prey availability; the need for increased energy expenditure. The annual variations in responses by Thick-billed Murres in Chapter 6 and the differences in results found by Paredes et al. (2005, 2006) demonstrate clearly the need for conducting physiological studies in real situations.

To my knowledge, the experiments described in this thesis represent the most methodologically demanding protocols and manipulations ever conducted on wild auks.

Though the challenging environmental conditions during the 2002 field season were an asset with respect to identifying significant changes in body composition and thresholds, the primary limitation of this study is that those birds sampled by the end of the season may have been older with more reproductive experience than those collected at the beginning of the season. I do not have direct evidence of this and it is also possible that there was no sampling bias. However, had younger, less experienced birds also been sampled at the end of the season, I predict that I would have seen larger variation in the data from the last sampling period and those younger birds would have been less likely to decrease their lipid stores to the level of older birds. This may be because older birds have a lower chance of successful reproduction in the following seasons than younger birds that may fare better by maintaining their own condition for future attempts, or (in a good year), that older birds are better able to minimise wing loading in favour of reducing the energy required for flight. Since it was not possible to identify the age or breeding experience of any individuals sampled, all of this is speculative. I do think that this potential sampling bias, if it occurred, would have allowed for the identification of the lower threshold in body lipid stores during chick-rearing. Had environmental conditions allowed for the successful reproduction of younger, inexperienced birds, this might not have been possible. I suggest that there may be a minimum body lipid mass threshold during chick-rearing below which current reproductive effort may cease. I support this with evidence that those Thick-billed Murres still engaged in breeding by the time the final collections were made, had chicks which were gaining very little mass compared with previous years and 63% of all chicks died before reaching 14 days old (Gaston et al. 2005).

The condition indices developed for Thick-billed Murres and Northern Fulmars using fatty acid analysis were an improvement on the traditional morphometric indices. However, I was unable to develop this concept further because samples from previous or following years were unavailable. Though changes in total body lipid mass were well correlated with the indices, it was not possible to test it on an independent data set. It is known that changes in fatty acid composition can occur with changes in diet (Kirsch et al. 1998, Iverson et al. 2002, Pierce and McWilliams 2005). Prey species composition delivered to chicks differs between Prince Leopold Island and Coats Island. On Prince Leopold Island, the predominant species is arctic cod. On Coats Island, the predominant species is capelin (Davidson 2005) and prey species diversity is higher than it is on Prince Leopold Island. There have been changes in the composition of the prey species delivered to chicks on those colonies among years (Coats Island; Davidson 2005). Consequently, condition indices based on plasma fatty acid composition would need to be colony-specific and may not be applicable if the abundance and distribution of prey changes annually. However, the fatty acid composition might be used as a detector for changes in the diet composition. Before the condition index can be applied or developed, a validation study needs to be conducted.

To overcome the effect of feeding rates variation among individuals and low sample sizes, I should have monitored feeding rates before adding the handicaps. Given the duration of the experiment, this was not possible. In a similar study, Paredes et al. (2005) deployed their handicaps for only 1-4 days, allowing them time to conduct feeding watches on individuals prior to the manipulation. During their pre-manipulation observations, they found no differences in feeding rates among groups or sexes. It is difficult to compare our data with other handicapping studies because there is no standard for handicaps. In addition, comparison with Paredes et al. (2005) is further complicated because, as mentioned earlier, female and male incubation shifts appear to be inverted when compared with data from Coats Island.

The manipulative experiments present evidence that adults, when presented with an increase in the cost of reproduction, preserve their own condition at a cost to their offspring. However, much more investigation is required. It is still unknown why males accompany their chicks for the long swimming migration, and not females. Our data hint that males are in better condition (Chapter 6, female plasma lipid concentrations increased during chick-rearing, suggesting that they are mobilising more lipids and, consequently, working harder; see also Paredes et al. 2006; Harding et al. 2004), but further study is required. Paredes et al. (2006) state that males are not in better condition than females just before chick fledging but they did not collect data on body condition or mass. Instead, they argue that though females brought more fish back to chicks during chick-rearing, males flew further to forage. Without completing an energy budget or estimating the costs of short-distance foraging versus long-distance foraging, they assume that the energy expenditure of the two sexes were equal. Harding et al. (2004) argue that because the cost of egg production by the female is high (Monaghan et al. 1989), females may be in poorer condition than males at the end of chick-rearing. Seabirds have high mate fidelity, and it is suggested that there is an adaptive advantage for each mate to maintain their partner's condition. Consequently, males taking over chick-rearing after departing the colony may increase the future reproductive success of the pair. We might therefore predict that in birds having high mate fidelity, males will contribute more effort to chick-rearing than in those species where mate fidelity is lower or extra pair copulations are more frequent. Further study is required and plasma fatty acids may be useful in answering not only this, but also other questions related to parental strategy.

Standardised analyses

There is need for standardizing the methods for lipid analyses to facilitate the comparison of data collected by independent researchers. There are several approaches to tissue lipid extraction using a variety of techniques, solvents, and apparatus. Extractions can be conducted on either wet or dry tissues. Though there are advantages to extractions from wet tissues, standardized methods should focus upon dry tissue extraction (Montevecchi and Piatt 1987). In drying a tissue, the researcher is able to directly measure the amount of water in the body that may complement a lipid analysis. In the case of murres, however, single prey items are carried in the length of the bill and there is less dehydration than in species which transport several prey items across the bill.

Discussions surrounding the appropriate solvent to use for the extraction of lipids from tissues are based primarily on the amount of non-lipid that they each extract (i.e. Dobush 1985). A combination of methanol and chloroform are most commonly used, though this combination has been shown to extract some non-lipid components. It is possible to remove these components just after extraction by 'washing' the solution with water and KCl. This highly polar wash, when separated from the solution, removes the polar, non-lipid components. It is important to note, however, that this method may influence (approximately by 5-10 %) any measurements of total protein content from the lipid-extracted tissues unless the non-lipid components in the KCl solution are returned to the lean tissue components prior to protein analysis. Irrespective, it seems that washing is the best method for removing any non-lipid material that may have been extracted with the lipids.

Gas chromatography and mass spectrometry are generally used in combination to identify the percent contribution of each fatty acid present in the sample. Mass spectrometry measures the molecular mass of each type of molecule within the sample, allowing for simple and accurate identification of length and degree of saturation of the fatty acids within the sample. This instrument was not available for the analyses presented; fatty acids were identified by comparing the retention times from the sample with those of known standards run prior to sample analysis. Since retention times are subject to slight changes over time, and for the ease of fatty acid identification, it is recommended that mass spectrometry be used if available.

Measuring percent contributions of fatty acids tells us nothing of changes in total concentrations within a tissue. The research presented in this thesis has shown that both the composition and the total concentration of lipids within a tissue can be used as potential indicators of diet and rate of lipid mobilization, respectively. Standard methods of fatty acid composition analysis should incorporate an internal standard of known concentration into each sample so that the concentrations of all the other fatty acids can be determined.

Prey availability, sea ice, and reproductive success

As those species under investigation here are adapted to foraging in waters with sea ice, they may be negatively affected by either early or late ice breakup. The extent of ice cover and the time of ice breakup have been found to be important influencing variables upon breeding in only a few Arctic seabirds and in Southern Ocean penguin species (Gaston and Hipfner 1998 – also the first study to demonstrate this relationship in on Arctic seabird species). Sea ice supports an ecosystem of small fish species feeding off plankton and zooplankton found on the under surface of floating ice. Consequently, sea ice surrounded by open areas of water is an ideal environment for foraging. Though there is relatively little information on the effects of sea ice breakup for either Black-legged Kittiwakes or Northern Fulmars, there is some information about the consequence of changing ice conditions upon the reproductive parameters of Thick-billed Murres. At the more southern colony, Coats Island, the timing of sea ice breakup has advanced and Gaston et al. (2005a) have shown that this has resulted in earlier egg laying which is correlated with a decline in chick growth rates and lower adult body mass. At Prince Leopold Island, reproduction is less successful when sea ice breakup occurs later in the season because chick growth rates and adult body condition were found to be lower in those years (Gaston et al. 2005b). Advances in the time of ice breakup due to environmental warming may be beneficial to these high Arctic populations in the short term. Foraging during years of later ice breakup, as in years with very early ice breakup, may require an increase in effort because it is likely for there to be a decline in prey availability. I simulated a decline in prey availability by increasing the cost of diving among Thick-billed Murres and showed that there is a subsequent decline in chick mass (and therefore potentially reproductive success) as the energy required for foraging increases. In the 2003 experiment, I associated this increase in energy expenditure with an increase in the mobilisation of lipid stores (in males) for a portion of the experiment.

The time of ice breakup varies across the Arctic largely with latitude, breaking up earliest at southern latitudes. Data by Gaston et al. (2005a and b) suggest that there is an optimum time of sea ice breakup for reproduction that does not currently coincide with breeding activities either at the high or low Arctic colony. I show that when the cost of reproduction increases, adults preserve their own condition at a cost to their offspring. Declining reproductive success is being witnessed at both colonies. On Prince Leopold Island, reproductive success declines during years of late sea ice breakup. On Coats Island, it declines in years of early ice breakup (Figure II).

Foraging mode, spatiotemporal variability in the food supply, or the cost of reproduction to the adult, are primary influences upon the response by different species to changes in food availability (Jodice et al. 2002). Studies on the way in which Thick-billed Murres and Black-legged Kittiwakes respond to changes in prey availability have shown a difference in response attributed to foraging limitations. Murres, deep water pursuit-divers, compensated for a decline in sandeel abundance by seeking other prey species

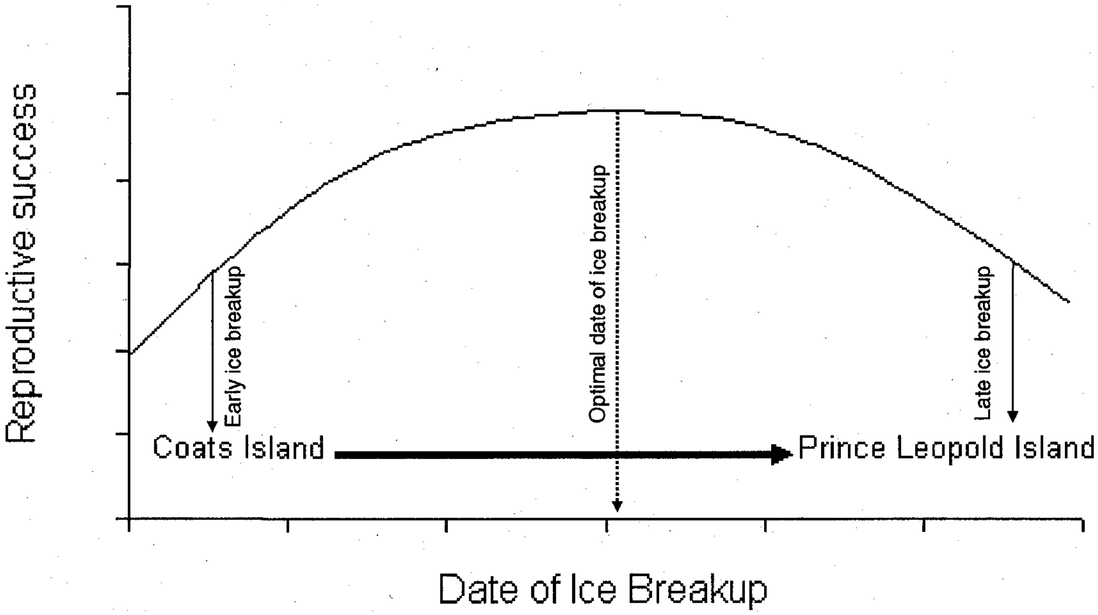

Figure II: A possible relationship between the timing of ice breakup (and consequently, prey availability) and reproductive success on Coats Island and Prince Leopold Island.

while surface-feeding Black-legged Kittiwakes, being more constrained by incubation shift length and foraging range, did not change foraging strategy (Rindorf et al. 2000). Though both species did demonstrate a decline in reproductive success (i.e. decreased chick mass at fledging), only Thick-billed Murres showed an attempt at compensation. Conversely, Black-legged Kittiwakes responded to an increase in abundance of prey species by increasing their daily energy expenditure, resulting in an increase in reproductive success (Jodice et al. 2006). In a study by Carscadden et al. (2002), a decline or shift in the vertical distribution of surface water prey species caused a decline in Black-legged Kittwak breeding success because they were unable to dive for prey. The same studied showed that Common Murres (*Uria aalge*) and puffins were not affected by the decline because they have more flexible diving ranges. Other seabird species may exhibit more flexibility and a greater ability to compensate for changes in prey availability due to a less restrictive breeding strategy. Thick-billed Murres and Northern Fulmars (until chick desertion) do not leave their nests unattended while Black-legged Kittiwakes do so for short and then longer periods as the chick grows. Hamer et al. (2007) found that during a decline in prey availability when several seabird species were experiencing a decline in reproductive success, Northern Gannets (*Morus bassanus)* were able to maintain their breeding success by increasing foraging effort and decreasing nest attendance. It was however predicted that should conditions deteriorate, soon there would be a threshold reached below which the risk of predation would outway the benefit of maintaining prey delivery rates.

A useful definition of prey availability must also take into consideration prey quality. The decline of of arctic cod in prey deliveries to chicks (Davidson 2005) is of great concern because I have shown that cod is of greater nutritional value per delivery than capelin (as a function of mass; Chapter 6). Rose (2005) has described capelin as the sea 'canary' for marine ecosystems and has shown that population distributions have responded quickly and predictably to changes in ocean temperatures. Capelin populations likely feed and migrate in areas where the Arctic and Atlantic waters converge (Vilhjalmsson 2002). The influence of the warmer Atlantic Ocean on Arctic waters has increased by up to 20% resulting in a northward shift in the distribution of capelin populations (Rose 2005). This trend is predicted to continue as water temperatures increase. A decline in the availability of larger prey items is likely to negatively impact chick growth, and therefore the reproductive success of Thick-billed Murres breeding in the Low Arctic.

The variability among individuals engaged in the same activity and the variability in responses to increases in the cost of reproduction (Chapter 5) suggests that individuals may be able to adapt to increases in the cost of reproduction by modifying different aspects of reproductive activity in varying combinations. Each individual may manage an increase in cost by compensating in a number of activities, all to a varying degree among individuals. An individual's strategy may be dependent upon prey availability, their own condition, and their reproductive experience, all of which may also interact. However, what this individual variability suggests is that Thick-billed Murres are generalists in their approach to cost management.

If Thick-billed Murres are able to adapt to increases in the cost of reproduction by modifying several activities, then it is important to measure variables which are affected by this more generalist strategy. Indeed, assessing the cost of egg production represents a similar challenge as studies have suggested that "costs are met through the reallocation of resources among different physiological systems" (Williams 2005). Consequently, a method of accounting for the possibility of resource sharing is needed which will not produce a zero net effect. In our case, measuring lipid utilisation was an appropriate variable because if there is a decline or increase in energy mobilisation, it will be detectable irrespective of which activity the change affects and can account for any compounded effects of a generalist strategy. However, I was not always able to measure cost sharing in behaviour (but see Chapter 6; feeding watches). Plasma lipids as indicators of energy mobilisation, coupled with direct observations of time budgeting should more clearly elucidate the individual strategies to managing increasing the cost of reproduction.

This study can be regarded as a preliminary investigation of the way in which analyses of lipids, and their constituent fatty acids, can improve our understanding of reproductive investment in marine birds.

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