REPRODUCTIVE FORAGING ECOLOGY
OF FIVE
SYMPATRICALLY BREEDING ALCID SEABIRDS

by

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Abstract

I used stable isotope methods to track the diets and habitat use of five sympatrically breeding Alcid seabirds throughout three stages of reproduction. Eggs, and nestling tissues were also collected in order to examine differences between items selected for provisioning offspring and those selected for self-feeding. This sampling protocol permitted an experimental field-based validation of isotopic discrimination for whole blood, which revealed that conventional lab-based estimates of this central parameter (~3.4‰) are >200% higher than the field-based estimates calculated here (~1.06‰). Using these field-based estimates, I show that the diets and habitat use of these five sympatric Alcids can differ between stages, as well as between adults and offspring. These results have implications for designing marine protected areas, and predicting the effects of climate-change, as well as for considering the evolutionary relationships between foraging and reproduction.
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Chapter 1   General Introduction

Linking foraging to reproduction is central to understanding how reproductive strategies function in nature (Pianka 1976). If we appreciate that the general function of reproductive strategies is to turn resources into offspring – two main questions concerning the role of foraging behaviour in reproduction arise: 1) how is interspecific variation in reproductive traits (e.g. egg-size) adaptively related to foraging behaviour, and 2) how is interspecific variation in reproductive success (e.g. among years, or among sites) influenced by the foraging environment. In sum, linking foraging behaviour to reproduction can tell us a great deal about why species differ in both their appearance and in their population dynamics (Stephens and Krebs 1986).

This area of study – linking foraging to reproduction – has progressed surprisingly little in the 30 years since Pianka (1976) highlighted its value as a major theme in evolutionary ecology (Boggs 1992, Ydenberg 1994, Ricklefs 2000). The main reason for the stunted progress of this topic is due to the simple fact that tracking foraging behaviour of wild animals is an exceptionally difficult task. In fact, there is still no group of species in nature for which good information exists on foraging behaviour during reproduction. Consequently, it has remained notably difficult to link interspecific variation in either reproductive traits, or reproductive success to environmental variation (Croxal et al. 2002).

Bridging the gap between foraging and reproduction has recently benefited from the advent of stable isotope methods. This developing method relies on the fact that that stable isotopes occur naturally in the environment, and become incorporated into the body tissues of animals through their diets. Accordingly, the body tissues of animals can be used as data recorders from which chronological accounts of diet and habitat use can be inferred (West et al. 2006). However, while easy in practice, the utility of this method hinges on accuracy of a physiological parameter, known as ‘diet-tissue discrimination’
(Gannes et al. 1998). Discrimination is basically a series of physio-chemical processes that cause the isotopic ratio in consumer tissue to become enriched relative to that of their prey, and must be corrected for prior to data analysis (Kelly 2000). However, very few estimates of discrimination are available in the literature (~100: see Vanderklift and Ponsard 2003), and the existing estimates display large interspecific variation (Vanderklift and Ponsard 2003). Consequently, although the stable isotope technique is well poised to help bridge the gap between foraging and reproduction, its utility is being held back by the limited availability of discrimination estimates.

This thesis contributes to the study of reproductive foraging ecology in two novel ways: 1) I develop new methods for calculating study-specific estimates isotopic discrimination in the wild, and 2) I apply this new method to conduct the most extensive isotope survey to date on the foraging behaviour of wild animals by tracking diet and habitat use of five sympatrically-breeding Alcid seabirds throughout three consecutive stages of reproduction (egg-formation, incubation, rearing).

In the first chapter, I take advantage of the unique reproductive behaviour of the 'burrow-nesting' Alcid seabirds studied in this thesis to provide the first estimates of isotope discrimination for wild animals. Burrow-nesting seabirds build nests in underground burrows, which allow for exceptionally controlled studies of physiological function in the wild. By monitoring, collecting, and analyzing the diets and blood of two species of burrow-nesting Alcids I was able to obtain precise study-specific estimates of isotope discrimination for my field site. Similar methods were carried out for egg-tissues. These study-specific discrimination estimates were found to be >50% less than the conventionally used estimates available in the literature, which was found to be the equivalent of an entire trophic level in his study system. In this first chapter I provide a general prospectus for using stable isotopes in the wild.

In the second chapter, I apply the study-specific discrimination estimates from Chapter 1 to an extensive survey of isotope samples that I collected at the same study site. By applying these study-specific discrimination estimates to my data set I was able to provide a level of data interpretation not yet matched in previous stable isotope studies.
My main results were to show that foraging behaviour differed both among reproductive stages, as well as between adult and offspring within stages. These results provide some of the best evidence yet that foraging behaviour is highly variable during reproduction, and exposes some striking interspecific patterns between foraging and reproductive traits. I offer some speculation of the adaptive significance of this interspecific variation, and suggest that there may be a general relationship between reproductive duration and reproductive rate.

1.1 References


Chapter 2  Taking Stable Isotopes into the Field: Methods for Calculating Field-Based Discrimination Factors.

2.1 Abstract

The application of stable isotopes in dietary studies is largely dependent on the accuracy of diet-tissue discrimination factors (also known as: fractionation, enrichment, or trophic shift). However, despite the importance of accurate discrimination estimates to the efficacy of this technique, very few estimates are available in the literature (~100: see Vanderklift and Ponsard 2003). This limited availability of estimates is problematic in three major ways: 1) limited taxonomic coverage, 2) limited tissue coverage, and 3) absence of field-based estimates. These shortcomings are exacerbated by marked inter-specific and inter-tissue variation. Consequently, isotope practitioners are often forced to use ‘best available’ estimates – a practice that has been shown to cause serious errors in the interpretation of data (Phillips and Koch 2002). In this study, I used the unique reproductive behaviour of burrow-nesting seabirds to provide the first study-specific discrimination estimates for wild animals. The study-specific estimates calculated here for whole-blood, egg-yolk, and egg-albumen were all found to be >50% less than the conventionally used estimates available in the literature. Based on the relative ease of the approach taken here, it is argued that more attention should be placed on designing stable isotope studies to permit the estimation of study-specific discrimination factors.

2.2 Introduction

Stable isotope analysis is becoming an increasingly popular technique for studying the diets of wild animals (Kelly 2000, Dalerum and Angerbjorn 2005). This simple technique is based on the tendency of animal body tissues to trap the isotopic signatures of the foods they eat (Deniro and Epstein 1979, 1981). Therefore, body tissues such as blood can be used as convenient data recorders from which chronological
accounts of diet can be inferred (West et al. 2006). However, while easy to carry out, interpretation of the data requires accurate estimates of isotopic discrimination, which is only available for a small number of species (~100: see Vanderklift and Ponsard 2003). Literature reviews of this key parameter show large interspecific variation (Vanderklift and Ponsard 2003, Dalerum and Angerbjörn 2005), and have demonstrated how small errors in its magnitude can generate misleading results (Phillips and Koch 2002, Robbins et al. 2002, Gaye-Siessegger et al. 2004a, b). Consequently, the efficacy of the stable isotope technique is currently restricted by the availability of discrimination estimates. One avenue of overcoming this restriction is to design isotope studies to permit the calculation of study-specific estimates (Robbins et al. 2005), however this approach has yet to be taken to the field. Accordingly, methods that permit the estimation of discrimination in the field will greatly improve the efficacy of this technique in ecological studies.

Isotopic discrimination (also: fractionation, enrichment, or trophic shift) is the process that causes consumer tissues to become isotopically enriched relative to their diets (Vanderklift and Ponsard 2003). Essentially, when dietary items are eaten they are discriminated into ‘enriched’ consumer tissue and ‘depleted’ dietary waste. The magnitude of this process (in parts per thousand: %o) is referred to as the diet-tissue discrimination factor, and forms the basis of most dietary stable isotope models (see Gannes et al. 1997, 1998, Phillips and Koch 2002). Because this discrimination process occurs between each successive trophic level, food webs become isotopically characterized in a predictable way, whereby each trophic level is separated by a magnitude approximately equal to that of the discrimination factor. It is therefore possible to determine which resources a consumer exploited by comparing its isotopic signature to that of potential resources in its environment; where in the simplest case a match indicates the exploited resource (see Kelly 2000). However, prior to carrying out this comparison procedure it is first necessary to correct the consumer’s isotope signature for the enrichment it incurred during the discrimination process. This correction serves to reduce the consumer’s signature to that of the resource it exploited, and makes the consumer-resource comparison meaningful.
The limited number of discrimination estimates available in the literature restricts the application of stable isotopes in three major ways: 1) limited taxonomic coverage, 2) limited tissue coverage, and 3) lack of field-based estimates. First and foremost, the taxonomic coverage is extremely limited, with less than 100 species-specific estimates available in the literature (Vanderklift and Ponsard 2003). This is problematic because discrimination shows pronounced interspecific variation, thereby making it difficult to select an appropriate estimate from the existing catalogues. The conventional approach is to either select an estimate from a closely related species, or use the average of some larger taxonomic group; both of which are clearly unfavourable in light of the high interspecific variation (Robbins et al. 2005). Secondly, tissue coverage is biased towards the commonly studied body tissues, and until recently it was uncommon for researchers to simultaneously measure discrimination of different types of body tissues in the same species. Consequently, studies that attempt to assemble chronological accounts of resource use by combining different body tissues must often select estimates from different species (see Dalerum and Angerbjorn 2005). Thirdly, all estimates of isotopic discrimination have been carried out on captive species in lab-based settings (Vanderklift and Ponsard 2003). This is problematic because field settings are often very different from the lab, and animal physiology is known to respond accordingly (see Goldstein and Pinshow 2002, 2006). Given the many ways in which discrimination is known to be affected by physiological condition (see Focken and Becker 1998, Gaye-Siessegger et al. 2004a,b) it is has been suggested that lab-based estimates cannot be reliably extended to the field (e.g. Gaye-Siessegger et al. 2004a,b). Here, it is suggested that these shortcomings can be overcome by designing isotope studies to permit the calculation of study-specific estimations of discrimination in the field.

In this study, I took advantage of the unique reproductive behaviour of burrow-nesting seabirds to calculate the first study-specific estimates of isotopic discrimination for wild animals. Burrow-nesting seabirds provide an ideal system for examining physiology in the field because their burrows serve as natural 'cages' from which diet and other parameters can be closely monitored and controlled. Reported here are study-specific estimates of isotopic discrimination for whole-blood, egg-yolk, and egg-
albumen. The suitability of these study-specific estimates was evaluated, and compared to the conventional estimates available in the literature by applying both types of discrimination factors to a large, concurrently collected data set of seabird stable isotope samples (see Davies Chapter 3). Suitability tests indicated that the study-specific estimates calculated here are approximately 50% less than the conventional estimates available in the literature, and were found to provide a much better interpretation of the data.

2.3 Methods

2.3.1 Accompanying Study

The purpose of this study was to produce study-specific discrimination estimates for an accompanying stable isotope survey of Alcid seabird foraging ecology (Davies Chapter 3). Therefore, reported here are primarily the methods used for estimating the study-specific discrimination estimates, and more detailed methods and sampling procedures for the study as a whole can be found in the accompanying study (see Davies Chapter 3).

2.3.2 Study Site

This study was carried out Triangle Island – a large seabird colony, located approximately 50km off the northwest tip of Vancouver Island, British Columbia, Canada (50° 52’ N, 129° 05’W). Breeding sympatrically at this colony are six species of Alcidae seabirds, totalling approximately 1.2 million individuals: Cassin’s Auklet (*Ptychoramphus aleuticus*), Tufted Puffin (*Fratercula cirrhata*), Rhinoceros Auklet (*Cerorhinca monocerata*, which is a ‘puffin’), Pigeon Guillemot (*Cepphus columba*), and Common Murre (*Uria aalge*). To avoid taxonomic confusion we hereafter refer to the Rhinoceros Auklet, as the Rhinoceros Puffin to maintain association with its true phylogenetic family (Storer 1945, Gaston and Jones 1998).
2.3.3 Estimation of discrimination factors: direct and indirect methods

Discrimination factors can be estimated in two ways, directly and indirectly – both methods were used in this study. Direct estimation requires that the consumers diet can be monitored, collected, and isotopically analyzed. Alternatively, when diet cannot be monitored it can be indirectly inferred, then collected, and analyzed. The former approach is superior in its accuracy and is the conventional method followed in lab-based studies. However, because the diet of wild animals is often difficult to monitor, the feasibility of direct methods is often limited, and in such cases indirect methods may offer a practical alternative. Despite the apparent value of indirect methods, there have been surprisingly few attempts to estimate discrimination in this way. To my knowledge, there have only been two previous attempts to indirectly estimate discrimination factors for wild animals: Hobson and Welch (1992) for polar bears, and Abend and Smith (1997) for pilot whales. This study is the first to indirectly estimate discrimination for birds.

2.3.4 Direct Estimation

Direct estimates of field-based $^{15}$N diet-tissue discrimination factors for whole-blood were carried out for nestlings of two species of burrow-nesting Alcid seabirds: the Cassin’s Auklet, and the Rhinoceros Puffin. Both species construct small burrows in the earth, which generally consist of a single entrance and a slightly enlarged chamber at the back where they raise their nestlings (Gaston and Jones 1998). These burrows are easy to monitor, and have relatively constant environmental conditions. As such, seabird burrows resemble the ‘cages’ of laboratories, and lend themselves well to the controlled study of animal function in the field. The methods used here for directly estimating discrimination factors were the same for both species. In general, nestling blood and nestling dietary items were: collected, isotopically analyzed, and subtracted from one another, with the resulting difference (in %o) representing the discrimination factor.

Specifically, nestling blood (ca. 0.5 mL) was drawn from the brachial vein of 15 nestlings of each species at approximately three weeks of age, transferred to labelled plastic vials, and placed in an on-site freezer (-10°C) for temporary storage. Nestling diet
was monitored throughout the rearing period, and dietary items (zooplankton and fish) delivered to nestlings were collected and stored at -10°C. Zooplankton was collected from the provisioning loads of adult Cassin’s Auklets. Upon capture Auklets were induced to regurgitate their provisioning loads from their throat pouches into plastic bottles, from which sub-samples (ca. 1 ml) were removed, placed into labelled plastic vials, and stored frozen. Taxonomic identification of zooplankton was carried out in a concurrent study (Hipfner et al. 2004), which found samples to be dominated (≥64% identifiable wet mass) by one of three alternative prey types: copepods (*Neocalanus cristatus*), euphausiids (*Thysanoessa spinifera*), or larval fish (species unidentifiable due to state of remains). For stable isotope analysis, I randomly selected five regurgitant samples having ≥64% proportion of each of these three dominant prey types. Similarly, fish were collected from the nestling provisioning loads of Rhinoceros Puffins (see Hipfner et al. 2004). For stable isotope analysis, I randomly selected ten individuals from each of the following dominant species: sandlance (*Ammodytes hexapterus*), pacific salmon (*Oncorhynchus* spp.), rockfish (*Sebastes* spp.), and Pacific saury (*Calolabis salia*). From each individual, ca.1g samples of white muscle tissue were cut from the mid-body, placed into labelled plastic vials and stored frozen. For estimating whole-blood discrimination factors, we used the average of all zooplankton species (hereafter: ‘zooplankton’) for the Cassin’s Auklet, and the average of all fish species (hereafter: ‘fish’) for Rhinoceros Puffins, as outlined in Tables 1, 2.

### 2.3.5 Indirect estimation

Indirect estimation of $^{15}$N diet-tissue discrimination was carried out for egg-yolk, and egg-albumen of Tufted Puffins. Tufted Puffins were selected, because in a concurrent study at the same field site (Davies Chapter 3) they were found to have nitrogen stable isotope values during both egg-formation and incubation that were so low, that they could have only been feeding on zooplankton. Therefore, because we were confident that Tufted Puffins were feeding on exclusively zooplankton during egg-formation it was possible to indirectly estimate discrimination factors for their egg tissues. Accordingly, the method for indirectly estimating discrimination was as follows: infer diet (as zooplankton), collect diet (as per direct method), collect consumer tissue,
isotopically analyze, with the resulting difference (in %) representing the discrimination factor. To error on the side of caution, we used the lowest valued zooplankton (i.e. copepod: δ¹⁵N 11.09 %) rather than the average of all zooplankton used for the whole-blood calculations. Ten eggs were collected from Tufted Puffins soon after laying and stored in an on-site freezer at -10°C. In a laboratory, eggs were cut in half while frozen with a mitre saw, and c.a. 1 mL samples of yolk and albumen were collected and separately placed into labelled plastic vials.

2.3.6 Stable isotope analysis

All samples were shipped frozen to the stable-isotope laboratory at University of Saskatoon for analysis. Egg, blood, and prey samples were freeze-dried and powdered. Egg yolk and prey samples then had lipids extracted by successively rinsing in a 2:1 chloroform: methanol solution, then air-drying under a fume hood (Bligh and Dyer 1959). Stable nitrogen isotope assays were performed on 1 mg sub-samples of powdered material by loading into tin cups and combusting in a Robo-Prep elemental analyzer at 1800°C. The resultant N₂ gases were separated and analyzed using an interfaced Europa 20:20 continuous-flow isotope ratio mass spectrometer, with every five samples separated by two laboratory standards. Measurement precision (SD) for δ¹⁵N was estimated to be ±0.3 %.

2.3.7 Isotope calculations: concentration and discrimination

In order to calculate diet-tissue discrimination, it is first necessary to calculate the stable isotope concentrations of the diet and consumer tissue, with the difference between these two values representing the discrimination factor. The isotopic concentration of a sample is calculated by combusting the sample in a mass spectrometer and measuring the ratio of heavy to light isotopes in the resulting gas (Kelly 2000). This ratio is then expressed in delta (δ) notation, which is the parts per thousand (‰) deviation of the sample from its international elemental standard, according to the following equation:
Equation 1:

\[ \delta X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \]

where \( X = ^{15}\text{N} \), and \( R_{\text{sample}} \), and \( R_{\text{standard}} = \) the ratio of \(^{15}\text{N}/^{14}\text{N}\) in the sample, and standard respectively. The standard for nitrogen is atmospheric air (AIR).

The discrimination factor between the diet and the consumer tissue is found with the following equation:

Equation 2:

\[ \Delta_{dt} = \delta_t - \delta_d \]

where \( \Delta_{dt} \) is the diet-tissue discrimination factor, \( \delta_t \) is the isotopic concentration of the consumer tissue, and \( \delta_d \) is the isotopic concentration of the diet.

2.4 Results

2.4.1 Prey stable isotope values

Stable isotope values (mean ± 2SE) of prey species collected from the seabird nestling burrows are as follows. Zooplankton: euphausiid (11.09 ± 0.66), copepod (11.36 ± 0.66), larval fish (11.73 ± 0.66). Fish: rockfish (13.13 ± 0.46), sandlance (13.65 ± 0.46), salmon (14.00 ± 0.46), saury (14.31 ± 0.46).

2.4.2 Seabird \(^{15}\text{N}\) diet-tissue discrimination factors: direct estimates

Cassin's Auklet nestlings had \(^{15}\text{N}\) diet-tissue discrimination factors for whole-blood of +1.53%, which is the difference between the average value of nestling whole-blood (12.92%) and the average value of the three species of zooplankton they were provisioned (11.39%). Rhinoceros Puffin nestlings had \(^{15}\text{N}\) diet-tissue discrimination factors for whole-blood of +0.60%, which is the difference between the average value of
nestling whole-blood (14.37%), and the average value of the four dominant species of fish that they were provisioned (13.77%). The average of these two discrimination estimates is +1.06%.

2.4.3 Seabird $^{15}$N diet-tissue discrimination factors: indirect estimates

Tufted Puffins had $^{15}$N diet-tissue discrimination factors for egg-yolk of +1.53%, which is the difference between the signatures of their egg-yolk (12.62%) and the zooplankton species with the lowest nitrogen signature (euphaussid: 11.09%). Tufted Puffins had $^{15}$N diet-tissue discrimination factors for egg-albumen of +0.80%, which is the difference between the signatures of their egg-albumen (11.89%) and the euphaussid zooplankton (11.09%).

2.4.4 Suitability Tests for Discrimination Factors

Currently, there is no standard method for testing the suitability of discrimination factors. Accordingly, I developed a simple graphical technique for testing the suitability of both the study-specific discrimination estimates calculated in this study, and the conventionally used estimates available in the literature. This technique, outlined in Figure 1, simply involves plotting discrimination-corrected consumer data against values of prey species known to be included in their diets. If the discrimination estimate is perfect, the consumer’s signature should sit exactly on top of its diet (i.e. have the same value); too strong (consumer below diet); or too weak (consumer above diet). The key requirement of this suitability test is that the isotope signature of the diet is known. We satisfied this requirement in that we monitored, collected, and isotopically analyzed nestling diet for two nestling species (i.e. Cassin's Auklet and Rhinoceros Puffin).

Below, I report the results of two tests – one for the suitability of the conventional discrimination estimates available in the literature, and the other for the study-specific estimates calculated here. For these suitability tests I plot consumer data from both the monitored nestling burrows reported in this study, as well as data from a concurrent study conducted at the same field site for five sympatrically breeding Alcid seabirds (see
Davies Chapter 3). The nestling burrows provide an exact test of the suitability of the conventional literature estimates (see Test 1 below). While the data from the concurrent study on the entire seabird community (Davies Chapter 3) provide a good test of the suitability of extending the study-specific discrimination estimates calculated here to closely related species at the same field site (see Test 2 below).

2.4.5 Test 1: Conventional Literature Discrimination Factors

The conventional discrimination estimates available in the literature at the date of this study, and those tested were as follows. Egg discrimination factors for carnivorous birds were only available for a single study on falcons by Hobson (1995). The average of the three species of falcons in Hobson (1995) was +3.53 ‰ for δ¹⁵N_{diet-yolk}, and +3.16 ‰ for δ¹⁵N_{diet-albumen}. Whole-blood discrimination factors were only available for ten species of birds (see Cherel et al. 2005), of which the average was +2.87 ‰ for δ¹⁵N_{diet-blood}; of these ten estimates, four were from fish-eating seabirds, of which the average was +2.7 ‰ for δ¹⁵N_{diet-blood}. Because this study involved fish-eating seabirds, we used the estimate for fish-eating birds given in Cherel et al. (2005) of +2.7 ‰. These discrimination factors were applied to the nestlings studied here, as well as to data from a concurrent study and plotted in Figure 1a.

As can be seen from Figure 1a, the suitability of the conventional estimates available in the literature is very poor. A few major points can be noticed. First, both of the monitored nestling species appear far below the prey species they were known to provisioned: Cassin’s Auklet nestlings (value: 10.22 ‰) appear 1.17 ‰ below the average value of their zooplankton prey (11.39 ‰), and Rhinoceros Puffin nestlings (value: 11.67 ‰) appear 2.10 ‰ below the average value of their fish prey (13.77 ‰). It should be noted that the magnitude of this error is comparable to the difference between zooplankton and fish (i.e. 2.38 ‰), or the equivalent of a whole trophic level. The second thing to note about Figure 1a is that the seabird community as a whole appears entirely below the value of the lowest fish species – which is known, on many accounts to be false. For example, nestlings of Pigeon Guillemot, Common Murre, and Tufted Puffin are all know to be provisioned exclusively fish – yet, when using the conventional
discrimination estimates the data suggests that they are being provisioned zooplankton. Similar arguments can be raised for many of the adults. The third thing to note about Figure 1a is that many species appear below the lowest zooplankton, having values that are similar to that reported for Particulate Organic Matter (POM) – and as such, is again likely to false and misleading. Taken together, the suitability tests for the conventional discrimination estimates available in the literature indicate that they are entirely inappropriate for the seabird community studied here.

2.4.6 Test 2: Study-Specific Discrimination Factors

For the second test, I extended the study-specific discrimination estimates calculated in this study to a concurrently collected data set of related Alcid seabird species at the same field site (see Davies Chapter 3). The specific values extended and tested were as follows. For whole-blood I used a $^{15}$N diet-tissue discrimination factor of $+1.06 \%$ (which is the average of Cassin's Auklet nestling $+1.53 \%$, and the Rhinoceros Puffin nestlings value of $+0.60 \%$). And for egg tissues, I used the values reported above for Tufted Puffins: egg-yolk $+1.53 \%$, and egg-albumen $+0.80 \%$.

As can be seen from Figure 1b, the study-specific discrimination estimates provide a much better fit to the data than do the conventional literature estimates. The main concerns addressed above for the conventional factors seem well rectified by applying the study-specific estimates: 1) the monitored nestlings (Cassin’s Auklet and Rhinoceros Puffin) now sit on top of their known diets, 2) the fish-eating species, most notably the fish-provisioned nestlings of Pigeon Guillemot, Tufted Puffin, and Common Murre now all sit on top of their fish prey, and 3) no species are lower than the lowest zooplankton prey. Therefore, although there is certain to be some degree of error inherent to extending the study-specific discrimination factors from nestlings to adults, and from one species to another, it is abundantly clear from the suitability tests that it nevertheless provides a much better fit.
2.5 Discussion

2.5.1 General findings

The study-specific discrimination estimates derived in this study differ greatly from the conventional estimates available in the literature. The whole-blood estimate (1.06%) differs by 1.64%, or 60% from the conventional standard (2.7%); the egg-yolk estimate (1.53%) differs by 2.00%, or 56% from the conventional standard (3.53%); and the egg-albumen estimate (0.80%) differs by 2.36%, or 75% from the conventional lab-based standard (3.16%). These findings reinforce the growing consensus that conventional lab-based estimates cannot be reliably extended to field settings without some form of validation test (e.g. Gannes et al. 1997, 1998, Gaye-Siessegger et al. 2004a,b) and underscore the value of designing isotope studies to permit the estimation of study-specific discrimination factors (Robbins et al 2005).

To put these results into context I refer to the work of Gaye-Siessegger et al. (2004b) who examined the implications of using inaccurate estimations of discrimination to reconstruct diet using standard mixing-models. In their analyses, Gaye-Siessegger et al. demonstrate how an error of 1% in the discrimination factor could lead to a 66% error in the estimation of the relative contribution of food types in the diet, when the difference between food types is 3%. The magnitude of difference detected here between the study-specific discrimination estimates and those available in the literature is 1.4% - 2.36%, and the difference between prey types was only 2.38%. As indicated by Gaye-Siessegger et al. (2004) the level of error increases as the error of the discrimination estimate increases and the difference between prey types decreases. Therefore, the consequence of using lab-based estimates to examine trophic relationships for the Alcids studied here would be much greater than 66%. This effect can be clearly observed in Figure 1a.

As outlined in the introduction, the three inherent limitations of using the conventional discrimination estimates available in the literature are: 1) limited taxonomic coverage, 2) limited tissue coverage, and 3) lack of field-based studies. The results of
this study, in reference to the work of Gaye-Siessegger et al. (2004) provide one example of how influential these restrictions can be on the ability to obtain quantitatively meaningful results. In light of these restrictions and findings, it is argued here that a shift in focus is needed - away from building increasingly larger lab-based catalogues of discrimination factors, to a more field-based approach of designing studies to permit study-specific estimates. The burrow-nestling seabirds studied here offer one obvious example, however it is likely that this approach will be of general use to isotope practitioners in a diverse array of systems. In the following sections, some general guidelines are provided for designing isotope studies to permit the estimation of discrimination in the field, and it is noted that similar methods could be used to estimate isotopic turnover.

2.5.2 Estimating discrimination in the field

As outlined in the methods section, discrimination can be estimated in two ways: directly, or indirectly. Direct estimations require that the diet can be monitored, while indirect methods are used when diet cannot be monitored and as such rely on inferring diet with some type of dietary proxy. Regardless of the approach, there are two key ecological features that will greatly simplify the estimation of discrimination in the field. First, species that have ‘sedentary’ life-history stages, or have reduced mobility will make the monitoring of diet relatively easy. And secondly, species that are ‘dietary specialists’ will make the calculations more tractable. This second point is especially important because discrimination is known to be affected by the chemical composition of the diet (see Focken and Becker 1998, Pearson et al. 2003, Hobson and Bairlein 2003, Robbins et al. 2005). Moreover, the estimation of discrimination is greatly complicated if the diet comes from a number of different types of dietary sources, such as plants and animals (see Phillips and Koch 2002). These ecological features are not requisites, although sedentary behaviour and dietary specialization will greatly simplify the estimation of discrimination in the field.
2.5.3 Direct estimation

Sedentary offspring are likely to provide a rich source of opportunity for estimating discrimination in the field. For example, many species of birds raise their offspring in nests from which they provision them relatively specialized diets (Ydenberg 1994). Birds such as passerines provisioning exclusively insects, raptors provisioning rodents, or seabirds provisioning fish are all good candidates. Likewise, mammals often cache their young in nests or burrows and deliver meals in addition to milk. The other obvious group of species that exhibits sedentary life-history stages are the insects, for which there are many species that raise young in nests or on stationary carcases. In all these examples diet can be easily monitored by behavioural observation from a hide, and or collection of feces and dietary remains.

Setting up feeding stations in the wild is another technique that could prove useful, especially for adults. Such feeding stations should contain a single type of food, and the food type should be one that is more profitable than those that could be found in the environment, thereby encouraging exclusive use of the feeding station. Such feeding stations would require both a monitoring device and some method of collecting samples from feeding individuals. This technique would be most suitable for tissues that have relatively short turnover durations, such as muscle, whole blood, plasma or breath (see Dalerum and Anjerbjorn 2005).

If neither of the above field-based approaches of direct estimation is possible, one should consider capturing individuals from the wild and housing them in captivity to obtain species, and tissue-specific discrimination factors. The methods would then follow the conventional lab-based approach (see Hobson and Clark 1992). Captive studies are becoming increasingly common (e.g. Haramis et al. 2001, Bearhop et al. 2002, Hobson and Bairlein 2003, Evans Ogden et al. 2004, Cherel et al. 2005, Yarnes et al. 2005, Seminoff et al. 2006). The study of Seminoff et al. (2006) is especially notable as it was the first study to provide nitrogen discrimination estimates for a turtle species, as and such exemplifies the need for more estimates of this basic isotopic parameter.
2.5.4 **Indirect estimation**

Estimating discrimination indirectly through the use of some type of dietary proxy offers a practical alternative to direct methods, however this approach has been generally overlooked, and attempted only three times (Hobson and Welch 1992, Abend and Smith 1997, this study). The basic requirement of indirect estimation is that the species under study has a relatively specialized diet, thereby insuring that the diet is coming from a known source. Apart from species that are true dietary specialists, there are a number ecological circumstances whereby generalists engage in dietary specialization due to seasonally or geographically abundant resources. Accordingly, estimating discrimination indirectly may be possible for a wide variety of species. The examples provided here are simply meant to spark the interest of isotope practitioners and impress the ease with which the estimation of discrimination can be carried out in the field.

2.5.5 **Conclusion**

In conclusion, the attention that the stable isotope technique is currently receiving in respect to its methodology is owed largely to the inspirational reviews of Gannes et al. (1997, 1998), who challenged practitioners to both scrutinize their data and bring something to the table. The continued expansion of this technique to elusive and threatened species (see Rubenstein and Hobson 2004), its refinement of modelling techniques (e.g. Phillips and Koch 2002), and its quality of reviews (e.g. Vanderkliift and Ponsard 2003, Dalerum and Angerbjörn 2005) insure its progress. As we suggest here, field practitioners can make a valuable contribution to the continued improvement of the stable isotope technique by designing their studies to permit field-based estimations of isotope discrimination and tissue turnover. Doing so will not only add estimates to the literature catalogue, but will permit isotope practitioners to take this technique to new species, new tissues, and new study systems.
2.6 Figures
Figure 2.1: Suitability tests for isotope discrimination factors. Figures contrast the suitability of applying: (A) conventional and (B) study-specific discrimination factors to a large isotope data set collected in a concurrent study (Davies Chapter 3), and to monitored nestling burrows (dotted lines 1: Cassin’s Auklet, 2: Rhinoceros Puffin). In both figures, prey isotope values (open circles) serve as reference points for comparing to consumer isotope values (solid circles). Data points represent mean ±95% CI (sample sizes reported in Davies Chapter 3). Suitability is high, when for a known dietary relationship, the consumer and prey values are not significantly different (i.e. within 95% CI). See Results for Interpretation. X-axis: 15N %. Y-axis legend: species (PIGU: Pigeon Guillemot, COMU: Common Murre, TUPU: Tufted Puffin, RHAU: Rhinoceros Puffin, CAAU: Cassin’s Auklet), reproductive stage (egg-form: egg-formation, yolk: egg-yolk, albumen: egg-albumen, inc: incubation, rearing: nestling-rearing, nestling: nestling).
2.7 References


Yarnes, C.T., Rockwell, J.N., and Boecklen, W.J. 2006. Patterns of trophic shift in $\delta^{15}N$ and $\delta^{13}C$ through a Cynipid gall wasp community (Neuroterus sp.) in Quercus turbinella. Community and Ecosystem Ecology 34: 1471-1476.

Chapter 3 Using stable isotopes to track the diets and habitat use of five sympatrically breeding Alcid seabirds.

Seabirds are among the best-studied vertebrate groups in the world (Croxall et al. 2002), however central aspects of their reproductive biology remain surprisingly unknown. In particular, there is a notable rift in understanding between the on-colony and off-colony aspects of their reproduction. This situation stems from the fact that seabirds are easy to study at their colonies, yet exceptionally difficult to study while away (Weimerskirch 2004). Accordingly, most on-colony aspects of seabird reproduction are very well understood, including the timing, form, and development of the three main stages of reproduction (egg-formation, incubation, and nestling-rearing). However, much less is known about the accompanying off-colony, foraging components of these stages. Consequently, understanding how foraging ecology at sea is adaptively related to the form and function of reproductive traits back at the colony is still an unresolved question in seabird biology (Ricklefs 2000).

Two off-colony aspects of seabird foraging remain especially pressing. First, for most species it is unknown whether seabirds forage in different marine habitats or exploit different types of prey during the various stages of reproduction (the ‘stage’ question). And secondly, within stages it is still largely unknown whether parents use different resources for self-feeding than they do for provisioning their offspring (the ‘allocation’ question). Resolving these issues have important implications for both the conservation of seabirds, as well as understanding the evolution of their life-history traits. With regard to conservation, there is increasing evidence to suggest that sympatrically breeding seabirds respond differently to environmental variation depending on the type of food resource they exploit (e.g. Kitaysky and Golubova 2000, Bertram et al. 2001, Jenouvrier et al. 2005). Therefore, establishing whether species shift resources from stage to stage, or between self-feeding and provisioning will allow us to determine which stages, and
from whose perspective (adult or offspring) species-specific responses are driven (see Croxall et al. 2002). Similarly, with regard to life-history theory it is still surprisingly unclear how interspecific variation in seabird life-history traits, such as egg-size, or incubation duration is adaptively related to the environment (Ricklefs 2000, Weimerskirch 2003). Understanding this life-history variation will require linking reproductive traits at the colony to foraging behaviour at sea (Ricklefs 1983, Ydenberg 1994).

Recently, major progress has been made on both the ‘stage’, as well as the ‘allocation’ questions in seabird foraging studies. However no study has yet tracked resource use of both parents and offspring consecutively throughout all stages of reproduction. Examination of ‘allocation-specific’ foraging started largely with the work of Chaurand and Weimerskirch (1994), which showed how dramatically different self-feeding and provisioning can be from one another. Since this seminal work many other species of seabirds have been found to employ so-called “bi-modal”, or “alternate” foraging tactics to suit the contrasting demands of self-feeding and provisioning (see Ropert-Coudert et al. 2004). However, while there is now widespread evidence for the use of allocation-specific foraging during the rearing stage, it remains largely unknown whether the alternation of such foraging tactics occurs during egg-formation or incubation.

‘Stage-specific’ foraging studies have been equally successful in elucidating the strategic relationships between seabird reproductive traits and foraging behaviour. Nevertheless, stage-specific studies are still relatively uncommon (see Charrassin et al. 1998). The goal of these studies has been to examine whether the great differences among reproductive stages in time, energy, and risk commitments are met with equally pronounced differences in foraging behaviour (e.g. Ricklefs 1983, Salamolard and Weimerskirch 1993, Weimerskirch 1995, Charrassin et al. 1998, Shaffer et al. 2003). Together, work on stage-specific and allocation-specific foraging has served to convince seabird biologists that there is indeed a very common and tight relationship between the specific aspects of reproduction and the role of foraging behaviour (Ricklefs 2000).
However, for most species of seabirds there is still no information available on stage-specific or allocation-specific foraging ecology during reproduction. Consequently it remains difficult to speculate on how off-colony selection pressures influence the form and function of life-history traits back at the colony.

One especially useful technique for bridging the gap between breeding at the colony and feeding at sea is the analysis of naturally occurring stable isotopes from the tissues of seabirds and their prey (Kelly 2000). This method is based on the fact that organisms incorporate into their body tissues the isotopic signals of the environments in which they live and the foods that they eat (Gannes et al. 1998). Accordingly, this technique has played a key role in exposing some of the finer details of seabird foraging strategies due its ability to distinguish both foraging habitats (with $\delta^{13}$C) and prey types (with $\delta^{15}$N) (Forero and Hobson 2003). For example, isotope studies have highlighted the fact that some species thought to be specialists on a single trophic level, such as zooplankton or fish, actually use a mixture of both trophic levels during reproduction (e.g. Hobson and Welch 1992, Hobson 1993, Hobson et al. 1996, Sydeman et al. 1997). However, because so few isotope studies have controlled for reproductive stage it still remains largely unknown exactly when and how often such resource shifts occur during reproduction (i.e. ‘stage’ question), or whether shifts occur between adults and offspring within stages (i.e. ‘allocation’ question).

In this study we use stable isotope analysis to track habitat use (with $\delta^{13}$C) and diet (with $\delta^{15}$N) of parents and offspring throughout three stages of reproduction (egg-formation, incubation, and nestling-rearing), for five species of sympatrically breeding Alcid seabirds: Cassin’s Auklet (*Ptychoramphus aleuticus*), Rhinoceros Puffin (*Cerorhinca monocerata*), Tufted Puffin (*Fratercula cirrhata*), Pigeon Guillemot (*Cepphus columba*), and Common Murre (*Uria aalge*). Accordingly, this is the first seabird study to examine resource use of parents and offspring throughout a complete reproductive cycle. The basic aim of this study was to characterize the diets and habitat use of these five Alcid seabirds throughout reproduction, and identify if and when shifts in resource use occur – either between stages (i.e. stage question) or within stages.
between adults and offspring (i.e. ‘allocation’ question). This isotope study is novel in three respects: 1) it is the first study to use field-based estimates of isotopic discrimination, 2) both the raw isotope data, and the discrimination-corrected data are reported, and 3) we develop a new technique for graphing isotope data that serves to facilitate the interpretation of data points.

3.1 Methods

3.1.1 Study Site

This study was carried out at Triangle Island, British Columbia, Canada (50° 52' N, 129° 05' W) where approximately 1.2 million Alcid seabirds breed annually. The island is approximately 1.5 km long, 120 ha, and is located 46 km offshore over shallow (<200 m) continental shelf waters, and inside of the deeper shelf-break waters by about 25 km (Mackas et al. 2004). Accounts of the islands: climate, flora, and fauna can be found in Carl et al. (1951); zooplankton (Mackas et al. 2004); and oceanography Robinson and Brink (1998). The Centre for Wildlife Ecology at Simon Fraser University operates a long-term research station on the island and organizes research permitting.

3.1.2 Study Species & Colony Populations

We examined the reproductive foraging ecology of five sympatrically breeding Alcid seabirds at Triangle Island: Cassin’s Auklet (Ptychoramphus aleuticus), Tufted Puffin (Fratercula cirrhata), Rhinoceros Auklet (Cerorhinca monocerata, which is a ‘puffin’), Pigeon Guillemot (Cepphus columba), and Common Murre (Uria aalge). To avoid taxonomic confusion we hereafter refer to the Rhinoceros Auklet, as the Rhinoceros Puffin to maintain association with its true phylogenetic family (Storer 1945, Gaston and Jones 1998). The colony at Triangle Island supports the world’s largest population of Cassin’s Auklets (1,096,000 birds; 40% of global population); internationally significant populations of Rhinoceros Puffins (83,000; 6.5%), and Tufted Puffins (52,000, 1.5%); and the largest provincial populations of Common Murres (8,200), and Pigeon Guillemots (350), (Rodway 1991, Nettleship 1996).
3.1.3 Stable Isotope Sampling Protocol

We collected: i) prey delivered to nestlings (zooplankton, fish), as well as seabird: ii) eggs (yolk, albumen), iii) adult blood, and iv) nestling blood from all five sympatric Alcid species during the 2002 breeding season. Our sampling protocol had the following target sample sizes: blood (15 samples/species/stage), eggs (10/species), and prey (fish: 10/species, zooplankton: 5/species). There were a few circumstances where these target sample sizes were not achieved, and these cases are indicated in Appendix 1. “Self-feeding” was estimated at all stages, by analyzing adult blood samples collected during egg-formation, incubation, and nestling-rearing. “Provisioning” was estimated during egg-formation (egg-yolk, egg-albumen), and nestling-rearing (nestling blood), but not during incubation. All individuals were banded with Government of Canada issued metal leg bands to prevent pseudoreplication.

3.1.4 Prey

Prey (zooplankton and fish) delivered to nestlings at the colony were collected and analyzed to provide local isotopic references for our model. Zooplankton was collected from the nestling provisioning-loads of Cassin’s Auklets. Upon capture, auklets were induced to regurgitate their throat pouch contents into plastic bottles, from which sub-samples (ca. 1 ml) were removed, placed into labelled plastic vials, and frozen at -10°C. Taxonomic identification of zooplankton was carried out in a concurrent study (Hipfner et al. 2004), which found most samples to be dominated (≥64% by wet mass) by one of three alternative prey types: copepods (Neocalanus cristatus), euphausiids (Thysanoessa spinifera), or larval fish (species unidentifiable due to state of remains). For stable isotope analysis, we randomly selected five regurgitant samples having ≥64% proportion of each of these three dominant prey types.

Fish and squid were collected from the nestling provisioning loads of Rhinoceros Puffins. For stable isotope analysis, we randomly selected ten individuals from the following dominant species: sandlance (Ammodytes hexapterus), pacific salmon (Oncorhynchus spp.), rockfish (Sebastes spp.), Pacific saury (Calolabis salia), and squid
(Gonatus spp.). Fish were weighed, measured to fork length, and ca. 1g samples of white muscle tissue were cut from the mid-body, placed into labelled plastic vials and frozen at -10°C. White muscle tissue has been recommended for use in isotopic dietary reconstruction studies due to its tendency to exhibit the low isotopic variability in comparison with other body tissues (Pinnegar and Polunin 1999). Squid were processed likewise, although tissue samples were removed from the body-mantel.

3.1.5 Eggs

Eggs were collected from each species soon after laying and immediately frozen at -10°C. In a laboratory, eggs were cut in half while frozen with a mitre saw, and 1 mL samples of yolk and albumen were collected and separately placed into labelled plastic vials. Yolk and albumen samples were removed from approximately midway between the start and end of their production to reduce possible sampling bias of egg formation strategies (see Hobson 1995).

3.1.6 Blood

Blood (0.5-1.0 mL) was drawn from the brachial veins of breeding adults, from each of the five species during: egg-formation, incubation, and nestling-rearing. Nestlings were sampled likewise (ca. 0.5 mL blood) at approximately three weeks of age; by which time isotopes derived from the egg tissues should have been metabolically turned-over with prey-provisions from adults. Blood was placed into labelled plastic vials and frozen at -10°C.

3.1.7 Stable Isotope Analysis

All samples were shipped frozen to the stable-isotope laboratory at University of Saskatoon for stable isotope analysis. Egg, blood, and prey samples were freeze-dried and powdered. Egg yolk and prey samples then had lipids extracted by successively rinsing in a 2:1 chloroform: methanol solution, then air-drying under a fume hood (Bligh and Dyer 1959). Stable-carbon and nitrogen isotope assays were performed on 1 mg sub-samples of powdered material by loading into tin cups and combusting in a Robo-
Prep elemental analyzer at 1800°C. The resultant CO₂ and N₂ gases were separated and analyzed using an interfaced Europa 20:20 continuous-flow isotope ratio mass spectrometer, with every five samples separated by two laboratory standards. Measurement precision (SD) for δ¹³C and δ¹⁵N was estimated to be ±0.1 %o and ±0.3 %o respectively.

3.1.8 Isotopic Calculations

The isotopic composition of a sample is calculated by measuring the ratio of heavier to lighter isotopes. This ratio is then expressed in delta (δ) notation, which is the parts per thousand (‰) deviation of the sample from its international elemental standard, according to the following equation:

\[
\delta X = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000
\]

where \(X = ^{15}\text{N} \text{ or } ^{13}\text{C}\), and \(R_{\text{sample}}\), and \(R_{\text{standard}}\) = the corresponding ratio of \(^{15}\text{N}/^{14}\text{N}\) or \(^{13}\text{C}/^{12}\text{C}\) in the sample, and standard respectively. The standard for nitrogen is atmospheric air (AIR), and the standard for carbon is a type of marine limestone fossil known as Pee Dee Belemnite (PDB). Positive isotope δ values simply mean that the sample has more of the heavy relative to lighter isotopes than the standard, and visa versa for a negative δ value; this is why carbon has a negative value.

3.1.9 Isotopic Turnover Rate

Body tissues can be metabolically inert or active. Inert tissues such as egg, hair, or feather maintain the isotope signature that they incorporate during their production. In contrast, metabolically active tissues such as blood, liver and bone are constantly regenerating, or 'turning-over' at rates specific to their metabolism. These turnover rates, which refers to the number of days required to completely replace all the cells in a tissue type, range from 1-2 days for blood plasma to years for bone (Hobson and Clark 1992a). It is important to have accurate estimations of tissue turnover in order to determine when samples should be collected, because samples collected on a given date represent
foraging behaviour prior to collection date by of a time period equal to that of the turnover period.

Here, we use the average blood turnover value from the three available studies which experimentally quantified whole blood turnover rate in birds: (1) Japanese Quail (Coturnix japonica) $^{13}\text{C} = 22.8$ days (Hobson and Clark 1992a), (2) Great Skua (Catharacta skua) $^{13}\text{C} = 31.4$ days, and $^{15}\text{N} = 28.8$ days (Bearhop et al. 2002), and (3) Dunlin (Calidris alpina pacifica) $^{13}\text{C} = 22.4$ days, and $^{15}\text{N} = 20$ days (Evans Ogden et al. 2004). The average value of these three studies is 25.5 days for $^{13}\text{C}$, and 24.4 days for $^{15}\text{N}$. Given the close proximity of these estimates to each other, we use a turnover value of 25 days for both $^{13}\text{C}$ and $^{15}\text{N}$ to simplify the model. Because egg tissue does not turnover per se, turnover rates are not relevant.

3.1.10 Isotopic Discrimination ($\Delta$)

A discrimination factor is the degree of isotopic enrichment, in parts per thousand ($\%\delta$) that occurs during the physiochemical transformation between a sample (e.g. fish) and the product (e.g. consumer tissue) (Lajtha and Michener 1994). For example, the nitrogen in a fish consumed by a seabird would be discriminated into $\delta^{15}\text{N}$ “depleted” uric acid waste, and $\delta^{15}\text{N}$ “enriched” consumer tissue. This discrimination process is what causes stable isotopes such as $\delta^{15}\text{N}$ to become “enriched” with increasing trophic level and makes the use of stable isotopes in ecological studies possible.

The discrimination factor between the diet, and the consumer tissue is found with the following equation:

$$\Delta_{dt} = \delta_t - \delta_d$$

where $\Delta_{dt}$ is the isotopic discrimination factor between the diet and the tissue, $\delta_t$ is the isotopic concentration of the consumer tissue, and $\delta_d$ is the isotopic concentration of the diet.
3.1.11 Use of Discrimination Factors: Lab and Field-based estimates

Here, as is standard in stable isotope studies, we initially compiled and employed lab-based estimates of egg and blood discrimination available in the literature for our modeling (for current discrimination estimate compilations see: Vanderklift and Ponsard 2003, Dalerum and Angerijörn 2005, and Robbins et al. 2005). However, these lab-based estimates (which are only available for ~10 avian species), when applied to our field system were found to be too strong, in that that they caused our consumer (seabird) signatures to come out below our diet (zooplankton and fish) signatures. Fortunately, our study design permitted us to calculate our own field-based discrimination estimates for whole blood, egg-albumen, and egg-yolk, which were all >50% less those available in the literature (see Davies Chapter 1). These study-specific discrimination estimates (reported below) provided a much better fit to the data, and are therefore used in all statistical tests and isotopic modelling procedures. This is the first stable isotope study to estimate and use field-based discrimination factors on wild animals, and is a practice which feel greatly improves the accuracy of isotopic dietary reconstructions. For a general methodology to designing field-studies to permit calculation of field-based discrimination factors see Davies (Chapter 1).

To determine field-based discrimination factor estimates of $\delta^{15}$N for our study system, we took advantage of the ‘lab-like’ reproductive behaviour of the burrow-nesting Alcid seabirds studied here (see Chapter 1 for full methodology). Burrow-nesting seabirds provide ideal study organisms for field-based experiments, because they are “caged-in” their burrows; all input (food provisions) can be easily monitored, while temperature and other environmental variables are fairly constant. We derived discrimination factor estimates from our field data, as the isotopic difference between nestling blood and their delivered prey for two species of seabirds nestlings: the plankton-provisioned Cassin’s Auklet, and fish-provisioned Rhinoceros Auklet. However, for our analysis we use the average of these two estimates, as all species of adults are known or suggested to feed on both plankton and fish. Cassin’s Auklet nestlings had whole-blood $\delta^{15}$N discrimination factors of $+1.53 \%$. Rhinoceros Puffin nestlings had whole-blood $\delta^{15}$N discrimination factors of $+0.60 \%$. The average of these
two $\delta^{15}N$ discrimination factor estimates, and the values we used in this study for our whole-blood samples are: $+1.05\%$.

Study-specific egg-yolk and egg-albumen discrimination estimates for $\delta^{15}N$ are derived from our field data as the difference between the mean values of the lowest $\delta^{15}N$-valued zooplankton we collected, the Euphausiid, *Thysanoessa spinifera* ($\delta^{15}N=11.09$), and both Tufted Puffin egg-yolk ($\delta^{15}N=12.62$), and Tufted Puffin egg-albumen ($\delta^{15}N=11.89$). These resulting differences, and by extension discrimination factors are $1.53\%$ for yolk, and $0.80\%$ for albumen.

We did not calculate $\delta^{13}C$ study-specific discrimination estimates for either whole-blood or egg tissues, because the prey species found at our study site (i.e. zooplankton and fish) are found in both inshore and offshore habitats (Figure 3); and we therefore could not make meaningful estimates. However, carbon has a very small discrimination magnitude in biogeochemical processes to begin with, so fortunately this is not a great concern (Kelly 2000). Instead we used discrimination estimates available in the literature. For whole-blood we used the average $\delta^{13}C$ discrimination value of the four species of fish-eating seabirds reported in Cherel et al. (2005) of $0.00\%$. For egg-tissues we relied on the only published account available for egg production in carnivorous birds, those of Hobson (1995). We report the average of the three species of falcons used in Hobson (1995), which for lipid-free yolk are: and $+0.2\%$, and for albumen are: $+0.86\%$.

Statistical tests were performed on dual isotope comparisons (combined $\delta^{15}N$ and $\delta^{13}C$) using MANOVA with the Wilks' Lambda statistic, and on single isotope comparisons ($\delta^{15}N$, or $\delta^{13}C$) using ANOVA, with the Tukey-Kramer HSD *post hoc* test. Data is presented in figures as mean values ± 95% Confidence Intervals (CI), thereby permitting visual examination of statistically significant relationships.
3.2 Results

3.2.1 Prey

Prey isotope data (mean, SE) appear in Appendix 2. Zooplankton and fish were cleanly separated in their $\delta^{15}N$ values (ANOVA: $F_{1,53}=91.4$, $P<0.0001$), with all fish species having $\delta^{15}N$ values higher by $\geq 1.4\%$ than all zooplankton species (Tukey’s test, all $P \leq 0.001$) (Figure 1a). Prey types were also differentiated by habitat type through carbon analysis into an inshore group (sand lance, rockfish, larval fish), and an offshore group (pacific saury, juvenile salmon, copepods), with a $\geq 1.7\%$ difference in $\delta^{13}C$ between inshore and offshore prey types (Tukey’s test, all $P \leq 0.001$) (Figure 1a). Although euphausiids exhibited large variation in $\delta^{13}C$ spanning both inshore and offshore groups, and squid was especially offshore (Figure 1a).

This clear isotopic separation between prey types permits a rough delineation of ‘prey-type regions’ in the isotope parameter space of the figures (Figure 1a,d, Figure 2 a-f). This new graphical technique permits a more immediate and accurate interpretation of data points within the isotopic parameter space. We delineated a zooplankton/fish boundary at $\delta^{15}N=12.14$; which is the midpoint between the highest $\delta^{15}N$ valued zooplankton (fish larva =11.7 $\delta^{15}N$), and the lowest $\delta^{15}N$ fish (rockfish = 13.13 $\delta^{15}N$). Similarly we delineated an inshore/offshore boundary at $\delta^{13}C=18.72$; this is the midpoint between the most offshore of the inshore prey (rockfish = -17.85 $\delta^{13}C$), and the most inshore of the offshore prey (juvenile salmon = -19.58 $\delta^{13}C$). These prey boundaries are overlaid on all figures as dashed lines: vertical (inshore/offshore), and horizontal (zooplankton/fish). The resulting isotopic regions serve as coarse approximations of four alternative foraging tactics: $T_{if}$ (inshore-fish), $T_{iz}$ (inshore-zooplankton), $T_{of}$ (offshore-fish), $T_{oz}$ (offshore-zooplankton) (Figure 1b).

3.2.2 Seabirds: Intraspecific contrasts among stages

Seabird isotope data (mean, SE) appear in Appendix 1. With data separated by stage, all five Alcid species exhibited significant among-stage differences in their stable isotope signatures, suggesting widespread use of alternative foraging tactics among
stages (Figure 1b-f). Cassin’s Auklet combined $\delta^{15}$N and $\delta^{13}$C (MANOVA: Wilks’ Lambda = 0.07, $F_{10,142}=39.28$, $P<0.0001$); $\delta^{15}$N (ANOVA: $F_{5,72}=21.66$, $P<0.0001$) and $\delta^{13}$C (ANOVA: $F_{5,72}=68.65$, $P<0.0001$). Rhinoceros Auklet combined $\delta^{15}$N and $\delta^{13}$C (MANOVA: Wilks’ Lambda = 0.07, $F_{10,142}=39.28$, $P<0.0001$); $\delta^{15}$N (ANOVA: $F_{5,72}=15.39$, $P<0.0001$); and $\delta^{13}$C (ANOVA: $F_{5,72}=11.88$, $P<0.0001$). Tufted Puffin combined $\delta^{15}$N and $\delta^{13}$C (MANOVA: Wilks’ Lambda = 0.07, $F_{10,142}=39.28$, $P<0.0001$); $\delta^{15}$N (ANOVA: $F_{5,70}=48.26$, $P<0.0001$); and $\delta^{13}$C (ANOVA: $F_{5,70}=18.06$, $P<0.0001$). Common Murre combined $\delta^{15}$N and $\delta^{13}$C (MANOVA: Wilks’ Lambda = 0.07, $F_{10,142}=39.28$, $P<0.0001$); $\delta^{15}$N (ANOVA: $F_{5,75}=69.64$, $P<0.0001$); and $\delta^{13}$C (ANOVA: $F_{5,75}=49.74$, $P<0.0001$). Pigeon Guillemot was the only species for which we did not meet the sample size objectives. However, based on our limited sample sizes we found that Pigeon Guillemots exhibited significant differences among reproductive stages in combined $\delta^{15}$N and $\delta^{13}$C isotope signatures (MANOVA: Wilks’ Lambda = 0.41, $F_{8,36}=2.53$, $P=0.267$), and in both $\delta^{15}$N (ANOVA: $F_{4,19}=2.74$, $P=0.05$), and in $\delta^{13}$C (ANOVA: $F_{4,19}=2.84$, $P=0.05$).

3.2.3 Egg-Formation

Three species had isotope signatures suggesting the use of alternate foraging tactics for self-feeding and provisioning during egg-formation: the Cassin’s Auklet, Common Murre, and Pigeon Guillemot. These three Alcids all provisioned their egg-yolk with higher trophic-level prey ($\delta^{15}$N) then they used for self-feeding (Cassin’s Auklet and Common Murre: Tukeys $P\leq0.0001$, Figure 2b,c; Pigeon Guillemot: Tukeys $P=0.5$, Figure 2d). A different pattern however was found for the two puffins, Rhinoceros and Tufted, which appeared to use the same trophic level of prey for self-feeding, egg-yolk, and egg-albumen (Tukeys all $P\geq0.31$); (Figure 2e,f). Examination of carbon isotope signatures reinforced this grouping, in that Cassin’s Auklet, Common Murre, and Pigeon Guillemot all had yolk signatures that were significantly more inshore in origin that of their albumen signatures, whereas the two puffins were indistinguishable in this respect (Figure 2b-f).
3.2.4 Incubation

Only "self-feeding" was examined during incubation, as the methodology for sampling "provisions" to incubation has yet to be fully developed; but see Discussion. However, in contrast to other reproductive stages, we found that all species except the Pigeon Guillemot (note, its limited sample size) had significantly distinct stable isotope signatures during incubation (Tukeys, all $P \leq 0.001$), (Figure 2b-f).

3.2.5 Nestling-Rearing

The same two species showing strong evidence of alternate foraging tactics for self-feeding and provisioning during egg-formation: the Cassin's Auklet, and Common Murre, again exhibited isotope signatures suggesting use of alternate foraging tactics during nestling-rearing. The Cassin’s Auklet provisioned nestlings with prey of a higher trophic level ($\delta^{15}N$), then they used for self-feeding (Tukeys $P=0.0001$), but had equal $\delta^{13}C$ signatures (Tukeys $P=0.38$), suggesting that they foraged for both prey types in the same “offshore” marine habitat (Figure 2b). Whereas Common Murres provisioned nestlings with prey of a lower trophic level ($\delta^{15}N$: Tukeys $P<0.0001$), and of more offshore origin ($\delta^{13}C$: Tukeys $P=0.0001$), then they used for self-feeding (Figure 2c). In contrast, the two puffins had isotope signatures consistent with using the same type of prey for self-feeding and provisioning during the rearing stage, just as they did during the egg-formation stage. The Tufted Puffin appeared to forage on the same prey types ($\delta^{15}N$: Tukeys $P=0.97$) and in the same marine habitats ($\delta^{13}C$: Tukeys $P=0.46$) for self-feeding and provisioning (Figure 2e). Similarly, the Rhinoceros Puffin foraged on the same types of prey ($\delta^{15}N$: Tukeys $P=0.25$) but appeared to collect nestling provisions and self-feed in slightly different locations ($\delta^{13}C$: Tukeys $P=0.03$), (Figure 2f).

3.3 Discussion

3.3.1 General Results

The novel aspect of this work is that is the first seabird study to track resource use of parents and offspring throughout an entire reproductive cycle: here for five sympatric
Alcids during egg-formation, incubation, and nestling-rearing. Inasmuch, we provide a distinctively comprehensive account of the diets and habitat use of these seabirds throughout reproduction, and are able to use these accounts to identify some general interspecific differences in their foraging strategies. Our main results show that, with regard to the ‘stages’ question: 1) three species (Cassin’s Auklet, Pigeon Guillemot, and Common Murre) appear to rely on a single trophic resource (i.e. zooplankton or fish) for all stages of reproduction. Consequently, these species do not appear to shift between trophic levels during the breeding season. In contrast, 2) the two puffins (Rhinoceros and Tufted) were found to engage in very pronounced trophic shifts, with both species relying heavily on zooplankton early in the season (egg-formation and incubation) and shifting to an exclusively fish-based diet during the rearing stage. With regard to the ‘allocation’ question, 3) the species using a single trophic resource (Cassin’s Auklet, Pigeon Guillemot, and Common Murre) were also found to use alternative foraging tactics within stages - using different types of prey, and different marine habitats for self-feeding and provisioning offspring. And 4), the species using multiple trophic resources (Rhinoceros and Tufted Puffins) were found to use similar foraging tactics within all stages – using similar prey types, and similar marine habitats the for both self-feeding and provisioning offspring.

Taken together, our results suggest that the five species of seabirds studied here may adopt one of two very different types of reproductive foraging strategies. Specifically, the Cassin’s Auklet, Pigeon Guillemot, and Common Murre all appear to time and confine their reproduction to a single trophic resource (zooplankton or fish), yet appear to use alternate foraging tactics for self-feeding and provisioning within each reproductive stage. Alternatively, the Rhinoceros and Tufted Puffins appear to stretch reproduction over the two consecutive trophic resources (zooplankton and fish), yet appear to use similar foraging tactics for self-feeding and provisioning during each reproductive stage. These different reproductive foraging strategies are elaborated on below.
3.3.2 Robustness of stable isotope data

From what is known of the marine distributions and trophic positions of the Alcid seabirds studied here and their prey, our stable isotope data fits very well once it is corrected with our field-based isotope discrimination factors (see methods). Species known to be 'inshore': rockfish (Love et al. 2002), sand lance (Field 1987), and Pigeon Guillemot (Gaston and Jones 1998) all had high $\delta^{13}C$ signatures; whereas species known to be 'offshore': copepods (Mackas et al. 2004), pacific saury (Tian et al. 2003), and early-season Tufted Puffins (Gaston and Jones 1998) had relatively low $\delta^{13}C$ signatures. Equally strong support was found for $\delta^{15}N$ as an indicator of the trophic level of seabird dietary items. Zooplankton prey types, and the planktivorous Cassin’s Auklet had low $\delta^{15}N$ values; whereas fish prey, and seabird nestlings fed exclusively fish (Rhinoceros and Tufted Puffins, Common Murres, and Pigeon Guillemots) all had high $\delta^{15}N$ values. This suggests that the isotopic patterns found in this study are real, and that $\delta^{13}C$ and $\delta^{15}N$ isotopes, when corrected with field-based discrimination factors are reliable indicators of seabird diet and movement among marine habitats.

3.3.3 New Isotope Graphing Technique

During the course of this study, we developed a new technique for graphing isotope data that assists in interpreting consumer data points. As outlined in the Methods section, and in Figure 1 this technique simply involves overlaying resource boundaries onto a graphs parameter space where the resulting iso-regions serve as coarse approximations of consumer foraging tactics (Figures 1a,b). When applied to our data, this graphical technique identifies four major tactical regions: $T_{if}$ (inshore-fish), $T_{iz}$ (inshore-zooplankton), $T_{of}$ (offshore-fish), $T_{oz}$ (offshore-zooplankton) (Figure 1b). The useful aspect of this graphical technique is that it emphasises the difference between 'coarse-shifts' (i.e. between tactical regions) and 'fine-shifts' (i.e. within tactical regions). For example, coarse-shifts would include a shift in resources between either 'inshore' and 'offshore', or 'zooplankton' and 'fish'. Whereas fine-shifts would include any statistically distinct shift within a tactical region; for example fish to different fish, or offshore-habitat to different offshore-habitat. In the following section we use the iso-
region graphing technique to highlight the major patterns of resource use during reproduction for each of the five species studied here.

3.3.4 Intraspecific Patterns

In the following species-specific sections we describe the isotope data in qualitative terms, hoping to provide a general characterization of the major patterns of prey exploitation and habitat use of these Alcids during reproduction. The species using a single trophic resource (Cassin's Auklet, Pigeon Guillemot, Common Murre) precede description of the species consecutive trophic resources (Rhinoceros Puffin, Tufted Puffin). Because this is the first study of Alcid seabirds to track habitat use and prey exploitation throughout all stages of reproduction, our results expose some new aspects of the foraging ecology that were previously unknown. We emphasize that the following resource characterizations are simplified to permit the major patterns to be appreciated, and to avoid over-emphasising differences of small magnitude that may be an artefact of the isotope technique. This is particularly important for interpretation of carbon isotope values, as limited work has been carried out on how describing carbon isotope gradients in marine systems.

Cassin's Auklet

$\delta^{13}C$ values indicate a coarse-shift in use of marine habitats from inshore during egg-formation to offshore during the incubation and rearing stages. This shift could be indicative of the ephemeral patchiness of their prey source, such as the appearance of offshore zooplankton during these later stages (Mackas et al. 2004). Alternatively, it may suggest that there is some benefit to residing among inshore habitats during the egg-formation stage. Nevertheless, this shift in marine habitats is pronounced and suggests that reproductive success of this species may depend on the accessibility and quality of both inshore and offshore habitats. With regards to the types of prey exploited, the $\delta^{15}N$ values show that, with the exception of egg-yolk provisions, Cassin's Auklets use exclusively zooplankton during all stages of reproduction. However, as advanced previously (Gauthier et al. 2003) we feel that the high nitrogen signature of egg-yolk
might indicate the use of a ‘capital-based’ egg-formation strategy, and not the use of fish prey. Collectively, these results suggest that the Cassin’s Auklet relies on a single trophic resource - zooplankton, yet engages in fine-scale alternation of foraging tactics within and between stages.

**Pigeon Guillemot**

Due to the small sample sizes obtained for this species (Appendix 1) it is difficult for us to reach firm conclusions. Nevertheless, δ¹³C signatures from all stages show extremely ‘inshore’ signals, and the 95% confidence intervals fall well within the inshore boundary. Similarly, δ¹⁵N values are all extremely high and the 95% CI’s are within the ‘fish’ boundary. Taken together, the δ¹³C and δ¹⁵N values suggest that this species is an ‘inshore-fish’ specialist. Additionally, as described above for the Cassin’s Auklet, the nitrogen signatures of egg-yolk were higher than the self-feeding signatures; suggesting use of a capital-based egg-formation strategy.

**Common Murre**

Based on δ¹³C and δ¹⁵N values it appears that this species, like that of the Pigeon Guillemot is an ‘inshore-fish’ specialist. However it is also evident that this species engages in fine-scale shifts within this tactical region, both between habitats (δ¹³C) and prey types (δ¹⁵N). It should be noted that the 95% CI’s are exceedingly small, which suggests that these small-scale shifts are likely biologically significant. Moreover, this species shows significant differences between self-feeding and provisioning signatures during both egg-formation and rearing. Accordingly, it also appears that this species, like the Cassin’s Auklet and Pigeon Guillemot uses a capital-based egg-formation strategy, and uses alternative foraging tactics for self-feeding and provisioning during the rearing stage.

**Rhinoceros Puffin**
Because this species’ carbon, as well as nitrogen isotope signatures are located so close to the tactical boundaries (Figure 1e), they are likely using a mixture of both habitats (inshore and offshore), as well as both trophic resources (zooplankton and fish) during the earlier stages of reproduction. $\delta^{13}$C values suggest a bias towards the use of offshore foraging habitats during all stages except incubation, during which time it appears they make a coarse-shift to inshore waters. Their $\delta^{15}$N values indicate a shift in the trophic level of prey from a mixed diet of zooplankton and fish during egg-formation and incubation, to an exclusively fish-based diet during the rearing-stage. This is suggested by the close proximity of the incubation, and especially egg-formation signatures to the zooplankton-fish boundary. During egg-formation, and offspring-rearing this species appears to forage in similar habitats, and on similar prey for both self-feeding and provisioning. Accordingly, Rhinoceros Puffins may use a more income-based egg-formation strategy than the capital pattern described for the above three species.

**Tufted Puffin**

$\delta^{13}$C values of Tufted Puffins suggest a steady inshore movement during reproduction, although it appears as though these birds do forage in offshore waters for all stages. Egg-formation apparently takes place quite far offshore, as is evidenced from the fact that they had the most offshore values found in this study. $\delta^{15}$N values show a single coarse-shift in the trophic level of prey, from an exclusively zooplankton diet during egg-formation and incubation to an exclusive fish diet during the rearing stage. There are some striking similarities to, and one notable difference from the foraging behaviour of Rhinoceros Puffins. Like Rhinoceros Puffins, the Tufted: a) shifts from a zooplankton-based diet during egg-formation and incubation to an exclusively fish-based diet during the rearing stage, b) use similar prey types and marine habitats for self-feeding and provisioning at all stages, and c) also appear to use an income-based egg-formation strategy. The notable difference occurs during incubation when Tufted Puffins appear to forage further offshore than the Rhinoceros Puffins. This marked difference,
may be related to their contrasting patterns of daily activity, whereby Tufted is diurnal, and Rhinoceros is nocturnal.

3.3.5 Conclusion

The results presented in this paper offer a uniquely comprehensive account of the foraging behaviour of five sympatrically breeding Alcid seabirds throughout reproduction. We have provided convincing stable isotope evidence that these Alcid species have different diets, and use different foraging habitats throughout the various stages of reproduction. Furthermore, it appears that some species also forage differently when self-feeding than when provisioning offspring. These results have interesting implications for both the efficacy of conservation initiatives as well as understanding the evolution of the reproductive strategies of these birds in general.

First, with regards to conservation initiatives there has recently been a strong push to understand why sympatric species respond differentially to environmental perturbation such as climate change or resource harvesting. Previous models examining seabirds have assumed that species are either exclusively planktivorous, or exclusively piscivorous (e.g. Kitaysky and Golubova 2000). However, the results from our study suggest that this common generalization may not be suitable for the Alcids – the group for which this generalization is most commonly applied (e.g. Kitaysky and Golubova 2000). In this study, the puffins were found to use both zooplankton and fish; they relied heavily on zooplankton early in the season, and switched to exclusively fish during the rearing stage. These data are quite surprising, and notable in that ‘puffins’ of all species are commonly referred to, and modelled as piscivores – yet, in this study both Rhinoceros and Tufted Puffins relied heavily on zooplankton during both the egg-formation as well as incubation stages. It is therefore likely that studies modeling the reproductive success of puffins in relation to food supplies should be broadened to include zooplankton, as well as fish resources.

Secondly, with regards to the evolution of life history traits, it appears as though species using a single trophic resource for reproduction (Cassin’s Auklet, Pigeon
Guillemot, and Common Murre) also use alternative foraging tactics for self-feeding and provisioning offspring during all stages. Quite oppositely, the species using two consecutive trophic resources during reproduction (the puffins) do not appear to use alternative foraging tactics within stages; at least not to the same extent. Interestingly, the life-history difference between these two groups seem to be reflected in their egg-formation strategies, whereby the trophic specialists appear to use a more capital-based strategy, whereas the trophic generalists appear to use a more income-based approach (Figure 2b-f). Although these results are preliminary, they offer a novel hypothetical framework with which to examine the striking, yet largely unexplained life-history differences among the Alcidae seabirds (see Gaston and Jones 1998).
3.4 Figures

Figure 3.1: New technique for interpreting stable isotope data based on characterizing the isotope parameter space into 'prey type' regions, shown here for the marine system surveyed in this study. Step 1 (as seen in A): separate prey types by habitat, by placing a dashed line at the mid-point between inshore and offshore prey types (here, for Carbon at: -18.72). Step 2 (as seen in A) separate prey types by trophic level, by placing a dashed line at the mid-point between zooplankton and fish (here, for Nitrogen at: 12.14). This technique provides a rough characterization of the different types of foraging tactics available to the consumers studied (as seen in B): 'inshore fish', 'inshore zooplankton', 'offshore fish', and 'offshore zooplankton'. To use this technique for interpreting consumer stable isotope data, simply overlay these prey type lines onto equally scaled figures of consumer data as shown in Figure 2.
Figure 3.2: Stable isotope signatures (mean ±95% CI) of prey species (A), and seabird species collected at three stages of reproduction (B-F). Dashed lines represent approximate isotopic boundaries between 'prey types' (horizontal: zooplankton below, fish above), and 'habitat types' (vertical: offshore to left, inshore to right), see Figure 1. Symbols for figures (B-F): self-feeding adult seabirds (uppercase) during: egg-formation (E), incubation (I), and nestling-rearing (R); and offspring provisions (lowercase) to: egg-yolk (y), egg-albumen (a), and nestlings (r). Prey Latin names appear in Methods.
3.5 References


Appendices
### Appendix 1: Seabird stable isotope data.

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</table>


2: C-Raw: raw carbon data.

3: C-Lit: raw carbon data corrected with discrimination estimates from the literature (whole-blood: 0.00‰, egg-yolk: +0.20‰, egg-albumen: +0.86).

4: N-Raw: raw nitrogen data.

5: N-Lit: raw nitrogen data corrected with discrimination estimates from the literature (whole-blood: +2.70‰, egg-yolk: +3.53‰, egg-albumen: +3.16‰).

6: N-Thesis: raw nitrogen data corrected with discrimination estimates from Davies thesis (whole-blood: +1.01‰, egg-yolk: +1.53‰, egg-albumen: +0.80‰).
Appendix 2: Prey stable isotope data.

<table>
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<th>Prey Type</th>
<th>Sample Size</th>
<th>Nitrogen</th>
<th>SE</th>
<th>Carbon</th>
<th>SE</th>
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