

# RECONSTRUCTING AVIAN DIETS USING STABLE-CARBON AND NITROGEN ISOTOPE ANALYSIS OF EGG COMPONENTS: PATTERNS OF ISOTOPIC FRACTIONATION AND TURNOVER<sup>1</sup>

KEITH A. HOBSON

Canadian Wildlife Service, 115 Perimeter Road, Saskatoon, Saskatchewan S7N 0X4, Canada, and  
Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada

**Abstract.** Because eggs are composed of nutrients that are ultimately derived from the diet of adult females, the relative abundance of naturally-occurring stable isotopes of carbon and nitrogen in eggs should be related to those in their diet and this may form the basis of a method for tracing diets. Before such dietary reconstructions can be established, however, it is necessary to know how isotopic signatures change (or fractionate) from the diet during the synthesis of various egg components. In this study, stable-carbon and nitrogen isotopic fractionation factors between diet and whole yolk, lipid-removed yolk, albumen, and shell membranes of eggs of captive-raised Mallards (*Anas platyrhynchos*), Japanese Quail (*Coturnix japonica*), Prairie Falcons (*Falco mexicanus*), Peregrine Falcons (*Falco peregrinus*), and Gyrfalcons (*Falco rusticolis*) were established. In addition, carbon isotope fractionation patterns between diet and eggshell carbonate and yolk lipid were determined for quail, Mallards and falcons. On average, yolk, albumen and membranes were enriched in <sup>15</sup>N relative to diet for all birds by 3.4‰, a value typical of processes associated with protein synthesis. For quail and Mallards, albumen, membranes, and shell carbonate were enriched in <sup>13</sup>C relative to diet by 1.5, 3.6, and 14.9‰, respectively, and whole yolk was depleted in <sup>13</sup>C by 2.6‰ due to the presence of yolk lipids. Falcons showed lower carbon diet-tissue fractionation for all tissues (albumen: +0.9, whole yolk: -1.9, membranes: +2.7, carbonate: +11.2‰) and this may be due to a greater reliance on dietary lipids vs. carbohydrates for the synthesis of egg components or on fractionation differences associated with fermentation of carbohydrates. Patterns of isotopic fractionation between diet and Mallard eggs were not influenced by clutch order. A diet-switch experiment using quail indicated that albumen, shell membrane and shell carbonate values reflect diet integrated over 3-5 days and yolk over eight days prior to laying. Because birds were fed ad libitum, these data provide baseline data on fractionation patterns for birds optimally mobilizing nutrients from diet to egg components. These values may differ for those wild birds that rely more heavily on endogenous reserves.

**Key words:** Stable isotopes; isotopic fractionation; eggs, diet; carbon-13; nitrogen-15; *Coturnix japonica*; *Anas platyrhynchos*, *Falco peregrinus*, *Falco mexicanus*, *Falco rusticolis*.

## INTRODUCTION

Stable-carbon and nitrogen isotopic analyses of avian tissues can provide valuable information on the diets of individuals and populations (Hobson 1987, 1990; Mizutani et al. 1990; Hobson and Sealy 1991; Hobson et al. 1994). This approach is based on the fact that stable-isotope ratios of these elements in a consumer's tissues are related ultimately to those in its diet (DeNiro and Epstein 1978, 1981). Advantages of using stable isotope analysis to augment more conventional dietary approaches include the evaluation of assimilated vs. ingested dietary contributions and the potential for time-integrated dietary es-

timates (Tieszen et al. 1983, Hobson 1993). Recent investigations have also indicated that a great deal of dietary information can be obtained from the isotopic analysis of feathers or blood fractions thereby reducing the need to sacrifice birds in cases where they may be captured alive (Mizutani et al. 1990, Hobson and Clark 1993).

Bird eggs are another potential source of material for isotopic investigation of avian diets because they are formed from nutrients that are derived ultimately from the diet of the laying female. Eggs are also usually readily available, either from the wild or through archived collections, and so are a convenient source of material for isotopic analysis. Previous studies have shown that stable-carbon and oxygen isotope analysis of the organic matrix (C) or carbonate (C and O) component of eggshells can reveal dietary infor-

<sup>1</sup> Received 4 January 1995. Accepted 22 March 1995.

mation from both archaeological and contemporary specimens (von Schirnding et al. 1982, Schaffner and Swart 1991). However, to date, no isotopic studies have used other components of eggs such as the yolk and albumen fractions and virtually no information exists on how isotopic signatures change or fractionate between diet and the various components of the avian egg (but see von Schirnding et al. 1982, Schaffner and Swart 1991). In order to understand more precisely the diets of females during egg formation it is useful to establish these isotopic fractionation factors for each component of interest, a process involving the captive rearing of individuals on diets of known and constant isotopic composition (e.g., Hobson and Clark 1992a). It would also be useful to know how quickly nutrients from the diet are incorporated into various egg components and the extent to which endogenous reserves are used in egg formation (Krapu 1981, Austin and Fredrickson 1987, Afton and Ankney 1991). This information would allow insight into the period over which isotopic information gained from egg components reflected the diet of the laying bird. I investigated isotopic fractionation between diet and various components of eggs from several species of birds raised in captivity on controlled diets. I chose Japanese Quail (*Coturnix japonica*) and wild-strain Mallards (*Anas platyrhynchos*) raised on grain-based diets and three species of falcon raised on quail to determine possible influences of diet on isotopic fractionation factors. In addition, I conducted a dietary-switch experiment using quail in order to determine how quickly new dietary information is incorporated into yolk, albumen, shell membrane and shell carbonate.

## METHODS

### ISOTOPIC FRACTIONATION

Eggs from nine laying Japanese Quail were obtained randomly from a colony at the University of Saskatchewan where all birds had been raised for several generations on a homogenized commercial grain-based diet. Birds had been raised from hatch to egg laying on a single batch of this food together with vitamin and mineral supplements. Quail were obtained from the same colony as that used previously to assess patterns of isotopic fractionation and turnover in various quail tissues (Hobson and Clark 1992a, 1992b). Food samples of quail used here were obtained

every two weeks for four months prior to egg collections.

The quail colony was also used to provide an exclusive diet for a captive breeding population of Peregrine Falcons (*Falco peregrinus*), Prairie Falcons (*Falco mexicanus*), and Gyrfalcons (*Falco rusticolis*) at the University of Saskatchewan. Single infertile eggs from six Peregrine Falcons, four Gyrfalcons and two Prairie Falcons were used. I reasoned that since fertilization is independent of the mechanisms of egg formation, my choice of infertile eggs would not influence the establishment of fractionation patterns for eggs in general. Falcons consumed primarily pectoral muscle tissue of the quail and so I considered this material to be largely representative of their diet. Three samples of quail muscle tissue used by falcons were obtained every two weeks for four months prior to egg laying by falcons.

The third sample consisted of infertile eggs obtained from captive wild-strain Mallards. These second-year birds had been raised in captivity from eggs salvaged from the wild under permit granted by the Canadian Wildlife Service. Mallards were fed a constant diet consisting of wheat and a commercial feed in the ratio of 1:1.4 by weight together with vitamin, grit and shell supplements. Single batches of feed were used for the entire rearing period prior to egg collections. Seven subsamples of food components were obtained for isotope analysis. Single eggs were taken from first clutches of eight females and an additional eight eggs from second clutches of another eight individuals.

### TURNOVER IN QUAIL EGGS

Four female quail raised on the homogeneous wheat-based diet described above were chosen randomly, housed in separate pens, and switched to a new corn-based diet. The new diet was formulated to have similar whole fat, carbohydrate and protein levels as the previous diet but was isotopically more enriched in  $^{13}\text{C}$  due to the presence of corn which has a C-4 photosynthetic pathway. This technique was used previously to determine isotopic turnover rates in other quail tissues (Hobson and Clark 1992a). The experimental quail were chosen from a group of birds that were laying eggs daily. Eggs were collected from these birds for two days prior to the diet switch (09:00 CDT on Day 0) and then each day thereafter for 10 days. As controls, on Day 10 eggs were obtained from three birds randomly

TABLE 1. Mean ( $\pm$ SD) stable-carbon and nitrogen isotope values for diets used in this study. Values for duck food were derived from measurements of wheat and pellet constituents. Other values represent samples taken from single homogenized batches prior to and during egg laying.

Species	Diet	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Mallard	Wheat/pellet mix	7	$-24.4 \pm 0.5$	$3.6 \pm 0.3$
Quail	Turkey starter (control)	10	$-24.0 \pm 0.2$	$2.8 \pm 0.2$
	Turkey starter (experimental)	9	$-16.7 \pm 0.9$	$2.4 \pm 0.3$
Falcons	Quail muscle	12	$-23.4 \pm 0.5$	$4.2 \pm 0.3$

selected from the colony that had remained on the wheat-based diet.

#### ISOTOPE ANALYSIS

Prior to isotope analysis, eggs were stored for up to a week in a refrigerator at  $+5^\circ\text{C}$ . Eggshell, albumen and yolk samples were separated by hand and subsamples of yolk and albumen taken using a syringe. Eggshells were rinsed thoroughly in distilled water and allowed to dry at room temperature after shell membranes were removed. They were then powdered using a mortar and pestle. Yolk and albumen were similarly powdered after freeze drying. Membranes were dried after washing in distilled water and then cut into fine fragments using stainless steel scissors. Lipids were removed from a subsample of yolks using the method of Bligh and Dyer (1959). Other than membranes, all food samples were powdered using an analytical mill.

For  $\delta^{15}\text{N}$  analysis, powdered samples were loaded into Vycor tubes with  $\text{CuO}$ , wire-form  $\text{Cu}$ ,  $\text{Ag}$  foil, and powdered  $\text{CaO}$ , evacuated and flame sealed. These samples were then combusted at  $850^\circ\text{C}$  for 6 hr and allowed to cool slowly overnight. The resultant  $\text{N}_2$  gas was then introduced directly into a VG OPTIMA isotope ratio mass spectrometer for  $^{15}\text{N}/^{14}\text{N}$  analysis. Powdered organic samples for  $\delta^{13}\text{C}$  analysis were loaded into pyrex tubes with wire-form  $\text{CuO}$ , flame sealed under vacuum, and combusted at  $550^\circ\text{C}$  for 6 hr. Resultant  $\text{CO}_2$  gas was cryogenically separated and then introduced into a VG 602E isotope ratio mass spectrometer for  $^{13}\text{C}/^{12}\text{C}$  analysis. Shell carbonates were reacted with phosphoric acid under vacuum to evolve  $\text{CO}_2$  for direct isotopic analysis using the VG OPTIMA mass spectrometer.

Stable isotope concentrations were expressed in  $\delta$  notation according to the following:

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

where X is  $^{13}\text{C}$  or  $^{15}\text{N}$  and R is the corresponding ratio  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ .  $R_{\text{standard}}$  for  $^{13}\text{C}$  and  $^{15}\text{N}$  are the PDB standard and atmospheric nitrogen (AIR), respectively. Using laboratory internal standards for organic material, we estimate measurement error to be  $\pm 0.1\text{‰}$  and  $\pm 0.2\text{‰}$  for carbon and nitrogen, respectively.

#### RESULTS

##### ISOTOPIC FRACTIONATION FACTORS

Stable-carbon isotope values of the foods of laying Mallards, quail and falcons were similar and reflected a C-3 plant base (Table 1; see Peterson and Fry 1987). Stable nitrogen isotope values for these foods were more varied and were highest for quail muscle which was in turn enriched over quail food by an average of  $1.4\text{‰}$ . Mean stable isotope values for dietary samples were subtracted from mean values for egg components in order to estimate mean diet-tissue fractionation factors (Table 2).

Relative to diet,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of egg albumen and shell membrane were enriched for all species (Table 2). For  $^{15}\text{N}$ , this pattern of enrichment was also maintained for whole and lipid-free yolk and  $\delta^{15}\text{N}$  fractionation values for all tissues were similar (mean enrichment:  $+3.4\text{‰}$ ; range:  $2.4\text{--}4.4\text{‰}$ ). Stable-carbon isotope fractionation values were more variable. The negative fractionation factor for  $^{13}\text{C}$  in whole yolk was due largely to the presence of lipids that were substantially depleted in  $^{13}\text{C}$  relative to diet (Table 2; mean depletion:  $-2.6\text{‰}$ ). Little fractionation of  $^{13}\text{C}$  occurred between diet and the protein component of the yolk. Highest  $^{13}\text{C}$  diet-tissue fractionation values were found for shell carbonate followed by shell membranes. With the exception of shell carbonate, Mallards and quail did not differ in isotopic fractionation factors for both carbon and nitrogen. However, compared to Mallards and quail, falcons typi-

TABLE 2. Carbon and nitrogen stable-isotope diet fractionation factors (mean  $\pm$  SD) for captive-raised birds, and the results of ANOVA tests for significant differences among species' means for each isotope. Results of Tukey's multiple comparison test are indicated by superscripts where means having at least one superscript in common are not significantly different ( $P > 0.05$ ).

Species (n)	Diet-tissue fractionation factor (‰)					
	Albumen	Whole yolk	Lipid-free yolk	Yolk lipid	Shell membrane	Shell carbonate
Quail (9)						
C	+1.6 $\pm$ 0.4 <sup>a</sup>	-1.1 $\pm$ 0.3 <sup>a</sup>	+0.1 $\pm$ 0.3 <sup>a</sup>	-2.6 $\pm$ 0.5 <sup>a</sup>	+3.5 $\pm$ 0.3 <sup>a</sup>	15.6 $\pm$ 0.5 <sup>a</sup>
N	+2.4 $\pm$ 0.2 <sup>a</sup>	+3.4 $\pm$ 0.3 <sup>a</sup>	+3.4 $\pm$ 0.3 <sup>a</sup>		+4.1 $\pm$ 0.4 <sup>a</sup>	
Mallard						
1st clutch (8)						
C	+1.4 $\pm$ 0.6 <sup>a</sup>	-1.4 $\pm$ 0.7 <sup>a</sup>	-0.1 $\pm$ 0.5 <sup>a</sup>	-2.7 $\pm$ 0.5 <sup>a</sup>	+3.7 $\pm$ 0.3 <sup>a</sup>	14.3 $\pm$ 0.7 <sup>b</sup>
N	+3.0 $\pm$ 0.4 <sup>a</sup>	+3.4 $\pm$ 0.3 <sup>a</sup>	+3.1 $\pm$ 0.4 <sup>a</sup>		+4.4 $\pm$ 0.3 <sup>a</sup>	
2nd clutch (8)						
C	+1.4 $\pm$ 0.6 <sup>a</sup>	-1.3 $\pm$ 0.7 <sup>a</sup>	+0.2 $\pm$ 0.5 <sup>a</sup>	-2.6 $\pm$ 0.5 <sup>a</sup>	+3.4 $\pm$ 0.4 <sup>a</sup>	14.6 $\pm$ 0.7 <sup>b</sup>
N	+2.8 $\pm$ 0.4 <sup>a</sup>	+3.0 $\pm$ 0.3 <sup>a</sup>	+3.2 $\pm$ 0.4 <sup>a</sup>		+4.0 $\pm$ 0.3 <sup>a</sup>	
Peregr. Falcon (6)						
C	+0.9 $\pm$ 0.5 <sup>b</sup>	-2.2 $\pm$ 0.5 <sup>b</sup>	0.0 $\pm$ 0.5 <sup>a</sup>	-3.5 $\pm$ 0.5 <sup>b</sup>	+2.6 $\pm$ 0.5 <sup>b</sup>	11.1 $\pm$ 0.5 <sup>c</sup>
N	+3.1 $\pm$ 0.4 <sup>ab</sup>	+3.3 $\pm$ 0.4 <sup>a</sup>	+3.5 $\pm$ 0.3 <sup>a</sup>		+3.5 $\pm$ 0.4 <sup>b</sup>	
Gyr Falcon (4)						
C	+0.8 $\pm$ 0.5 <sup>b</sup>	-1.8 $\pm$ 0.5 <sup>ab</sup>	+0.1 $\pm$ 0.5 <sup>a</sup>	-3.2 $\pm$ 0.4 <sup>b</sup>		11.2 $\pm$ 0.5 <sup>c</sup>
N	+3.3 $\pm$ 0.3 <sup>b</sup>	+3.1 $\pm$ 0.4 <sup>a</sup>	+3.6 $\pm$ 0.4 <sup>a</sup>			
Prairie Falcon (2)						
C	+0.9	-1.4	+0.1	-3.6	+3.0	11.6
N	+3.1	+3.5	+3.5		+3.2	
Species ANOVA C:	17.49	10.23	1.11	11.99	13.82	211.11
P	<0.001	<0.001	NS	<0.001	<0.001	<0.001
F	4.35	0.64	0.81		12.61	
N:	<0.01	NS	NS		<0.001	

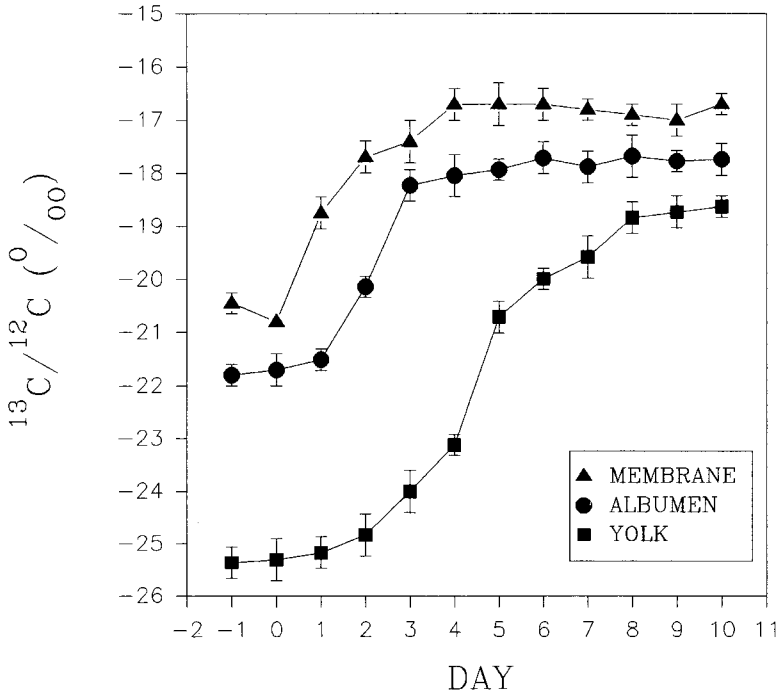


FIGURE 1. Response of quail whole yolk, albumen and shell membrane to a  $^{13}\text{C}$  isotopic dietary switch on Day 0. Data are means  $\pm$  SD and sample sizes are  $n = 4$  eggs from different individuals for each point.

cally showed less enrichment in  $^{13}\text{C}$  relative to diet for all tissues (Table 2). For all egg components, Mallards showed no significant difference in isotopic fractionation values between first and second clutches (Table 2).

#### ISOTOPIC TURNOVER IN QUAIL EGGS

Quail that were switched to a  $^{13}\text{C}$ -enriched diet showed a rapid positive response in their yolk, albumen, shell membrane, and shell carbonate  $\delta^{13}\text{C}$  values (Figs. 1, 2). Following the diet switch, albumen, shell membrane and shell carbonate reached a new equilibrium value in 3–5 days whereas whole yolk took approximately eight days. The experimental food was enriched in  $^{13}\text{C}$  over the control food by  $+7.3\text{‰}$  (Table 1) but only egg yolk and shell carbonate showed a similar change in  $\delta^{13}\text{C}$  values at equilibrium following the dietary switch (i.e., mean  $\delta^{13}\text{C}$  enrichment of 6.7 and 7.0‰, respectively). Albumen and shell membrane reached a new equilibrium with a mean  $\delta^{13}\text{C}$  enrichment over that of the control diet of only  $+3.8\text{‰}$ . Egg albumen and yolk samples for three control birds that were not switched

to the new diet, did not differ in their  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values at Day 10 compared with experimental birds at Day 2 ( $t = 1.3$ ,  $p > 0.3$ ) indicating that the isotopic change observed in the experimental eggs was due entirely to the new diet switch.

#### DISCUSSION

##### ISOTOPIC FRACTIONATION IN EGG COMPONENTS

This isotopic study of bird eggs indicates fairly uniform patterns of isotopic fractionation between diet and yolk, albumen, yolk lipid, shell membranes and shell carbonate. This is consistent with previous captive-rearing studies that have confirmed the general predictability of departures of tissue isotope values from that of diet, particularly for some mammalian tissues (DeNiro and Epstein 1978, Tieszen et al. 1983, see also Hobson and Clark 1992b). However, the magnitude of fractionation differed substantially between egg components, a reflection of differences in the biochemical and metabolic processes involved in tissue synthesis (Tieszen and Boutton

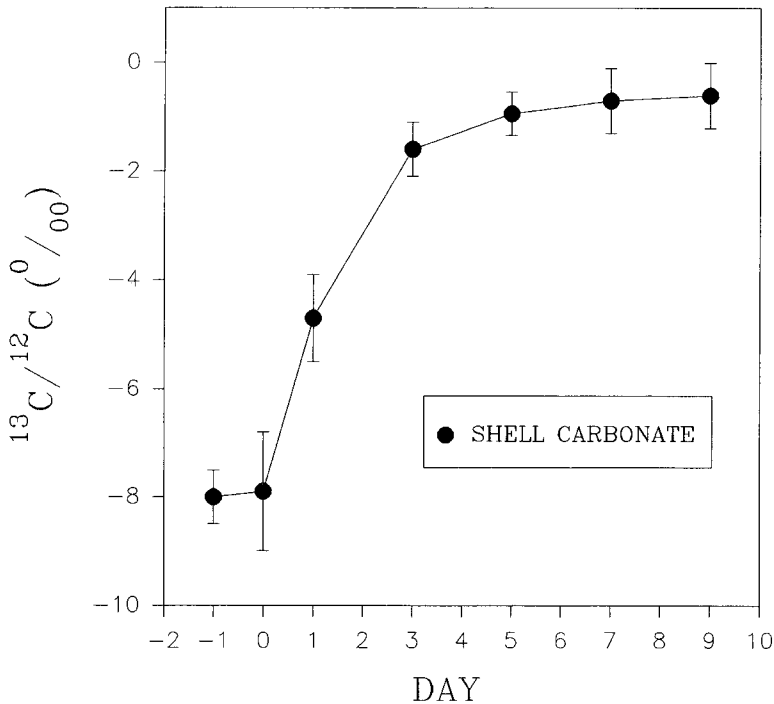


FIGURE 2. Response of quail egg carbonate to a  $^{13}\text{C}$  isotopic dietary switch on Day 0. Data are means  $\pm$  SD and sample sizes are  $n = 4$  eggs from different individuals for each point.

1988, Nakamura et al. 1982, Krueger and Sullivan 1984).

The magnitude of nitrogen isotope fractionation between diet and albumen, yolk and shell membrane were similar, being close to +3.4‰. This value is in agreement with the 3–5‰  $^{15}\text{N}$  trophic enrichment factor seen in terrestrial and marine food webs (Schoeninger and DeNiro 1984, Fry 1988, Hobson et al. 1994). Nitrogen in yolk, albumen and membranes occurs primarily as protein and  $^{15}\text{N}$  fractionation between diet and protein generally occurs during processes of amino acid amination and transamination (Macko et al. 1982). As such, protein synthesis and its corresponding isotopic fractionation for egg components is similar to the synthesis of other proteins in the adult bird.

A comparison of  $\delta^{13}\text{C}$  values of whole yolk, yolk with lipids extracted, and yolk lipid demonstrates how lipid content in egg yolk dramatically influences stable carbon isotope values. Lipids tend to be much less enriched in  $^{13}\text{C}$  than other tissues (McConnaughey and McRoy 1979, Tieszen and Boutton 1988). Also, lipid content

in many tissues including yolk can vary between species and individuals. For this reason, it is advisable to remove lipids from yolk when making broad comparisons.

Shell carbonate was highly enriched in  $^{13}\text{C}$  compared to diet for all species. Von Schirnding et al. (1982) similarly found a large positive enrichment in  $^{13}\text{C}$  relative to diet in the eggshell carbonates of the Ostrich (*Struthio camelus*) and Schaffner and Swart (1991) recorded the same trend in three species of seabirds. Animal shell carbonates are typically enriched over substrates (Fritz and Poplawski 1974, Fry and Wainright 1991) and, in the case of birds, it is possible that major carbon isotope fractionation occurs during the formation of carbonate ions by carbonic anhydrase of oviduct fluids (Simkiss and Tyler 1958, von Schirnding et al. 1982). The diet-carbonate isotopic enrichment factor of +16.2‰ estimated by von Schirnding et al. (1982) is in close agreement with our average value of +14.3 to +15.6‰ for Mallards and quail, respectively. However, falcons showed a consistently lower carbonate enrichment factor of about +11.2‰. In their iso-

topic study of seabird eggshells, Schaffner and Swart (1991) also reported smaller differences between eggshell carbonate and diet than those reported by von Schirnding et al. (1982) and suggested that such differences may be linked to the fact that seabirds are mostly protein feeders whereas Ostriches are mostly carbohydrate feeders. Interestingly, similar differences in carbon isotopic fractionation have been recorded between herbivore and carnivore bone apatite and collagen. The inorganic fraction of bone is primarily calcium phosphate crystallized as hydroxyapatite. Inorganic carbon in bone occurs as carbonate ions that substitute for phosphate groups. These carbonate ions are presumably incorporated into the apatite structure from dissolved bicarbonate in body fluids during crystal growth (Krueger and Sullivan 1984) and, in this sense, carbonates in bone apatite are analogous to carbonates in the eggshell matrix. Similarly, bone collagen, the organic component of bone, is analogous to the protein component of eggshell.

Krueger and Sullivan (1984) proposed an explanation for the greater diet-tissue isotopic enrichment in  $\delta^{13}\text{C}$  for bone apatite observed for herbivores vs. carnivores that may be appropriate also for the pattern observed in eggshell in this study. In general, herbivore diets consist of relatively more carbohydrates and fewer proteins and lipids than carnivore diets. Carbohydrates in herbivore diets are allocated primarily to energy metabolism whereas protein is allocated mainly to growth and maintenance of tissues such as collagen. Bone apatite (and shell carbonate) is derived from blood bicarbonate which is in turn generated from the metabolism of energy substrates (DeNiro and Epstein 1978). Carnivores depend relatively more on lipids than on carbohydrates for their energy metabolism and since lipids are depleted in  $^{13}\text{C}$  relative to proteins and carbohydrates, the carbon available for bone and shell formation in carnivores should be, on average, more depleted relative to diet than for herbivores. More recently, Ambrose and Norr (1993) and Tieszen and Fagre (1993) investigated the relationships of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate by manipulating the isotope composition of macronutrients in the diets of laboratory rats and mice. Both studies demonstrated that carbonates in bone apatite are de-

rived from whole diet whereas bone collagen is derived primarily from dietary protein (see also Ambrose 1993). Dietary protein not used in tissue maintenance and growth is apparently used in energy metabolism. Considering these results and the findings of Krueger and Sullivan (1984), a model of possible nutrient flow and isotopic fractionation during egg formation in herbivorous and carnivorous birds is presented in Figure 3.

In the herbivore model (Fig. 3a), only bulk diet has been depicted since the precise roles of various macronutrients are poorly understood. However, carbohydrates make up the bulk of the diet and will contribute to the formation of shell carbonate and possibly to yolk lipid and the proteinaceous components of eggs. Undoubtedly, dietary lipids and proteins will also contribute to egg lipid and protein components, respectively. In carnivores (Fig. 3b), the hypothetical model emphasizes the potential contribution of both carbon derived from dietary lipids as well as proteins to the formation of egg components, especially yolk lipids and shell carbonate. Lipids in quail muscle are depleted by 3.2‰ relative to lipid-free muscle (Hobson, unpub. data), a value almost identical to the mean depletion of yolk lipid relative to lipid-free quail muscle. This suggests that yolk lipids are derived without fractionation either from the diet or from lipid stores. Although somewhat speculative, the models presented here could be tested and refined by switching laying birds between diets of known lipid, protein, and carbohydrate isotopic composition (Ambrose and Norr 1993, Tieszen and Fagre 1993).

Schaffner and Swart (1991) provided an alternate explanation for the fractionation differences observed between high protein vs. carbohydrate feeders. These authors noted that digestive gasification or fermentation associated with the digestion of carbohydrates might contribute to the more positive carbon fractionation values associated with herbivore vs. carnivore eggshell formation. Digestive gasification might result in the export of lighter (i.e.,  $^{13}\text{C}$  depleted) carbon and the overall enrichment of the carbon reservoir available within the bird for carbonate precipitation. It is, of course, possible that carbohydrate gasification in herbivores and the greater reliance of carnivores on dietary lipids for energy metabolism both contribute to carbon isotope fractionation differences between diet and egg

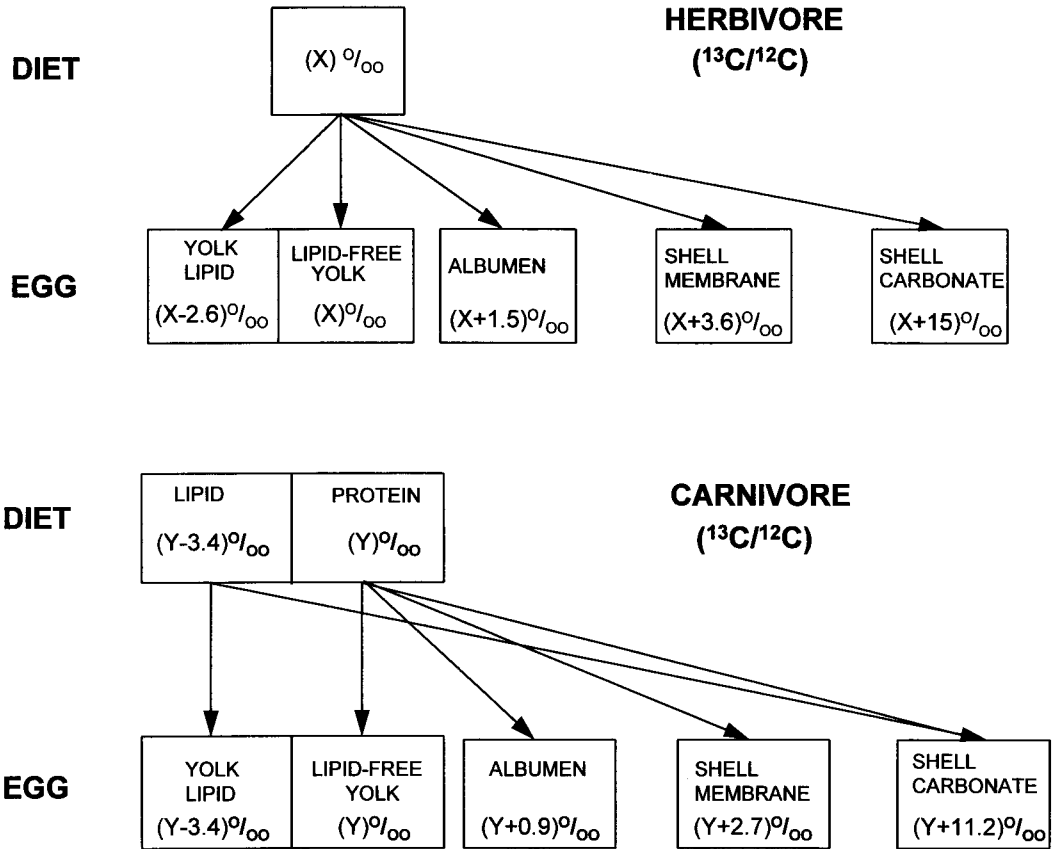


FIGURE 3. Hypothetical model for carbon isotope fractionation between diet and egg components of (a) herbivores consuming a diet consisting primarily of carbohydrates and (b) carnivores consuming diets consisting primarily of lipids and proteins in meat.

components for these two groups of consumers. Both explanations are consistent with the reductions in carbon isotopic fractionation factors observed in falcons vs. quail and Mallards.

This study has provided the first estimates of isotopic fractionation between *whole* diet and several components of eggs. However, it is important to also consider fractionation patterns associated with dietary macromolecules such as carbohydrates, lipids, proteins and even individual amino acids (Tieszen and Boutton 1988, Macko et al. 1982). The diet-switch experiment underscores this point, since diet-tissue isotopic fractionation factors for carbon remained the same under the experimental and control diets for yolk and carbonate but changed for albumen and shell membranes. This suggests that the shell carbonate and egg yolk were derived from *whole*

diet whereas membranes and albumen were derived from subcomponents of the whole diet (i.e., proteins) that may have changed in relative composition between the two diets. Again, further investigations are required to elucidate the metabolic and biochemical processes that result in isotopic fractionation and how these are influenced by dietary substrates such as protein, lipids and carbohydrates (Galimov 1985, Tieszen and Boutton 1988). Current research into patterns of fractionation associated with the formation of bone collagen and apatite will also undoubtedly contribute to our understanding of similar processes associated with the formation of the organic and inorganic components of eggs in birds and vice versa (von Schirnding et al. 1982, Schaffner and Swart 1991, Ambrose 1993, Ambrose and Norr 1993, Tieszen and Fagre 1993).



### ISOTOPIC TURNOVER DURING EGG FORMATION

The results of the diet-switch experiment are generally consistent with our current understanding of the processes of egg formation. The laying of each egg is preceded by *rapid follicle growth* (RFG) during which yolk accumulates in each ovarian follicle (reviewed by Alisauskas and Ankney 1992). If yolk is formed rapidly from dietary sources then the contribution of the new diet to the isotopic signal in the yolk should be proportional to the additional mass of yolk formed using the new diet. In this sense, the diet uptake curve for yolk shown in Figure 1 should resemble the growth curve for the mass of developing follicles in quail. The shape of the isotopic response curve for yolk agrees with King's (1973) assumption that follicle growth curves in birds are sigmoidal (but see Gilbert 1971, Grau 1984). These results also suggest a period of RFG to be about eight days (i.e., the time for egg yolks to reach a new isotopic equilibrium following the diet switch), a value consistent with that estimated for chickens (Gilbert 1971) and medium-sized ducks (see review by Bluhm 1992).

Albumen proteins are synthesized in the magnum from amino acids removed directly from the blood (Taylor 1970). Albumen is also deposited rapidly around the yolky ovum at this stage (about 2.5 hr) as are eggshell membranes and these factors account for the rapid response of both albumen and membranes to the new isotopic diet. The isotopic response of shell carbonate following the diet switch was also rapid. Unlike calcium that may be mobilized from medullary bone during the process of shell formation, carbon for the calcium carbonate fraction of shell is derived directly from plasma through the metabolism of recently assimilated food (Simkiss and Tyler 1958). Carbonates in food do not contribute to carbonates in eggshell since they react with digestive acids to produce carbon dioxide gas which is lost (Simkiss 1961, Mongin 1968, Schaffner and Swart 1991). For this reason, the carbonate component of the diet of captive birds (e.g., shell supplements) were not expected to influence derived isotopic fractionation factors.

### APPLICATION TO AVIAN DIETARY STUDIES

The isotopic diet-tissue fractionation values determined here for yolk, albumen, yolk lipid,

membrane and eggshell carbonate using captive birds provide a first approximation of those values for wild birds. For the moment, the values obtained for quail and ducks approximate those for wild herbivorous birds consuming C-3 plant-based diets and values for falcons approximate those for piscivorous and carnivorous species. Fractionation values derived here for shell membranes should be interpreted with some caution since the membrane material used were from freshly laid or infertile eggs. Membranes change in composition throughout incubation by becoming thicker and more collagenous and so membranes obtained from hatched eggs may differ in isotopic composition compared to the same material obtained earlier in incubation (Schaffner and Swart 1991). Early membranes may thus be better diet indicators of the laying female than later (i.e., incubated) membranes. The protein fraction of eggshells is another material that may be used for isotopic analysis (see von Schirnding et al. 1982) and it would be useful to conduct similar studies on this component. Here, eggshell protein is analogous to bone collagen and refers to the proteinaceous matrix within the eggshell and not the shell membranes. As with shell carbonates and membranes, an advantage to using this material is that it is often readily available from nest sites as shell fragments and does not involve destructive sampling.

Fractionation values derived in this study were based on birds that had ad libitum access to food and they may not be applicable in those situations where birds mobilize significant amounts of stored nutrient reserves. Nutrient storage prior to reproduction is particularly well known in waterfowl (reviewed by Alisauskas and Ankney 1992) and it is possible that isotopic fractionation would be different if lipids and proteins are mobilized from somatic reserves vs. those produced directly from the conversion of dietary carbohydrate or protein. On the other hand, the process of mobilization of stored lipids may involve very little fractionation if lipids are mobilized whole without further biochemical breakdown. Nonetheless, isotopic studies using egg yolks should remove lipids and analyze them separately.

Systematic declines in somatic protein in response to protein demands during egg production have also been demonstrated in wild waterfowl (reviewed by Alisauskas and Ankney 1992). In these cases, it is possible that the amino

acid pool may become enriched in  $^{15}\text{N}$  due to processes of catabolism or nutrient mobilization (Hobson et al. 1993). This in turn may result in further  $^{15}\text{N}$  enrichment of the protein components of eggs relative to diet. Should somatic reserves make up a significant portion of the protein used for egg formation, then an increase in the  $\delta^{15}\text{N}$  value of egg proteins would be expected. Moreover, the magnitude of the enrichment effect would be expected to change with egg order within and between clutches, with those eggs requiring a greater proportion of somatic reserves showing higher  $\delta^{15}\text{N}$  enrichment. While I was unable to demonstrate any changes in  $\delta^{15}\text{N}$  enrichment between first and second clutches in captive Mallards, researchers using eggs for isotopic investigations should be aware of the potential for such influences on patterns of isotopic fractionation in eggs.

#### ACKNOWLEDGMENTS

I thank J. Hochbaum, R. George, and M. Smith for laboratory assistance and C. van Kessel and L. Wasenaar for stable-isotope analyses. P. Thompson and L. Oliphant made available quail and falcon material and C. Bluhm made available duck eggs and dietary material. C. Bluhm, R. G. Clark, L. Tieszen and F. Schaffner made valuable comments on an earlier draft of this manuscript. Partial funding and laboratory facilities were provided by the Prairie and Northern Wildlife Research Center (CWS) in Saskatoon. C. Bluhm thanks the Canadian Wildlife Service for granting permits to conduct studies on captive mallards. The Mallards were housed in the breeding pens of the Gaylord Avicultural Laboratory of the Delta Waterfowl and Wetlands Research Station. Funding for the waterfowl was provided by the Delta Waterfowl Foundation.

#### LITERATURE CITED

- AFTON, A. D., AND C. D. ANKNEY. 1991. Nutrient-reserve dynamics of breeding Lesser Scaup: a test of competing hypotheses. *Condor* 93:89-97.
- ALISAUSKAS, R. T., AND C. D. ANKNEY. 1992. The cost of egg laying and its relationship to nutrient reserves in waterfowl, p. 30-61. *In* B.D.J. Batt, A. D. Afton, M. G. Anderson, C. D. Ankney, D. H. Johnson, J. A. Kadlec, and G. L. Krapu [eds.], *Ecology and management of breeding waterfowl*. Univ. of Minnesota Press, Minneapolis.
- AMBROSE, S. H. 1993. Isotopic analysis of palaeodiets: methodological and interpretive considerations, p. 59-130. *In* M. K. Sandford [ed.], *Investigations of ancient human tissue*. Gordon and Breach, Langhorne, PA.
- AMBROSE, S. H., AND L. NORR. 1993. Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate, p. 1-37. *In* J. Lambert and G. Grupe [eds.], *Prehistoric human bone: archaeology at the molecular level*. Springer-Verlag, Berlin.
- AUSTIN, J. E., AND L. H. FREDRICKSON. 1987. Body and organ mass and body composition of post-breeding female Lesser Scaup. *Auk* 104:694-699.
- BLIGH, E. G., AND W. J. DYER. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37:911-917.
- BLUHM, C. K. 1992. Environmental and endocrine control of waterfowl reproduction, p. 323-364. *In* B.D.J. Batt, A. D. Afton, M. G. Anderson, C. D. Ankney, D. H. Johnson, J. A. Kadlec, and G. L. Krapu [eds.], *Ecology and management of breeding waterfowl*. Univ. of Minnesota Press, Minneapolis.
- DENIRO, M. J., AND S. EPSTEIN. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* 42:495-506.
- DENIRO, M. J., AND S. EPSTEIN. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim. Cosmochim. Acta* 45:341-351.
- FRITZ, P., AND S. POPLAWSKI. 1974.  $^{18}\text{O}$  and  $^{13}\text{C}$  in the shells of freshwater molluscs and their environments. *Earth and Plan. Sc. Letters* 24:91-98.
- FRY, B. 1988. Food web structure on Georges Bank from stable C, N, and S isotopic compositions. *Limnol. Oceanogr.* 33:1182-1190.
- FRY, B., AND S. C. WAINRIGHT. 1991. Diatom sources of  $^{13}\text{C}$ -rich carbon in marine food webs. *Mar. Ecol. Progr. Ser.* 76:149-157.
- GALIMOV, E. M. 1985. *The biological fractionation of isotopes*. Academic Press, New York.
- GILBERT, A. B. 1971. The ovary, p. 1163-1202. *In* D. J. Bell and B. M. Freeman [eds.], *Physiology and biochemistry of the domestic fowl*, Vol III. Academic Press, New York.
- GRAU, C. R. 1984. Egg formation, p. 33-57. *In* G. C. Whitton and H. Rahn [eds.], *Seabird energetics*. Plenum Press, New York.
- HOBSON, K. A. 1987. Use of stable-carbon isotope analysis to estimate marine and terrestrial protein content in gull diet. *Can. J. Zool.* 65:1210-1213.
- HOBSON, K. A. 1990. Stable isotope analysis of Marbled Murrelets: evidence for freshwater feeding and determination of trophic level. *Condor* 92:897-903.
- HOBSON, K. A. 1993. Trophic relationships among high Arctic seabirds: insights from tissue-dependent stable-isotope models. *Mar. Ecol. Progr. Ser.* 95:7-18.
- HOBSON, K. A., AND S. G. SEALY. 1991. Marine protein contributions to the diet of Northern Saw-whet Owls on the Queen Charlotte Islands, British Columbia: a stable-isotope approach. *Auk* 108:437-440.
- HOBSON, K. A., J. PIATT, AND J. PITOCHELLI. 1994. Using stable isotopes to determine seabird trophic relationships. *J. Anim. Ecol.* 63:786-798.
- HOBSON, K. A., AND R. G. CLARK. 1992a. Assessing avian diets using stable-isotope analysis. I: Turnover of carbon-13. *Condor* 94:181-188.
- HOBSON, K. A., AND R. G. CLARK. 1992b. Assessing avian diets using stable-isotope analysis. II: Factors influencing diet-tissue fractionation. *Condor* 94:189-197.

- HOBSON, K. A., AND R. G. CLARK. 1993. Turnover of  $^{13}\text{C}$  in cellular and plasma fractions of blood: Implications for nondestructive sampling in avian dietary studies. *Auk* 110:638-641.
- HOBSON, K. A., R. T. ALISAUSKAS, AND R. G. CLARK. 1993. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analyses of diet. *Condor* 95:388-394.
- KING, J. R. 1973. Energetics of reproduction in birds, p. 78-107. *In* D. S. Farner [ed.], *Breeding biology of birds*. National Academy of Sciences, Washington, DC.
- KRAPU, G. L. 1981. The role of nutrient reserves in Mallard reproduction. *Auk* 98:29-38.
- KRUEGER, H. W., AND C. H. SULLIVAN. 1984. Models of carbon isotope fractionation between diet and bone, p. 205-220. *In* J. R. Turnland and P. E. Johnson [eds.], *Stable isotopes in nutrition*. ACS Symp. Ser. 258. American Chemical Society, Washington.
- MACKO, S. A., W. Y. LEE, AND P. L. PARKER. 1982. Nitrogen and carbon isotope fractionation by two species of marine amphipods: laboratory and field studies. *J. Exp. Mar. Biol. Ecol.* 63:145-149.
- MCCONNAUGHEY, T., AND C. P. McROY. 1979. Food web structure and the fractionation of carbon isotopes in the Bering Sea. *Mar. Biol.* 53:257-262.
- MIZUTANI, H., M. FUKUDA, Y. KABAYA, AND E. WADA. 1990. Carbon isotope ratio of feathers reveals feeding behavior of cormorants. *Auk* 107:400-403.
- MONGIN, P. 1968. Role of acid-base balance in the physiology of egg shell function. *World's Poult. Sci. J.* 24:200-230.
- NAKAMURA, K., D. A. SCHOELLER, F. J. WINKLER, AND J. H. SCHMIDT. 1982. Geographical variations in the carbon isotope composition of the diet and hair in contemporary man. *Biomed. Mass Spec.* 9:390-394.
- PETERSON, B. J., AND B. FRY. 1987. Stable isotopes in ecosystem studies. *Ann. Rev. Ecol. Syst.* 18:293-320.
- SCHAFFNER, F. C., AND P. K. SWART. 1991. Influence of diet and environmental water on the carbon and oxygen isotopic signatures of seabird eggshell carbonate. *Bull. Mar. Sci.* 48:23-38.
- SCHOENINGER, M. J., AND M. J. DENIRO. 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochim. Cosmochim. Acta* 48:625-639.
- SIMKISS, K. 1961. Calcium metabolism and avian reproduction. *Biol. Rev.* 36:321-367.
- SIMKISS, K., AND C. TYLER. 1958. Reaction between eggshell matrix and metallic cations. *Quart. J. Microscop. Sci.* 99:5-13.
- TAYLOR, T. G. 1970. How an eggshell is made. *Sci. Am.* 222:89-97.
- TIESZEN, L. L., AND T. FAGRE. 1993. Effect of diet quality and composition on the isotopic composition of respiratory  $\text{CO}_2$ , bone collagen, bioapatite, and soft tissues, p. 1-37. *In* J. Lambert and G. Grupe [eds.], *Prehistoric human bone: archaeology at the molecular level*. Springer-Verlag, Berlin.
- TIESZEN, L. L., T. W. BOUTTON, K. G. TESDAHL, AND N. A. SLADE. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for  $\delta^{13}\text{C}$  analysis of diet. *Oecologia* 57:32-37.
- TIESZEN, L. L., AND T. W. BOUTTON. 1988. Stable carbon isotopes in terrestrial ecosystem research, p. 167-195. *In* P. W. Rundel, J. R. Ehleringer, and K. A. Nagy [eds.], *Stable isotopes in ecological research*. Springer-Verlag, Berlin.
- VON SCHIRNDING, Y., N. J. VAN DER MERWE, AND J. C. VOGEL. 1982. Influence of diet and age on carbon isotope ratios in Ostrich eggshell. *Archaeometry* 24:3-20.