Using stable isotopes to quantitatively track endogenous and exogenous nutrient allocations to eggs of birds that travel to breed

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Conventional approaches to measuring endogenous nutrient allocations to reproduction in birds have relied on the sampling of several individuals within populations in order to contrast mass gains of the clutch with mass loss of the laying female's tissues. The reasoning has been that mass loss of female endogenous tissues during laying can be attributed to 'capital' investment into reproduction. Apart from the destructive nature of this work, problems associated with this approach involve uncertain conversion efficiencies between endogenous reserves and eggs and the general loss of information on individual strategies. As a result, the role of endogenous reserves have likely been overestimated in most cases. Stable isotope measurements of endogenous reserves and egg components can help to trace quantitatively the relative allocation of endogenous vs. exogenous reserves to eggs in cases where local (breeding season) diets differ isotopically from endogenous reserves typically acquired on the wintering or staging grounds prior to arrival to breed. Fortunately several avian species change 'isoscapes' when travelling to breed and so are amenable to isotopic tracking of their body nutrients to eggs. For example, birds wintering in coastal marine biomes and travelling to terrestrial or freshwater biomes experience a general depletion in the isotopes of several elements (C, N, H, O and S) in their local foodwebs. Previous captive studies have allowed us to estimate the isotopic ($\delta^{13}C$ and $\delta^{15}N$) fractionation factors between diet and several egg components (lipid-free yolk, albumen, yolk lipid, shell carbonate) for herbivorous and carnivorous 'income' breeders. Currently, we are using the carnivore model to also estimate fractionation expected between endogenous reserves and eggs. The net result of this work has been an isotopic multi-source mixing model to predict the relative allocation of endogenous vs. exogenous protein and lipid capital to eggs. While refinement is needed for this model, it provides a firm basis for future avian isotopic tracking studies involving nutrient allocations.

Key words: stable isotopes, endogenous nutrients, reproductive investment

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INTRODUCTION

Tracing the relative allocation of endogenous and exogenous nutrients to reproduction in migratory and resident animals, including birds that travel to breed, is of fundamental importance to an understanding of the evolution of life history strategies (Drent & Daan 1980, Jönsson 1997, Bonnet *et al.* 1998). In addition, species that are exposed to different factors influencing their fitness at different locations may require special management considerations (Hobson *et al.* 1997). Recently, such interest has been framed in terms of hypotheses related to migratory connectivity which postulate that events during one period of the annual cycle can influence events at a subsequent period (Webster *et al.* 2001). Understandably then, in birds, a great deal of research has been conducted that has attempted to quantify the relative allocations of resources to reproduction by the laying female. This has been particularly the case with waterfowl in North America where it has previously been assumed that species fall along a continuum between *capital* breeders like eiders at one extreme to smaller bodied *income* species like teal at the other (Drent & Daan 1980, Meijer & Drent 1999). Unfortunately, methods used to quantify such nutrient allocations were almost certainly flawed since they relied on correlating mass gains to the clutch with mass loss of macronutrients in the laying female, including losses to her reproductive tissues (e.g. Alisauskas & Ankney 1992). This approach assumed that maintenance costs of the female are negligible during reproduction and that there is a conversion efficiency of 100% from endogenous nutrient sources to the eggs. Both of these assumptions are almost certainly invalid. The net result has been a likely overestimation of the role of endogenous nutrients to reproduction (Meijer & Drent 1999, Morrison & Hobson 2004). In addition to these drawbacks, the conventional approach has relied on the sequential killing of birds throughout the reproductive period so that their endogenous nutrient depots (i.e. muscle and fat) could be measured. Apart from the destructive nature of this work, information is only provided at the population level and not at the level of the individual where it is typically more informative. The recent advent of stable isotope techniques to more directly trace nutrient allocations to eggs represents a fundamental improvement over the more indirect conventional technique. In this paper, I will review the principles behind the stable isotope tracing technique, review the studies using this approach to date, and conclude by reviewing the kind of future research directions we need to better refine this exciting new development.

STABLE ISOTOPES AND ISOSCAPES

Elements in nature occur in more than one stable form due to varying numbers of neutrons in the nucleus. These various forms are known as stable isotopes of an element and have identical chemical but varying kinetic properties due to their mass differences. Compounds containing isotopes of elements thus behave differently in various biogeochemical processes that ultimately result in changes in the relative abundance of the heavier to the lighter isotope. The most common isotopes measured in ecological studies are those of C, N, O, S and H. We depict the relative abundance of the heavier to lighter isotope of elements (e.g. $^{13}C/^{12}C$) according to comparisons of this ratio in a sample to those occurring in international standards by means of mass spectrometry. This results in a so-called delta (δ) notation in parts per thousand deviation from the standard. Since the choice of standards has been largely arbitrary, stable isotope values can be positive and negative. The important consideration is simply that modern technology allows us to detect extremely small variations in the relative abundance of one isotope species over another in inorganic and organic materials.

For application to ecological studies, it is important to know that isotopic ratios in diet are reflected in consumer tissues. Any changes in the isotopic ratio between diet and tissues, known as 'isotopic discrimination' (previously referred to as

isotopic fractionation), once known, can be used to reconstruct nutrient pathways and trophic positions of consumers and this realization has recently resulted in an exponential increase in isotopic applications to wildlife research (Kelly 2000, Rubenstein & Hobson 2004). Thus

$$\delta X_t = \delta Xd + \Delta\delta_{dt} \quad (1)$$

where δX_t is the stable isotope value of consumer tissue t (and X might be ^{13}C, ^{15}N, ^{34}S etc.), δX_d is the stable isotope value of diet and $\Delta\delta_{dt}$ is the isotopic discrimination factor between diet and tissue *t*. Isotopic discrimination factors between diet and consumer tissues have been derived empirically primarily through captive studies on animals raised on known isotopic diets (e.g. Hobson & Clark 1992a,b).

The next important aspect of dietary reconstructions using stable isotope analyses is to realize that different macromolecules undergo different metabolic routing from diet to ultimate incorporation into consumer tissues (Martinez del Rio & Wolf 2004). Whereas nitrogen in proteins typically ends up in proteins in the consumer, carbon can be routed anywhere in consumers, including lipids, proteins or glycogen in the consumer. Carbon in glycogen may in turn be metabolised directly to breath CO_2 and so go undetected isotopically in tissues (e.g. Podlesak *et al.* 2005). Thus dietary tracking with stable isotopes may involve careful consideration of several factors that depend in part on the ecology of the species of interest (Hobson & Stirling 1997). Finally, stable isotope abundance in metabolically active tissues represents an integration of inputs over a time window inversely correlated with the metabolic rate of that tissue. Thus for high metabolic rate tissues like liver, the window of integration is comparatively short whereas for bone proteins with very slow metabolism, it is relatively long (Hobson & Clark 1992a).

It is our good fortune that stable isotope abundance for several elements differs across food webs due to a variety of biogeochemical processes. This provides a mosaic of 'isotopic landscapes' or 'isoscapes' in nature that can ultimately provide information on source of nutrients to consumers. Several reviews are now available that list such isotopic situations and how these might be used to track animal diets and movements (Hobson 1999, Kelly 2000, Rubenstein & Hobson 2004). One of the most important examples corresponds to the general enrichment in the heavy isotopes of C, N, S, O, and H in marine vs. terrestrial and freshwater biomes. Within terrestrial systems, we also find isotopic structure in C due primarily to differences in photosynthetic pathway and, along with N, through land-use practices and climate. Recently, the realization that deuterium in consumer tissues can ultimately be related to weighted average patterns of deuterium content in precipitation during the growing-season at continental scales (Bowen *et al.* 2005) has revolutionized our ability to infer origins of migrating birds and other wildlife in North America and Europe (Hobson 2005). Another practical consideration in the use of stable isotopes in field studies is the ease with which tissues can be preserved and stored for later laboratory analyses (Gloutney & Hobson 1998).

Although most applications of isotopic dietary tracing to date involving birds have dealt with foraging ecology of individuals and populations, the same basic principles should apply to tracing nutrient pathways *within* birds. It was precisely this realization that motivated Hobson (1995) to examine the nature of isotopic discrimination between diet and various components of the avian egg thereby forming the basis of quantitative tracking of endogenous and exogenous nutrient allocations to eggs.

BEHAVIOUR OF STABLE ISOTOPES IN EGG FORMATION

Recognizing that nutrient pathways between diet and egg components likely differ for high carbohydrate vs. high protein diets, Hobson (1995) raised Japanese Quail *Coturnix japonica* on a plant-based diet and Peregrine Falcons *Falco peregrinus* on a quail diet and then related dietary lipid and lipid-

free $\delta^{13}C$ and $\delta^{15}N$ values in diet to corresponding isotope values in egg yolk lipid, lipid-free yolk, albumen, and shell carbonate. In order to separate the isotopic effects of differential lipid content in various egg components, and to account for possible differential routing of carbon from dietary lipids versus proteins and it was generally assumed that dietary lipids would be preferentially routed to egg lipids and dietary proteins to egg proteins. Isotopic measurement of mixed lipid and protein components of diet and eggs would thus only obscure such differential routing. It was also assumed that both captive groups of birds, fed *ad libitum*, were both essentially income feeders. This was tested with quail by switching their diets isotopically and then measuring how quickly the new dietary signal appeared in their eggs. As expected, shell carbonate and albumen showed the most rapid response to the diet switch, followed by yolk protein. Overall rate of change in yolk isotope values conformed to expectations based on patterns of rapid follicular growth (Hobson 1995). Interestingly, isotopic discrimination factors between diet and egg components differed between the herbivore and carnivore diets reflecting, in part, differential macromolecular routing of lipids, carbohydrates, and proteins. Lipids from the carnivore diet actually contributed to more depleted shell carbonate $\delta^{13}C$ values relative to diet than in the herbivore model.

An unexpected benefit of considering the carnivore income breeding model was the fact that it provided the first estimate of isotopic discrimination factors that were likely applicable to the capital breeding strategy. The reasoning here is simply that conversion of dietary protein (muscle) and lipids to eggs during an income process of egg formation should be kinetically and thus isotopically similar to the process of the production of eggs from endogenous muscle and lipid stores. Gauthier *et al.* (2003) were the first to apply this reasoning to generate the first fully quantitative estimate of the role of endogenous and exogenous nutrients to reproduction in Greater Snow Geese *Chen caerulescens atlantica*. In addition, they made use of a concentration-dependent isotopic mixing model developed by Phillips & Koch (2002) which provides a unique mathematical solution to *n* inputs based on *n–1* stable isotopes. This model also has the advantage of recognizing that should the various endogenous and exogenous inputs differ in their concentration of the elements considered (in this case [C] and [N]), then such differences will be accounted for in the model. A current weakness of the model is that it does not explicitly deal with different assimilation efficiencies of elements among different inputs and, if known, these need to be weighted accordingly (e.g. Gauthier *et al.* 2003).

TRACING NUTRIENT PATHWAYS IN WILD BIRDS

By now it will be clear to the reader that there are three basic principles to the successful application of stable isotope methods to quantitatively trace endogenous and exogenous nutrients to eggs (Fig. 1). The first is that these two endpoint pools of nutrient sources to eggs differ isotopically so that relative contributions can be resolved statistically. The second is that we know how stable isotopes discriminate between each macromolecular pool and the ultimate formation of egg components. Finally, it is necessary to realize that pathways between pools and egg components differ and that overall assimilation of elements into eggs can differ based on dietary substrate. To date, with the exception of Gauthier *et al.* (2003) and Schmutz *et al.* (2006) few studies have tackled these sorts of issues explicitly and have been forced to rely to a large extent on inference. Nonetheless, the application of stable isotope measurements to this interesting field has developed rapidly (Table 1) and the following is a brief overview of progress to date. In all of these examples, it is implicit in applying stable isotope methods that *capital* necessarily refers to the mobilization of endogenous reserves obtained in an isoscape different from that related to the breeding grounds. Only if endogenous reserves differ isotopically from those that would be formed from local breeding ground

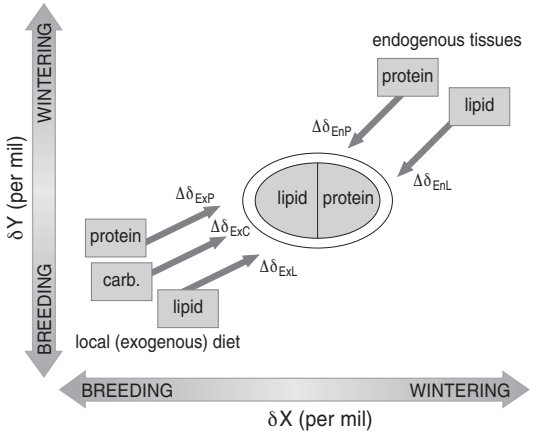

Figure 1. Schematic depiction of a hypothetical two-isotope analysis of dietary and endogenous tissue allocation to egg components. Here endogenous tissues are isotopically distinct from local breeding area foodwebs since they were formed on the wintering grounds. The isotopic discrimination factors that link dietary and endogenous macromolecules with egg components are depicted where ExL, ExP, and ExC subscripts correspond to exogenous lipid, protein and carbohydrate, respectively, and subscripts EnL and EnP correspond to endogenous lipid and protein sources.

diet can the method be used quantitatively. Moreover, endogenous or capital reserves could be obtained on the wintering grounds, *en route*, or anywhere prior to breeding as long as an isotopic distinction exists between breeding (*income*) diet and reserves formed from these other diets.

To my knowledge, the first researcher to use stable isotope measurements of egg components to infer origins of nutrients was Trust (1993) who examined $\delta^{13}C$ and $\delta^{34}S$ values in eggs of Redhead Ducks *Aythya americana* breeding in Manitoba, Canada. She determined that there was a poor correlation between $\delta^{13}C$ values in albumen and yolk suggesting different sources of nutrients. However, since lipids were not removed from the yolk it is not possible to easily decipher these results. Possibly, only the lipid fraction of the yolk was derived from different sources which may have been more, or less, isotopically variable than those for yolk protein, or yolk could have contained different amounts of ^{13}C-depleted lipids among individuals. These explanations are supported by the strong correlation Trust (1993) found between $\delta^{34}S$ values of albumen and whole yolk, as sulphur is found in protein but not in lipid.

Hobson *et al.* (1997) measured isotopically eggs of three colonial waterbirds breeding on Lake Ontario, Canada, in order to determine if there was evidence for the transfer of marine nutrients acquired on the wintering grounds to eggs laid on the freshwater breeding grounds. That study was motivated by the fact that the eggs of Double-crested Cormorants *Phalacrocorax auritus* had been used for decades as indicators of contaminant loads on the Great Lakes but it was never demonstrated unequivocally that endogenous nutrients, and hence endogenously held contaminants from the wintering grounds, had not entered these eggs. That cormorant eggs were isotopically equivalent in $\delta^{34}S$ and $\delta^{13}C$ values as those expected and found based on a purely local (freshwater) food web indicated that cormorants were not capital breeders and that contaminant loads in eggs were almost certainly derived form the local source. This study lead to a more ambitious examination of 5 species of gulls, 4 species of terns, and one skua breeding on Great Slave Lake, Canada (Hobson *et al.* 2000). As indicated by $\delta^{13}C$ and $\delta^{15}N$ measurements of tissues, birds arrived with largely marine-derived endogenous nutrients but only 3 species (*L. delawarensis, Sterna paradisaea, S. hirundo*) showed a strong indication of transferring marine-derived lipids to eggs and 4 species (*L. delawarensis, S. hirundo, S. caspia, Stercororarius parasiticus*) showed evidence of marine-derived protein contributions to eggs. Thus, despite there being some expectation that these high-latitude breeders should show signs of a capital breeding strategy, this proved to be the exception rather than the rule.

An interesting development in using stable isotopes to examine nutrient transfer to eggs was provided by the first use of deuterium (δD) measurements. Hobson *et al.* (2004) examined δD and $\delta^{13}C$ values of endogenous reserves (muscle and fat) and egg components in Redhead Ducks breeding in

Table 1. Summary of estimates of endogenous reserve inputs to egg components based on stable isotope analyses. Some studies were not designed to provide quantitative estimates but instead relied on inference from isotopic data (indicated by *).

Species	Location	Tissue	Isotope	% Endogenous	Source
Double-crested Cormorant	Lake Ontario	protein	$\delta^{13}C$, $\delta^{34}S$	0	Hobson *et al.* 1997*
Phalacrocorax auritus		lipid	$\delta^{13}C$	0	
Herring Gull	Lake Ontario	protein	$\delta^{13}C$, $\delta^{34}S$	0	Hobson *et al.* 1997*
Larus argentatus		lipid	$\delta^{13}C$	0	
Ring-billed Gull	Great Slave Lake, NWT	lipid	$\delta^{13}C$	12	Hobson *et al.* 2000
L. delawarensis		protein	$\delta^{13}C$	29	Hobson *et al.* 2000
Bonapartes Gull *L. philadelphia*	Great Slave Lake, NWT	lipid/protein	$\delta^{13}C$, $\delta^{15}N$	0	
California Gull *L. californicus*	Great Slave Lake, NWT	lipid/protein	$\delta^{13}C$, $\delta^{15}N$	0	
Mew Gull *L. canus*	Great Slave Lake, NWT	lipid/protein	$\delta^{13}C$, $\delta^{15}N$	0	
Arctic Tern	Great Slave Lake, NWT	lipid	$\delta^{13}C$	14	Hobson *et al.* 2000
Sterna paradisaea		protein	$\delta^{13}C$, $\delta^{15}N$	0	Hobson *et al.* 2000
Black Tern *Chlidonias niger*	Great Slave Lake, NWT	lipid	$\delta^{13}C$, $\delta^{15}N$	3	Hobson *et al.* 2000
		protein	$\delta^{13}C$, $\delta^{15}N$	0	Hobson *et al.* 2000
Caspian Tern *S. caspia*	Lake Ontario	protein	$\delta^{13}C$, $\delta^{34}S$	0	Hobson *et al.* 1997*
		lipid	$\delta^{13}C$	0	
	Great Slave Lake	protein	$\delta^{13}C$, $\delta^{15}N$	41	Hobson *et al.* 2000
Common Tern *S. hirundo*	Great Slave Lake	lipid	$\delta^{13}C$	75	Hobson *et al.* 2000
		protein	$\delta^{13}C$, $\delta^{15}N$	49	Hobson *et al.* 2000
Arctic Skua *Stercorarius parasiticus*	Great Slave Lake	protein	$\delta^{13}C$	12	Hobson *et al.* 2000
Redhead Duck	Minnedosa, Manitoba	protein	$\delta^{13}C$, δD	0	Hobson *et al.* 2004
Aythya americana		lipid	$\delta^{13}C$, δD	0	
Barrow's Goldeneye	Riske Creek,	protein	$\delta^{13}C$	23.7–28.7 (1^{st} egg)	Hobson *et al.* 2005
Bucephala islandica	British Columbia			9.3–12.3 (8^{th} egg)	
		lipid	$\delta^{13}C$	4.9 (^{1st} egg)	
				0 (8^{th} egg)	
Harlequin Duck	Pemberton,	protein	$\delta^{13}C$,$\delta^{15}N$	0	Bond *et al.* in press
Histrionicus histrionicus	British Columbia	lipid	$\delta^{13}C$	0	
Greater Snow Goose	Bylot Island, NWT	protein	$\delta^{13}C$,$\delta^{15}N$	33	Gauthier *et al.* 2003
Chen caerulescens		lipid	$\delta^{13}C$	20	
Ruddy Turnstone	Alert, Nunavut	protein	$\delta^{13}C$,$\delta^{15}N$	+ (early eggs)	Morrison & Hobson
Arenaria interpres		lipid	$\delta^{13}C$	++ (early eggs)	2004*
	Greenland/Canada	protein	$\delta^{13}C$	0	Klaassen *et al.* 2001*
Red Knot *Calidris canutus*	Greenland/Canada	protein	$\delta^{13}C$	0	Klaassen *et al.* 2001*
	Alert, Nunavut	protein/lipid	$\delta^{13}C$,$\delta^{15}N$	0	Morrison & Hobson 2004*
Grey Plover *Pluvialis squatarola*	Greenland/Canada	protein	$\delta^{13}C$	0	Klaassen *et al.* 2001*
Ringed Plover *Charadrius hiaticula*	Greenland/Canada	protein	$\delta^{13}C$	0	Klaassen *et al.* 2001*
Semipalmated Plover *Ch. semipalmatus*	Greenland/Canada	protein	$\delta^{13}C$	0	Klaassen *et al.* 2001*
Sanderling *C. alba*	Greenland/Canada	protein	$\delta^{13}C$	0	Klaassen *et al.* 2001*
	Alert, Nunavut	protein/lipid	$\delta^{13}C$,$\delta^{15}N$	0	Morrison & Hobson 2004*
Purple Sandpiper *C. maritima*	Greenland/Canada	protein	$\delta^{13}C$	0	Klaassen *et al.* 2001*
Dunlin *C. alpina*	Greenland/Canada	protein	$\delta^{13}C$	0	Klaassen *et al.* 2001*
Semipalmated Sandpiper *C. semipalmatus*	Greenland/Canada	protein	$\delta^{13}C$	0	Klaassen *et al.* 2001*
White-rumped Sandpiper *C. fuscicollis*	Greenland/Canada	protein	$\delta^{13}C$	0	Klaassen *et al.* 2001*

Manitoba, Canada. Deuterium measurements provide the advantage that not only is there a very large isotopic difference between terrestrial and marine food webs but that within terrestrial food webs, deuterium can be a powerful indicator of latitude in North America (Hobson & Wassenaar 1997). The study showed striking patterns of isotopic change in endogenous tissues throughout the season for both isotopes but, once again, there was little evidence for any endogenous nutrient inputs to eggs for this species.

In a similar study using both $\delta^{13}C$ and $\delta^{15}N$ analyses of females and their eggs, Hobson *et al.* (2005) examined Barrow's Goldeneye *Bucephala islandica* breeding in central British Columbia, Canada. Although there was overall little evidence for a capital breeding strategy in Goldeneyes, compared to later-laid eggs, the first-laid egg showed the greatest probability of including endogenously derived nutrients of both protein and lipid. However, rather than there being an evolved strategy *per se* for directing such nutrients into eggs, the most parsimonious explanation appears to be that in a species laying an average of 9.8 eggs per clutch on that study site, the first follicle simply was the most advanced during the early arrival period on the breeding grounds and so received some wintering ground nutrients in the central portion of the follicle. Future studies could examine this further by sampling sequential yolk growth rings for the first, and subsequent eggs. Another good stable isotope study on Harlequin Duck *Histrionicus histrionicus* in British Columbia has similarly shown no evidence for the use of marine, winter derived, endogenous reserves to eggs (Bond *et al.* in press).

Klaassen *et al.* (2001) provided a creative application of stable isotope analyses to nutrient allocations among arctic waders by measuring $\delta^{13}C$ values in natal down of newly-hatched individuals together with those values of feathers from adults growing feathers on the marine wintering grounds and juveniles later growing feathers on the breeding grounds. The authors reasoned that if eggs contained marine-derived endogenous nutrients, natal down should reflect that input and would thus be more enriched in ^{13}C compared to juvenile feathers that were presumably based entirely on local foods. That study introduced a fairly tantalizing and convenient means of quickly assaying the probability of endogenous nutrients to eggs for birds moving between marine and terrestrial biomes (see also Klaassen 2003). However, to date, no ground truthing has been done to investigate the isotopic discrimination in ^{13}C between egg proteins and natal down (i.e. the formation of adult and juvenile feathers from diet on wintering and breeding grounds, respectively, is not necessarily isotopically equivalent to the formation of natal down from egg proteins). Klaassen *et al.* (2004) did find a good correlation between whole eggs of Black-headed Gulls *Larus ridibundus* and the natal down they produced but that, on average, down was 3.1‰ more positive than the egg material. This is expected since whole yolk material contains isotopically negative lipids that do not route to feathers. Issues related to not knowing isotopic discrimination factors for natal down and the specific egg constituents involved in down production would be more of a problem when trying to distinguish between isoscapes that may be more similar (e.g. between a terrestrial and an estuarine environment) compared to those that are more different (e.g. a terrestrial vs. purely marine environment). More importantly, it must be stressed that the isotopic measurement of feathers might only give information on protein and not lipid contributions to offspring, a point emphasized earlier with respect to metabolic routing. Finally, isotopic values corresponding to a given avian breeding population may differ significantly regionally and temporally and so it may be hazardous to associate nutrient isotopic endpoints with feathers formed on the wintering grounds or en route to the breeding grounds. Similarly, juvenile feathers grown late in the breeding season may not be equivalent to the isotope endpoint corresponding to a female's exogenous dietary endpoint during egg formation earlier in the season. Several studies have attempted to avoid this problem by tracking isotopic signatures of endogenous nutrients for each population of interest by

sampling adult females following arrival on the breeding grounds and through incubation (e.g. Hobson *et al.* 2000, Morrison & Hobson 2004, Hobson *et al.* 2004). Contrary to the inference of Klaassen *et al.* (2001), in their isotopic study of Red Knot *Calidris canutus islandica* and Ruddy Turnstone *Arenaria interpres interpres* breeding in the high Arctic, Morrison & Hobson (2004) found evidence for endogenous nutrient allocation to early laid eggs. Thus it appears premature to label all arctic-breeding waders as purely income breeders, at least without further study through a variety of spring weather conditions and without full acknowledgement of the differential roles of stored proteins and lipids in reproductive strategies.

The study on arctic waders by Morrison & Hobson (2004) also raised an important consideration when using stable isotopes to track endogenous nutrient contributions to eggs. Clearly, the best situation will be cases where birds lay early, soon after arrival. In these cases, endogenous tissues will be most different from local diet. As the season progresses, female endogenous reserves will become more similar to local diets as the arrival signature of the endogenous tissues becomes diluted due to routine elemental turnover in tissues (Morrison & Hobson 2004). This is not a problem if considering strictly the fate of winter- or stopover-derived nutrients that differ from local signals. However, if we are interested in any endogenously derived nutrient contribution to eggs, including those nutrients derived locally on the breeding grounds and incorporated into later-laid eggs, then such an effect of temporal changes in endogenous signals needs to be taken into account.

To date, the most quantitative attempt to ascertain the extent of endogenous and exogenous nutrient allocations to eggs has been conducted by Gauthier *et al.* (2003). That study used isotopic discrimination factors corresponding to capital vs. income models in multisource mixing models for protein and lipid contributions to eggs of Greater Snow Geese. Because the two groups of local foods available to geese, graminoids and forbs, were isotopically different, these authors used a 3 input (i.e. graminoids, forbs, endogenous tissue), two isotope mixing model that accounted for differences in the [C] and [N] values of the foods. The mathematical solution space and the unique solution for this scenario are presented in Fig. 2.

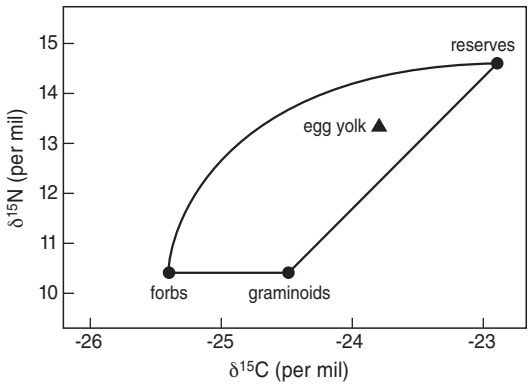

Figure 2. Results of the two isotope, three source concentration-dependent mixing model used to calculated the relative dietary and endogenous protein (muscle) input to the eggs of Greater Snow Geese in Gauthier *et al.* (2003). The various endpoints correspond to the positions egg protein would be expected to occupy if formed entirely from that source (i.e. isotopic discrimination factors have been applied). The triangle corresponds to the solution space possible with these model inputs (see Phillips & Koch 2002).

The study by Gauthier *et al.* (2003) was also interesting in that it contrasted the isotopic variance associated with the regressions of isotope values in muscle and liver with protein components of eggs. The regressions between liver isotope values and egg proteins were always stronger (higher R^2) than between muscle isotope values and egg proteins. This was interpreted as additional evidence for local foods being directed into eggs compared with endogenous (muscle) protein and suggests a potentially useful means of examining isotopically tissues of female birds that have been collected.

FUTURE RESEARCH NEEDS

There is no question that one of the most important areas of research will be to refine the isotopic

discrimination factors corresponding to capital vs. income reproductive strategies. Hobson (1995) has provided a template for this using quail and falcons but it would be useful to conduct similar captive studies on species of interest like the waterfowl. For herbivores consuming high carbohydrate diets, delineating isotopic discrimination factors for carbohydrate pathways alone (e.g. Fig. 1) will likely not be achieved but empirically determined factors corresponding to lipid-free whole diet will provide a reasonable approximation (e.g. Hobson 1995, Gauthier *et al.* 2003). The most difficult to test will be the use of the falcon carnivore model as a proxy for the formation of egg nutrients from endogenous sources simply because it is very difficult to force birds to lay eggs in captivity without any access to food (i.e. to produce eggs entirely from their tissues). A more feasible approach might be to contrast isotopically the tissues of laying females and their egg components for species known or suspected of being mostly capital breeders. Thus, we can imagine the derivation of isotopic discrimination factors corresponding to an eider or penguin model.

There is considerable interest in refining stable isotope applications to use compound-specific analyses instead of the analysis of bulk tissues or macromolecules. Thus, instead of examining whole muscle, yolk, or albumen, stable isotope analysis of individual amino acids could be employed. Similarly, instead of examining bulk lipids, the measurement of individual fatty acids would allow much more careful tracing of nutrients. Such approaches may help refine specific nutrient pathways to egg formation.

The potential for using natal down of newly-hatched birds for a measure of endogenous vs. exogenous protein allocation to reproduction suggested by Klaassen *et al.* (2001) should be developed precisely because it represents a very convenient means of assaying birds non-destructively. Here, I encourage researchers to conduct the necessary captive studies to establish isotopic discrimination factors between egg components and natal down for a variety of elements. Additional captive studies could be conducted to determine the extent of metabolic routing of carbon from dietary macromolecules to egg components as well as to feathers of newly hatched birds.

Finally, while unique solutions exist for the case of n isotopic inputs using $n–1$ stable isotopes, probabilistic models can be used in the case where the number of inputs exceeds the number of isotopes measured by more than one (Phillips & Greg 2003). Such multi-source mixing models could be applied to a two isotope model if say, three or more isotopically different local foods were available to laying females in addition to their endogenous nutrient pools. However, in all isotope mixing models, it will be important for researchers in future to conduct sensitivity analyses on how model predictions are influenced by changes in discrimination factor estimates as well as error associated with dietary and endogenous endpoint values.

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SAMENVATTING

Het maken van eieren kost veel energie en voedingsstoffen. Om te onderzoeken welke delen van het lichaam de bouwstoffen daarvoot leveren wordt van oudsher een weinig subtiele manier gevolgd: vrouwen worden tijdens de eileg opgeofferd, de bestanddelen van het lichaam worden gewogen, waarna de afname van gewicht – op basis van metingen aan vele dieren – wordt vergeleken met de massa van het legsel. Er kleven nogal wat bezwaren aan deze methode want – afgezien van ethische bedenkingen – zijn er onder meer onzekerheden over de efficiëntie waarmee lichaamsweefsel wordt omgezet in eieren. Vermoedelijk wordt de betekenis van lichaamsweefsel bij de productie van eieren overschat want bouwstenen kunnen niet alleen uit lichaamsreserves worden betrokken, maar ook uit in het broedgebied opgenomen voedsel. Tegenwoordig is een alternatieve methode in zwang om het aandeel te schatten van lichaamsreserves en additioneel in het broedgebied opgenomen voedsel om de eieren te produceren. Dit kan wanneer de isotoopsamenstelling in het voedsel in het broedgebied afwijkt van voedsel dat opgenomen is in de eraan voorafgaande trekperiode. Een dergelijke verschuiving in voedselsamenstelling komt bijvoorbeeld voor bij vogels die langs de kust of op zee overwinteren en in het binnenland of in zoetwatergebieden broeden. Op grond van eerdere studies met vogels in gevangenschap – zowel planten- als vleeseters – kunnen we schatten in welke verhoudingen stabiele isotopen van koolstof en stikstof uit het voedsel ingebouwd worden in het ei (dooier, eiwit, vet, schaal). Op het moment wordt bij vleeseters onderzocht in welke verhoudingen isotopen vanuit de lichaamsreserves ingebouwd worden in de eieren. Dit onderzoek is een belangrijke stap voorwaarts om op basis van verhoudingen van isotopen te schatten in welke mate lichaamsvoorraden en additioneel opgenomen voedsel in het broedgebied gebruikt worden voor de aanmaak van de eieren.

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