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ISOTOPIC EVIDENCE FOR SOURCES OF NUTRIENTS ALLOCATED TO CLUTCH FORMATION BY HARLEQUIN DUCKS

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Abstract. Waterfowl employ a broad array of strategies for acquiring the energy and nutrients needed for egg formation, ranging from storage of endogenous reserves prior to arrival on breeding areas to complete reliance on exogenous food sources available at breeding sites. We used stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) to quantify the relative nutrient inputs to Harlequin Duck (*Histrionicus histrionicus*) eggs and, therefore, to identify the strategy of nutrient acquisition and allocation used by females to meet the demands of egg production. Marine-derived endogenous nutrients are isotopically more enriched than freshwater dietary nutrients for Harlequin Ducks that migrate between marine wintering grounds and terrestrial breeding grounds. There was little evidence that endogenous reserves stored on marine wintering areas were allocated to clutch formation. Therefore, Harlequin Ducks relied on food available in streams on breeding grounds for egg formation, and reserves stored on marine areas were likely used during other energetically and nutritionally demanding periods.

Key words: capital breeding, endogenous reserves, income breeding, stable isotope analysis.

Evidencia Isotópica sobre Fuentes de Nutrientes Destinadas a la Formación de la Nidada en *Histrionicus histrionicus*

Resumen. Las aves anseriformes utilizan una amplia gama de estrategias para adquirir la energía y los nutrientes necesarios para la formación de los huevos, desde el almacenamiento de reservas endógenas anteriores a la llegada al área de cría hasta una dependencia completa de fuentes exógenas de alimentos disponibles en los sitios de cría. Empleamos un análisis de isótopos estables ($\delta^{13}C$ y $\delta^{15}N$) para cuantificar el aporte relativo de nutrientes a los huevos de *Histrionicus histrionicus* y, de este modo, identificar la estrategia de adquisición y distribución de nutrientes empleada por las hembras para satisfacer las demandas de la producción de huevos. Los nutrientes endógenos derivados del mar presentan mayor enriquecimiento isotópico que los nutrientes de agua dulce de la dieta de los individuos de *H. histrionicus* que migran entre las áreas marinas de invernada y las áreas terrestres de cría. Encontramos poca evidencia de que las reservas endógenas almacenadas en las áreas marinas de invernada fueran destinadas a la formación de la nidada. De este modo, *H. histrionicus* dependió de los alimentos disponibles en los arroyos de las áreas de cría para la formación de los huevos, por lo que las reservas almacenadas en las áreas marinas fueron probablemente usadas durante otros períodos con demandas energéticas y nutricionales.

Animals maximize fitness through optimal resource use, which involves employing various strategies of nutrient acquisition and allocation throughout the annual cycle (Calow and Townsend 1981, Jönsson 1997). Egg production in birds can be an energetically and nutritionally demanding stage, particularly for precocial species like waterfowl that lay large clutches of energy-dense eggs (Alisauskas and Ankney 1992). Waterfowl exhibit an array of strategies for meeting the demands of clutch formation. One strategy is to store nutrients prior to breeding that can subsequently be invested into reproduction. These nutrient stores are known as endogenous reserves, and use of these reserves for reproduction has been referred to as 'capital' breeding (Drent and Daan 1980, Jönsson 1997, Meijer and Drent 1999). Alternatively, reliance on locally available food sources, also known as exogenous resources, to acquire energy and nutrients for reproduction has been termed 'income' breeding (Drent and Daan 1980, Jönsson 1997, Meijer and Drent 1999).

Nutrient acquisition and allocation for clutch formation by waterfowl spans a continuum from capital to income strategies, and can be variable even within species. Although strict capital breeding is now considered rare (Meijer and Drent 1999), some species, such as Common Eiders (*Somateria mollisma*), have been reported to rely heavily on endogenous reserves for egg production (Parker and Holm 1990). In contrast, Northern Shovelers (*Anas clypeata*) and Greater Scaup (*Aythya marila*) in Alaska rely almost entirely on dietary intake (MacCluskie and Sedinger 2000, Gorman 2005), and many

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species apparently use intermediate proportions of endogenous reserves for clutch formation, including Lesser Scaup (*Aythya affinis*; Esler et al. 2001), Canvasbacks (*Aythya valisineria*; Barzen and Serie 1990), and Ruddy Ducks (*Oxyura jamaicensis*; Tome 1984, Alisauskas and Ankney 1994). Esler and Grand (1994) demonstrated that there can also be intraspecific variation in endogenous reserve use. Identifying the nutrient allocation strategy for different species and populations offers insight into resource use and the suitability of differing habitats for meeting the requirements of reproduction. Additionally, in a conservation context this information allows recognition of when and where nutritional constraints might be expressed, which has important population and habitat management ramifications (Anteau and Afton 2004).

Stable isotope analysis has been used to directly trace nutrient allocation for reproduction (Hobson et al. 2000, 2005, Klaassen et al. 2001, Gauthier et al. 2003). This method uses naturally occurring stable isotope signatures in the environment as a means of identifying nutrient sources for egg formation. The isotopic signatures of consumer tissues are related to their diets (DeNiro and Epstein 1978); therefore, the stable isotope technique is ideal for identifying nutrients acquired from different environments, either directly through diet or through tissues of laying females. Marine ecosystems are typically enriched in ^{13}C and ^{15}N relative to terrestrial or freshwater ecosystems (Michener and Schell 1994). Birds that migrate between wintering and breeding grounds that differ in isotopic composition, such as marine and freshwater areas, will have stored nutrients that differ isotopically from locally available nutrient sources (Hobson 2006).

Harlequin Ducks (*Histrionicus histrionicus*) winter in marine environments and breed along freshwater streams, and feed on invertebrate prey in both areas (Robertson and Goudie 1999). Adult females increased their mass by an average of 7% (40 g) on the wintering grounds immediately prior to migration and reproduction, and it is unknown if they transfer these marine nutrients, or marine nutrients acquired prior to spring hyperphagia, to egg production (Bond and Esler 2006). After accounting for costs of migration, we estimated that females in our breeding study site retained at least 30 g of endogenous reserves acquired during hyperphagia in marine areas (Bond 2005), which potentially could represent the majority of the lipid component of a clutch. Lipids are the most common nutrients stored for clutch formation (Ankney and Alisauskas 1991), and roughly 15% of the wet mass of duck eggs is estimated to be lipid, corresponding to 35–50 g of lipid in a Harlequin Duck clutch. Hence, it is plausible that females could allocate considerable amounts of marine nutrients to egg production. In this study, our objective was to identify the nutrient allocation strategy for clutch formation of Harlequin Ducks by quantifying the relative input of nutrients from wintering and breeding sites into eggs using stable isotope analysis. This information is important for understanding nutritional requirements and factors influencing reproductive performance.

METHODS

STUDY AREA AND CAPTURE TECHNIQUES

Harlequin Ducks were captured in streams on breeding grounds using mist nets from 9 to 20 May 2003 and from 21 April to 20 May 2004 in the southern Coast Mountains of British Columbia, Canada. The study area near the towns of Pemberton (50°19′N, 122°48′W) and Lillooet (50°41′N, 121°56′W) included the following streams: Bridge River, Seton River, Cayoosh Creek, Yalakom River, Ryan River, Rutherford Creek, and Brandywine Creek. Wintering ducks were captured from 27 February to 11 April 2004 in the Strait of Georgia, British Columbia, using a floating mist-net capture method adapted for inshore ocean use (Kaiser et al. 1995). Captured birds were immediately removed from the net, then weighed, banded, and assigned to an age class based on the depth of the bursa of Fabricius (Mather and Esler 1999).

SAMPLE COLLECTION

Blood samples were taken from captured wintering females for stable isotope analyses to represent the signature of marine nutrients. A 1.5 ml blood sample was taken from the jugular vein using a heparinized 5.0 ml syringe with a 21-gauge needle. For a small number of females, a 1.0 ml syringe with a 24-gauge needle was used to take 0.5 ml of blood from the tarsal vein instead. Collected blood was transferred to a heparinized vial and stored on ice until the plasma was separated from cellular blood components using a centrifuge (within 12 hr). These samples were stored frozen.

A subcutaneous lipid biopsy was also taken from each wintering female. Feathers in the area of lipid deposits on the belly were parted using isopropyl alcohol and the site was sterilized with Betadine solution. Using forceps, the skin was lifted and a small (~5 mm) incision was made with surgical scissors. Several milligrams of lipid in the region of the incision were removed with forceps. The incision site was sealed with veterinary-grade adhesive. Lipid samples were kept frozen until analysis.

To find nests for egg collection, each female captured in breeding areas was fitted with a radio-transmitter using a subcutaneous anchor and glue. The transmitters (model RI-2B, Holohil Systems Ltd., Carp, Ontario, Canada) weighed 6 g and had a motion-sensitive mortality sensor and a battery life of 3–9 months. Radio-marked females were monitored at least once a week to locate nests, and one egg was removed at random from each discovered clutch. Clutches ranged from five to seven eggs. One egg was provided for our study from the Skagit River (49°00′N, 121°05′W) in southern British Columbia. We also opportunistically collected two full clutches; in 2003, one female abandoned her nest due to rain and flooding, and in 2004 a female was depredated, leaving her clutch unattended. All collected eggs were hard-boiled and then frozen (Gloutney and Hobson 1998).

Prey samples were collected from each stream in this study to represent freshwater nutrient sources. We collected 10–20 freshwater invertebrate larvae such as stoneflies, mayflies, and caddisflies by

picking them off rocks; these are the major diet items of Harlequin Ducks in streams (Wallen 1987, Wright et al. 2000). These samples were frozen in vials and later analyzed as aggregate prey samples for each stream.

LABORATORY TECHNIQUES

Samples for carbon and nitrogen stable isotope ratio analyses were separated into lipid and nonlipid components, if necessary, because of potential differences in allocation of these nutrients to eggs. Samples analyzed for this study included abdominal lipid ($n = 18$), cellular fraction of blood ($n = 60$), freshwater invertebrates (lipid, $n = 5$; nonlipid, $n = 9$), lipid-free egg yolk ($n = 23$), egg yolk lipid ($n = 23$), and egg albumen ($n = 23$). Cellular blood samples were dried at 60°C in an oven and homogenized by grinding to a fine powder with a mortar and pestle. Freshwater diet samples were rinsed with distilled water, dried, and then homogenized. Lipids were removed from the diet samples using a 2:1 chloroform:methanol solution (Bligh and Dyer 1959) and retrieved by evaporating the solvent in a fume hood. The lipid-free, homogenized samples were treated with a few drops of 0.1N HCl solution without rinsing to remove carbonates. Abdominal fat was subsampled and analyzed directly. Eggs were easily separated into yolk and albumen because they had previously been hard-boiled. Albumen was dried and ground into a fine powder. The egg yolk was dried, and yolk lipid and lipid-free yolk were separated using the same extraction methods described above.

The carbon and nitrogen stable isotope signatures of samples were determined by loading 1 mg of each sample into tin cups and combusting them at 1200°C in a Robo-Prep elemental analyzer (Europa Scientific, Crewe, UK). Isotopic ratios were then measured in a Europa (Crewe, UK) 20:20 mass spectrometer using continuous-flow isotope ratio mass spectrometry (CFIRMS). Analytical error for each isotope measurement was estimated to be 0.1‰ and 0.3‰ for carbon and nitrogen, respectively. All isotope values per sample are expressed in delta (δ) notation, a ratio of the heavier to lighter isotope relative to a standard, in parts per thousand. This ratio is written as:

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

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The standard for carbon is PeeDee Belemnite (PDB) and for nitrogen is atmospheric nitrogen.

STATISTICAL ANALYSES

To evaluate the relative contributions of source nutrients (marine or freshwater) to eggs, we used single-isotope linear mixing models for carbon and nitrogen (Phillips and Gregg 2001). We were only interested in considering two nutrient sources for eggs, so we did not look at both isotopes simultaneously in a dual-isotope mixing model. Also, because lipid contains very little nitrogen, only carbon isotope values were used to model origins of this macronutrient (Hobson et al. 2005). Values are reported as means ± SE unless otherwise noted.

The mean lipid ($\delta^{13}C = -29.7‰ \pm 1.4‰$) and nonlipid ($\delta^{13}C = -25.4‰ \pm 0.8‰$, $\delta^{15}N = 2.2‰ \pm 0.4‰$) isotopic values from the freshwater invertebrate samples collected in our study area were used for the freshwater food source endpoint in the mixing model. For endogenous marine sources, the isotopic values of cellular blood collected in winter ($\delta^{13}C = -13.9‰ \pm 0.1‰$, $\delta^{15}N = 14.0‰ \pm 0.2‰$) were used for nonlipid samples and of abdominal adipose tissue collected in winter ($\delta^{13}C = -19.7‰ \pm 0.3‰$) were used for lipid samples. These body tissues do not appear to turn over quickly; therefore, they represent an average winter diet (Hobson and Clark 1992a, Hobson 2005). Using the mixing model, relative contributions of these marine and freshwater sources to egg albumen, lipid-free yolk, and yolk lipid were determined.

A critical assumption implicit in the application of the stable isotope technique is that stable isotope concentrations of consumer diets can be related to those of consumer tissues in a predictable fashion (Hobson and Clark 1992b). The changes in isotope values from diet to tissue, also known as isotopic discrimination (previously fractionation) factors, have been experimentally determined for various somatic tissues (Hobson and Clark 1992b, Bearhop et al. 2002) and for egg components (Hobson 1995). Although there is debate regarding the applicability of discrimination factors among species and different tissues (Dalerum and Angerbjörn 2005), use of tissue-specific factors determined for closely related species with similar diets and habitats has been recommended to minimize errors associated with incorrect discrimination factors (Vanderklift and Ponsard 2003). Because Hobson (1995) published the only report of discrimination factors between diet and egg tissues, we used his estimates for the carnivore model as being most similar to Harlequin Ducks. In this model, based on exogenous sources, carbon discriminates from diet to tissues by 0‰ for yolk lipid and nonlipid and by +0.9‰ for albumen, and nitrogen discriminates by +3.4‰ for egg yolk nonlipid and albumen. Discrimination factors for endogenous sources to eggs have not been experimentally determined, so we followed Gauthier et al. (2003) and Schmutz et al. (2006) in using discrimination factors from the same carnivore model (Hobson 1995), assuming that mobilization of nutrients from somatic tissues is similar to a carnivorous diet.

The mixing model results reflect the mean contribution to eggs over the entire clutch because only one randomly chosen egg was analyzed from each clutch. However, because two full clutches were opportunistically collected, it was also possible to calculate intraclutch variation using a coefficient of variation and compare this to the variation among clutches.

RESULTS

By monitoring 34 female Harlequin Ducks captured in the southern Coast Mountains of British Columbia, we discovered 22 nests. We collected single eggs

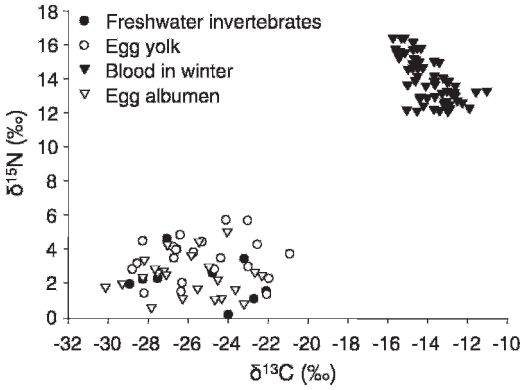

FIGURE 1. Lipid-free carbon and nitrogen stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) were more enriched in Harlequin Duck blood sampled in winter than in freshwater invertebrate prey from breeding areas. Isotopic values for egg components (egg yolk and egg albumen) were similar to freshwater prey values, indicating allocation of freshwater nutrients to egg production. Note that isotopic discrimination (fractionation) factors were applied to egg components.

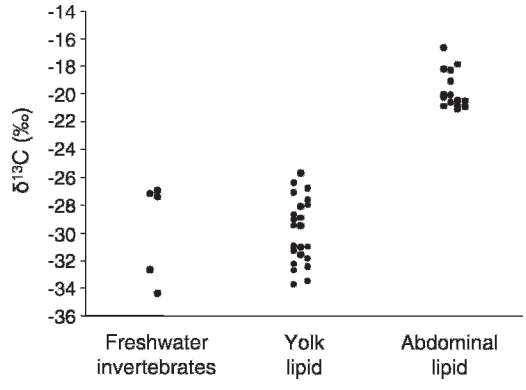

FIGURE 2. Carbon stable isotope values ($\delta^{13}C$) for lipid were more enriched in Harlequin Duck abdominal lipid sampled in winter than in freshwater stream invertebrates. Yolk lipid stable isotope values were similar to freshwater prey values, indicating allocation of freshwater nutrients to egg production.

from 20 of these nests, complete clutches from the remaining two, and a single egg was received from a nest on the Skagit River, British Columbia.

Female Harlequin Ducks allocated almost entirely freshwater nutrients to egg formation. The carbon mixing model estimated that 100% (±8%) of the nutrients for egg albumen and lipid-free yolk and 100% (±15%) of nutrients for yolk lipid were derived from freshwater sources. The nitrogen mixing model produced similar results, with freshwater sources making up 98% (±4%) of albumen and 89% (±4%) of lipid-free yolk. We determined that reasonable changes in discrimination factors did not significantly alter our results and, if anything, made our results more indicative of freshwater inputs to eggs.

Plots of isotopic signatures from the marine and freshwater biomes show that carbon and nitrogen isotope values for nonlipid components of eggs are clearly clustered with the values for freshwater invertebrates, and are distinct from the cluster of values for blood collected at wintering sites (Fig. 1). Similarly, carbon values for yolk lipid matched those of lipid in freshwater invertebrates and were dissimilar from carbon isotope values of abdominal lipid collected from Harlequin Ducks in wintering areas (Fig. 2).

When interclutch and intraclutch stable isotope signatures were contrasted, variation within clutches was considerably smaller than variation among clutches (Fig. 3). The interclutch coefficient of variation (CV) for egg yolk nonlipid was 18% and 9% for nitrogen and carbon, respectively. In contrast, intraclutch CVs for nitrogen and carbon were 4% and 3% for one complete clutch and 2% and 1% for the second complete clutch. Similarly, the interclutch CV for carbon in egg yolk lipid was 8%, compared to an intraclutch CV of 1% for each complete clutch. Even with the higher variation among clutches, our results are strongly indicative of freshwater rather than marine contributions to egg formation.

DISCUSSION

Our stable isotope data indicate that freshwater breeding site nutrient allocation is the predominant strategy of Harlequin Ducks for clutch formation in the southern Coast Mountains of British Columbia. However, our results are based on a randomly chosen egg from each clutch; thus, they represent the average strategy of nutrient allocation to egg production.

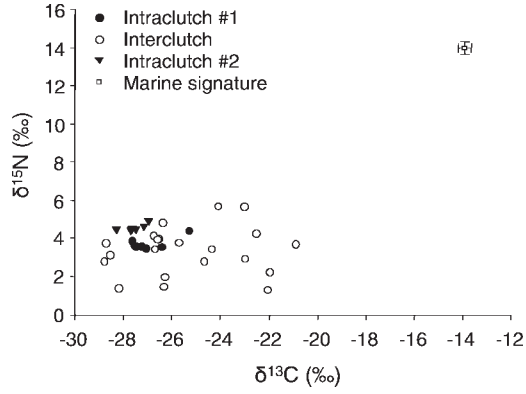

FIGURE 3. Interclutch variation in carbon and nitrogen stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) for lipid-free egg yolk was greater than intraclutch variation. Intraclutch #1 and #2 represent two full clutches that were opportunistically collected. The mean marine stable isotope values for carbon and nitrogen from female blood samples taken during winter (with 95% confidence intervals) are also shown.

There is some evidence to suggest that there can be significant intraclutch variation in sources of egg nutrients. Both Barrow's Goldeneye (*Bucephala islandica*; Hobson et al. 2005) and Redhead (*Aythya americana*; Hobson et al. 2004) females allocated more endogenous stores to eggs laid early in the sequence than to later-laid eggs. Our data do not support hypotheses of opportunistic marine endogenous transfer to eggs by Harlequin Ducks because variation within clutches was small and entirely within the freshwater range of isotope values. This suggests that values from a single egg per clutch for Harlequin Ducks are representative of each individual's strategy and, when pooled, allow appropriate inference about the average strategy of the population.

Some differences in isotopic values were evident among clutches in our data. We suggest that this is not related to variation in strategies of nutrient acquisition and allocation among females, but is due to differences in isotopic signatures across streams and freshwater diets. Other studies have found a lack of consistency in freshwater isotopic endpoints (Hobson et al. 2004, Hobson et al. 2005), which is likely caused by different processes affecting each of the freshwater sources. In our study system, isotopic signatures among streams differed slightly and the exact prey composition of females' diets likely also differed. These dissimilarities would lead to variation among eggs laid by different individuals. However, despite variation among clutches, the isotopic signatures of egg components fell almost entirely within the isotopic range of freshwater stream invertebrates.

If female Harlequin Ducks use freshwater nutrients to form eggs, why do they store endogenous reserves on wintering grounds prior to migration? As shown by Bond (2005), flight costs for Harlequin Ducks from the Strait of Georgia to the breeding study site near Lillooet are slight and would result in only a 2%–3% reduction in body mass. It has been suggested that females may store nutrients as a buffer against unfavorable conditions on the breeding grounds when they arrive. Alternatively, they could strategically store limiting nutrients required for the physiological changes associated with reproduction (Rohwer 1992, Morrison and Hobson 2004). Another possibility is that stored nutrients are used for the female's own maintenance during clutch formation or during subsequent stages of reproduction, such as incubation. Nesting success has been related to body condition because the depletion of endogenous reserves may lead to nest failure through nest abandonment or decreased nest attentiveness (Gloutney and Clark 1991, Arnold et al. 1995). Therefore, the condition of females prior to incubation can be influential to breeding success. In Harlequin Ducks, females are the sole incubators and are thought to leave the nest only once a day to feed and preen (Robertson and Goudie 1999). Hunt (1998) found that female Harlequin Duck body mass decreased by 11% from laying to brood rearing, which indicates significant energy demands during incubation. Thus, it may be beneficial for females to store endogenous marine nutrients to meet these energy requirements.

Harlequin Ducks may be simultaneously balancing the demands of clutch formation and the benefits of nutrient stores for incubation. Physiologically, it may be more efficient for females to convert exogenous, dietary nutrients directly into eggs rather than mobilizing endogenous reserves. Schmutz et al. (2006) determined that arctic-nesting geese used a mixed strategy of endogenous and exogenous resources for egg production, but also found isotopic evidence of significant endogenous marine reserve use during incubation. This suggests that waterfowl may 'save' some endogenous resources for incubation and supplement egg production with exogenous sources.

Because the nutrient allocation strategy for clutch formation by Harlequin Ducks in the southern Coast Mountains of British Columbia involves mostly freshwater sources, they are highly dependent on stream productivity and access to invertebrate food items for egg production. This should be taken into consideration when altering or managing stream habitats. Reduced food levels in streams during the clutch formation stage may impede the ability of females to produce eggs, or decrease reserves intended for use later in reproduction. Thus, broad-scale changes in stream conditions could lead to consequences for productivity and subsequently population demographics. Because of current concerns regarding low productivity of Harlequin Ducks in southern British Columbia (Smith et al. 2001, Rodway et al. 2003), future research could investigate how food limitation in streams in breeding areas may influence egg production. Previous work has linked diving duck productivity and food availability (Gardarsson and Einarsson 2004, but see Goudie and Jones 2005), but more research is required to address the issue of food limitation and resultant effects on productivity in Harlequin Ducks.

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