Capital versus income breeding in a migratory passerine bird: evidence from stable-carbon isotopes

K.M. Langin, D.R. Norris, T.K. Kyser, P.P. Marra, and L.M. Ratcliffe

Abstract: Birds meet the energetic demands of egg formation by using either endogenous reserves (capital breeding) or recently ingested nutrients (income breeding). Examining these strategies in migratory birds has been difficult because of the inability to assign the origin of egg nutrients. We used stable-carbon isotopes ($\delta^{13}C$ values) to determine whether American Redstarts (*Setophaga ruticilla* (L., 1758)) form eggs using endogenous reserves acquired on tropical wintering areas or local dietary sources. Redstart diet tends to be enriched in ^{13}C on tropical wintering areas; therefore, we predicted that if endogenous reserves are used to form eggs, then ^{13}C would be enriched in first clutches relative to replacement clutches. We analyzed yolk ($\delta^{13}C_{YK}$) samples from successive first, second, and third clutches and blood plasma ($\delta^{13}C_{PL}$) sampled from females over the same time period. Values of $\delta^{13}C_{YK}$ in first-clutch and second-clutch eggs were significantly more positive than those in third-clutch eggs. Although the isotopic shift in yolk was in the direction predicted for a mixed capital–income strategy, $\delta^{13}C_{PL}$, which represents the locally derived diet, varied seasonally in accordance with the shift in $\delta^{13}C_{YK}$. Our findings indicate female Redstarts are primarily income breeders, forming eggs from an isotopically variable diet during the breeding season.

Résumé : Pour satisfaire les besoins énergétiques requis pour la formation des œufs, les oiseaux utilisent ou bien leurs réserves endogènes (reproduction à partir du capital) ou les nutriments récemment ingérés (reproduction à partir du revenu). L'étude de ces stratégies chez les oiseaux migrateurs est difficile lorsqu'il n'est pas possible de déterminer l'origine des nutriments des œufs. Nous utilisons les isotopes stables de carbone (valeurs de $\delta^{13}C$) pour déterminer si les parulines flamboyantes (*Setophaga ruticilla* (L., 1758)) forment leurs œufs à partir de réserves endogènes acquises dans les aires d'hivernage tropicales ou à partir de sources alimentaires locales. Les régimes alimentaires des parulines flamboyantes dans les aires d'hivernage tropicales ont tendance à être enrichis en ^{13}C; notre prédiction est donc que, si les réserves endogènes servent à la formation des œufs, la teneur en ^{13}C sera plus élevée dans les premières portées que dans les portées de remplacement. Nous avons analysé des échantillons de jaunes ($\delta^{13}C_{YK}$) des premières, secondes et troisièmes portées et des échantillons de plasma sanguin ($\delta^{13}C_{PL}$) de femelles prélevés au cours de la même période. Les valeurs de $\delta^{13}C_{YK}$ dans les œufs des premières et secondes portées sont significativement plus élevées que celles des troisièmes portées. Bien que le changement isotopique dans le jaune se fasse dans le sens prévu pour une stratégie mixte de capital–revenu, $\delta^{13}C_{PL}$, qui représente le régime d'origine locale, varie au cours de la saison en parallèle avec le changement de $\delta^{13}C_{YK}$. Ces résultats indiquent que les parulines flamboyantes sont surtout des reproducteurs qui utilisent leur revenu et qui forment leurs œufs à partir d'un régime alimentaire variable du point de vue isotopique durant la saison de reproduction.

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Introduction

The tactics birds use to acquire and allocate resources for reproduction are an important component of their life-history strategy and can influence the amount of resources available for growth, maintenance, and survival (Stearns 1989). The concepts of capital breeding and income breeding have been used to describe alternative strategies by which birds allocate resources for reproduction (Drent and Daan 1980). Specifically, capital breeders use endogenous reserves (body stores), whereas income breeders rely on exogenous sources (recently ingested nutrients; Jönsson 1997). Since many birds likely use a combination of endogenous and exogenous sources to fuel egg formation, nutrient acquisition tactics may be more accurately described as falling along a continuum between the two strategies (Meijer and Drent 1999).

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Pinpointing where along that continuum a species falls has been challenging because of the difficulty in assigning the origin of egg nutrients. The degree to which endogenous reserves are mobilized during egg formation has traditionally been studied by examining changes in female body mass and composition (Krapu 1981; Choinière and Gauthier 1995; Houston et al. 1995). Such methods have been criticized because of their indirect nature and inability to distinguish the use of stored reserves for forming eggs from their use for general maintenance (Meijer and Drent 1999). Recently, stable-isotope analysis has been applied to this problem because, unlike traditional methods, researchers can directly trace the pathway from resource acquisition to the allocation of those resources for egg formation (Hobson et al. 1997, 2000, 2004; Klaassen et al. 2001; Gauthier et al. 2003).

Stable isotopes can be used as an ecological tracer because their abundance varies naturally in relation to a variety of ecological and environmental factors (Lajtha and Michener 1994). For example, stable-carbon isotope ($\delta^{13}C$) values vary among plants with different photosynthetic pathways (C_3 vs. C_4) and water-use efficiencies; $\delta^{13}C$ values in plants are, in turn, transferred up the food chain through phytophagous insects to insectivorous animals (Lajtha and Michener 1994). Such patterns are fortuitous for tracking nutrient allocation since, in general, ^{13}C in animal tissues becomes more depleted with increasing latitude, largely because the ratio of C_3 to C_4 plants increases with latitude (Kelly 2000; Still et al. 2003).

For migratory birds whose breeding and overwintering diets are isotopically distinct, stable-isotope signatures in eggs laid upon arrival at the breeding grounds can be used to infer the origin of egg-forming nutrients. For example, Klaassen et al. (2001) found that $\delta^{13}C$ values in egg and natal down of 10 species of Arctic-breeding shorebirds were entirely reflective of the tundra breeding grounds, which was in direct contrast to the hypothesis that these species were capital breeders (Drent and Daan 1980). In a similar study, a comparison of the $\delta^{13}C$ values in eggs, body tissues, and food sources of Greater Snow Geese (*Chen caerulescens atlantica* Kennard, 1927) breeding in the high Arctic revealed that endogenous reserves contribute only 33% of yolk proteins and 20% of yolk lipids — lower contributions than what was previously assumed (Gauthier et al. 2003).

Thus far, stable isotopes have only been used to examine the origin of nutrients in eggs of non-passerine bird species. In this paper, we used stable-carbon isotopes and experimental clutch removals to infer the origin of egg-forming nutrients in a small (8 g), long-distance migratory songbird, the American Redstart (*Setophaga ruticilla* (L., 1758)). The prevailing view, based on traditional methods, has been that small passerines are exclusive income breeders (Meijer and Drent 1999), although Zebra Finches (*Taeniopygia guttata* (Vieillot, 1817)) are thought to mobilize substantial endogenous stores during egg formation (Houston et al. 1995). Redstarts breed in deciduous forests in North America and winter in the Caribbean and Central America (Sherry and Holmes 1997). They offer an excellent opportunity to examine the origin of egg nutrients in a migratory songbird because their tropical overwintering diet is enriched in ^{13}C (by approximately 2‰) relative to their temperate breeding diet (Marra et al. 1998; Norris et al. 2005). We predict that if egg formation in Redstarts is partially supported by endogenous reserves acquired in the tropics, as would be the case for a mixed capital–income breeding strategy, $\delta^{13}C$ values in egg yolk ($\delta^{13}C_{YK}$) will be more positive in the first clutch relative to replacement (second and third) clutches laid by the same female. Alternatively, if Redstarts form eggs primarily from locally derived nutrients, we predict that $\delta^{13}C_{YK}$ values will not vary across clutches, assuming no variation in yolk composition (lipid to protein ratio) and dietary $\delta^{13}C$.

Materials and methods

Sample collection

Field research was conducted on five forest plots (60 ha total) at the Queen's University Biological Station (44°34′N, 76°19′W) near Chaffey's Lock, Ontario, Canada, during May and June of 2003 and 2004. The study site is a mixed deciduous forest that is composed largely of sugar maple (*Acer saccharum* Marsh.), hophornbeam (*Ostrya virginiana* (P. Mill.) K. Koch), American beech (*Fagus grandifolia* Ehrh.), bitternut hickory (*Carya cordiformis* (Wangenh.) K. Koch), white ash (*Fraxinus americana* L.), American elm (*Ulmus americana* L.), and northern red oak (*Quercus rubra* L.). Each plot was intensively surveyed on a daily basis to look for newly arrived male and female American Redstarts. Twenty-one Redstart pairs (2003: $n = 15$; 2004: $n = 6$) were sampled to represent the widest possible range of arrival dates. Most males ($n = 19$) and some females ($n = 10$) were caught in mist nets, weighed (to the nearest 0.1 g) using an electronic balance, given a unique color-band combination, and fitted with a single Canadian Wildlife Service (CWS) leg band. At least one member of each pair was banded. Pairs were monitored daily to locate nests and to determine laying date; all eggs were collected once the clutch was complete (CWS permit CA0087; protocol approved by the Queen's University Animal Care Committee). For a subset of experimental pairs, eggs from second (2003: $n = 10$; 2004: $n = 5$) and third (2003: $n = 7$; 2004: $n = 4$) clutches were also collected. The mean number of days separating the onset of first-clutch and second-clutch laying and second-clutch and third-clutch laying was 9.6 ± 2.4 (mean ± SD; $n = 15$) and 8.8 ± 0.8 ($n = 11$), respectively. In 2003, eggs were individually weighed (to the nearest 0.0001 g) on the day of collection using an electronic balance. Eggs were stored frozen at –20 °C within 3 h.

Previous research has shown blood plasma carbon ($\delta^{13}C_{PL}$) has a relatively fast turnover rate (half-life = 24.8 ± 12.3 h (mean ± SD) in Yellow-rumped Warblers (*Dendroica coronata* (L., 1766)); Podlesak et al. 2005) and thus reflects recent diet. To test the assumption that dietary $\delta^{13}C$ remains constant during the formation of first and replacement clutches, 10–50 μL of blood was collected in heparinized micro-hematocrit capillary tubes from the brachial vein of laying and non-laying female Redstarts during May and June of 2004. Blood samples were stored in a cooler at approximately 5 °C until they were centrifuged (within 4 h) for 8 min at 14 000 r/min (12 700*g*). The plasma component of the blood was immediately separated from the

packed cell fraction using a Hamilton syringe and stored frozen at –20 °C until isotopic analysis. All female Redstarts had been on the breeding grounds for longer than 1 day prior to blood sampling. Few females (4 of 21) were those from whom clutches were experimentally removed.

Stable-isotope analysis

Eggs were separated while frozen to obtain samples of pure egg yolk. Yolk and blood plasma samples were freeze-dried, powdered, and weighed to 0.25–0.50 mg in tin capsules. All isotopic analyses were performed at the Queen's Facility for Isotope Research in Kingston, Ontario. The samples were combusted in a NCS 2500 elemental analyzer and introduced online into a Delta Plus XP isotope ratio mass spectrometer. Stable-carbon isotope ratios are expressed according to the formula:

$$\delta^{13}C = \left[\left(\frac{^{13}C}{^{12}C} \right)_{sample} / \left(\frac{^{13}C}{^{12}C} \right)_{standard} - 1 \right] \times 1000$$

where $\delta^{13}C$ (in ‰) is the isotope ratio of the sample relative to the international standard Vienna Pee Dee Belemnite. In addition to $\delta^{13}C$, the carbon to nitrogen ratio (C/N) was analyzed for each yolk sample. One standard was run for every five unknowns. $\delta^{13}C$ values were repeatable to within 0.2‰ for $\delta^{13}C_{YK}$ (n = 10) and $\delta^{13}C_{PL}$ (n = 6), while yolk C/N values were repeatable to within 0.9 (n = 10).

Statistical analysis

Arrival fat (in grams) was estimated by subtracting the average fat-free body mass of after-hatch year American Redstarts (from Odum 1993) from the average body mass of individuals captured within 4 days of arrival in 2003 and 2004; males and females were analyzed separately. All birds captured within 4 days of arrival were included in this analysis, not just individuals whose eggs were sampled. Total yolk and yolk lipid content (in grams) were estimated for first-clutch eggs based on egg mass data from Redstarts in this population and egg composition data for typical altricial bird species (Sotherland and Rahn 1987).

Values of $\delta^{13}C_{YK}$ in two eggs from the same clutch had the same repeatability as two measurements within the same sample (0.2‰; n = 10); therefore, the isotopic composition of one randomly selected egg was taken to be representative of the entire clutch. In instances when more than one egg was analyzed per clutch, the average values of $\delta^{13}C_{YK}$ and C/N were used. Mixed-model repeated-measures ANOVA was used to test for variation in isotopic and elemental ratios across clutches laid by the same female. This procedure permits the inclusion of missing data (i.e., females from whom less than three clutches were collected; n = 10). Clutch order and year were included as fixed effects in the model; there was no interaction between these effects for $\delta^{13}C_{YK}$ or C/N ($P > 0.67$ for both). A mixed linear model was used to test for seasonal variation in yolk isotopic values, with laying date as a fixed effect and female as a random effect, thereby controlling for the lack of independence among clutches laid by the same female. Parametric correlations were used when appropriate. Our data met the assumptions of all statistical analyses. Data were analyzed using JMP® IN (SAS Institute Inc. 2003).

Results

Arrival fat and yolk lipid content

Lipids constitute 58% of avian egg yolk (Sotherland and Rahn 1987); therefore, the carbon isotopic composition in Redstart yolk samples is largely reflective of the origin of lipids for egg formation. The body mass of male and female Redstarts captured within 4 days of arrival was 8.1 ± 0.4 g (mean ± SD; n = 74) and 7.9 ± 0.5 g (n = 25), respectively. From this, we estimate that both male and female Redstarts arrive to breed with 1.5 g of fat, on average (using data from Odum 1993; see Methods). Most females (15 of 21) whose eggs were collected proceeded to lay a first clutch consisting of 4 eggs, with those clutches having a total mass of 5.37 ± 0.34 g (n = 10). By multiplying this value by 0.2 (proportion of yolk in eggs) and 0.58 (proportion of lipids in yolk), we estimate that female Redstarts in this population produce 1.07 g of yolk and 0.62 g of yolk lipids when forming first clutches.

$\delta^{13}C$ in yolk

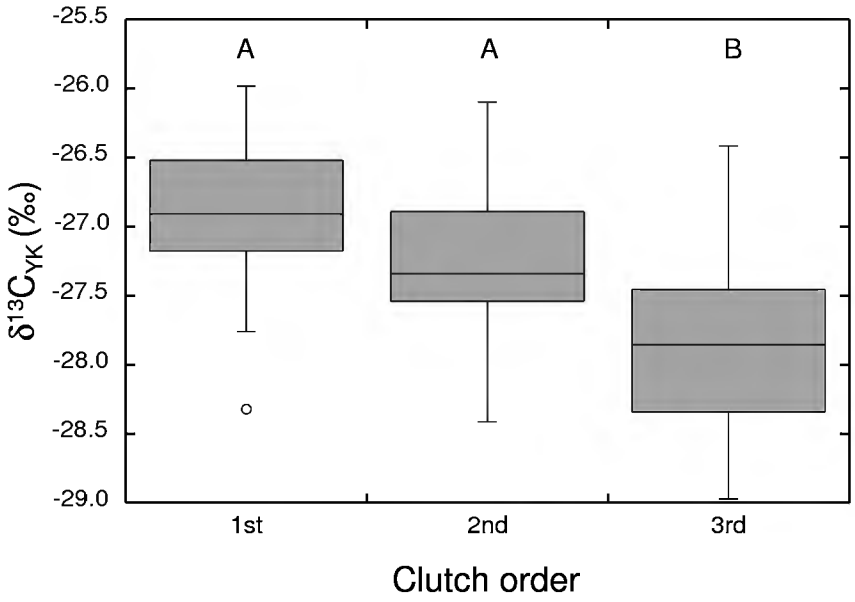
With all clutches combined, $\delta^{13}C_{YK}$ values became more negative in relation to laying date (mixed linear model; r^2 = 0.55, $P < 0.001$, n = 47). Values of $\delta^{13}C_{YK}$ in first-clutch (n = 21) and second-clutch (n = 15) eggs were significantly more positive than those in third-clutch (n = 11) eggs (repeated-measures ANOVA; $F_{[2,23]} = 12.7$, $P < 0.001$; Tukey's multiple comparison test) (Fig. 1). There was a significant ^{13}C-enrichment in the yolks in 2003 than in 2004 ($F_{[1,23]} = 4.3$, $P = 0.05$). Restricting this analysis to 2004 (the year for which blood-plasma samples were collected), the result remains unchanged: first-clutch (n = 6) and second-clutch (n = 5) eggs were more enriched in ^{13}C than third-clutch (n = 4) eggs (repeated-measures ANOVA; $F_{[2,7]} = 11.5$, $P = 0.006$; Tukey's multiple comparison test).

Protein and lipid components of the egg yolk were analyzed together. Since lipids are depleted in ^{13}C relative to proteins (Hobson 1995), variation in the lipid to protein ratio across first, second, and third clutches should produce corresponding variation in $\delta^{13}C_{YK}$. The C/N ratio, which serves as an approximation of the lipid to protein ratio (Matthews and Mazumder 2005), did not vary significantly among clutches laid by the same female (repeated-measures ANOVA; $F_{[2,23]} = 1.2$, $P = 0.31$) and did not vary with year ($F_{[1,23]} = 1.3$, $P = 0.27$). However, there was a slight negative trend in the C/N ratio (i.e., decreasing lipid content) from first to third clutches, which is opposite to what would be predicted if the $\delta^{13}C_{YK}$ values reflected changes in yolk composition. The C/N ratio was also not related to $\delta^{13}C_{YK}$ (ordinary least squares (OLS); all clutches combined: r^2 = 0.03, P = 0.23, n = 47; first clutches: r^2 = 0.05, P = 0.35, n = 21; second clutches: r^2 = 0.15, P = 0.16, n = 15; third clutches: r^2 = 0.19, P = 0.19, n = 11), confirming that the variation in $\delta^{13}C_{YK}$ values across sequential clutches was not due to corresponding variation in yolk composition.

$\delta^{13}C$ in blood plasma of females

Values of $\delta^{13}C_{PL}$ in females who were laying eggs (n = 7) did not differ from those undertaking pre-laying (n = 8; e.g., nest building) or post-laying (n = 7; e.g., feeding nestlings)

Fig. 1. Comparison of $\delta^{13}C$ in egg yolk ($\delta^{13}C_{YK}$) across first (n = 21), second (n = 15), and third (n = 11) clutches laid by American Redstarts (*Setophaga ruticilla*) in southeastern Ontario during May and June of 2003 and 2004. Clutches with different letters were significantly different from one another. The shaded area within each box represents the middle 50% of the data, the central line is the median value, and the vertical whiskers extend to minimum and maximum values, with the exception of one outlier (○) in the first-clutch group.



activities (ANOVA; $F_{[2,21]}$ = 2.7, P = 0.10). All females sampled for blood plasma, regardless of reproductive stage, were therefore included when testing for seasonal variation in dietary isotopic signatures. In May and June of 2004, $\delta^{13}C_{PL}$ values in female Redstarts became significantly more negative in relation to capture date (OLS; r^2 = 0.44, P < 0.001, n = 22), declining at a rate of 0.04‰ per day (Fig. 2). This relationship remained the same after excluding females that were laying eggs (OLS; r^2 = 0.66, P < 0.001, n = 15), feeding young (OLS; r^2 = 0.37, P = 0.001, n = 17), and after excluding the relatively ^{13}C-depleted female sampled on 28 June (OLS; r^2 = 0.36, P = 0.004, n = 21). The seasonal shift in $\delta^{13}C_{PL}$ (magnitude = 2.9‰) was in the same direction and of a similar magnitude as the shift in $\delta^{13}C_{YK}$ from first to third clutches (3.0‰) over the same time period.

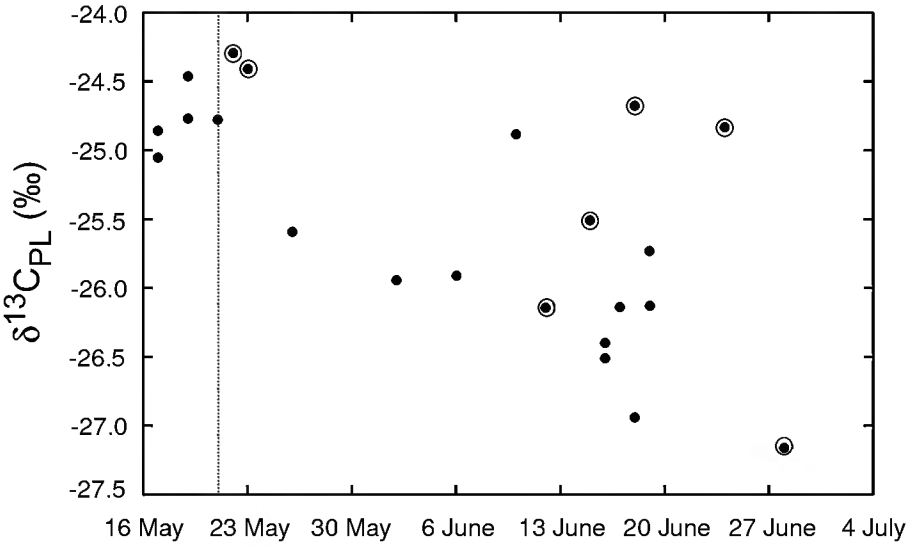
Discussion

Female Redstarts arrived with more fat than was required to produce first-clutch yolk lipids; however, our results suggest that yolk material is primarily derived using exogenous nutrients. Although first-clutch and second-clutch $\delta^{13}C_{YK}$ values were significantly higher than those in third-clutch eggs (Fig. 1), we found a parallel shift in $\delta^{13}C_{PL}$ values (Fig. 2), which reflect recent diet (Podlesak et al. 2005). We interpret the seasonal variation in $\delta^{13}C_{YK}$ values, therefore, to result from diet-related changes in $\delta^{13}C$ rather than the use of stored reserves. Seasonal variation in dietary isotopic signatures may be due to changes in the $\delta^{13}C$ values of insect prey or to changes in the types of prey females consume, as discussed further below. Regardless, our results indicate that female Redstarts use an income breeding strategy, forming eggs from an isotopically variable diet on the breeding grounds. Such a conclusion supports the prevailing view that small passerines are primarily income breeders (Meijer and Drent 1999).

Our experimental approach of removing eggs and sampling subsequent clutches within the same female represents a powerful approach for testing nutrient sources in migratory birds. Previous isotopic studies investigating capital breeding versus income breeding have only analyzed eggs from one clutch, comparing them to female tissues and local food sources (Gauthier et al. 2003; Hobson et al. 2004), feathers grown on breeding and wintering areas (Klaassen et al. 2001), or to eggs of year-round resident species (Hobson et al. 1997, 2000). Sampling successive clutches within the same female allowed us to examine changes in the source of nutrients over time while controlling for potential variation between individuals. It is possible that the experimental removal of clutches may have influenced $\delta^{13}C_{YK}$ values through increased physiological stress, but the parallel shift in $\delta^{13}C_{PL}$ values in non-experimental females (i.e., did not have their eggs removed) is consistent with dietary changes in $\delta^{13}C$ that influenced the whole population.

The successful use of stable isotopes to track nutrient allocation depends partly on isotopic differences between different geographic areas. Greater Snow Geese and Arctic-breeding shorebirds, for example, arrive on tundra breeding areas with estuarine-derived body stores that are enriched in ^{13}C by approximately 5‰–10‰ relative to local dietary sources (Klaassen et al. 2001; Gauthier et al. 2003). For American Redstarts, ^{13}C is enriched in the overwintering diet relative to the breeding-season diet by approximately 2‰ (Marra et al. 1998; Norris et al. 2005). It is possible that body stores of Redstarts arriving at breeding areas would be enriched by less than 2‰, considering birds may utilize some stores acquired on tropical wintering areas during migration and subsequently replenish them at stopover sites. Nevertheless, the carbon isotopic shift we measured

Fig. 2. $\delta^{13}C$ in blood plasma ($\delta^{13}C_{PL}$) sampled from female American Redstarts in various stages of breeding in relation to capture date in 2004. Circles represent females whose blood was sampled during the laying stage of breeding. Plasma samples collected before the first egg was laid (represented by the vertical dotted line) are still representative of the diet during egg formation because females begin follicle growth several days before egg laying (Houston et al. 1995). Plasma $\delta^{13}C$ was negatively related to capture date (ordinary least squares (OLS); $r^2 = 0.44$, $P < 0.001$, $n = 22$), declining at a rate of 0.04‰ per day.



from first to third clutches closely mirrored the shift in blood plasma over the same period, a tissue that reflects recent diet (Podlesak et al. 2005). Had first-clutch yolk material been derived from a substantial contribution of endogenous stores, we would have expected the range in $\delta^{13}C_{YK}$ values (3.0‰) to exceed the range in $\delta^{13}C_{PL}$ values (2.9‰).

Laboratory studies on birds have demonstrated that experimental changes in the carbon isotopic composition of the diet are closely followed by corresponding changes in blood-plasma $\delta^{13}C$ values (Hobson and Clark 1993; Podlesak et al. 2004). Contrary to these studies, birds in natural populations may not have ad libitum access to food and thus some of the blood-plasma carbon may originate from endogenous stores, particularly for individuals undergoing a period of high energetic demand (e.g., egg laying). Even so, the seasonal variation in female $\delta^{13}C_{PL}$ values does not appear to be reflective of changes in the amount of stored lipids circulating in the bloodstream of egg-laying females, as the pattern was also observed in females who were not laying eggs (Fig. 2). In addition, if the circulation of endogenously derived carbon were responsible for the seasonal isotopic shift, we would have expected $\delta^{13}C_{PL}$ values to vary by much less than 2‰, given that endogenous inputs are likely mixed with a substantial contribution from recently ingested nutrients. The seasonal shift in $\delta^{13}C_{PL}$ values, therefore, is most likely reflective of changes in the isotopic composition of the diet, not changes in the utilization of endogenous stores acquired in the tropics.

Storing endogenous reserves in excess of those required to fuel the migratory journey may entail substantial energetic and ecological costs (Witter and Cuthill 1993; Jönsson 1997; Kullberg et al. 2005). Redstarts in this study population migrate upwards of 3000 km from wintering sites in the Caribbean before arriving to breed in Ontario (Norris et al. 2006). Despite this lengthy migratory journey, Redstarts arrive with enough fat to meet the lipid requirements of egg formation. Our estimates of arrival fat are comparable with those found in a Redstart population in Michigan, where females with fat on arrival experience reproductive gains in the form of increased clutch size, egg volume, and nestling mass (Smith and Moore 2003). Our data suggest that endogenous fat reserves, at least in this Ontario population, are not the primary nutrient source for egg formation. Instead, we concur with Smith and Moore (2003) who suggested that fat stores on arrival may provide indirect benefits to reproduction, such as contributing to general maintenance while females forage for nutrient-limiting resources like calcium during egg formation (Bures and Weidinger 2003). Individuals arriving early on the breeding grounds also face potential costs in the form of lower food supplies and bouts of cold weather increasing thermoregulatory demands. Fat stores may help buffer individuals from these hazards while allowing the benefits of early arrival (i.e., higher quality territories and mates).

The local food supply may be sufficient to meet the energetic demands of egg formation when Redstarts begin laying, at least in terms of the protein and lipid requirements. Female Redstarts in the study population lay first-clutch eggs in late May and early June, the time period over which caterpillars — the most energy-rich prey item available to Redstarts (Lovette and Holmes 1995) — were found to be most abundant in 2004 (K.M. Langin, unpublished data). The additional energetic cost of egg formation, over and above daily maintenance costs, may also be minimal in small species like Redstarts that have high basal metabolic rates. The short egg-laying interval that is typical for small passerines (i.e., 24 h) is consistent with the view that these species can acquire enough resources for egg formation within a short period of time (Meijer and Drent 1999). Female Redstarts therefore appear to be capable of forming first-clutch eggs using local dietary sources, without mobilizing a substantial amount of endogenous reserves.

The seasonal shifts in $\delta^{13}C_{YK}$ and $\delta^{13}C_{PL}$ values suggest that there is variation in the isotopic composition of the female Redstart diet during May and June. Thus far, few isotopic studies using carbon and nitrogen (n = 20; Dalerum and Angerbjörn 2005) have identified temporal variation in the diet of birds, none of which were conducted on a passerine during a stationary period (i.e., breeding or wintering). The seasonal variation in $\delta^{13}C$ values in Redstarts could be related to a seasonal isotopic shift in one or more prey items, or to foraging shifts between prey items that are isotopically distinct. Resolving the nature of the diet shift in Redstarts has proven challenging, however, because of considerable $\delta^{13}C$ variation within many insect orders that are potential prey (e.g., 10‰ variation in Diptera, n = 10; K.M. Langin, unpublished data), coupled with our inability to identify insects below order using traditional methods of analyzing diet (e.g., stomach contents, faeces, foraging observations). Further research is therefore needed to determine the underlying cause of the seasonal variation in dietary $\delta^{13}C$.

Regardless of our ability to pinpoint the mechanism driving seasonal isotopic variation, our assumption of constant dietary $\delta^{13}C$ was tested easily and effectively using a metabolically active tissue that integrates isotopic signatures from the whole diet. By testing this assumption, we were led to the opposite interpretation of our yolk isotopic data than what would have otherwise been the case. Stable-isotope techniques are now being applied to a variety of ecological questions and, as is the nature of any burgeoning field, many assumptions go untested. For example, Morrison and Hobson (2004) reported a seasonal decline in carbon isotopic signatures in the eggs of shorebirds breeding in the high Arctic, concluding that ^{13}C-enriched body stores derived from marine wintering areas may be used to form early clutches; however, seasonal variation in dietary $\delta^{13}C$ was not tested as a possible alternate explanation. Our findings emphasize the need for thorough testing of assumptions before reaching conclusions based on isotopic data.

This study is the first to apply stable-isotope techniques to distinguish endogenous from exogenous inputs to egg formation in a migratory passerine bird. Although small inputs of endogenous stores may have gone undetected, our results suggest that the majority of nutrients used to fuel egg formation were derived from an isotopically variable local dietary source. Such a conclusion is consistent with the prevailing view that small passerines are primarily income breeders. Future studies on small passerines may be better conducted in a laboratory setting, where dietary isotopic signatures can be held constant and large isotopic differences between endogenous and exogenous sources can be artificially created. Zebra Finches would be an ideal study species, since they are easily bred in captivity and are suspected of mobilizing a substantial amount of endogenous stores during egg formation (Houston et al. 1995). Laboratory studies, however, will not be able to elucidate breeding strategies used by migratory birds that frequently travel thousands of kilometres between periods of the annual cycle. For these species, stable isotopes remain an effective method to track the relative input of endogenous versus exogenous nutrients given large enough differences between successive stages of the annual cycle. In addition, compound-specific isotopic analysis (e.g., O'Brien et al. 2003) shows promise as a means of identifying the origin of specific amino acids, particularly if specific amino acids or their concentrations are indicative of different geographic locations.

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