

# Isotopic spiking and food dye experiments provide evidence that nestling Canada Jays (*Perisoreus canadensis*) receive cached food from their parents

R. Derbyshire, D.R. Norris, K.A. Hobson, and D. Strickland

**Abstract:** While many animals rely on stored food to survive periods of no or few resources, some of these species may also use cached food to feed young. The Canada Jay (*Perisoreus canadensis* (Linnaeus, 1766)) is a territorial, food-caching resident of North American boreal forests. Canada Jays have high winter survival when fresh food is rarely available and achieve high fledging rates before the return and breeding of most sympatric migratory passerines. Stored food must account for the Canada Jay's winter survival, but it is less certain that stores are also used to feed nestlings. We conducted two experiments seeking evidence of such use, one using food marked with dye and a second using  $^{15}\text{N}$ -enriched glycine. Breeding pairs were supplemented with marked food in the pre-incubation period, followed by our attempts to find the marker in nestling feces (dye experiment) or feathers ( $^{15}\text{N}$ -enrichment experiment). Both experiments provided evidence that at least some nestlings had ingested the marker, although the importance of this behaviour within the population remains unknown. Our study demonstrates how food markers can be used to infer feeding behaviour that has not been directly recorded in the wild, even after over 50 years of close observation.

**Key words:** caching, Canada Jay, food supplementation, nitrogen-15, stable isotopes, *Perisoreus canadensis*.

**Résumé :** Si de nombreux animaux ont recours à de la nourriture emmagasinée pour survivre à des périodes durant lesquelles les ressources sont rares ou inexistantes, certaines de ces espèces pourraient aussi cacher de la nourriture dans le but de nourrir leur progéniture. Le mésangeai du Canada (*Perisoreus canadensis* (Linnaeus, 1766)) est un résident territorial des forêts boréales de l'Amérique du Nord qui cache de la nourriture. Les mésangeais du Canada présentent un taux de survie hivernale élevé alors que des aliments frais sont rarement disponibles et des taux d'envol du nid élevés avant le retour et la reproduction de la plupart des passereaux migrateurs sympatriques. Si la nourriture emmagasinée doit expliquer la survie hivernale des mésangeais du Canada, il est moins certain qu'elle soit également utilisée pour nourrir les oiseaux niais. Nous avons mené deux expériences pour tenter de trouver des preuves d'une telle utilisation, dont une avec de la nourriture marquée avec un colorant et l'autre avec de la glycine enrichie en  $^{15}\text{N}$ . Les couples reproducteurs ont reçu de la nourriture marquée durant la période précédant la couvaison, puis nous avons tenté de trouver le marqueur dans les fèces (expérience avec le colorant) ou les plumes (expérience avec enrichissement en  $^{15}\text{N}$ ) des oiseaux niais. Les deux expériences ont produit des preuves qu'au moins certains des oiseaux niais avaient ingéré le marqueur, bien que l'importance de ce comportement dans la population demeure inconnue. L'étude démontre comment des marqueurs alimentaires peuvent être utilisés pour inférer un comportement d'alimentation qui n'a pas été observé directement dans la nature, même après 50 années d'observation soigneuse. [Traduit par la Rédaction]

**Mots-clés :** emmagasinage de nourriture, mésangeai du Canada, supplément de nourriture, azote-15, isotopes stables, *Perisoreus canadensis*.

## Introduction

Food caching is observed in numerous vertebrate taxa and may serve at least one of three general functions: (1) to dampen short-term variations in food availability; (2) to permit survival through seasons when food is otherwise unavailable; or (3) to support reproduction (Vander Wall 1990; Hitchcock and Houston 1994; Sutton et al. 2016). Normally (64 of 68 species cited by Vander Wall 1990), species that cache food for use in feeding offspring do so shortly before, and (or) during, the reproductive period. However, in four food-caching corvids (Spotted Nutcrackers, *Nucifraga caryocatactes* (Linnaeus, 1758); Clark's Nutcrackers, *Nucifraga*

*columbiana* (A. Wilson, 1811); Canada Jays, *Perisoreus canadensis* (Linnaeus, 1766); Siberian Jays, *Perisoreus infaustus* (Linnaeus, 1758)), the late-winter nesting season (e.g., incubation beginning as early as February in Canada Jays; Strickland and Ouellet 2011) is not immediately preceded by the late-summer and fall food-storage season. The recovery of autumn-stored food and its use in feeding nestlings 4–6 months later has been documented in the two nutcracker species (Swanberg 1956; Mewaldt 1956; Tomback 1998), but similar evidence has been lacking in *Perisoreus* spp. (Strickland and Ouellet 2011).

The Canada Jay is a year-round resident of North American boreal and subalpine forests (Strickland and Ouellet 2011). Individ-

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ual Canada Jays have been estimated to store over 1000 food items a day throughout their territories (Waite 1991). Although most caching behaviour occurs during the warm months when more food is available (berries, invertebrates, mushrooms, vertebrate flesh; Strickland and Ouellet 2011), Canada Jays also store food opportunistically throughout the otherwise apparently foodless boreal winter whenever it becomes available (e.g., a large-mammal carcass, human handouts). Strong circumstantial evidence suggests that recovery of stored food must be critical for the Canada Jay's winter survival. In particular, breeding pairs occupy large permanent territories (27–146 ha; Strickland and Norris 2015) and have high winter survival (>90%; Norris et al. 2013), and yet this performance cannot be attributed to either of the only two plausible winter sources of fresh food in boreal forests: carcasses and cones from conifers. Canada Jays do not attempt to open cones (Dow 1965) and we have seen no evidence that adult abundance or nesting success are influenced by occasional bumper tree seed crops. Although Canada Jays will feed on animal carcasses, their year-round territories are still too small to provide a plausibly uninterrupted carcass supply and, in any case, Canada Jays are typically excluded very quickly from carcasses by larger mammalian and avian scavengers (Strickland and Ouellet 2011). Therefore, food cached the previous summer and fall is likely a critical component of the Canada Jay's winter diet and would certainly help explain the species' conspicuous summer and fall food-storage activities.

Despite this putatively causal link between food storage and adult winter survival, the possible use of stored food to feed nestlings is less compelling. Unlike the non-perishable seeds stored by nutcrackers, foods stored by Canada Jays in late summer and fall are perishable. These foods are therefore subject to potentially significant degradation before the winter's-end breeding season because even subfreezing temperatures are not necessarily low enough to halt bacterial activity, and degradation could be exacerbated by food-damaging freeze–thaw events and the associated occasional mid-winter thaws (Sechley et al. 2015; Sutton et al. 2016). Moreover, until the studies reported here, there was no unambiguous evidence that stored food is ever fed to nestlings. In more than 50 years of Canada Jay studies in Ontario and Quebec, for example, we have never been able to identify any food item brought to active nests or recovered from nestlings that could be unequivocally tied to stored food. On the contrary, all the arthropod remains from nestling stomach contents or castings that we have referred to experts for identification have been attributed to life stages that, in the opinion of those experts, could have been available during typical nestling periods of Canada Jays (i.e., between early April to early May in our study areas). Moreover, aquatic insect larvae have sometimes been among such remains and this is consistent with occasional observations of Canada Jays arriving at nests with sodden belly feathers and with other observations of Canada Jays wading, and apparently foraging, at the edges of open creeks in the still snow-covered landscape at that time of year. Furthermore, in cases of replacement, or otherwise late nests when we have observed adults foraging after the disappearance of snow cover, such foraging always involved birds flying from perch to perch ca. 2–4 m above the ground, peering downwards, and obtaining food items exclusively from moss or leaf litter on the forest floor. Those items, although almost never identified, can safely be categorized as fresh food because Canada Jays have never been observed to store food on or in the ground.

Notwithstanding our failure to observe the use of stored food to feed nestlings and the evidence that fresh food is used when available, we maintain that only the former can explain the dramatically early, and yet highly successful, late-winter nesting characteristic of Canada Jays (Strickland and Ouellet 2011). There have been no previous experimental studies to test this hypothesis in Canada Jays, which are relatively unique among passerines due to the perishability of their cached food paired with their very

early breeding behaviour. Since the initial and surviving quantities of stored food on territories of Canada Jays are unknown, we had no expectation of quantifying the use of stored food to feed nestlings, but instead wished to determine if such use occurred in the wild. In this paper, we report the results of two experiments designed to test whether Canada Jays ever feed stored food to their nestlings. Specifically, we predicted (i) that food dye added to supplemental food and fed to Canada Jays before the initiation of incubation would later appear in the fecal sacs of nestlings and (ii) that the distinct stable isotope signature of food enriched with  $^{15}\text{N}$  glycine powder and supplied to adult Canada Jays in the pre-breeding season would later appear in the feathers of nestlings.

## Materials and methods

We conducted this study in Parc de la Vérendrye, Quebec, Canada (47°N, 76°W), and Algonquin Provincial Park, Ontario, Canada (45°N, 78°W). Details of the ecology of each study area can be found in McNeil (1961) and Wilson (1971) for Parc de la Vérendrye, and Rutter (1969) and Strickland (1987) for Algonquin Park. The reproductive dynamics of Canada Jays in Algonquin Park have been monitored since the 1960s (Rutter 1969; Strickland and Waite 2001; Norris et al. 2013). Each Canada Jay is individually banded with a unique combination of coloured leg bands, as well as a standard Canadian Wildlife Service aluminum band (Banding Permit No. 10416), either as a nestling (7–14 days) or as an adult as soon as it is discovered in the study population. Approximately 20–30 pairs have been monitored annually since the 1980s in this population (Waite and Strickland 2006; Derbyshire et al. 2015; D. Strickland and D.R. Norris, unpublished data).

### Food dye experiment

This experiment was performed in 1967–1968 by D. Strickland in Parc de la Vérendrye. In January 1967, a Canada Jay was held in captivity and fed ad libitum with bread marked with the dye Brilliant Blue FCF (Fisher Scientific, Ottawa, Ontario, Canada) to test the efficacy of this dye as a marker that could later be detected in the feces of Canada Jays. The subject was not detectably harmed by the experience and its fecal matter was made conspicuously blue–green, as had been shown in an earlier study on human digestion (Lutwak and Burton 1964).

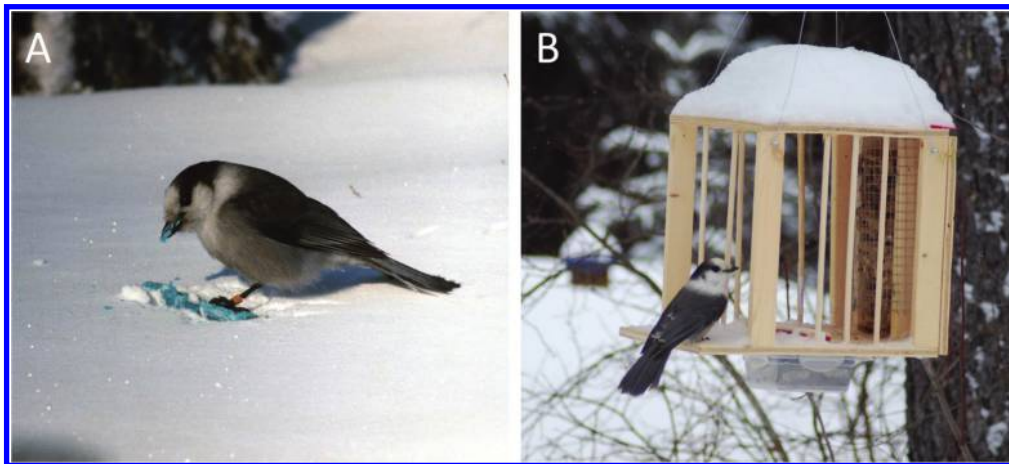
In 1968, four of eight adult breeding pairs in the study area were supplemented with approximately 170 g each (4–6 slices) of dyed bread per day (Fig. 1A; colour version of figure online), from 5 February to 19 March (the day before clutch initiation in the only ultimately relevant treatment pair). Bread was dyed the evening before presentation to the Canada Jays by dipping each slice into a Brilliant Blue dye solution containing approximately 400 mg of dye per slice. During the study, each pair was supplemented with approximately 5.1 kg of bread containing 3 g of dye. The intention was to monitor nests of each pair through the incubation and nestling periods and if possible to observe and collect fecal sacs removed by adults (Strickland and Ouellet 2011). Due to nest failures (likely caused by predation), only two breeding pairs were observed during the nestling period.

Although no official animal care guidelines existed at the funding institution at the time, great care was taken to reduce both the use of animals and the effects of experimental protocols on the animals used in this experiment, as would be required by current guidelines under the Canadian Council on Animal Care.

### Nitrogen-15 spiking experiment

Forty-five years later, we conducted a second experiment in Algonquin Park in which adult breeding pairs were provided with  $^{15}\text{N}$ -enriched supplemental food in the pre-breeding stage (31 January – 26 February) for 2 years (2013 and 2014). Thus, food was provided both before incubation and during a time when Canada Jays would plausibly have access to winter food resources, such as meat from animal carcasses. In 2013, 10 Canada Jay terri-

**Fig. 1.** Method for administering the food marker in each of our two supplementation experiments. (A) Canada Jay (*Perisoreus canadensis*) feeding on bread that had been dyed with Brilliant Blue FCF. Four breeding pairs were fed from 5 February to 19 March 1968 in the food dye experiment. (B) Feeder design for the 2013–2014 nitrogen-15 spiking experiment. This Canada Jay is about to enter the feeder to gain access to the cat food, which is behind the mesh at the back of the feeder. For additional details on feeder design refer to Derbyshire et al. (2015). Colour version online.



territories were selected for the food supplementation treatment and 16 received no additional food and served as controls. In 2014, 10 territories were once again selected for the supplementation treatment; 9 of these were either control territories or vacant territories in 2013 and 1 of these had been supplemented in 2013 but had a new female in 2014. Fifteen territories were selected as controls in 2014, seven of which had been supplemented in 2013 (Supplementary Table S1).<sup>1</sup> Over the two seasons of the experiment, 15 territories received both control and supplementation treatments, with 14 of these territories containing the same female between years (Supplementary Table S1).<sup>1</sup>

To provide food to the Canada Jays, a single feeder was deployed in each territory (Fig. 1B; colour version of figure online), at least 100 m inside the territory boundary (territory boundaries estimated based on nest locations in previous years). This placement helped ensure that Canada Jays from neighbouring territories were unlikely to access the feeders. Our feeder design included a method for tracking female entries (Derbyshire et al. 2015). Every day we stocked each feeder with enough food such that the total amount of food supplied throughout the entire food supplementation period was more than twice the amount needed to sustain the breeding pair, three nestlings, and an extra bird (which sometimes accompanies the pair; Strickland and Ouellet 2011) for the entire breeding season, based on the estimated daily energy requirement of 47 kcal (Shank 1986). Dry kitten food (IAMS Proactive Health Kitten, Mason, Ohio, USA) was selected in 2013 and canned cat food (Authority Adult Weight Management Canned Food, Phoenix, Arizona, USA) was selected in 2014. We switched from dry kibble to canned food in 2014 because we were concerned that the addition of water to the dry kibble (to add isotopically enriched nitrogen, see below) may have caused the dry kitten food to spoil more quickly than the canned food.

Glycine enriched in <sup>15</sup>N (Isotec Stable Isotopes, Sigma-Aldrich, St. Louis, Missouri, USA) was added to the food in both years. First, 1.6 mg of enriched glycine was dissolved in a small amount of water. Previous experiments demonstrated that this dose of glycine powder resulted in high values of <sup>15</sup>N in the food supplement (e.g., ~60%; D. Strickland and D.R. Norris, unpublished data). For the dry kibble, this isotope solution was then blended for at least 60 s with 350 g of a 1:1 mixture of water : kitten food (by volume). The food was then dried on a stovetop for >1 h by stirring the

mixture in a pot on low heat until most liquid had evaporated, then packaged into 800 g individual portions for daily supplementation to the jays. For the canned food, the same method was employed, but without the addition of water to the food. A similar method has been employed with canned dog food by D.R. Norris (unpublished data), and strong isotopic signals (e.g., 157‰) were subsequently detected in feathers of both adult and juvenile Canada Jays.

After food supplementation, reproduction was monitored by locating the nest in each territory and monitoring the females daily until they reached the egg-laying stage. Once the female has laid the first egg, she rarely leaves the nest (Strickland and Ouellet 2011). Therefore, we estimated the date of first egg as the day when a female was known to be sitting on her nest for the first time or, in ambiguous cases, as the midpoint of possible first days. We banded nestlings at 7–12 days of age and collected a feather sample from each nestling at that time. This timing ensured that a very small percentage (likely <1%) of maternal nitrogen would be present in nestling feathers (K.A. Hobson, unpublished data), making feather  $\delta^{15}\text{N}$  a reliable measure of dietary  $\delta^{15}\text{N}$ .

To prepare nestling feathers for isotope analysis, samples were first washed and rinsed in 2:1 chloroform:methanol solution and then allowed to air dry for 48 h. Small clippings of the feather were then wrapped within tin capsules and sent to the Stable Isotope Facilities at the Department of Soil Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, for analysis. Usually, two feather samples (from two different nestlings) were sent for analysis from each successful nest (range = 1–4, mean = 1.9; samples were not analysed for all nestlings in each nest). Samples were combusted using a Costech elemental analyser (Thermo, Bremen, Germany) coupled with a Thermo delta V mass spectrometer using continuous-flow isotope ratio mass spectrometric analyses. Resultant N<sub>2</sub> was analysed for  $\delta^{15}\text{N}$  using in-house calibration standards (egg albumen and BWB keratin). All values are reported in the standard delta notation in parts per thousand (‰) deviation from atmospheric (AIR) nitrogen. Measurement error based on within-run measurements of replicate standards was estimated as  $\pm 0.2\%$ .

Nitrogen-15 spiking experiments were approved by the University of Guelph Animal Care Committee (eAUP # 1842) and comply with guidelines of the Canadian Council on Animal Care.

<sup>1</sup>Supplementary Table S1 is available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjz-2018-0158>.



## Statistical analyses

For the food dye experiment, presence of the marker was determined by visual inspection (see Results below). Similarly, for the nitrogen-15 spiking experiment, we were interested in testing whether any of our supplemented pairs had used our food supplement to feed nestlings. Therefore, a test of whether mean  $\delta^{15}\text{N}$  was different between supplemented and control nests was not appropriate; this type of test would only inform us whether Canada Jays, on average, provided our food supplement to nestlings and may have masked our detection of this behaviour if it occurred in only a subset of individuals. To understand how our treatment influenced individual pairs within the supplemented group, we used a statistical approach that allowed us to examine whether nestling  $\delta^{15}\text{N}$  was influenced by statistical interactions between territory and treatment. We were interested in a territory  $\times$  treatment interaction, and not a nestling  $\times$  treatment interaction, because, based on a Pearson's correlation test of 25 nests (for which we had samples from two or more nestlings), feather  $\delta^{15}\text{N}$  values within nests were highly correlated ( $r = 0.97$ ,  $t_{[23]} = 18.0$ ,  $p < 0.0001$ ; for nests with  $>2$  nestling samples ( $n = 5$ ), 2 samples were chosen randomly for the test). In addition, this statistical design allowed us to account for variation among territories while simultaneously testing the main effect of each territory on our response variable.

Our statistical analyses occurred in three stages. First, we conducted a preliminary analysis to determine which parameters should be included in our model. Then, we modeled the influence of these parameters on nestling  $\delta^{15}\text{N}$ . We have termed this second model our "main analysis", as it allowed us to model the effects of several parameters on our response variable of interest (nestling  $\delta^{15}\text{N}$ ). Finally, we conducted a bootstrap analysis that was used to further test for differences between supplemented and control nestlings based on the results of the main analysis. Challenges relating to sample size and model assumptions are detailed at the end of this section.

Since the experiment took place across years, it was important to first understand whether there was any effect of year (2013 vs. 2014) on our experimental results. To test this, we built a two-way ANOVA, with year and treatment (supplemented or control) as predictor variables and nestling  $\delta^{15}\text{N}$  as the response variable. We were not able to construct a mixed model with territory as a random effect because a paucity of observations resulted in the failure of mixed models to converge. However, as we sought only to determine whether year should be included in later models, we decided to proceed without including territory as a random effect. Based on our ANOVA, nestling feather  $\delta^{15}\text{N}$  was not influenced by year ( $F_{[1,66]} = 0.03$ ,  $p = 0.9$ ), although treatment was a significant predictor of  $\delta^{15}\text{N}$  values ( $F_{[1,66]} = 12.6$ ,  $p = 0.0007$ ). We therefore excluded year from all subsequent analyses.

For our main analysis, we built a linear model with territory, treatment, and a territory  $\times$  treatment interaction to test the effects of these variables on nestling  $\delta^{15}\text{N}$ . We included territories only if we had nestling feather samples for both treatments (control and supplemented) and if we knew that the female had accessed the food during the supplementation treatment ( $n = 10$ ; Supplementary Table S1<sup>1</sup>). Of these 10 territories, 9 territories had the same female for both control and supplemented treatments, whereas 1 territory had a different female between the 2 years (Mile 36; Supplementary Table S1<sup>1</sup>); therefore, Female ID was not included as a random effect because it was almost exactly correlated with territory. In this analysis, territory was not coded as a random effect because we were interested in testing the main influence of territory on the response variable, as well as identifying which territories might exhibit a significant interaction with the treatment. In other words, as the purpose of the statistical test was to compare the influence of different territories on nestling  $\delta^{15}\text{N}$  (and not to control for differences between territories), territory was coded as a fixed factor.

Lastly, we generated an accelerated, bias-corrected (BCa; Efron and Tibshirani 1993) 95% confidence interval (95% CI) of bootstrapped means using the R package "boot" (Canty and Ripley 2017). To do this, a single  $\delta^{15}\text{N}$  nestling value was taken from each control nest ( $n = 22$ ); for nests with multiple nestlings, a single nestling was randomly selected. These values were sampled with replacement 1000 times. The mean of each bootstrapped sample was then calculated and a BCa 95% CI was generated based on these bootstrapped means ( $n = 1000$ ). This allowed us to confirm whether the mean  $\delta^{15}\text{N}$  values of nestlings in supplemented territories with a significant territory  $\times$  treatment interaction were outside the 95% CI of control values.

In both our ANOVA and linear model, some assumptions of the model were violated. The most obvious violation was the assumption of homogeneity of variance in the residuals. We ameliorated this problem slightly by square-root-transforming our response variable; however, the violation remained a concern. Because we suspected the problem might be caused by two high-value outliers within the data set (two nestlings from one nest), we removed the outliers and found that the problem was greatly diminished. However, we did not wish to remove these outliers from our main analysis, as we believed they were likely nestlings that had received the marked food supplement. Nor could we employ mixed models or nonparametric tests because of sample-size limitations. Therefore, we did not remove the outliers in our main analysis, but we warn the reader to interpret our results with caution.

All statistical analyses were performed in the program R version 3.4.4 (R Core Team 2018).

## Results

### Food dye experiment

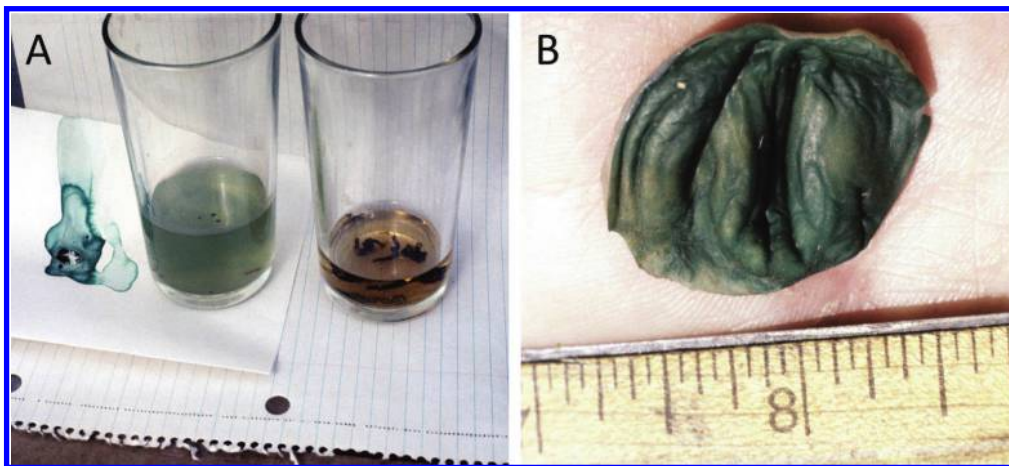
In one of the two experimental nests that survived to the nestling period, the nestlings disappeared (likely due to predation) on successive days when they were 8–9 days old, with no sign of green-coloured feces before this time. However, at the remaining nest, on 26 April, an adult was observed removing a fecal sac from one of the 17-day-old nestlings; the observed fecal sac clearly had green areas. A similar sac was observed on 27 April (along with two others whose dark portions were dark brown or black). On 28 April, a fecal sac was recovered after an adult placed it on a log near the nest; although the fecal material within it appeared black, green colour immediately diffused from the sac when it was placed in a glass of water (Fig. 2A; colour version of figure online). The green-containing fecal sacs that were observed on these 3 days appeared 38, 39, and 40 days after the last day when the adults were supplied with dyed bread.

On 29 April, this last remaining ongoing treatment nest was attacked by a red squirrel (*Tamiasciurus hudsonicus* (Erxleben, 1777)) and one of the nestlings was killed. This nestling was collected and dissected, revealing that the stomach lining was unmistakably green (Fig. 2B; colour version of figure online). This nestling was collected 41 days after the last provision of dyed bread to its parents.

### Nitrogen-15 spiking experiment

In 2013, of the 10 supplemented territories, 4 were excluded from the analyses because the female did not access the feeder (but we could not confirm that the male had not;  $n = 2$ ), or the pair did not successfully reproduce ( $n = 2$ ). Five control territories were also excluded from the 2013 analyses because the pairs did not reproduce successfully ( $n = 4$ ), or on the day that we accessed the nest, nestlings were too small to take a usable feather sample ( $n = 1$ ). Two supplemented pairs were excluded from analysis in 2014 because the female did not access the feeder ( $n = 1$ ), or the pair did not reproduce successfully ( $n = 1$ ). Five control territories were excluded in 2014 because the pair did not reproduce successfully (Supplementary Table S1).<sup>1</sup> In total, 33 nestling feather samples

**Fig. 2.** Appearance of Brilliant Blue FCF dye in fecal samples (A) and stomach tissue (B) of Canada Jay (*Perisoreus canadensis*) nestlings. (A) On the left (on paper) are blue-coloured adult feces collected from a supplemented territory compared with two nestling fecal sacs dissolved in water. The glass on the left contains a fecal sac (blue–green colouration) from a supplemented territory, whereas the glass on the right contains a fecal sac (brown colouration) from a control territory. (B) The dyed-green interior of the stomach of a deceased Canada Jay nestling that was collected 41 days after the last day that the adult pair was provided with dyed bread. Colour version online.



were analysed from control nests (2013:  $n = 14$  from 12 nests; 2014:  $n = 19$  from 10 nests) and 36 feather samples were analyzed from food-supplemented nests (2013:  $n = 20$  from 6 nests; 2014:  $n = 16$  from 8 nests).

Our linear model to test the effects of territory, treatment, and territory  $\times$  treatment on nestling feather  $\delta^{15}\text{N}$  values was significant ( $F_{[19,21]} = 42.83$ ,  $p > 0.0001$ , adjusted  $R^2 = 0.95$ ; but see discussion on model assumptions above). Based on this model, treatment was not a significant predictor of  $\delta^{15}\text{N}$  ( $B \pm \text{SE} = 0.092 \pm 0.052$ ;  $t = 1.76$ ,  $p = 0.09$ ; Fig. 3A; colour version of figure online). However, three territories displayed a significant interaction with treatment: TDS South ( $B \pm \text{SE} = 0.22 \pm 0.073$ ;  $t = 2.931$ ,  $p = 0.008$ ); Mile 36 ( $B \pm \text{SE} = 0.23 \pm 0.075$ ;  $t = 3.089$ ,  $p = 0.006$ ; we noted that a different female occupied this territory in the second year of the experiment; Supplementary Table S1<sup>1</sup>), and Boardwalk ( $B \pm \text{SE} = 1.13 \pm 0.090$ ;  $t = 12.53$ ,  $p < 0.0001$ ). In all three territories,  $\delta^{15}\text{N}$  values during the supplemental food treatment were higher than during the control treatment (Fig. 3B; colour version of figure online). In one territory (Boardwalk),  $\delta^{15}\text{N}$  values in the supplementation treatment were more than twice the mean for all control samples (11.54‰ vs. a mean value of 5.28‰ for control samples; range for control samples = 4.29‰–6.63‰). In addition, mean  $\delta^{15}\text{N}$  values in these three nests during the supplementation treatment fell outside the BCa 95% CI generated from bootstrapped control values (95% CI = 5.04‰–5.57‰;  $\delta^{15}\text{N}$  values (mean  $\pm$  SD): TDS South = 5.87‰  $\pm$  0.36‰, Mile 36 = 6.02‰  $\pm$  0.18‰, Boardwalk = 11.54‰  $\pm$  0.96‰). Nestling feathers from these nests were collected 39–51 days after the last day of the food supplementation treatment.

## Discussion

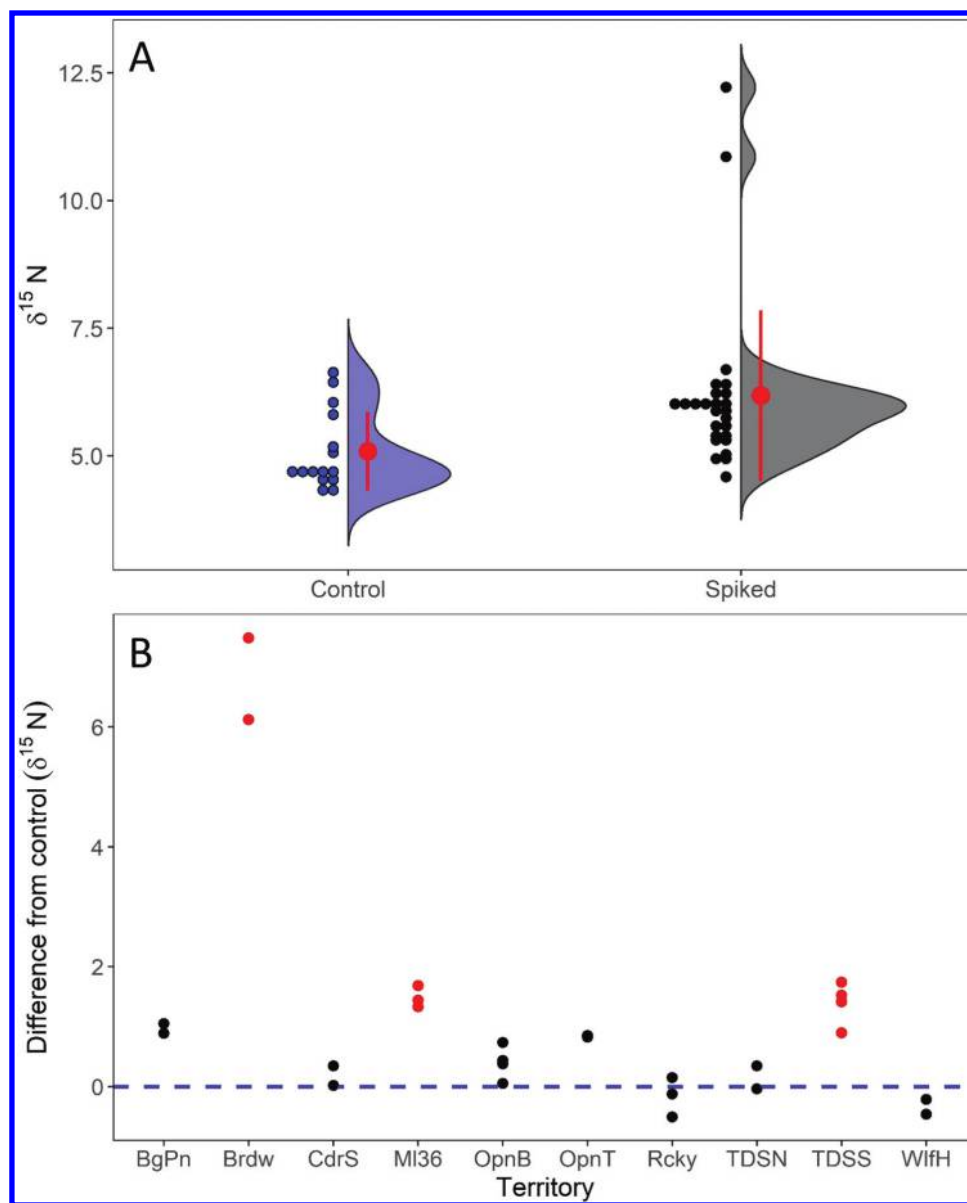
The results from our food dye and isotope spiking experiments provide the first direct evidence that Canada Jays at least occasionally provide cached food to their nestlings, although the extent and frequency of this behaviour within the general population remain unknown. In the dyed bread experiment, our dye was clearly detected within nestling feces and stomach lining 38–41 days after the conclusion of food supplementation. In the nitrogen-15 spiking experiment, the interaction between treatment and territory was significant for 3 of 10 territories analyzed across both years, with 1 of these territories having nestlings with  $\delta^{15}\text{N}$  values more than two times greater than in the control treatment (Fig. 3A; colour version of figure online). We interpret these

findings as evidence that Canada Jays at least occasionally use cached food to feed nestlings.

Despite this evidence for the use of cached food to provision nestlings, it is important to note that the results of our nitrogen-15 spiking experiment were equivocal in some cases. We believe the highly significant interaction and large effect size (more than twice the control value) for the Boardwalk territory constitutes strong evidence that the pair for this territory provided the marked food supplement to nestlings. However, the values for the other two territories with significant interactions are less persuasive. Due to our small sample size and violations of model assumptions, the associated significance values and confidence intervals have questionable reliability (Lewis-Beck and Lewis-Beck 2015). For very large effect sizes (as seen for the Boardwalk territory), this problem may not be serious; however, the significance values associated with smaller effect sizes should be interpreted with caution. Moreover, although both small-effect territories showed higher mean  $\delta^{15}\text{N}$  values during the supplementation experiment (TDS South: control = 4.48‰ vs. supplemented = 5.87‰; Mile 36: control = 4.53‰ vs. supplemented = 6.02‰) and mean supplemented values for these nests were outside the BCa 95% CI of bootstrapped controls (5.04‰–5.57‰), the mean supplementation values for these pairs were still within the range of control values for this study (4.29‰–6.63‰ for all control nestlings). There are two possibilities to explain this pattern. The first is that nestlings in these territories did receive our food supplement, but in such small quantities that it did not cause the  $\delta^{15}\text{N}$  values to exceed the maximum value within the range of controls. Canada Jays can store over 1000 caches in a single day (Waite 1991) and could be relying on a variety of stored food items to provision nestlings (i.e., not only the stored food provided by us); for this reason, our treatment may have increased  $\delta^{15}\text{N}$  values only slightly for these territories. The second possibility is that the  $\delta^{15}\text{N}$  values increased for some other reason, such as another dietary change unrelated to our supplement. For example, animal protein has higher values of  $\delta^{15}\text{N}$  (Deniro and Epstein 1981); it is possible that the observed  $\delta^{15}\text{N}$  values in TDS South and Mile 36 nestlings were caused by a higher level of dietary animal protein during the supplementation treatment (for instance, if the pair had access to a carcass).

Based on the above considerations, we conservatively suggest that only one of our supplemented pairs in the nitrogen-15 spiking experiment clearly provided nestlings with our marked food

**Fig. 3.** Values of feather  $\delta^{15}\text{N}$  for Canada Jay (*Perisoreus canadensis*) nestlings in final paired analyses for nitrogen-15 spiking experiment. (A) Actual measured  $\delta^{15}\text{N}$  values (‰) in control versus supplemented nests represented as a split violin and dot plot. The individual dots represent individual measured values, whereas the violin plot displays the relative distribution of dots for each value of  $\delta^{15}\text{N}$  (wider areas of the plot represent a higher number of data points at that y value; the plot is smoothed between y values). The single red dot within the violin plot displays the mean value for that treatment, whereas the red vertical bars display standard deviation from the mean. (B) Difference between mean control values (blue horizontal broken line) and  $\delta^{15}\text{N}$  values (‰) for individual nestlings following the supplementation treatment for each territory. Red dots are from territories for which the territory  $\times$  treatment interaction was significant. Territory name codes are as follows: BgPn, Big Pines; Brdw, Boardwalk; CrdS, Cedar Swamp; MI36, Mile 36; OpnB, Opeongo Bridge; OpnT, Opeongo Turn; Rcky, Rockyard; TDSN, TDS North; TDSS, TDS South; WlfH, Wolf Howl Pond. Colour version online.



supplement. Our data are insufficient to test whether the other pairs in our nitrogen-15 spiking experiment also provided cached food to nestlings, but in quantities that were too small for the enriched nitrogen-15 signal to be clearly apparent. We also acknowledge the possibility that, since the feather samples we used for analysis did not always incorporate an entire feather, we may have reduced the chances of detecting a single feeding event because feathers represent a time series of multiple feeding events (Thompson and Furness 1995). Therefore, although we have provided evidence in both our food dye and nitrogen-15 spiking experiments that Canada Jays at least occasionally provide cached

food to nestlings, the extent of this behaviour remains unknown, as does its importance for nestling survival.

The principle that breeding seasons have been selected to coincide with the period of maximum food abundance to feed developing offspring is well established (Lack 1954). In the two corvid genera *Nucifraga* and *Perisoreus*, however, the breeding season is conspicuously shifted from the typical summer nesting seasons of almost all sympatric boreal and subalpine passerines (Benson and Winker 2001) to late winter, when the supply of obvious fresh food is poor or nonexistent. In *Nucifraga*, the advantage of early nesting has been attributed to the greater time available for juveniles to



acquire and improve their food-caching and other survival skills ahead of the late-summer and fall food-storage season (Tomback 1978). This may also apply to *Perisoreus* spp., but other advantages are likely as well (Strickland and Ouellet 2011). First, early-nesting adults, by initiating reproduction in late winter and thereby bringing their young to nutritional independence early in the season, will be able to invest more post-nesting caching effort in their territories before the onset of freezing temperatures and the disappearance of fresh storable food. This potential advantage of early nesting should be especially important in the far northern parts of the Canada Jay's range (e.g., the Mackenzie River delta north of the Arctic Circle) where, compared with our la Vérendrye and Algonquin study areas, there is a much longer winter period of presumed obligatory reliance on stored food and a correspondingly much shorter period when the stored food can be reliably acquired. Second, given the partial juvenile dispersal system of Canada Jays (Strickland 1991), ejected juveniles of early-nesting pairs should have a competitive advantage over late-produced ejected juveniles in gaining acceptance from unrelated breeders on other territories. Third, by nesting before other boreal passerines, Canada Jays may avoid some nest losses to young red squirrels, a major predator in subalpine and boreal forests (Martin 1988; Sieving and Willson 1998; Boulet et al. 2000), and one that apparently must learn from experience that bird nests can be nutritionally rewarding (Pelech et al. 2010).

Clear evidence exists that the food which makes early nesting possible in *Nucifraga* is often, or even predominately, retrieved from caches made the previous late summer or fall, as it typically consists of seeds from a few large-seeded, mostly coniferous species that could not be acquired or stored at any other time (Swanberg 1956; Vander Wall 1990; Tomback 1998). Conversely, until our study, there was no concrete evidence that Canada Jays use cached food to feed their nestlings, however plausible or even necessary such use might appear in explaining the occurrence and success of the early-nesting behaviour in this species. One hypothesis to explain this lack of evidence is that fresh food items are preferred by breeding Canada Jays, but caches remaining in late winter are used in emergency situations. In our food dye experiment, the weather had been sunny with signs of insect activity in the days preceding 25 April (the day before the first green fecal sac was observed), but then gave way to 2 days of cold temperatures and intermittent rain and snow with no evident signs of insect activity. Therefore, stored food may have been used only because no fresh food was available. If this emergency food hypothesis is correct, then stored food might play a crucial, risk-mitigating role in permitting early nesting in Canada Jays, even though its actual use might be intermittent and weather-dependent. This risk-mitigation strategy has been hypothesized to be important for the survival of other food-hoarding species, such as the Acorn Woodpecker (*Melanerpes formicivorus* (Swainson, 1827)) (Hitchcock and Houston 1994), so its use during the nestling period in Canada Jays is plausible.

The use of stored food to feed nestlings is a fundamental assumption of the hoard-rot hypothesis, which has been invoked to explain the observed decline in Canada Jay populations at the edge of their southern range (Waite and Strickland 2006; Menebroeker et al. 2016). Several lines of evidence now support this hypothesis, which proposes that increased temperatures and consequently poorer preservation of caches lead to poorer fledgling production and (or) quality and finally to declining recruitment (Waite and Strickland 2006; Sechley et al. 2015; Derbyshire et al. 2015). Canada Jays in our study area have already been shown to be food-limited during the breeding season (Derbyshire et al. 2015), and this food limitation could be related to the increased perishability of caches due to the warmer, and more widely fluctuating temperatures associated with climate change. A similar negative influence of warmer temperatures on reproduction has also been proposed for the wolverine (*Gulo gulo* (Linnaeus, 1758)), another boreal species

that caches perishable food (Inman et al. 2012). Future work should address the persisting uncertainties about the quantities and types of cached or fresh food that are provided to Canada Jay nestlings, as well as investigate how climate change may affect cache survival and hoarding behaviour in other food-storing species known or suspected to cache perishable food items to support reproduction.

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