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USING STABLE-ISOTOPE ANALYSIS AS A TECHNIQUE FOR DETERMINING CONSUMPTION OF SUPPLEMENTARY FOODS BY INDIVIDUAL BIRDS

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Abstract. The amount of supplementary food humans provide to wild animals is increasing, yet the full effects of this provisioning remain unclear. For these effects to be assessed at the levels of both the population and the individual, the degree to which individuals are using this resource must be quantified. Traditional approaches rely on observations of feeding animals and analysis of gut contents and feces, but these have several limitations. Stable-isotope analysis can overcome some of these. If supplementary food items are isotopically distinct from natural ones, the relative contribution of supplementary food to the diet may be quantified accurately. We demonstrate how the isotopic signature of supplementary foods can be manipulated to increase their discrimination from natural food sources and provide an example of the utility of this approach in a supplementary feeding study. We provided supplementary food over a winter, then sampled birds during the following breeding season and analyzed their claws for their isotopic signature to estimate diet choices. The results highlight considerable variation in individuals' use of supplementary food, both within a study site and between different sites. Often the results from supplementation experiments are inconclusive. Even within the same species there can be an effect in one year or location but not in others, so a method for quantifying variation in food uptake could help in interpretation of the results. Stable-isotope analysis allows the effects of experimentally increased food supplies on ecology and behavior to be assessed accurately.

Key words: *breeding success, foraging ecology, mixing models, stable isotope, SIAR, supplementary feeding.*

Uso de Isótopos Estables como Técnica para Determinar el Consumo Individual de Alimentos Suplementarios en Aves

Resumen. La cantidad de alimento suplementario que la humanidad está proporcionando a los animales silvestres es cada vez mayor; sin embargo, los efectos de este suministro no están completamente claros. Para que estos efectos puedan ser evaluados tanto a nivel poblacional como individual, debe cuantificarse el grado en que las aves están utilizando ese recurso. Los enfoques tradicionales se han basado principalmente en observaciones de animales que se encuentran alimentándose y en el análisis de contenidos intestinales y de las heces; sin embargo, estos estudios presentan una serie de limitaciones. Los análisis de isótopos estables pueden superar algunos de estos problemas. Si los alimentos suplementarios son isotópicamente diferentes de los naturales, la contribución relativa de los alimentos suplementarios a la dieta puede cuantificarse con precisión. En este estudio, demostramos cómo la señal isotópica de los alimentos suplementarios puede ser manipulada para aumentar la distinción entre los alimentos suplementarios y los alimentos naturales. Alimentamos aves durante el invierno y consecutivamente realizamos el muestreo en la época de cría, analizando la señal isotópica de sus garras para estimar las preferencias de dieta de cada individuo. Los resultados evidencian una considerable variación entre los individuos en el uso de alimentos suplementarios, tanto dentro de un sitio de estudio como entre los diferentes sitios. Frecuentemente los resultados

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de los experimentos de suplemento de alimento no son concluyentes. Incluso dentro de la misma especie puede haber un efecto en un año o en una localidad, pero no en otros, por lo que un método para cuantificar la variación en el consumo de alimentos podría ayudar en la interpretación de los resultados. Los análisis de isótopos estables permiten determinar con precisión cuales son los efectos del suplemento de alimento sobre la ecología y el comportamiento de las aves.

INTRODUCTION

Experimental food supplementation in which animals are provided with resources over and above their natural foods provides a powerful approach for assessing the role of food supply in shaping behavior (Boutin 1990, Robb et al. 2008a). It may also be used to investigate how populations respond to changes in resource availability. For example, increasing urbanization provides animals with more anthropogenic resources, either intentionally through bird feeders (Chamberlain et al. 2005, Jones and Reynolds 2008) or unintentionally through domestic and industrial sources such as rubbish dumps and fishery discards (Tortosa et al. 2002), respectively. Food supplements may be provided directly to targeted individuals, allowing their consumption to be monitored (Grieco 2002), or in a more diffuse manner to multiple individuals or groups (Grubb and Cimprich 2002, Robb et al. 2008b). In the latter case the amount of supplementary food consumed by an individual can be difficult to quantify. Such information is important for researchers interested in the consequences of supplementary feeding, where use of supplements is related to variables such as individual survival or breeding success as well as to population-level effects such as variation in productivity by year. Many previous supplementation studies have not examined supplement use with rigor sufficient to account for the fidelity of focal individuals to food supplements or to assess their competitive exclusion by conspecifics and heterospecifics (Smith et al. 1980, Meretsky and Mannan 1999). An ability to quantify the use of supplementary food could help these individual differences be evaluated. In addition, it is difficult to measure variation in the extent to which individuals are using natural food sources, whose consumption often remains unmonitored, in addition to the supplementary food. Traditional methods of calculating the use of supplementary food include direct observation of the food supply (e.g., time individuals spend on a feeder; Wilson 2001) and fecal and gut-content analysis (Rosenberg and Cooper 1990). Direct observation is problematic as it is both time consuming and requires that results be extrapolated over long, unobserved periods (Wilson 2001). Fecal, pellet, and gut analyses (Trewick 1996) also have limitations, being biased toward certain food types and providing only a snapshot of dietary intake (González Solís et al. 1997). More recently, passive integrated transponder (PIT) tags have been used. In a study of the mountain hare (*Lepus timidus*) using PIT tags, Newey et al. (2009) found that only 50% of the target population made use of supplementary food and there was considerable variation in the number of visits and amount of time an individual spent at the feeders. Radio

telemetry has also been used in studies of supplementary feeding; for example, to monitor behavior following supplementary feeding of Oriental White-backed Vultures (*Gyps bengalensis*) (Gilbert et al. 2007) and changes in females' nest attendance with supplementary feeding of Northern Goshawks (*Accipiter gentilis*) (Dewey and Kennedy 2001). However, these studies used telemetry to assess changes in behavior rather than to estimate rates of consumption of the supplementary food or to measure the time an individual spent at a feeder.

Measuring the ratios of stable isotopes in consumers' tissues offers a potential solution to a number of these problems (Inger and Bearhop 2008). When applied appropriately, this approach can accurately reflect all food sources in the diet since the isotope ratio is derived from all nutrients assimilated and, depending on the turnover of the tissue chosen for analyses, reflects diet over various temporal scales (Hobson and Clark 1992). Different food items often have distinct isotopic signatures, and these are incorporated into the tissues of consumers in a predictable manner (DeNiro and Epstein 1978, 1981). Thus, by measuring the stable-isotope ratios of consumers' tissues, the relative importance of isotopically distinct food sources, such as those used in supplementary feeding studies, can be assessed (Davis et al. 2005).

A prerequisite is that supplementary food has an isotopic signature distinct from that of natural food sources. In many instances this is likely to be the case because most commercially available foods are grown via intensive agriculture (Lin 2005) and tend to be isotopically distinct from foods from nonagricultural sources such as woodlands (Hobson 1999). In addition, supplementary foods are often distinct from natural prey as they have frequently been through refining and manufacturing processes that can also alter stable-isotope ratios (Lin 2005). As a consequence, supplementary foods may be "naturally" labeled, carrying distinctive isotopic signatures that can be relatively easy to trace in the tissues of consumers.

Here, we examine the potential for using stable isotopes in food-supplementation studies by comparing the signature of food supplements commonly provided to birds with that of natural food sources. We show the potential for creating "natural" tracers by changing the isotopic signature of food items in instances where supplements have values similar to, or do not differ significantly from, natural sources. We then demonstrate the efficacy of this approach with a case study in which the individual and site-level variation in use of supplementary versus natural food is estimated by means of the isotopic ratios of claws from birds fed supplementary food over the winter. We

use the results from the data to link supplementary food consumption with characteristics such as age and sex. We might predict that older, more dominant individuals (males) may be able to monopolize food supplies and make more use of supplementary food. Meretsky and Mannan (1999) found that older adult Egyptian Vultures (*Neophron percnopterus*) exclude younger birds from supplementary food. Previous studies have also demonstrated that supplementary feeding can affect, mostly positively, measures of breeding such as date of laying and clutch size (Boutin 1990). We might, therefore, predict that the more an individual consumes, the greater the positive or negative effect on breeding success. Clamens and Isenmann (1989) found supplementary feeding of Blue Tits (*Cyanistes caeruleus*) increased clutch size in the first year of the study but not in the second, and Källander (1981) found the provision of food affected Great Tit (*Parus major*) populations positively only after a severe winter; it had no effect in a milder year when the crop of beech (*Fagus sylvatica*) mast was richer. Knowledge of the amount of supplementary food consumed may help explain such variation in results. We assess whether the amount of supplementary food consumed affects measures of breeding such as date of laying, clutch size, and brood size by comparing consumption of peanuts (*Arachis hypogaea*) over winter with breeding success in the following spring.

METHODS

CREATING TRACERS

We fed mealworms (*Tenebrio molitor*), often provided as a food supplement for birds, on a diet of 20% fish meal and 80% cereal, rather than a 100% cereal-based diet as is common practice in commercial mealworm production, to alter their isotopic signature. The $\delta^{13}\text{C}$ signatures and nitrogen-isotope signatures (expressed as $\delta^{15}\text{N}$) of marine animals are much higher than those of terrestrial animals occupying similar trophic positions (Bearhop et al. 1999). Therefore, mealworms fed fish meal will have isotopic values distinct from those of natural invertebrate prey. We also manipulated the signature of another commonly fed food item, peanuts. As C4 plants have $\delta^{13}\text{C}$ signatures higher than those of the C3 plants that dominate the vegetation in temperate regions, stable-isotope analysis can distinguish between plants using the two different photosynthetic pathways, and the animals that feed on them. To alter the C3 signature of peanuts, we soaked them in corn oil, which comes from a C4 source (*Zea mays*), resulting in them taking up the C4 signature and making them distinct from natural sources of dietary plants such as beech mast that may be important in the winter diet of birds in beech-dominated woodland (Betts 1955).

FIELD EXPERIMENT

We provided supplementary food in the form of peanuts throughout the winter (1 November–3 March 2006) at five woodland sites in County Down, Northern Ireland. All sites were predominantly deciduous woodland, were approximately 12–14 ha in

size, and were located in isolated blocks of fragmented woodland in an area dominated by agriculture. This ensured that access by birds to other sources of supplementary food (e.g., from gardens) was kept to a minimum. Trees dominating all sites were beech, sycamore (*Acer pseudoplatanus*), and oak (*Quercus robur*), and all sites were at least 3.5 km apart. Food was provided in feeders positioned at least 100 m apart along transect lines at a density of 1 ha⁻¹, averaging 12 feeders per site. We replenished feeders regularly so that food was available ad libitum. We observed and mist-netted around feeders to investigate which species were eating the supplementary food and weighed feeders approximately every 2 weeks to monitor the rate at which food was consumed. We added nest boxes to all sites at a density of 3 ha⁻¹, allowing us to monitor subsequent breeding success. It also aided our trapping of supplemented individuals following the cessation of feeding. The focal species using both feeders and occupying the majority of the nestboxes was the Blue Tit. The Blue Tit is a common resident woodland passerine in the United Kingdom, frequently observed in both urban and suburban areas and one of the species most commonly observed at feeders (Chamberlain et al. 2005).

We determined date of laying, clutch size, brood size, and fledging success by inspecting the nest boxes every 2 or 3 days from April to June. Double brooding is rare in this population of the Blue Tit, and we recorded no second broods during our study. Dates of laying were back-calculated on the assumption that one egg is laid per day (Perrins 1979) and all nests were found prior to clutch completion. At the end of the breeding season, we searched all nest boxes for unhatched eggs and dead chicks to calculate the rate of fledging success (defined as the percentage of brood successfully fledging the nest) (Svensson and Nilsson 1995).

We carried out initial analyses to confirm that the supplementary food (i.e., unmanipulated peanuts) was isotopically distinct from natural food sources and to assess whether there was a need to manipulate the signature of the supplement further. At all sites, we collected natural seeds such as beech mast as well as invertebrates, by “tree beating” (Southwood and Henderson 2000). Tree beating was an appropriate method because Blue Tits are generally arboreal, feeding mainly on insects found on the twigs, buds, and leaves of trees rather than on the ground (Betts 1955). We selected natural foods for analysis on the basis that Blue Tits feed on them (Betts 1955) and that they were present at all sites.

We trapped adult Blue Tits inside nest boxes during chick rearing as they entered to provision chicks. We recorded their age and sex and sampled a claw (~3 mm tip only) by clipping it with scissors. Claws of the Blue Tit grow at a rate of 0.04 (± 0.01 SD) mm per day, and their isotope ratios represent the diet consumed during the period of growth (Bearhop et al. 2003). Therefore, by analyzing the tip (the oldest part) of the claw removed in May or early June, we should find the isotope ratio representing food consumed over the previous winter during the time when the supplementary feeding was taking

place. We categorized the birds into two age classes, those that definitely hatched during the previous calendar year (i.e., first-year breeding birds) and older birds that hatched before the previous calendar year. We sampled the claw of each bird only once, and, to minimize disturbance and to avoid bias as a result of a pair foraging together, we sampled only one of each pair from a nest.

STABLE-ISOTOPE ANALYSIS

We dried all samples to constant mass in an oven at 80 °C, then pulverized them with a pestle and mortar and weighed a standard weight (0.7 mg) into tin capsules (except for claw material, which was cut into smaller pieces). We then measured the ratios of the stable isotopes of carbon (C) and nitrogen (N) by continuous-flow isotope-ratio mass spectrometry (CF-IRMS) involving a Costech ECS 4010 (Costech, Milan, Italy) interfaced with a Thermo Fisher Scientific (Bremen, Germany) Delta XP Plus IRMS. Results for stable-isotope ratios are expressed in the standard δ -notation as ‰. Replicate analyses of gelatine and two isotopically disparate alanine internal reference materials suggested that the reproducibility was 0.25‰ for $\delta^{15}\text{N}$ and 0.10‰ for $\delta^{13}\text{C}$.

ISOTOPE MODELING

We used the SIAR (stable-isotope analysis in R) package (Jackson et al. 2009, Parnell et al. 2010) to determine the proportions of different food sources in the diet. The output produces a range of solutions regarding the proportional contribution of each food to the diet for every individual (or claw sample). SIAR produces true probability-density functions, hence, the median value of the probability-density function represents the most probable solution and we used this value in the subsequent analysis. Trophic enrichment factors, the difference in signature between the source and the consumer as the food is incorporated into the tissues, were controlled for with estimates provided in the literature from past studies of similarly sized passerines (Hobson and Bairlein 2003, Pearson et al. 2003). Values used were +3‰ for $\Delta^{13}\text{C}$ and +4‰ for $\Delta^{15}\text{N}$. We used mean C and N isotope ratios for the peanut, natural seed, and invertebrate signatures (± 1 SD) and concentration-dependent models to incorporate variation in elemental concentration dependence in the different sources.

STATISTICAL ANALYSES

To test for isotopic differences in the samples used in the tracer experiments, we carried out multivariate analyses (MANOVAs) in SPSS 15.0 with C and N isotope values as the dependent variables. Individual variables that were significant in the MANOVAs were subjected to post hoc Tukey tests. Values presented in the Results are means ± 1 SD.

We examined the influence of age, sex, and site, as well as individual variation, on consumption of supplementary food. We tested for differences by site in the use of supplemental

food with a Kruskal–Wallis test and ran general linear mixed models (GLMMs) with the median value for the amount of supplementary food in the diet for each of the 27 individuals as the dependent variable. Then, we fitted age and sex as fixed factors and included site as a random factor. Also, we investigated the influence of the amount of supplementary food consumed upon measures of breeding (date of laying, clutch size, brood size, and number fledged) with the medium amount of peanuts consumed as an explanatory variable and site as a random factor. When looking at clutch, brood size, and numbers fledged, we also included date of laying as a covariate in order to control for timing of breeding. We used a Poisson distribution to fit the model for clutch and brood size and numbers fledged, as they were count data.

RESULTS

CREATING TRACERS

Mean values of $\delta^{15}\text{N}$ were considerably higher for mealworms fed a diet of fishmeal ($10.92 \pm 0.19\text{‰}$) than for those fed a diet of cereal only ($5.75 \pm 0.16\text{‰}$), with no overlapping values; mean values of $\delta^{13}\text{C}$ were also different (fish meal: $-26.18 \pm 0.17\text{‰}$; cereal: $-28.68 \pm 0.27\text{‰}$). Therefore, feeding mealworms a diet including fish meal rather than one of cereal only altered their isotopic signatures significantly (MANOVA: Wilk's lambda, $F_{1,8} = 1097.9$, $P < 0.001$) (Table 1). Addition of corn oil to peanuts also altered their isotopic signatures significantly (MANOVA: Wilk's lambda, $F_{1,8} = 47.28$, $P < 0.001$). Prior to the addition of corn oil, the $\delta^{13}\text{C}$ signature of the peanuts ($-28.19 \pm 0.61\text{‰}$) was not significantly different from that of natural seeds ($-28.41 \pm 0.26\text{‰}$) (post hoc Tukey comparison test: $P = 0.765$), but the addition of oil increased the $\delta^{13}\text{C}$ so that it could be distinguished from natural seeds ($-25.79 \pm 0.55\text{‰}$) (Table 1, post hoc Tukey comparison test: $P < 0.01$).

FEEDING EXPERIMENT

In terms of both the C and N isotope ratios, the peanuts provided in the feeding experiment were isotopically distinct from natural foods (seeds and invertebrates) (Fig. 1: ^{13}C ,

TABLE 1. Mean isotopic values for commonly fed food items and natural food sources.

Food item	$\delta^{15}\text{N}$	SD	$\delta^{13}\text{C}$	SD
Natural				
Seeds	-1.70	0.18	-28.41	0.26
Invertebrates	1.65	0.65	-24.32	0.57
Supplemental				
Peanuts	0.83	0.71	-28.19	0.61
Peanuts with oil	0.50	0.33	-25.79	0.55
Mealworms (corn-based diet)	5.75	0.16	-28.68	0.27
Mealworms (corn and fish-based diet)	10.92	0.19	-26.18	0.17

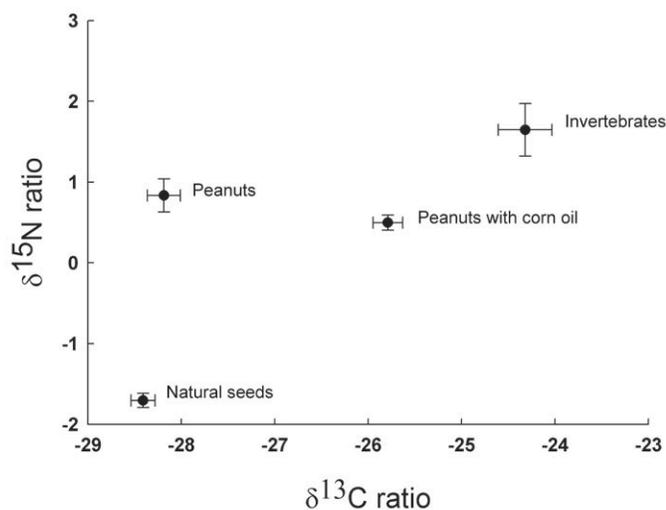


FIGURE 1. Isotopic signature of peanuts and peanuts with corn oil compared to those of the natural food sources used in the experiment. The ratios (mean \pm SE) of the stable isotopes of C and N in peanuts are significantly different from those in both natural seeds and wild invertebrates. Addition of corn oil, of C_4 origin, to peanuts, a C_3 plant, significantly changes the carbon-isotope signature (mean \pm SE).

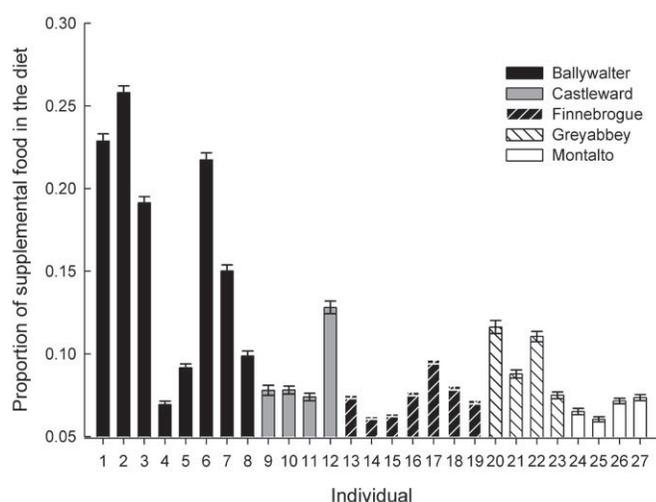


FIGURE 2. Variation in the estimated proportional use of supplementary food by individual Blue Tits during the winter of 2006, as calculated by SIAR (stable-isotope analysis in R). Bars represent the median value of the range of potential solutions regarding the contribution of supplementary food in the diet. Error bars show ± 1 SE.

$F_{3,20} = 79.14, P < 0.001$; $^{15}\text{N}, F_{3,20} = 32.18, P < 0.001$). We took claw samples from 27 breeding Blue Tits (9 males and 18 females) across all five sites, trapping four to eight individuals at each. Results from the SIAR analysis showed the contribution of supplementary food to the diet differed by individual with supplementary food constituting between 6 and 26% of an individual's diet (Fig. 2). Although sample sizes were small, there was evidence of individual variation in the use of supplementary food both between and within different sites. Site had a significant effect upon the amount of supplementary food consumed (Kruskal–Wallis test: $H_{4, \text{d.f.}} = 13.75, P = 0.008$), with overall higher use of supplementary food at one of the five sites (Ballywalter). Within-site variation was also highest at Ballywalter, although this could be due partly to increased sample size. However, at Finnebrogue a similar number of birds was sampled but less food was consumed overall and there was much less variation in consumption rates between individuals. Estimating consumption by stable-isotope analysis can be compared to estimating food consumption by weighing the amount of food removed from feeders at each of the sites. This method also showed the Ballywalter site to have one of the highest consumptions of supplementary foods. By this method, however, another site (Castle ward) also showed similar high levels of consumption (522 g of peanuts per day, compared to 511 g at Ballywalter), whereas according to stable isotopes supplementary food made up on average 16% of the diet at Ballywalter and only 9% at Castle ward.

We also considered a bird's age and sex in its consumption of supplementary food. We identified 5 birds as in their

first year and 22 as older, 18 as female and 9 as male. We found no relationship, however, between the amount of supplementary food in the diet (predicted from SIAR) and a bird's age (GLMM: $F_{1,20} = 0.002, P = 0.97$) and only a marginally significant relationship with sex (GLMM: $F_{1,20} = 3.98, P = 0.06$), with males consuming more supplemental food than females. Within this model, however, site did have a significant effect (GLMM: $F_{1,20} = 6.70, P = 0.001$).

We also considered how supplementary feeding influenced measures of breeding such as date of laying and clutch size, but we found no significant relationship between the amount of supplementary food consumed and any of these variables (date of laying: GLMM $F_{1,20} = 0.55, P = 0.47$; clutch size: GLMM $F_{1,20} = 0.30, P = 0.76$; brood size: GLMM $F_{1,20} = -1.41, P = 0.16$; number of fledglings: $F_{1,20} = 0.94, P = 0.35$). However, the sample was small (27 individuals over 5 sites).

DISCUSSION

This study highlights the potential variation between individual animals in their use of supplementary foods and describes an effective means of measuring this variation. If inferences are being made about the effects of supplementary feeding at the level of either the individual or the population, it is important that this variation be quantified and taken into account. If it is not accounted for, the effects of supplementary feeding could be masked. The results of our experiment show the potential of stable-isotope analysis for examining the foraging behavior of individuals and generating estimates of usage of supplementary food as well as

the relative use of other natural food sources. This technique offers advantages over traditional methods of investigating use of supplementary food, as it is nondestructive, all ingestion of food is accounted for, and multiple periods can be examined in a single sample. The technique minimizes the number of times animals need to be trapped and handled, which is beneficial if the importance of supplementary food during different periods of the year (e.g., during winter or breeding) is being examined.

However, the full benefits of stable-isotope analysis rely on the degree of separation of isotopic signatures of food sources. The results of the initial experiments show the potential for altering the isotopic signatures of commonly fed supplementary foods so that this can be achieved. Initial trials highlighted the importance of testing sources for isotopic separation prior to further trials. We changed the stable-isotope ratios of both the mealworms and peanuts significantly. Such alterations could be particularly useful in studies where multiple food sources are being compared where there is more potential for overlap in isotopic signatures. For the contribution of different foods to a diet to be assessed, isotopic separation between sources must be sufficient (Gannes et al. 1998, Phillips and Gregg 2001).

The most striking results are those from the over-winter provisioning experiment showing the considerable variation among individuals in their use of supplementary food (Fig. 2). This variation highlights the importance of being able to quantify intake, even if only in terms relative to other available food sources, in order to make inferences about how such supplements might influence the ecology of consumers. For example, von Brömssen and Jansson (1980) observed that birds that had second broods occupied nests that were nearer feeders than birds that had single broods, and they suggested that this could be as a result of females with nests nearer feeders using them more frequently. Stable-isotope analysis would be useful in validating this prediction.

One problem with quantifying contributions of food sources in the diet by mixing models is the issue of metabolic routing (Phillips and Koch 2002). This issue arises when different macronutrients from the diet are metabolized by different routes as they are incorporated in the tissues. These differences can lead to inaccuracies if "bulk" diet is used to estimate diet composition. Knowledge of the macronutrient composition of the food sources as well as of how these can be incorporated in the tissues is required, especially if the signatures of supplementary foods are being changed by the addition of natural tracers. For example, the addition of corn oil or fish will alter the lipid and protein composition of the supplementary foods. These changes will then be reflected in different tissues to different degrees depending on how these substances are incorporated into the body. Knowledge of both the composition of the supplemental food or diet and which parts of the diet the tissue being analyzed, for example, fur or claw, are being reflected by the isotope data is required. In particular, it is unclear how much carbon from corn oil

might be routed into keratin, and more studies are required for these types of processes to be understood and quantified. However, this problem can be reduced to a certain extent with concentration-dependent models, like those we used (Phillips and Koch 2002). A lack of understanding of the manner in which routing occurs without doubt increases the uncertainty in a model's output. A further way of dealing with this, while not addressing the issue in a mechanistic manner, would be to increase the variation associated with trophic enrichment factors used in the SIAR models. This would in turn produce outputs that had less certainty in their estimates.

As well as differences in uptake among individuals, variation among species has also been observed. Grubb and Cimprich (2002) observed that supplementary feeding increased the nutritional condition of wintering woodland birds. However, some species such as the Tufted Titmouse (*Baeolophus bicolor*) were affected more than others such as the Downy Woodpecker (*Picoides pubescens*) (Grubb and Cimprich. 2002). An ability to predict the relative contributions of supplementary foods in the diet with stable-isotope analysis would allow us to assess differences in reliance between species as well as between individuals. This may be particularly important when food is being supplemented in a diffuse and nontargeted way as is often the case in feeding experiments.

In our study we found the amount of supplementary food consumed differed by site, being highest at Ballywalter. Increased consumption at this site could be due to lower availability of natural food or a higher population density creating increased competition for resources. There was no effect of age on the amount of the supplementary food in the diet and a marginally significant effect of sex. However, this effect could have been significant if our sample size had been bigger. Previous studies have found a relationship between both age and sex and the use of supplementary food. For example, Smith et al. (1980) found young female Song Sparrows (*Melospiza melodia*) to use feeders less than either males or older females (Smith et al. 1980). This difference was due to competitive exclusion, young females suffering a higher number of displacements at feeders. An ability to correlate factors such as age and sex with the use of supplementary food, through a technique such as stable-isotope analysis, would add to our understanding of factors driving how individuals utilize food resources.

Use of stable isotopes to estimate supplementary food use could be applied to other ecological questions. For example, access to supplementary food has been linked to the expansion of the range of some species; stable-isotope analysis could be used to investigate whether this expansion is directly linked to dietary reliance on supplementary food (Orell 1989). We could also learn more about not only the consumption of supplementary food but also its nutritional and energetic quality. For example, Davis et al. (2005) were able to link the amount of the supplementary food in the diet to chick-growth rates.

Supplementary feeding studies often rely on testing the effects of the food supplement(s) through comparisons with control (i.e., unfed) animals. Although differences related to feeding site (e.g., habitat quality) can be partly alleviated through good experimental design and randomization, they may require the use of multiple sites to make the results more conclusive and to demonstrate a biological effect. An ability to quantify supplementary food consumption within a site reduces this requirement for experimental elaboration, especially if a proportion of the population within a site does not consume the supplementary food (Newey et al. 2009), making comparisons within a site possible.

Comparisons of stable-isotope analysis with a more traditional approach (i.e., weighing the amount of food removed from feeders) revealed potential differences between the methods. At the Ballywalter study site consumption of supplementary food was higher according to the stable-isotope analysis. Predicting reliance on food purely on food-depletion rates at feeders is unreliable if individuals and species using the feeder are not identified and because of marked variation in individuals' use of feeders. While advances in PIT-tag technology now allow us to identify individuals at feeders (and nest boxes), that method is still unreliable in the surveillance of food quantities consumed.

Despite its many benefits, using stable isotopes to examine the use of supplementary food does have some drawbacks. For example, it does not allow us to study the subtle behaviors of individuals at feeding stations or, if individuals are trapped and sampled after provisioning, the condition of the animals during the time of feeding. There are also potentially high costs associated with running the samples through stable-isotope analysis and the requirements for access to a laboratory with the facilities necessary for such analyses. In addition, if tissues are being used to represent food preferences during an earlier period, as in our study, there is also the problem that effective sampling is dependent on the retrapping of former feeder users. It is likely that combining stable-isotope analysis with more traditional approaches, such as observations of the foraging behavior of focal individuals, will yield the most reliable and detailed data.

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