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Characterising individual consumption of supplemental food by Mauritius parakeets as a predictor of reproductive performance and viral infection intensity

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Abstract

1. Supplemental food is often provided to threatened species in order to maintain or enhance reproductive fitness and thus population growth, but it is rarely evaluated for its impact on individual reproductive fitness and has been associated with both positive and negative consequences.
2. We used stable isotope analyses to characterise the relative proportional consumption of supplemental food and quantitative PCR to assess beak and feather disease viral infection intensity among parakeets. Life-history and nest-site data from a long-term monitoring effort was incorporated.
3. Older females benefitted the most from supplemental feeding, demonstrated by a greater reproductive uplift than younger females and there were no strong predictors of viral infection levels among nestlings.
4. Reproductive fitness, measured by the number of fledglings produced per brood, was positively associated with proportional dietary content of supplemental food among adult parakeets and breeding pairs that nested closer to feeding stations consumed more supplemental food than those nesting further away.

Synthesis and applications

Our study demonstrates that providing supplemental food can lead to an overall increase in population growth but by characterising individual consumption we reveal subtle patterns of use and differential benefits on reproductive fitness within a population. Manipulating the delivery of supplemental food may fulfil objectives associated with reducing demand on finite resources or targeting the proportion of the population that derive the most benefit, but is associated with trade-offs in population growth. This knowledge can be incorporated into adaptive management strategies that aim to fulfil specific objectives associated with species recovery and long-term viability but the relative importance of each objective must be considered.

44 **Keywords:** BFDV, diet, Mauritius, parrot, qPCR, reproductive fitness, stable isotope analysis,
45 supplemental feeding

Introduction

The provision of supplemental food to threatened species is a widely used method intended to manipulate elements of population dynamics, usually with the intention of aiding population recovery. The benefits of this practice are well documented and it has been implicated in the success of high-profile avian conservation programmes including that of the kakapo (*Strigops habroptilus*) (Clout and Craig, 1995) and California condor (*Gymnogyps californianus*) (Walters et al., 2010). Supplemental feeding is however, often implemented as a default management action under the assumption that it will benefit population recovery, but the precise costs and benefits have rarely been evaluated (Ewen et al., 2015). For example, the provision of supplemental food (hereafter "SF") has been associated with negative consequences such as increased dependence upon it, genetic adaptation, poor nutrition, disease transmission and a tendency for maintaining less productive individuals (Boutin, 1990, Blanco et al., 2011, Crates et al., 2016). Furthermore, providing food *ad-libitum* to a growing population places a high demand on finite resources leading to questions over long-term sustainability (Chauvenet et al., 2012) and provisioning is often not targeted towards those individuals in a population that are most likely to benefit from it (Ewen et al., 2015).

Providing food at communal feeders is therefore often accompanied by the simplistic assumptions that the access to, consumption, and resulting benefits of it are equally shared among individuals. Newey et al., (2009) however, discovered that only 50% of a target population of mountain hares (*Lepus timidus*) used SF and that substantial variation occurred among individuals in the number of visits to, and time spent at feeders. More recently, Crates et al., (2016) estimated individual consumption of SF in great tits (*Parus major*) and blue tits (*Cyanistes caeruleus*) revealing that younger individuals consumed more SF than adults. To fully understand how SF affects populations it is therefore essential to characterise individual use and understand how that variation predicts reproductive fitness (Robb et al., 2008).

Where local population densities increase as a result of SF use, viral transmission also becomes an important component of fitness as natural host-pathogen dynamics become altered, and the risks of

density-dependent and frequency-dependent pathogen transmission increase (Adelman et al., 2015). For example, in a recent review of supplemental feeding studies, Murray et al. (2016) noted that in 95% of 115 cases, pathogen transmission risk increased due to elevated contact rates, 77% of the studies promoted pathogen accumulation around feeders and in 80% of cases SF was considered an immunosuppressive contaminant. However, Wilcoxen et al., (2015) demonstrated that while prevalence of disease may be higher at feeders, many individuals using them revealed a greater propensity to tolerate infection due to increased immunocompetence as a result of high quality SF. In order to achieve the long-term objective of species recovery, it is therefore important to consider the implications of supplemental feeding on individual health and population viability.

Quantifying individual consumption of SF in free-living populations presents a considerable challenge for biologists. Where multiple feeding stations are employed, direct observations of individual birds are labour intensive and rely on the extrapolation of results over unobserved time and space (Robb et al., 2011). Furthermore, observations are often made from distance at feeding stations where individuals congregate and need to be identified simultaneously from individual tags such as leg rings. Alternatively the evaluation of blood and tissue samples using stable isotope analysis (SIA) can provide detailed, individual-level dietary information (Hobson and Clark 1992, Parnell et al., 2013) but requires the *a-priori* identification of potential dietary sources and relies on the existence of sufficient variation between those sources and the resulting post-assimilation ratios in body tissues (Hobson and Clark 1992, Inger and Bearhop, 2008). Used with care, SIA can effectively partition and quantify dietary sources thus providing valuable insight on individual-level patterns of SF use and the immediate and long-term population-level implications.

The Mauritius ‘echo’ parakeet (*Psittacula echo*) is an intensively studied, island-endemic species and the subject of a successful, long-term conservation recovery programme (Jones and Merton 2012; Raisin et al, 2012; Tollington et al., 2015). Supplemental food is offered *ad-libitum* and year round to this population and whilst evidence demonstrates that it increases fledging success (Tollington et al., 2013)

96 little is known regarding levels of individual consumption and how this relates to reproductive output.
97 Individuals breed for the first time in their second or third year, forming monogamous pairs that may last
98 more than 10 years. Each pair produces a single clutch of between one and four eggs and can fledge up to
99 four young. An outbreak of beak and feather disease virus (BFDV) in 2005 (Kundu et al., 2012) led to a
100 decrease in reproductive fitness in this population characterised by a marked decline in hatch success that
101 was short-lived and only apparent among breeding pairs that used SF (Tollington et al., 2015). BFDV
102 prevalence was however, not higher among individuals that used SF when compared to those that did not,
103 indicating that perhaps individual infection intensity may be an important factor.

104 Here, we use SIA to quantify (relative) individual consumption of SF among nestlings in a free-living
105 population of endangered parakeets. Further, we use these values to estimate the combined diet of each
106 breeding pair and identify the factors that predict variation in SF use. We then investigate how variation
107 between breeding pairs predicts reproductive output and test whether the benefits of this resource are
108 equally distributed. Finally, we use quantitative PCR (qPCR) to characterise individual nestling infection
109 load in order to investigate relationships between parental SF use and BFDV infection.

110 More precisely, we address the following hypotheses:

111 1. The variation in SF consumption between breeding pairs will be explained by nest-site and life-
112 history variables.
113 2. The number of fledglings produced per nesting attempt will be positively predicted by the
114 proportion of SF consumption derived from SIA of feather samples.
115 3. Nestling viral load will be positively associated with parental SF use and negatively associated
116 with nest-site distance from feeding stations.

117

Methods

Stable Isotope Analysis

Vegetation (leaf) samples from 37 plant and tree species, including all of those known to be important food species for the Mauritius parakeet (Jones et al., 2013), were collected from across their range within the Black River Gorges National Park, Mauritius. Ten independent samples of SF ('KayteeExact Parrot Pellets'; Kaytee Products Inc.) were also collected. This commercially produced parrot food consists of maize (*Zea spp*.) as the main ingredient and we therefore expect, owing to the variation in carbon fixation strategies between the two food groups (C_4 for *Zea* and C_3 for upland tropical vegetation), that $\delta^{13}C$ isotope analyses will reveal distinct signatures.

During the 2014/2015 breeding season, as part of the ongoing monitoring procedures, a small ($5mm^2$) sample of primary feather was taken from each nestling at ~45 days old. A total of 194 individuals from 85 different broods were sampled. Additionally, 20 feather samples were collected from captive individuals as a control measure. The diet of the captive individuals was closely monitored and comprised almost exclusively of the maize-based food; captive birds do not have access to natural vegetation but their diet is supplemented periodically with fruit and browse. All samples of these potential food items and feathers were subjected to stable isotope analyses as detailed in Appendix S1.

Quantification of viral load

To quantify individual BFDV infection intensity we designed a TaqMan probe-based, qPCR assay (*c.f.* Eastwood et al., 2015). We used a published sequence of the replicase gene (HQ641502.1, Kundu et al 2012), derived from this host population, and the software Beacon Designer to design primers and a fluorescent probe that amplified a 120 bp fragment of the viral genome (full details can be found in Appendix S1). Genomic host (and thus viral) DNA was extracted from whole blood samples taken from nestlings using an ammonium acetate precipitation method (Nichols et al., 2000) and qPCR to determine individual viral load was performed according to details in Appendix S1.

142 *Measures of reproductive fitness and nest-site variables*

143 Individual life-history and nest-site data were collected as part of the routine monitoring procedures
144 undertaken by the Mauritian Wildlife Foundation. During the breeding season (2014/2015) a total of 113
145 breeding attempts were identified. After discounting breeding attempts that were either a) 'recycle'
146 attempts (where initial broods had failed), b) broods that failed at egg stage, or c) inaccessible, our final
147 dataset consisted of 85 breeding attempts. Blood and feather samples were collected from a total of 194
148 nestlings from these breeding attempts. Pair reproductive fitness was characterised by the number of
149 fledglings per breeding attempt. This was determined by accessing each nest box ~10 days after the
150 predicted date of fledging and we therefore assume that any chicks that were not found deceased had
151 indeed successfully fledged.

152 Nest-site and individual life-history variables included in the dataset were: a unique identifier for each
153 nest-site, the estimated lay-date of the first egg (number of days after September 1st, determined by
154 accessing each nest to candle eggs and confirm hatch success and age of chicks), the hatch order of each
155 chick in each brood, the age and studbook identification of each female parent and the Euclidian distance
156 of each nest-site to the nearest SF station (in km). Given the lack of anthropogenic obstacles and small
157 distances involved in our study we assume that individuals do not deviate from a Euclidian path to
158 feeders.

159

Statistical procedure

Stable isotope analyses

All statistical procedures were performed using R version 3.4.3 (R core development team 2017). Initially, we used simple MANOVAs to investigate the isotopic variation between the two dietary sources by pooling values derived from SF and vegetation in order to verify that they were isotopically distinct. Raw isotopic ratios from all feather samples were then similarly analysed to confirm variation between captive and wild individuals. We used a Bayesian approach to source partitioning within a two-source stable isotope mixing model (SIMM) implemented in the R package SIAR (Parnell et al., 2008) to estimate the relative proportional contribution of different food sources to feather samples (see Supplementary Methods for details).

Brood-level variation in dietary composition and viral load

We used intraclass correlation tests on dietary proportions and viral load values to establish the within and among-brood variation in order to satisfy the assumption that siblings would reveal similar values. Nestlings were fed exclusively by their parents and therefore isotopic signatures from their feathers can be used to directly infer the combined parental diet. If intraclass correlations reveal higher within-brood than among-brood variation then this inference would not be possible. Values of individual viral load determined by qPCR were log-transformed to improve residual normality and also subjected to intraclass correlation tests to determine the within and between-brood variation. Since one of our hypotheses predicts that nestling infection load will be associated with parental SF use, we expect both of these variables to correlate highly among siblings.

Predictors of SF consumption, reproductive success and viral load

In order to address our first hypothesis, predictors of proportional SF consumption were analysed at the brood-level by using 'Nest-site ID' as a random effect in a GLMM (Generalised Linear Mixed Model) to account for the pseudoreplication of siblings. The response variable was individual dietary proportion

attributable to SF derived by SIMM and was arc-sin square-root transformed (Crawley 2012). The main explanatory predictors included were: female age (incorporating the quadratic term (Møller 2006)) and distance from nest-site to nearest feeding station. We also included as fixed covariates, the estimated lay-date of the first egg in order to reveal any relationships between supplemental feeding and breeding phenology (Arcese and Smith 1988), and subpopulation (north or south) based on previous research that supports the existence of spatially independent subpopulations (Raisin et al., 2012, Tollington et al., 2013).

We used the number of birds fledged per breeding attempt as our measure of reproductive success in a simple GLM (McDonald and White 2010) to determine the exact reproductive benefit of SF and to examine any differential effects associated with our other fixed covariates. Our main predictor variable therefore, was proportional dietary contribution of SF, averaged across nest-mates and included as a first-order interaction term with our other predictors: female age, distance to feeding station, lay date and subpopulation. We also included a brood-level value of viral load by taking the mean value of siblings.

GLMMs were used to investigate the predictors of nestling viral load using 'Nest-site ID' as a random effect. We used our previously described value of individual viral load as the response variable. The proportional consumption of SF derived from SIA, distance from each nest-site to the nearest feeding station and subpopulation were included as our main explanatory variables. We also included the following fixed covariates to account for demonstrable predictors of immunocompetence and disease susceptibility in birds: female age (Møller 2006), estimated lay date of first egg, (Hasselquist, et al. 2001) and hatch order (Saino et al. 2001).

Prior to analyses we performed extensive data exploration and derived Variance Inflation Factors (VIFs) following the protocols of Zuur et al., (2009). We standardised our predictors to avoid any biases associated with multicollinearity according to Cade (2015), and then used an information-theoretic approach to model selection (Burnham and Anderson 2002; Whittingham et al. 2006) to examine the fit of each candidate model. The R packages lme4 (Bates et al., 2010) and MuMIn (Bartoń 2016) were used

209 to perform GLMMs and model averaging respectively. Candidate models were evaluated using AIC_c and
210 final model sets were restricted to $\Delta AIC_c < 7$ before model averaging (Bolker et al., 2009, Burnham,
211 Anderson and Huyvaert 2011). Furthermore, goodness-of-fit was assessed by calculating marginal R-
212 squared values for each of our candidate models (Johnson 2014). We derived the relative importance of
213 our model covariates by calculating the AIC_c-weighted absolute t-statistic values of each model-averaged
214 coefficient (Cade 2015, Robinson et al., 2016).

215

Results

Stable isotope values

Mean values of $\delta^{13}C$ and $\delta^{15}N$ varied significantly between the two dietary sources confirming the existence of distinct isotopic signatures (MANOVA; Pillai, $F_{2,40} = 16.03$, $P < 0.001$). Supplemental pellets revealed significantly higher values of both $\delta^{13}C$ and $\delta^{15}N$ when compared to vegetation (Figure 1 and Table 1). Values derived from feathers revealed significant separation between captive and wild collected samples and substantial variation among wild individuals (MANOVA; Pillai, $F_{2,211} = 73.17$, $P < 0.001$). Values from captive individuals for both $\delta^{13}C$ and $\delta^{15}N$ were significantly greater than those for wild individuals (Figure 1 and Table 1).

The SIMM revealed that the relative dietary contribution of SF in feathers collected from captive individuals ranged from 76% to 91% and from 29% to 91% among wild-collected samples. As expected, the mean individual dietary contribution of supplemental pellets from the captive population was significantly higher than that among samples collected from the wild (captive mean = 82% ±5, wild mean = 67% ±15; Welch's T = 9.08, d.f. = 72.6, $P < 0.001$).

Brood-level variation in dietary composition and viral load

Intraclass correlations on proportional contribution of supplemental pellets demonstrated a high correlation coefficient among wild siblings and low within-brood variation (ICC = 0.97 (0.95 – 0.98), variance within = 0.001, variance among = 0.02) indicating as expected, that the diets of siblings were indistinguishable. Intraclass correlation coefficients for nestling viral load revealed surprising results suggesting that it was not associated with brood. The coefficient was effectively zero; the within-brood variance was considerably higher than the between-brood variance (ICC = 0.016 (-0.16 – 0.20), variance within = 1.77, variance among = 0.03).

Tests for multicollinearity between our model predictors revealed no correlation greater than 0.7, VIFs were < 3 for all of our variables indicating no reason to remove any of our covariates (Zuur et al., 2009).

1. *The variation in SF consumption between breeding pairs will be explained by nest-site and life-history variables.*

Our initial SIA results revealed substantial variation in dietary proportion of SF among breeding pairs (Figure 1). Model selection to identify the important predictors of this variation revealed that the top performing model by > 6 ΔAICc, with an R^2 of 0.36, contained distance to feeding station as the single explanatory covariate (Table S1). Model averaged coefficients confirmed that nest-site distance to feeding station was a strong predictor of individual SF consumption (t-statistic = 6.22) demonstrating that nestlings' dietary contribution of SF increased with proximity to the nearest feeding station (Figure 2 and Table 2). There was a small but noteworthy difference in SF consumption between the two subpopulations and the R^2 for the model containing both of these covariates was 0.40 (Tables S1 and 2). No other variables appeared in our model selection table (Table S1).

2. *The number of fledglings produced per nesting attempt will be positively predicted by the proportion of SF consumption derived from SIA of feather samples.*

Twenty-six models remained after model selection but these did not feature a clear 'best model'. The R-squared values ranged from 0.29 to 0.38 (Table S2). Model averaged coefficients revealed that the number of fledglings per breeding attempt was positively associated with proportional consumption of SF (t-statistic = 3.78). Female age also positively predicted the number of fledglings (t-statistic = 3.78). Moreover, the interaction between female age and proportion SF consumption was a significant predictor of fledgling productivity representing the third most important predictor (t-statistic = 2.87). The positive relationship between SF consumption and fledgling number was more pronounced as female age increased, indicating that supplemental feeding is disproportionately beneficial to older females (Figure 3). Lay-date was negatively associated with the number of fledglings; females that laid earlier clutches raised more fledglings (t-statistic = 1.62). Distance to feeding hoppers and subpopulation were not strong predictors of reproductive output. Full model averaged coefficients can be found in Table 3.

264 *3. Nestling viral load is positively associated with parental SF use and negatively associated with*
265 *nest-site distance from feeding stations.*

266 Viral load among nestlings was not predicted by any of our predictors. In the final candidate model set, R-
267 squared values ranged from zero (for the null model) to 0.07 reflecting the equally poor fit of all 64
268 candidates (Table S3). Model averaging demonstrated a lack of clear predictors of individual viral
269 infection intensity; model averaged coefficients were small, all had confidence intervals that included
270 zero and the model averaged t-statistics for all covariates were < 1.4 (Table 4). Of all the predictors,
271 subpopulation and lay date were the strongest, indicating that viral load tended to be higher in the
272 northern population and higher among broods produced earlier in the breeding season.

273

Discussion

Supplemental feeding increases productivity

Female age and relative proportional consumption of SF per breeding pair were both strong, positive predictors of the number of fledglings produced per breeding attempt. Importantly however, we found no relationship between SF consumption and female age suggesting that older females were able to maintain increased reproductive fitness over younger females without the need to increase their consumption of SF. Age-dependent effects on reproductive success in birds have been well-studied and have largely been associated with increased experience of older individuals in securing sufficient high quality nest sites, mates and food (Newton 1989, Oro et al., 2014). Presumably, this result reflects an element of greater experience among older females in brood provisioning, regardless of food availability.

This general result however, concealed a subtle but nonetheless significant pattern in SF use that revealed a disproportionate benefit to some individuals. The reproductive uplift provided by SF consumption was more pronounced with increasing female age; females of all ages demonstrated increased productivity with increased SF consumption but older females revealed the greatest benefit. This result perhaps suggests that food availability is less of a limiting factor for reproductive fitness among younger females than it is among older females. Providing SF to this population has clearly increased reproductive output, contributing to the recovery of this species and the ultimate objective of population growth. The implications of this strategy on long-term population viability are however, less clear. For example, some evidence suggests that offspring of older parents reveal reduced survival and recruitment (Torres et al., 2011) whilst others have shown that enlarged broods can lead to reduced individual survival (Naguib et al., 2004), increased levels of stress (Salleh Hudin et al., 2017) and that SF may in fact increase reproductive success without resulting in viable populations recovery (Peach et al., 2015). Our results are limited to data from a single breeding season and therefore an ongoing assessment of juvenile quality and long-term survival is required in this system in order to evaluate the implications of individual supplemental feeding on population-level fitness.

299

300 *Nest-site distance to feeding stations predicts SF consumption*

301 Relative dietary proportion of SF increased with proximity of nest-site to feeding stations; birds that
302 nested closer to the feeding stations consumed more SF than those nesting further away. No other
303 variables explained considerable variation in SF consumption and we found no indicators of distance
304 associated with female age, or that food provisioning advances egg laying dates as in other studies (e.g.
305 Harrison et al., 2010). Perhaps counterintuitively, in the model to describe reproductive output, nest-site
306 distance to feeders was not a strong predictor (Table 3). We were initially concerned that we had
307 introduced an element of collinearity in this model by including both the '*distance*' and '*proportion SF*'
308 variables. However, we were reassured by our extensive efforts that the level of collinearity between
309 these two variables was low enough to avoid misinterpretation in our model. To provide further
310 reassurance we repeated the model, omitted '*proportion SF*', and '*distance*' remained unimportant (Table
311 S4). This highlights unavoidable complexity in such studies: SF consumption positively predicted
312 reproductive output; distance to feeders strongly predicted SF consumption but pairs nesting closer to
313 feeders did not produce more fledglings. This initially confusing result suggests that, even though
314 distance to feeders was identified as the strongest predictor of SF consumption in our study, there are
315 likely to be numerous other, unmeasured and unknown factors that contribute to levels of supplemental
316 feeding and reproductive output including pairs' home ranges, habitat quality, seasonal variation and
317 competition at feeding stations. Furthermore, our measure of relative SF consumption was derived from
318 feather samples of nestlings and therefore describes the combined parental diet. It is therefore possible
319 that within a breeding pair there exists variation in feeding strategy (and chick provisioning) between the
320 male and female that we were unable to explain in this study.

321 *Parental consumption of SF does not predict viral load among nestlings*

322 BFDV infection intensity among nestlings was not associated with parental consumption of SF nor was it
323 strongly related to any of our nest-site or life-history variables. Our results suggested that viral load may

be weakly associated with subpopulation and lay date, relationships that may strengthen if a larger, multi-season dataset is considered.

Our approach to characterising consumption cannot precisely predict frequency of visitation to feeders or contact rates with other individuals, but one can reasonably expect individuals that consume high levels of SF to also spend more time at the feeders; especially since those that do so also occupy nest-sites that are closer to the feeders. Ideally, a range of methods should be employed in supplementary feeding studies that directly characterise the variables of interest. However, characterising both consumption and visitation or contact frequency simultaneously in a free-living bird population is accompanied by logistical constraints (such as the deployment of individual PIT tags) that often prevent comprehensive studies.

The lack of a strong relationship between supplemental feeding and pathogen infection may be explained by the results we observed for within and between-brood variation in infection intensity. These results suggest that infection intensity is not brood-related and is not associated with hatch order, perhaps indicating that infection intensity of nestlings is more closely aligned with individual life-history variables such as immunogenetic condition. Alternatively, perhaps a single assessment of viral load at a specific moment in time reflects the transient nature of BFDV infection and does not infer a current clinical infection associated with disease. Regnard et al., (2015) demonstrated that infection intensity was associated with clinical signs in Cape parrots (*Poicephalus robustus*); none of the nestlings in our study displayed signs of clinical infection and observational accounts of condition post-fledging were not recorded. Infection loads of individuals at the nestling stage may well predict post-fledging survival and future reproductive abilities but investigating this is not within the scope of this study.

Conclusion

In our study, the success of breeding pairs in terms of numbers of fledged offspring was predicted by their relative proportional consumption of supplemental food. Supplemental feeding was introduced to counter low productivity of parakeets as a result of diminished natural resources (Jones and Merton 2012) and has

fulfilled its main objective having played a significant role in preventing the extinction of this species (Butchart et al., 2006).

When food provisioning was initially introduced in this system however, there was little consideration of any indirect effects and therefore feeders were placed close to release aviaries for monitoring purposes. Our approach to quantifying variation in SF consumption among individuals has revealed that individual parakeets do not use this resource equally and do not derive equal benefit from it. Our study therefore, supports a growing recognition that the provisioning of food requires a more detailed evaluation of benefits and consequences in order that it can be applied in a more strategic manner (Ewen et al., 2015). These evaluations are difficult to implement in free-living populations owing to a variety of logistical constraints but our study has provided evidence to inform them. Reducing the overall volume of SF provided would reduce the demand on finite resources, whilst targeting supplementary feeding toward a specific portion of the population might be an appropriate management consideration if the long-term objective is to maintain population viability without the use of SF. Ultimately, any manipulation in food provisioning is associated with important trade-offs and a reduction in food provisioning will likely lead to a reduction in fecundity. It is therefore vital that the relative importance of the different objectives are considered and the conservation implications of each evaluated accordingly.

Our single-season analysis provides a snapshot of the factors that predict levels of SF use and the implications on productivity and viral infection in this population. It is therefore difficult to reach conclusions on long-term impacts of supplemental feeding in our system given these patterns because a much more comprehensive approach is needed that incorporates multiple seasons and an assessment of habitat quality. Nonetheless we have shown here that our methods, if incorporated into a multi-season study, could make valuable contributions to informing long-term strategies for recovering populations where SF is provided. By analysing stable isotopes of feathers we characterised the relative proportional consumption of SF by Mauritius parakeets to a level of detail previously unobtainable. This analysis has

373 enabled us to reveal subtle patterns in the parental use of this resource, identify the potential impacts of
374 providing SF and to offer recommendations for future research.

375

376

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Author Contributions

ST and JG conceived the idea.

ST, CJ, AG and VT designed the methodology

ST, DS and AH collected field samples

ST, JN, RM and DF performed laboratory analyses

ST analysed the data

ST and JE led the writing of the manuscript

Data Accessibility

TBC

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Table 1 Variation in raw mean isotopic signatures between dietary sources and target populations

Source	**$\delta^{13}C$**	**±SD ($\delta^{13}C$)**	**$\delta^{15}N$**	**±SD ($\delta^{15}N$)**
Supplemental pellets (n=10)	-19.52	1.19	3.07	1.43
Vegetation (n=37)	-29.73	4.31	-0.95	2.95
Target				
Wild (n=194)	-20.73	1.81	4.42	0.57
Captive (n=20)	-19.21	0.69	5.60	0.32

Table 2 Model averaged coefficients, standard errors (S.E.), confidence intervals and t-statistics (absolute, ratio and variance) from GLMM to predict relative individual dietary proportion of supplemental food. Predictors are ordered by weighted t-statistics as a measure of relative variable importance, those in bold feature coefficient estimates where confidence intervals do not cross zero.

	Estimate	**S.E.**	**CI 2.5%**	**CI 97.5%**	**T abs**	**T ratio**	**T var**
(Intercept)	0.08	0.00	0.08	0.08			
Distance	**-0.01**	**0.00**	**-0.02**	**-0.01**	**6.22**	**0.96**	**0.39**
Subpop	-0.01	0.00	-0.02	0.00	0.33	0.07	1.42

Table 3 Model averaged coefficients, standard errors (S.E.), confidence intervals and variable t-statistics (absolute, ratio and variance) from GLM to predict number of fledglings per brood. Predictors are ordered by weighted t-statistics as a measure of relative variable importance, those in bold feature coefficient estimates where confidence intervals do not cross zero.

	Estimate	**S.E.**	**CI 2.5%**	**CI 97.5%**	**T abs**	**T ratio**	**T var**
(Intercept)	2.27	0.08	2.1	2.43			
Supp food	**0.84**	**0.25**	**0.33**	**1.34**	**3.78**	**0.97**	**0.05**
Dam age	**0.61**	**0.16**	**0.28**	**0.93**	**3.78**	**0.97**	**0.05**
Lay date	**-0.33**	**0.16**	**-0.66**	**-0.01**	**1.62**	**0.42**	**0.16**
Distance	0.40	0.24	-0.09	0.89	1.10	0.28	0.30
Subpop	0.32	0.24	-0.16	0.79	0.69	0.18	0.29
Dam age: Supp	**0.92**	**0.32**	**0.29**	**1.55**	**2.87**	**0.73**	**0.02**
Lay date: Supp	-0.35	0.39	-1.12	0.42	0.21	0.06	0.12
Distance: Supp	-0.19	0.38	-0.96	0.58	0.08	0.02	0.03
Subpop: Supp	0.05	0.44	-0.82	0.92	0.02	0.01	0.01

Table 4 Model averaged coefficients, standard errors (S.E.), confidence intervals and t-statistics (absolute, ratio and variance) from GLMM to predict individual viral load. Predictors are ordered according weighted t-statistics as a measure of relative variable importance.

	Estimate	S.E.	CI 2.5%	CI 97.5%	T abs	T ratio	T var
(Intercept)	-7.02	0.09	-0.15	0.15			
Subpop	-0.31	0.29	-0.94	0.03	1.39	0.67	0.31
Lay date	-0.21	0.21	-0.67	0.02	1.25	0.62	0.29
Hatch order	-0.09	0.15	-0.54	0.13	0.51	0.26	0.22
Distance	0.10	0.20	-0.26	0.76	0.44	0.21	0.25
Dam age	-0.07	0.14	-0.55	0.17	0.40	0.22	0.19
Supp food	0.05	0.15	-0.31	0.61	0.25	0.15	0.17

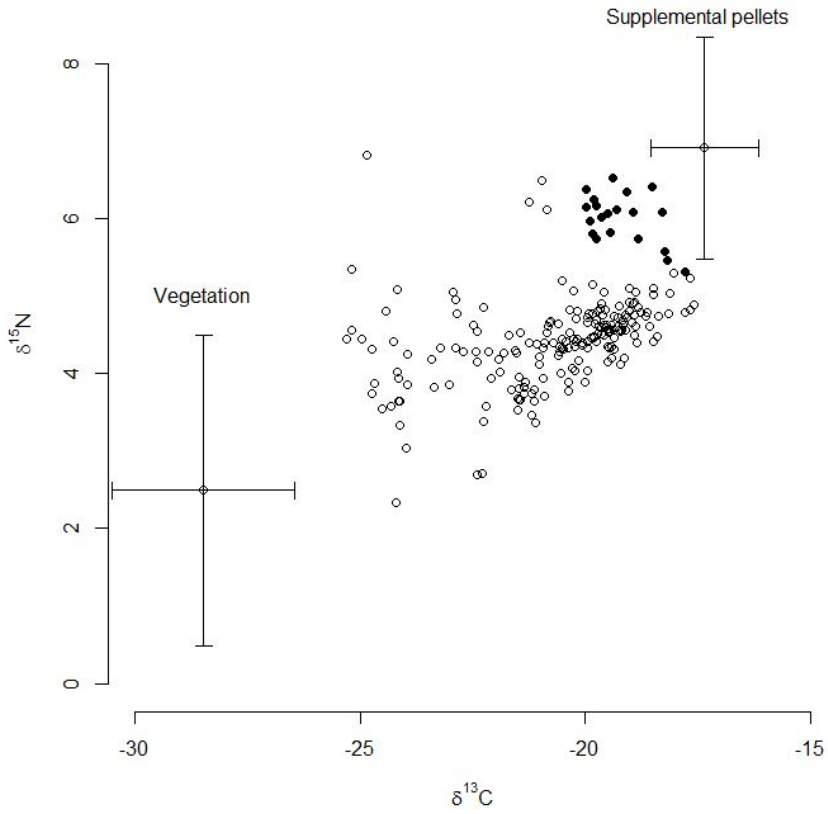

Figure 1. Mean (±SD) of raw isotopic values from dietary sources and individual values from feather samples. Captive individuals are shown in filled black circles.

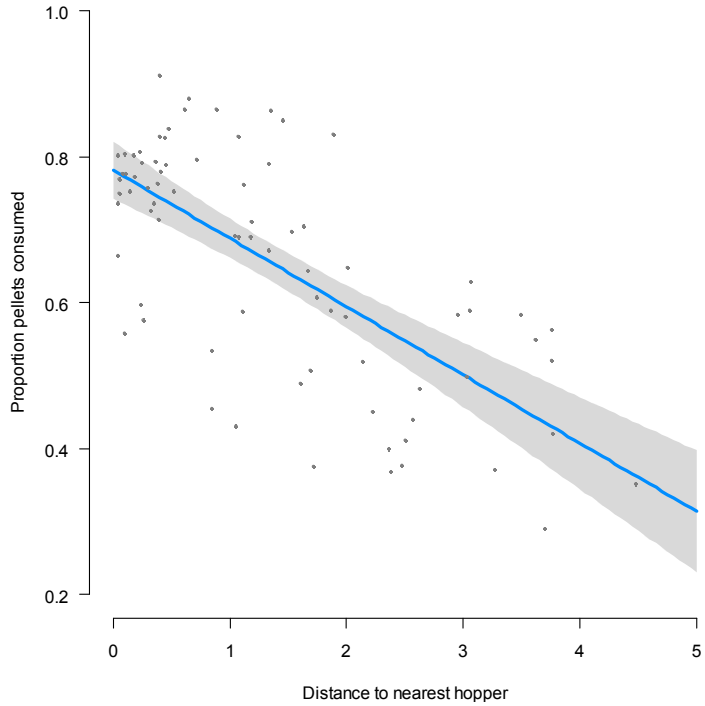

Figure 2. Average proportional consumption of supplemental food and distance in km to nearest feeding station. Each datapoint represents the combined diet of a breeding pair. (β = -0.1, S.E = 0.01, P = < 0.001, R^2 = 0.48).

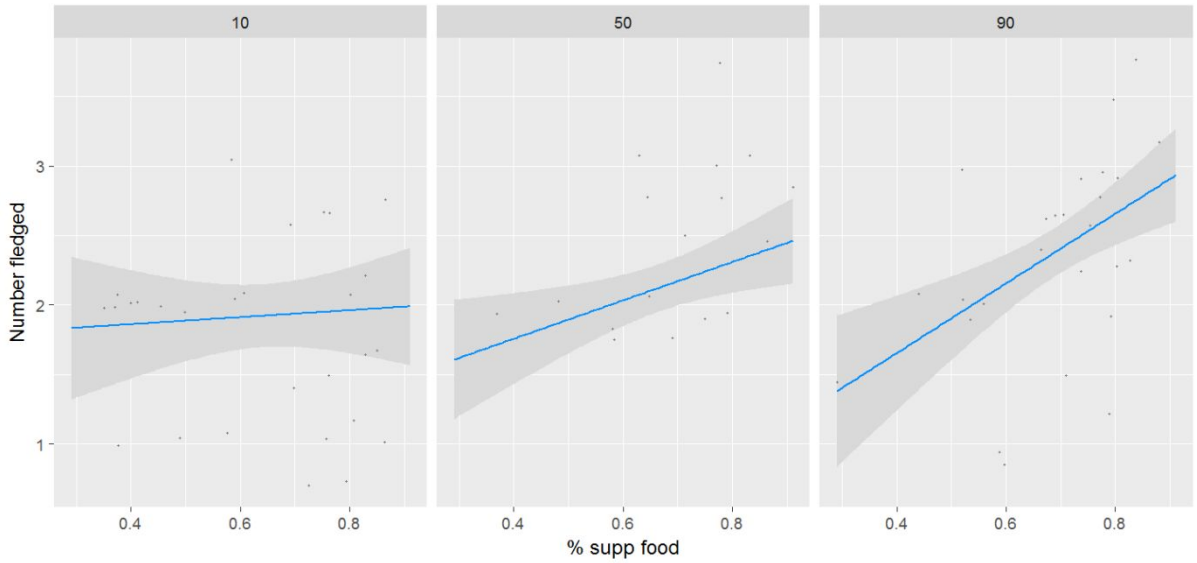

Figure 3. Interactive effect from GLMM of proportional supplemental food consumption and female age on the number of fledglings produced. Plots illustrate the relationship at the 10th, 50th and 90th quantile of female age illustrating the increasing benefit of supplemental food to productivity as female age increases.

Electronic Supplementary Material for Tollington et al., "Characterising individual consumption of supplemental food by Mauritius parakeets as a predictor of reproductive performance and viral infection intensity"

Appendix S1 – Supplementary Methods

Stable Isotope Analysis

Each dried leaf was crushed and 1.9mg weighed into tin capsules for analysis, samples of supplemental pellets weighing 0.7mg were similarly prepared. Each feather sample was washed in a 2:1 choloroform:methanol solution to remove excess dirt and oils and 0.7mg was weighed into tin capsules. Samples were analysed via continuous-flow mass spectrometry in order to derive the ratios of the stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). Replicate analyses of gelatin (n=20) in each run implied a precision (s.d.) of 0.08 ‰ for both $\delta^{13}C$ and $\delta^{15}N$. Analysis was conducted at the NERC Life Sciences Mass Spectrometry Facility, East-Kilbride, using a Costech ECS 4010 interfaced with a Thermo Fisher Scientific Delta XP Plus IRMS.

We applied isotope discrimination factors (+2.16$^{0}/_{00}$ for $\delta^{13}C$ and +3.84$^{0}/_{00}$ for $\delta^{15}N$) to our raw results following the recommendations of Caut et al., (2009). Discrimination (or trophic enrichment) factors describe the variation in isotopic composition between the tissue sample of a consumer and the dietary source as a result of trophic enrichment and physiological assimilation (Hobson and Clark 1992, Pearson et al., 2003). Estimates of fractionation factors vary according to nutritional status, taxon, tissue etc and are inherently difficult to accurately determine in free-living populations (Caut et al., 2009). Furthermore, estimates are often applied across a variety of food sources under the assumption that isotopes are enriched in a similar fashion. Studies invariably use estimates derived from controlled laboratory experiments in the absence of more accurate values which can only be calculated from exhaustive sampling of dietary sources and are therefore often unknown. Mean values (± 1 SD) for $\delta^{13}C$ and $\delta^{15}N$ isotope ratios were used for both vegetation and SF sources.

Relative proportional contribution of different food sources to feather samples was implemented in the R package SIAR (Parnell et al., 2008). SIAR uses a hierarchical Markov Chain Monte Carlo model fitting

procedure to estimate dietary proportions from a Dirichlet probability distribution (Parnell et al., 2013). Models were run for 500000 iterations with a burn-in period of 50000 and we took the mean values for each sample, from the resulting probability-density functions, to represent the individual proportional dietary contribution of SF and natural vegetation in subsequent analyses.

Quantification of viral load

The primers (Fwd: 5'-TGGGTGGCTACCTTATTG-3' and Rev: 5'-GGCTTATTGCTCGTGATAA-3') were first optimised and assay performance evaluated using serial dilutions of a positive control in a SYBR Green reaction according to a detailed Bio-Rad protocol (Bio-Rad 2006). A FAM-labelled fluorescent probe (5'FAM-CTCTGCGACCGTTACCCACA-3'TAM) incorporating a TAMRA quencher was then designed and optimised using the same protocol.

Concentrations were standardised to 10ng/µl using a Qubit 2.0 fluorometer and a high specificity assay kit (Invitrogen, ThermoFisher Scientific Inc). The qPCR protocol was designed according to iTaq Universal Probes Supermix (Bio-Rad Inc.) guidelines. Each reaction was performed in 20µl volumes and contained:

iTaq Universal Probes Supermix	10µl
Forward primer (10µM)	0.8µl
Reverse primer (10µM)	0.8µl
Probe (10µM)	0.2µl
DNA (10ng/µl)	5.0µl
DDH_2O	3.2µl

Samples were arranged in 96-well plates, each sample was duplicated and each plate contained at least two negative and two positive controls. A Bio-Rad CFX Connect real-time thermal cycler was then used

to perform qPCR analysis on each plate with the following conditions: initial denaturation of 5 min at 95°C; followed by 40 cycles of: 5 s at 95°C and 30 s at 60°C.

To calculate individual viral load we adhered to the protocol outlined by Eastwood et al., (2015) by repeating samples where the duplicate C_T values differed by more than one cycle and by normalising the difference between the plates by using the positive controls. We then used the comparative C_T method (Schmittgen and Livak 2008) to determine individual infection intensity (where ΔCT = average C_T between sample duplicates – average C_T of positive control duplicates):

$$\text{Viral load} = 2^{(-\Delta CT)}$$

Any sample that returned an average C_T of >38 cycles was attributed a viral load of zero (Eastwood et al., 2015).

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Supplementary Tables

Table S1 Model selection table and R-squared values using standardised predictors of supplemental feeding where ΔAICc <7

Model ID	Intercept	Subpop	Dam age	Hatch order	Lay date	Distance in Km	Df	logLik	AICc	ΔAICc	Weight	R^2
17	0.08					-0.01	4	665.34	-1322.43	0.00	0.92	0.36
2	0.08	-0.01					4	662.25	-1316.24	6.19	0.04	0.30
18	0.08	-0.01				-0.01	5	663.18	-1315.99	6.43	0.04	0.40

Table S2 Model selection table and R-squared values using standardised predictors of numbers of fledglings per breeding attempt where ΔAICc <7.

Model ID	Intercept	Subpop	Dam age	Distance in Km	Lay date	Supp food	Subpop: Supp food	Dam age: Supp food	Distance: Supp food	Lay date: Supp food	df	logLik	AICc	ΔAICc	Weight	R^2
95	2.25		0.64	0.46	-0.34	0.85		0.90			7	-66.20	148.18	0.00	0.17	0.37
92	2.25	0.37	0.60		-0.31	0.74		0.83			7	-66.85	149.47	1.30	0.09	0.36
96	2.25	0.24	0.64	0.37	-0.32	0.92		0.89			8	-65.58	149.48	1.30	0.09	0.38
351	2.23		0.63	0.44	-0.35	0.89		1.04		-0.33	8	-65.79	149.90	1.72	0.07	0.37
91	2.25		0.57		-0.33	0.52		0.82			6	-68.45	150.21	2.04	0.06	0.34
223	2.21		0.63	0.40	-0.36	0.87		0.91	-0.22		8	-66.00	150.33	2.15	0.06	0.37
87	2.25		0.59	0.45		0.92		0.95			6	-68.57	150.45	2.27	0.05	0.33
84	2.25	0.41	0.56			0.83		0.88			6	-68.76	150.83	2.65	0.04	0.33
348	2.23	0.36	0.59		-0.32	0.80		1.00		-0.39	8	-66.26	150.85	2.67	0.04	0.37
88	2.25	0.29	0.59	0.34		1.00		0.94			7	-67.73	151.23	3.06	0.04	0.35
352	2.23	0.25	0.63	0.34	-0.33	0.97		1.03		-0.33	9	-65.15	151.24	3.07	0.04	0.38
347	2.23		0.57		-0.35	0.60		1.00		-0.41	7	-67.83	151.44	3.26	0.03	0.34
224	2.21	0.26	0.63	0.29	-0.34	0.95		0.90	-0.25		9	-65.32	151.59	3.41	0.03	0.38
124	2.25	0.36	0.60		-0.31	0.74	-0.03	0.83			8	-66.84	152.01	3.83	0.02	0.36
83	2.25		0.53			0.60		0.87			5	-70.59	152.10	3.93	0.02	0.30
128	2.25	0.24	0.64	0.37	-0.32	0.92	-0.02	0.89			9	-65.58	152.10	3.93	0.02	0.37
479	2.21		0.63	0.41	-0.36	0.90		1.02	-0.13	-0.28	9	-65.73	152.41	4.23	0.02	0.37
215	2.24		0.59	0.43		0.93		0.95	-0.06		7	-68.55	152.88	4.70	0.02	0.33
116	2.26	0.43	0.56			0.82	0.11	0.88			7	-68.72	153.22	5.04	0.01	0.33
380	2.24	0.38	0.59		-0.32	0.80	0.07	1.01		-0.40	9	-66.25	153.45	5.27	0.01	0.36
120	2.27	0.32	0.59	0.34		1.00	0.13	0.93			8	-67.68	153.67	5.50	0.01	0.34
216	2.23	0.30	0.59	0.31		1.02		0.94	-0.11		8	-67.68	153.69	5.51	0.01	0.34
480	2.20	0.25	0.63	0.30	-0.34	0.98		1.02	-0.16	-0.28	10	-65.05	153.77	5.59	0.01	0.38
384	2.24	0.26	0.63	0.34	-0.33	0.97	0.07	1.04		-0.35	10	-65.13	153.93	5.75	0.01	0.38
256	2.22	0.32	0.62	0.26	-0.33	0.95	0.24	0.91	-0.38		10	-65.19	154.05	5.88	0.01	0.38
31	2.27		0.59	0.37	-0.38	0.72					6	-70.91	155.13	6.96	0.01	0.29

Table S3 Model selection table and R-squared values using standardised predictors of viral load where ΔAICc <7

Model ID	Intercept	Subpop	Supp food	Dam age	Hatch order	Lay date	Distance in Km	Df	logLik	AICc	ΔAICc	Weight	R^2
18	0.001498439	-0.32				-0.30		5	-229.90	470.19	0.00	0.06	0.04
50	0.000825429	-0.52				-0.29	0.28	6	-228.95	470.44	0.25	0.06	0.06
26	0.000580952	-0.34			-0.21	-0.33		6	-228.97	470.48	0.29	0.05	0.05
22	0.00075291	-0.35		-0.19		-0.27		6	-229.19	470.92	0.73	0.04	0.05
58	0.000195651	-0.52			-0.19	-0.31	0.26	7	-228.16	471.02	0.84	0.04	0.06
17	0.001112492					-0.31		4	-231.56	471.37	1.18	0.04	0.02
30	0.000256289	-0.36		-0.16	-0.19	-0.30		7	-228.47	471.65	1.46	0.03	0.06
34	0.000470614	-0.54					0.30	5	-230.68	471.75	1.56	0.03	0.03
2	0.001482964	-0.32						4	-231.75	471.75	1.57	0.03	0.02
6	0.000470529	-0.36		-0.23				5	-230.71	471.80	1.62	0.03	0.03
54	0.000522435	-0.50		-0.14		-0.27	0.23	7	-228.60	471.92	1.73	0.03	0.06
25	0.000208981				-0.18	-0.33		5	-230.84	472.06	1.87	0.02	0.03
20	0.001421686	-0.34	-0.03			-0.31		6	-229.89	472.31	2.13	0.02	0.04
52	0.000934486	-0.49	0.09			-0.28	0.32	7	-228.86	472.43	2.25	0.02	0.06
10	0.000176404	-0.34			-0.17			5	-231.11	472.61	2.42	0.02	0.03
28	0.000612182	-0.32	0.02		-0.21	-0.32		7	-228.97	472.64	2.46	0.02	0.05
19	0.001639751		0.15			-0.28		5	-231.13	472.65	2.46	0.02	0.03
21	0.000561339			-0.14		-0.29		5	-231.17	472.72	2.53	0.02	0.03
27	0.000677812		0.19		-0.22	-0.30		6	-230.11	472.74	2.56	0.02	0.04
38	7.55E-05	-0.52		-0.18			0.23	6	-230.11	472.75	2.56	0.02	0.04
60	0.000284124	-0.47	0.14		-0.21	-0.29	0.32	8	-227.92	472.76	2.57	0.02	0.07
62	8.19E-05	-0.51		-0.11	-0.18	-0.30	0.22	8	-227.92	472.77	2.59	0.02	0.07
42	-0.000490763	-0.54			-0.16		0.29	6	-230.15	472.84	2.66	0.02	0.04
1	0.000530946							3	-233.39	472.94	2.75	0.02	0.00
14	-0.00030762	-0.37		-0.21	-0.15			6	-230.26	473.05	2.86	0.02	0.04
24	0.000749302	-0.35	0.00	-0.19		-0.27		7	-229.19	473.10	2.91	0.01	0.05
36	0.000954266	-0.48	0.17				0.37	6	-230.35	473.23	3.04	0.01	0.04
49	0.001190099					-0.31	-0.02	5	-231.55	473.48	3.29	0.01	0.02
3	0.001916606		0.19					4	-232.68	473.61	3.43	0.01	0.01
23	0.000942551		0.18	-0.17		-0.25		6	-230.55	473.63	3.45	0.01	0.04
29	-4.49E-05			-0.11	-0.17	-0.31		6	-230.59	473.72	3.53	0.01	0.04
5	-0.000283729			-0.18				4	-232.76	473.76	3.58	0.01	0.01
32	0.00029368	-0.33	0.04	-0.16	-0.19	-0.29		8	-228.44	473.81	3.62	0.01	0.06
4	0.001664852	-0.30	0.04					5	-231.73	473.84	3.66	0.01	0.02
8	0.00067354	-0.32	0.06	-0.24				6	-230.66	473.85	3.67	0.01	0.03
44	-1.91E-05	-0.47	0.22		-0.19		0.38	7	-229.59	473.90	3.71	0.01	0.05
7	0.000899533		0.22	-0.22				5	-231.78	473.93	3.74	0.01	0.02
56	0.000611004	-0.47	0.09	-0.14		-0.26	0.27	8	-228.51	473.94	3.75	0.01	0.06

31	0.000356887		0.22	-0.15	-0.21	-0.27		7	-229.69	474.09	3.90	0.01	0.05
Model ID	**Intercept**	**Subpop**	**Supp food**	**Dam age**	**Hatch order**	**Lay date**	**Distance in Km**	**Df**	**logLik**	**AICc**	**ΔAICc**	**Weight**	**R^2**
9	-0.000825829				-0.15			4	-232.92	474.09	3.91	0.01	0.01
46	-0.000629383	-0.53		-0.16	-0.14		0.23	7	-229.69	474.09	3.91	0.01	0.05
11	0.000551619		0.24		-0.20			5	-231.87	474.12	3.93	0.01	0.02
57	0.000333074				-0.19	-0.33	-0.04	6	-230.80	474.14	3.95	0.01	0.03
40	0.000477348	-0.46	0.16	-0.17			0.30	7	-229.79	474.29	4.11	0.01	0.05
51	0.001468069		0.22			-0.27	0.11	6	-230.97	474.47	4.29	0.01	0.03
59	0.000535994		0.27		-0.23	-0.29	0.12	7	-229.92	474.55	4.36	0.01	0.04
64	0.000165948	-0.46	0.14	-0.11	-0.20	-0.27	0.28	9	-227.69	474.55	4.36	0.01	0.07
12	0.000483692	-0.28	0.09		-0.19			6	-231.01	474.56	4.37	0.01	0.03
53	0.000693187			-0.16		-0.28	-0.07	6	-231.08	474.68	4.50	0.01	0.03
15	2.79E-05		0.26	-0.20	-0.18			6	-231.12	474.78	4.59	0.01	0.03
16	-3.14E-05	-0.31	0.11	-0.22	-0.16			7	-230.11	474.94	4.75	0.01	0.04
35	0.001597489		0.29				0.17	5	-232.33	475.03	4.84	0.01	0.01
33	0.000594642						-0.01	4	-233.39	475.03	4.85	0.01	0.00
13	-0.001231071			-0.16	-0.13			5	-232.42	475.21	5.02	0.01	0.01
48	-0.000214865	-0.45	0.21	-0.15	-0.17		0.32	8	-229.17	475.26	5.08	0.01	0.05
43	0.000276321		0.35		-0.21		0.18	6	-231.47	475.46	5.28	0.00	0.02
61	0.000115498			-0.14	-0.17	-0.31	-0.08	7	-230.46	475.64	5.46	0.00	0.04
37	-3.37E-07			-0.21			-0.07	5	-232.66	475.70	5.51	0.00	0.01
55	0.000922837		0.21	-0.16		-0.25	0.06	7	-230.50	475.72	5.54	0.00	0.04
39	0.000855177		0.28	-0.19			0.10	6	-231.65	475.84	5.65	0.00	0.02
63	0.000319507		0.27	-0.13	-0.21	-0.27	0.08	8	-229.61	476.15	5.96	0.00	0.05
41	-0.000684096				-0.15		-0.03	5	-232.91	476.19	6.00	0.00	0.01
47	-4.77E-05		0.33	-0.17	-0.19		0.12	7	-230.96	476.62	6.44	0.00	0.03
45	-0.00090978			-0.19	-0.13		-0.08	6	-232.29	477.12	6.93	0.00	0.01

Table S4 Model averaged coefficients, standard errors (S.E.), confidence intervals and variable t-statistics (absolute, ratio and variance) from GLM to predict number of fledglings per brood omitting proportional consumption of supplemental food. Predictors are ordered according to the weighted t-statistics as a measure of relative variable importance and those in bold feature coefficient estimates where confidence intervals do not cross zero.

	Estimate	**SE**	**CI 2.50%**	**CI 97.50%**	**T abs**	**T ratio**	**T var**
(Intercept)	2.27	0.09	2.10	2.44			
Dam age	**0.58**	**0.18**	**0.22**	**0.93**	**3.21**	**0.98**	**0.03**
Lay date	**-0.46**	**0.18**	**-0.81**	**-0.11**	**2.39**	**0.72**	**0.07**
Distance	-0.14	0.19	-0.52	0.25	0.23	0.08	0.09
Subpop	-0.05	0.21	-0.47	0.37	0.08	0.03	0.02