Oecologia (2013) 172:1031–1039
DOI 10.1007/s00442-012-2570-y

POPULATION ECOLOGY - ORIGINAL RESEARCH

Food use is affected by the experience of nest predation: implications for indirect predator effects on clutch size

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Received: 23 February 2012 / Accepted: 14 December 2012 / Published online: 6 January 2013

Abstract Indirect predator effects on prey demography include any effect not attributable to direct killing and can be mediated by perceived predation risk. Though perceived predation risk clearly affects foraging, few studies have yet demonstrated that it can chronically alter food intake to an extent that affects demography. Recent studies have used stable isotopes to gauge such chronic effects. We previously reported an indirect predator effect on the size of subsequent clutches laid by song sparrows (*Melospiza melodia*). Females that experienced frequent experimental nest predation laid smaller clutches and were in poorer physiological condition compared to females not subject to nest predation. Every female was provided with unlimited supplemental food that had a distinctive ^{13}C signature. Here, we report that frequent nest predation females had lower blood $\delta^{13}C$ values, suggesting that the experience of nest predation caused them to eat less supplemental food. Females that ate less food gained less fat and were in poorer physiological condition, consistent with the effect on food use contributing to the indirect predator effect on clutch size. Tissue $\delta^{15}N$ values corroborated that clutch size was not likely constrained by endogenous resources. Finally, we report that the process of egg production evidently affects egg $\delta^{13}C$ values, and this may mask the source of nutrients to eggs. Our results indicate that perceived predation risk may impose food limitation on prey even where food is unlimited and such predator-induced food limitation ought to be added to direct killing when considering the total effect of predators on prey numbers.

Keywords Non-consumptive effects · Non-lethal predator effects · Perceived predation risk · Predator–prey interaction · Risk effect

Communicated by Stuart Bearhop.

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Introduction

Predator effects on prey demography have traditionally been attributed solely to direct killing. Recent research, however, suggests that direct killing may be only part of the equation and the total effect that predators have on prey numbers could often be due largely to indirect effects (Preisser et al. 2005; Creel and Christianson 2008; Zanette et al. 2011). Indirect predator effects on prey demography include any effect that does not involve direct killing, such as a negative effect on the 'birth rate' (i.e. number of propagules produced) or an increase in deaths from other causes, such as starvation (Creel et al. 2007; Sheriff et al. 2009; Travers et al. 2010; Zanette et al. 2011). Indirect predator effects may be mediated by perceived predation risk. Predators scare prey, and wildlife respond with a wide variety of anti-predator defences (Brown and Kotler 2004; Caro 2005) that are expected to carry nutritional, energetic,

or physiological costs that could potentially affect prey numbers (Creel et al. 2007; Sheriff et al. 2009; Zanette et al. 2011). Though long suggested by theory, few studies have yet experimentally demonstrated indirect predator effects on the demography of free-living wildlife, because of the many logistical challenges involved (reviewed in Creel and Christianson 2008; Lima 2009; Martin and Briskie 2009; Martin 2011). Only three experiments (Eggers et al. 2006; Travers et al. 2010; Zanette et al. 2011), for example, have so far demonstrated indirect predator effects on the annual 'birth rate' (number of eggs laid; Martin 1995; Zanette et al. 2006b) in birds (reviewed in Lima 2009; Martin and Briskie 2009; Martin 2011), and only one experiment to date (Zanette et al. 2011), on any wild bird or mammal, has unambiguously demonstrated that the perception of predation risk alone can also affect the death rate (reviewed in Martin 2011).

Determining how perceived predation risk can mediate prey demography can be difficult to assess because increases in anti-predator defences can be manifest in many ways leading to a variety of alterations in behaviour and physiology (Creel et al. 2009). Two recent studies have used stable isotopes to provide a signature of perceived predation risk (Christianson and Creel 2010; Hawlena and Schmitz 2010). Christianson and Creel (2010) showed that faecal nitrogen levels of elk (*Cervus elaphus)* were affected by the presence of wolves (*Canis lupus*), and Hawlena and Schmitz (2010) used faecal and body carbon and nitrogen levels to demonstrate that food intake by grasshoppers (*Melanoplus femurrubrum*) was affected by the presence of predatory spiders (*Pisurina mira*). Analysing stable isotopes in prey tissues provides a means of quantifying the cumulative net effect that predator-induced behavioural and physiological changes have on prey food intake (quantity and/or quality), because stable isotopes give an integrative, overall picture of an animal's diet (reviewed in Inger and Bearhop 2008). Stable isotopes can thus be used to track whether the perception of predation risk causes prey to chronically alter food intake to an extent that is likely to be pertinent to prey demography (Christianson and Creel 2010; Hawlena and Schmitz 2010).

In this paper, we use stable isotopes to evaluate whether and how perceived predation risk may affect the annual 'birth rate' (number of eggs laid; Martin 1995; Zanette et al. 2006b) by affecting the size of subsequent clutches laid by songbirds. To do so, we compare stable isotope values in the blood and eggs of female song sparrows (*Melospiza melodia*) that were subject to different levels of experimental nest predation. The stable isotope data reported here were collected during the course of an experiment previously described by Travers et al. (2010). Travers et al. (2010) demonstrated that females subject to frequent experimental nest predation laid smaller subsequent clutches and were in poorer physiological condition compared to females not subject to nest predation. These results are what would be expected if the experience of nest predation affects the female's perception of predation risk (Slagsvold 1982; Martin 1995; reviewed in Lima 2009; Chalfoun and Martin 2010; Travers et al. 2010). A second, non-mutually-exclusive hypothesis proposed by Travers et al. (2010) is that the indirect predator effect on clutch size that they demonstrated may have resulted from non-resource-based physiological constraints on egg production caused by the reproductive process itself (e.g. oxidative stress or hormone pleiotropy; Williams 2005).

We were able to use stable isotopes in the current study to track whether the experience of nest predation caused females to chronically alter their food intake to an extent likely to be pertinent to demography, because Travers et al. (2010) controlled for food supply by providing every individual in the experiment with ad libitum, easily accessible, high quality, supplemental food—that had a very distinctive $\delta^{13}C$ signature (since it was composed partly of millet, a C4 tropical plant; see "Materials and methods"). Because animals "are what they eat" with respect to stable isotopes (Inger and Bearhop 2008), we could thus test for treatment differences in $\delta^{13}C$ values to evaluate whether the experience of nest predation affected use of the supplemental food (others have similarly used stable isotopes to gauge the use of C4-based supplemental food; Gloutney et al. 1999; Féret et al. 2003). The degree of supplemental food use is definitely pertinent to demography because we have previously shown that this positively affects both the reproductive success and physiological profile of adults in this system (Clinchy et al. 2004; Zanette et al. 2006a, b). We also examined $\delta^{15}N$ values to corroborate physiological results reported in Travers et al. (2010) that indicated endogenous resource constraints were not likely responsible for the demonstrated indirect predator effect on clutch size. If females were catabolizing endogenous protein reserves to produce eggs (Monaghan et al. 1998; Veasey et al. 2001; but see Williams 2005), one might expect to see an increase in $\delta^{15}N$, because this effectively constitutes 'eating oneself', and thus moving up a trophic level (Hobson et al. 1993; Cherel et al. 2005; Inger and Bearhop 2008).

Materials and methods

Study area, species and general field procedures

We studied wild, free-living female song sparrows in the southern Gulf Islands in BC, Canada. The experiment described here was conducted over a single breeding season, as part of a long-term project. Details regarding sites

and sparrows can be found in Travers et al. (2010) and Zanette et al. (2006b, 2011). Briefly, egg-laying occurs from April to July, incubation normally lasts 13 days, and females may lay 2–8 clutches depending primarily on the probability of nest predation. Nests were located using behavioural cues from the mother, and we are confident we found every nest begun by every experimental subject because every subject was individually colour-banded and there were no inter-nest intervals long enough to suggest that we missed a nest (details in Travers et al. 2010).

Experimental design

The risk of nest predation was experimentally manipulated to create two treatments, females in one treatment ($n = 14$) experienced frequent artificial nest predation (FNP), whereas those in the other treatment ($n = 11$) experienced no nest predation (NNP) over the same experimental period (details in Travers et al. 2010). In both treatments, all the eggs were removed from each female's first and subsequent nests on about the 6th day of incubation. In the FNP treatment, every nest was left empty to simulate natural nest predation, causing the female to initiate a subsequent breeding attempt. In the NNP treatment, incubation of an unviable clutch was simulated by substituting artificial eggs for the female's own eggs which she then incubated for a further 11.5 ± 0.8 (mean ± SE) days, after which she abandoned and initiated a subsequent breeding attempt. This ensured that females in both treatments experienced nest-building, egg-laying and incubation, while none experienced brood-rearing. Natural nest predation was controlled for by selecting sites with low levels of nest predation (Zanette et al. 2006a) and by live-trapping to remove the two most important nest predators (raccoons, *Procyon lotor*, and brown-headed cowbirds, *Molothrus ater*). There were no significant treatment differences in initiation date, the size of the first clutch, its total mass or average egg mass (Travers et al. 2010). Both treatment groups were represented at most sites, and there was correspondingly no significant treatment difference (Mann–Whitney $U_{11,14} = 56.5$, $P = 0.27$) in the distance from the centre of each female's territory and the nearest shoreline.

To control for variation in food supply every female in the experiment was provided with ad libitum supplemental food dispensed from a feeder located at the center of her territory, from 31 March to the end of the study. The food was composed of a mixture that was high in fat, protein, carbohydrates and calcium, consisting of equal parts white proso millet and Purina Mills Aquamax Grower 400 (Purina Mills, St. Louis, MO, USA). To every kg of feed, we added 20 g of crushed oyster shell, 2.6 g of Oro Glo ® (containing 0.04 g xanthophyll carotenoids; Kemin Industries, Des Moines, IA, USA) and 60 g of whole egg powder. As mentioned in the "Introduction", we have previously demonstrated that use of this supplemental food affects the number of eggs laid (Zanette et al. 2006b), egg mass (Zanette et al. 2009), hatching success and nestling survival (Zanette et al. 2006a), and adult physiological condition (Clinchy et al. 2004).

Assessment of supplemental food use and endogenous protein catabolism using stable isotopes

Inger and Bearhop (2008) recently provided a comprehensive review of how and why $\delta^{13}C$ values from animal tissues can be used to identify diet. C4 plants have a distinctively different $\delta^{13}C$ signature compared to C3 plants (Inger and Bearhop 2008), and previous studies have taken advantage of this to directly trace the consumption of supplemental food, by provisioning birds with a predominantly C4 food in an otherwise C3 biome (e.g. Gloutney et al. 1999; Féret et al. 2003). Millet made up roughly half of the supplemental food mixture we provided. Millet is a tropical C4 plant with a $\delta^{13}C$ signature of about −13 (Yang et al. 2011), and the $\delta^{13}C$ signature of the supplemental food (−18.7 ± 1.3 ‰) clearly reflected its presence. In the region in which we worked, native plants may be expected to have an average $\delta^{13}C$ value of about −27 according to a recent review by Kohn (2010). We verified that we could use our $\delta^{13}C$ values to directly trace the consumption of the supplemental food, by comparing the blood $\delta^{13}C$ values of all of the females in our experiment (all of whom were food supplemented) with 10 non-food-supplemented birds, sampled at the same stage of the nesting cycle (8–10th day of incubation) at the same time of year.

We did not expect $\delta^{15}N$ values to vary with use of the supplement and we tested this by comparing between the food-supplemented and non-food-supplemented sparrows described above. Because $\delta^{15}N$ values did not vary with supplemental food use (see "Results"), we could use $\delta^{15}N$ values to test whether females in the two treatments were differentially catabolising endogenous protein reserves to produce eggs. Birds that fast for extended periods or experience nutritional stress in association with egg production (e.g. penguins, geese) frequently show elevated $\delta^{15}N$ values consistent with their catabolising endogenous protein reserves (Hobson et al. 1993; Cherel et al. 2005; Inger and Bearhop 2008). Travers et al. (2010) reported various physiological results which indicated that endogenous resource constraints were not likely responsible for the indirect predator effect on clutch size, and our objective

in testing for treatment differences in $\delta^{15}N$ values was to help corroborate whether this was the case.

Sampling blood and eggs and assessment of physiological condition

The blood assayed for stable isotopes was collected by capturing and sampling every female on the 8th–10th day of incubation of her last nest of the experimental period. There was no treatment difference in the date on which females were sampled (Travers et al. 2010). Upon capture, up to 300 µl of blood was collected from the brachial vein and visible subcutaneous fat was scored using a 7-point scale (Travers et al. 2010). Assays for stable isotopes were conducted on red blood cells collected following centrifugation of the blood. Centrifugation separates the red blood cells and plasma, so effects reported regarding blood $\delta^{13}C$ and $\delta^{15}N$ values are not attributable to variation in plasma lipid content (cf. Cherel et al. 2005). Whole blood and plasma were used to assess an array of physiological variables (details below). Eggs assayed for stable isotopes were collected from every subsequent clutch each female laid.

Travers et al. (2010) evaluated the effect of the experiment on 15 physiological variables: oxidative status, antioxidant capacity, carotenoids, total corticosterone, corticosteroid binding globulin (CBG), immunoglobulin, polychromasia, haematocrit, haemoglobin, free fatty acids, triglycerides, glucose, uric acid, body mass and fat score. To control for individual variation Travers et al. (2010) employed a repeated-measures approach of testing for effects on the before-treatment versus after-treatment *change* in each subject's physiological condition. The before-treatment assessment of condition was conducted before breeding began, from 6 to 28 March, and there was no treatment difference in the date on which this was conducted. To evaluate the effect of the experiment on overall condition, Travers et al. (2010) used the multivariate procedure for assessing "physiological dysregulation" developed by Seeman et al. (2001), calculated as the sum per subject of the number of physiological variables for which that subject's score falls within a specified quartile.

Food use, fat, physiological dysregulation and endogenous protein catabolism

Tissue ^{13}C enrichment indicative of supplemental food use was associated with increased fat deposition in the aforementioned studies on geese by Gloutney et al. (1999) and Féret et al. (2003), and perceived predation risk has previously been experimentally demonstrated to affect fat reserves in songbirds (Gentle and Gosler 2001). Consequently, food use and fat reserves may be expected to co-vary for both these reasons, if the experience of nest predation affects a female's perception of predation risk. Females in the frequent nest predation treatment demonstrated significantly greater physiological dysregulation and all of the physiological changes observed were consistent with this being a response to the perception of predation risk (Travers et al. 2010). If the experience of nest predation also affected supplemental food use, then food use and physiological dysregulation may also be expected to co-vary. If females are forced to 'feed' on themselves by catabolising endogenous protein reserves, as indicated by their demonstrating elevated $\delta^{15}N$ values, they may be expected to have already drawn-down their fat reserves and be in poor physiological condition (Hobson et al. 1993; Cherel et al. 2005). In which case, $\delta^{15}N$ values may be expected to co-vary with both fat and physiological dysregulation.

Stable isotope assays

Samples of supplemental food were stored dry until isotopic assays. Whole food samples were powdered in an analytical mill. Following centrifugation of whole blood, the compacted red blood cells were retained and stored frozen until being freeze-dried and powdered in preparation for isotopic analysis. Eggs were stored frozen until contents were homogenised and freeze-dried. Lipids were removed from egg homogenates using an overnight soak and multiple rinsing in a 2:1 chloroform:methanol solvent solution. The remaining lipid-free egg protein was then dried under a fume hood and powdered.

All powdered samples were loaded into tin cups and analysed for $\delta^{13}C$ and $\delta^{15}N$ via continuous flow isotope ratio mass spectrometry (CFIRMS). Specifically, between 0.5 ($\delta^{13}C$) and 1.0 mg ($\delta^{15}N$) of material was combusted using a Eurovector 3000 (Eurovector, Milan, Italy) elemental analyzer. The resulting CO_2 and N_2 analyte gas from the samples was separated by gas chromatography (GC) and introduced into a Nu Horizon (Nu Instruments, Wrexham, UK) triple-collector isotope-ratio mass-spectrometer via an open split and compared to a pure CO_2 or N_2 reference gas. Stable carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratios were expressed in delta (δ) notation, as parts per thousand (‰) deviation from the primary standards: VPDB (Vienna Pee Dee Belemnite carbonate) and atmospheric nitrogen, respectively. Using previously calibrated internal laboratory C and N standards (powdered keratin, peagrain and gelatin) within run precisions for $\delta^{13}C$ and $\delta^{15}N$ were better than ± 0.15 ‰.

Statistical analyses

We used 1-way ANOVAs to compare blood $\delta^{13}C$ and $\delta^{15}N$ values between food-supplemented and non-food-supplemented

sparrows, and to test for treatment effects. We used Spearman rank correlations to compare each female's blood $\delta^{13}C$ and $\delta^{15}N$ values with the change in her fat score and degree of physiological dysregulation. Physiological dysregulation was calculated both with fat included, as done by Travers et al. (2010), and with fat excluded, to ensure that any correlation between blood isotope values and condition reflected more than just variation in fat accumulation. We tested for treatment effects on egg $\delta^{13}C$ and $\delta^{15}N$ values using mixed model ANOVAs that included female identity as a random effect. All females laid two subsequent clutches while only those in the frequent nest predation treatment laid more. We thus used two methods to explore whether egg isotope values changed in each subsequent clutch. First, we considered only the first two subsequent clutches and used a 2-way, repeated-measures, mixed model ANOVA to test for effects due to both treatment and clutch number. Next, considering just females in the frequent nest predation treatment, we used 1-way, repeated-measures, mixed model ANOVAs to test for changes in egg isotope values across all subsequent clutches. Finally, we used Spearman rank correlations to compare each female's blood $\delta^{13}C$ and $\delta^{15}N$ values with those in the eggs of the clutch she was incubating when her blood was sampled (i.e. her final clutch). Prior to parametric analyses all data were tested for normality and homogeneity of variances. Degrees of freedom vary because of occasional sample loss. Descriptive statistics reported are mean ± SE.

Results

Supplemental food use clearly affected blood $\delta^{13}C$ values. Blood $\delta^{13}C$ values in food-supplemented sparrows (−20.6 ± 0.34 ‰) closely resembled the distinctive $\delta^{13}C$ signature of the supplemental food they were eating (−18.7 ± 1.3 ‰), and were significantly different ($F_{1,33} = 32.4$, $P < 0.001$) from that in non-food-supplemented sparrows (−24.2 ± 0.53 ‰) sampled at the same time.

Consistent with their eating significantly less of the ^{13}C-enriched supplemental food, females that experienced frequent experimental nest predation had lower blood $\delta^{13}C$ levels compared to those that did not experience nest predation (Fig. 1; $F_{1,23} = 7.0$, $P = 0.014$). Females with lower blood $\delta^{13}C$ values, indicating that they ate less of the supplemental food, gained less fat (Fig. 2a; Spearman $r = 0.51$, $t_{18} = 2.5$, $P = 0.023$), and were in poorer physiological condition (Fig. 2b); the relationship to condition being significant whether the calculation of physiological dysregulation included fat (Spearman $r = -0.71$, $t_{18} = -4.3$, $P < 0.001$) or not (Spearman $r = -0.67$, $t_{18} = -3.8$, $P = 0.001$).

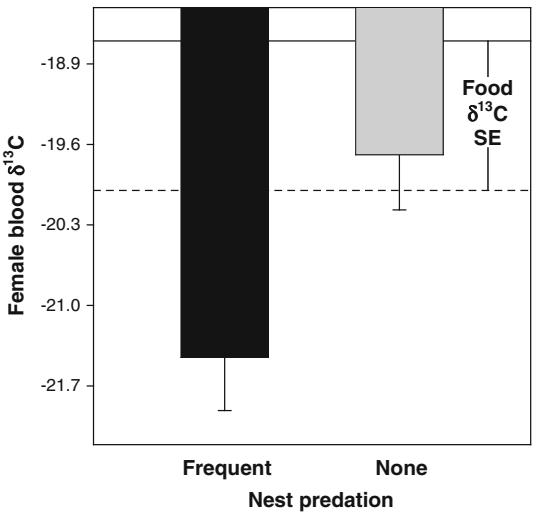

Fig. 1 Blood $\delta^{13}C$ values compared between female song sparrows (*Melospiza melodia*) that experienced frequent experimental nest predation (*black*) and those that did not experience nest predation (*grey*). Values are mean ± SE. The *horizontal solid* and *dashed lines* indicate the mean and SE of the $\delta^{13}C$ signature of the supplemental food

Though the eggs of the females in the experiment were enriched in ^{13}C (cf. blood $\delta^{13}C$ values in non-food-supplemented females), there was no significant treatment effect on egg $\delta^{13}C$ values (Fig. 3a; $F_{1,23} = 0.4$, $P = 0.533$), and no treatment by clutch number interaction ($F_{1,16} < 0.1$, $P = 0.890$). Clutch number itself affected egg $\delta^{13}C$ values, as there was a significant decrease in egg $\delta^{13}C$ values between the first and second subsequent clutches in both treatments (Fig. 3a; clutch number, $F_{1,15} = 7.5$, $P = 0.015$), and egg $\delta^{13}C$ values decreased significantly with clutch number considering all of the subsequent clutches laid by females in the frequent nest predation treatment (Fig. 3a; $F_{3,24} = 5.6$, $P = 0.005$). There was no correlation between a female's blood $\delta^{13}C$ and the $\delta^{13}C$ of the eggs of the clutch she was incubating when her blood was sampled (i.e. her final clutch; Spearman $r = 0.22$, $t_{21} = 1.0$, $P = 0.329$).

We did not expect blood $\delta^{15}N$ values to vary with use of the supplemental food and this was verified by our finding no significant difference ($F_{1,33} = 1.6$, $P = 0.215$) in blood $\delta^{15}N$ values between the food-supplemented (6.5 ± 0.33 ‰) and non-food-supplemented (6.0 ± 0.52 ‰) sparrows. The supplemental food had a $\delta^{15}N$ value of 5.7 ± 0.4 ‰.

We found no significant treatment effect on blood $\delta^{15}N$ values (FNP, 6.5 ± 0.47 ‰; NNP, 7.0 ± 0.49 ‰; $F_{1,23} = 0.5$, $P = 0.487$) and blood $\delta^{15}N$ was not correlated with either fat (Spearman $r = 0.08$, $t_{18} = 0.4$, $P = 0.725$)

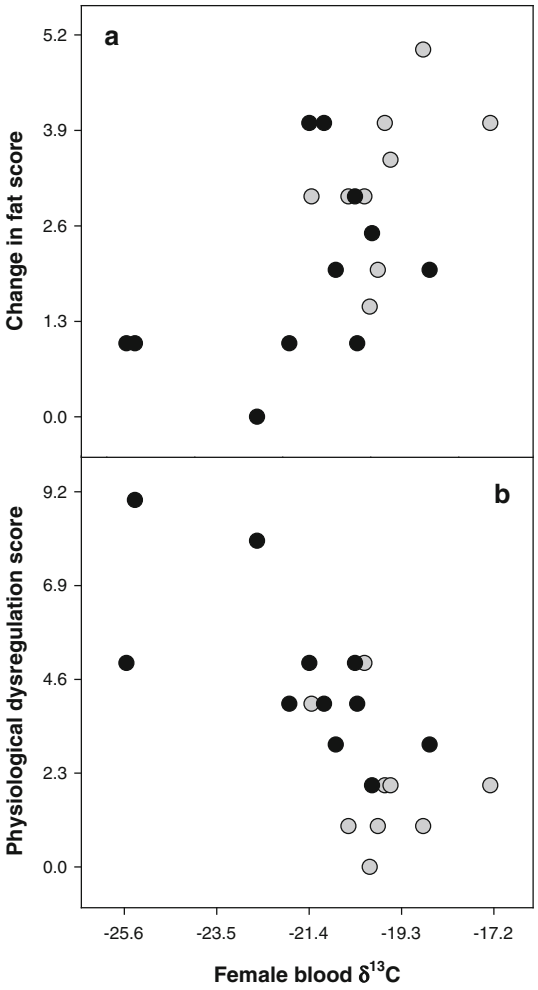

Fig. 2 Each female's blood $\delta^{13}C$ value compared to **a** the change in her fat score over the experimental period and **b** her physiological dysregulation score calculated as described by Travers et al. (2010). *Black circles* represent females that experienced frequent experimental nest predation while *grey circles* signify those that did not experience nest predation

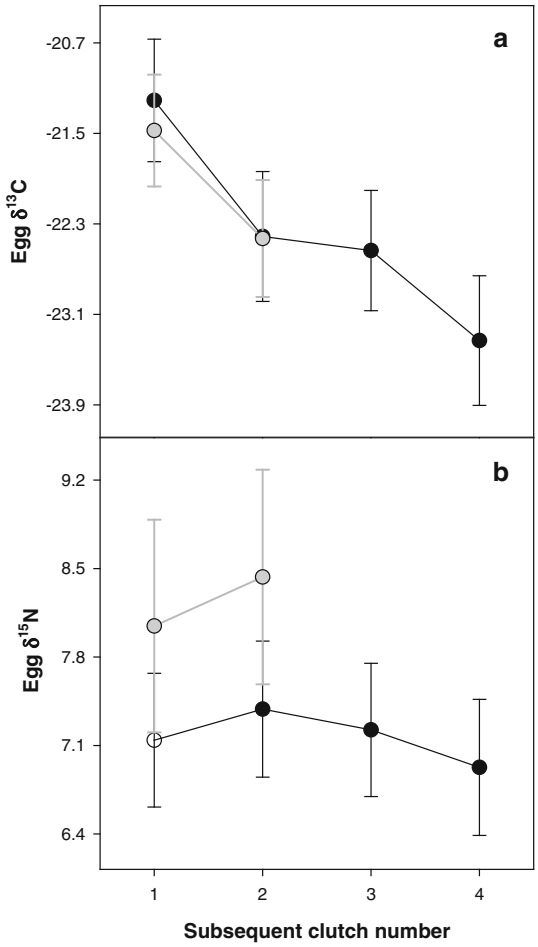

Fig. 3 Egg **a** $\delta^{13}C$ and **b** $\delta^{15}N$ values in successive subsequent clutches laid by females that experienced frequent experimental nest predation (*black*) and those that did not experience nest predation (*grey*). Values are mean ± SE

or physiological dysregulation (fat included, Spearman $r = 0.02$, $t_{18} = 0.1$, $P = 0.931$; fat excluded, Spearman $r < -0.01$, $t_{18} < -0.1$, $P = 0.972$). There was also no significant treatment effect on egg $\delta^{15}N$ (Fig. 3b; $F_{1,23} = 1.2$, $P = 0.285$), nor any effect of clutch number on egg $\delta^{15}N$, whether considering the first two subsequent clutches in both treatments (Fig. 3b; clutch number, $F_{1,15} = 2.4$, $P = 0.142$; treatment, $F_{1,22} = 0.7$, $P = 0.412$; interaction, $F_{1,15} < 0.1$, $P = 0.780$), or all of the subsequent clutches laid by females in the frequent nest predation treatment (Fig. 3b; $F_{3,24} = 0.9$, $P = 0.456$). Female blood $\delta^{15}N$ values were significantly correlated with the $\delta^{15}N$ value of the eggs of the clutch she was incubating when her blood was sampled (i.e. her final clutch; Spearman $r = 0.81$, $t_{22} = 6.5$, $P < 0.001$).

Discussion

Our experimental results indicate that food use by songbirds is affected by the experience of nest predation (Fig. 1), and that this effect on food use is associated with both reduced fat gain (Fig. 2a) and increased physiological dysregulation (Fig. 2b). This evident effect on food use is striking because: (1) there was no difference between the treatments in the physical accessibility, quality or quantity of the supplemental food provided; and (2) foregoing use of the supplemental food may be expected to come at a significant cost to an individual's reproductive success and physiological condition, as we have previously demonstrated (Clinchy et al. 2004; Rastogi et al. 2006; Zanette et al. 2006a, b, 2009). Our results are consistent with perceived predation risk being the mechanism responsible for the indirect predator effect on clutch size shown by Travers et al. (2010), but do not preclude the possibility that physiological constraints on egg production caused by

the reproductive process itself (Williams 2005) were also involved. Our $\delta^{15}N$ results corroborate the physiological results reported in Travers et al. (2010), indicating that endogenous resource constraints are not likely the mechanism responsible for the demonstrated effect on clutch size.

Because of the many logistical challenges involved, few manipulative experiments have yet tested for indirect predator effects on demography in birds and mammals (reviewed in Creel and Christianson 2008; Lima 2009; Martin and Briskie 2009; Martin 2011). To our knowledge, only two experiments (Eggers et al. 2006; Zanette et al. 2011) have demonstrated an indirect predator effect on the size of the first clutch of the season laid by birds, and Travers et al. (2010) was the first to demonstrate an indirect predator effect on the size of subsequent clutches (reviewed in Lima 2009; Martin and Briskie 2009; Martin 2011). The perception of predation risk alone was responsible for the effect on first clutches reported by Eggers et al. (2006) and Zanette et al. (2011), because in both studies it was solely due to hearing playbacks of predator calls that caused females to lay fewer eggs. Our results are consistent with the perception of predation risk also being responsible, either in part or in whole, for the effect on subsequent clutches demonstrated by Travers et al. (2010). Neither Eggers et al. (2006) nor Zanette et al. (2011) explored *how* perceived predation risk could affect the number of eggs laid, and our results suggest that effects on both food use (Fig. 1) and physiological stress (Fig. 2b) may be involved.

The perception of predation risk has been shown to affect foraging in literally hundreds of behavioural studies on numerous diverse taxa (Lima 1998; Brown and Kotler 2004; Caro 2005). What is less well established is whether perceived predation risk can chronically alter food intake to an extent that is likely to be pertinent to prey demography (Creel and Christianson 2008; Christianson and Creel 2010; Hawlena and Schmitz 2010). Christianson and Creel (2010) recently used stable isotopes to show that the presence of wolves caused a chronic alteration in food use by elk significant enough to account for a reported decrease in the birth rate of the elk (Creel et al. 2007; Creel and Christianson 2008). Our results, like Christianson and Creel's (2010), are consistent with the perception of predation risk causing a chronic alteration in food intake significant enough to result in a decrease in the birth rate. As noted in "Materials and methods", we have previously demonstrated that food-supplemented sparrows lay more eggs than non-food-supplemented birds (Zanette et al. 2006b). Consequently, the reduction in supplemental food use caused by the experience of nest predation (Fig. 1) may be expected to have contributed to the decrease in the birth rate (number of eggs laid) demonstrated by Travers et al. (2010). That the death rate may be affected by a chronic alteration in feeding behaviour caused by the perception of predation risk was demonstrated in our system by Zanette et al. (2011). Females exposed to predator playbacks fed their nestlings significantly less often, their nestlings were accordingly lighter, and a greater proportion consequently starved to death.

Physiological stress caused by the perception of predation risk has been proposed to affect the birth rate in both mammals (Boonstra et al. 1998; Sheriff et al. 2009) and birds (Scheuerlein et al. 2001; Clinchy et al. 2004). Travers et al. (2010) reported that their results were consistent with predator-induced stress being responsible for the demonstrated indirect predator effect on clutch size. Our results indicate that food use and physiological dysregulation were correlated (Fig. 2b). This correlation could be due to food use affecting physiological condition, or it could be the consequence of food use and physiological condition co-varying in response to perceived predation risk. We have previously experimentally demonstrated that supplemental food use affects physiological condition (Clinchy et al. 2004), and have shown that physiological condition is poorer where predators are more abundant (Clinchy et al. 2004, 2011). Some of the 15 physiological variables measured by Travers et al. (2010; carotenoids, total corticosterone, polychromasia) demonstrated changes that could have been due to food use affecting physiological condition, but others did not (haematocrit, free fatty acids, glucose; Clinchy et al. 2004). Thus, the relationship between food use and physiological dysregulation (Fig. 2b) was at most probably only partly a causal one, and both physiological dysregulation and food use likely contributed to the demonstrated effect on clutch size (Travers et al. 2010).

Rather than being due to a difference in supplemental food use, the treatment difference we observed in blood $\delta^{13}C$ values (Fig. 1) could conceivably have been due to there being a treatment difference in the use of some other food source, or it could be wholly a consequence of the treatment effect on physiological dysregulation. Supplemental food use clearly did affect blood $\delta^{13}C$ values, as demonstrated by the significant difference between food-supplemented and non-food-supplemented females, so neither alternative provides as parsimonious an explanation of the treatment effect on blood $\delta^{13}C$ values as does there being a treatment effect on supplemental food use. Furthermore, use of another food source appears improbable, because, although our study was conducted on continental islands, and marine and terrestrial food sources do differ in $\delta^{13}C$ (Inger and Bearhop 2008), there was no treatment difference in where female territories were located relative the nearest shoreline, as noted in "Materials and methods". Finally, though we cannot rule out physiological dysregulation itself as a cause there is no apparent reason to expect

this, in contrast to the vast literature (Lima 1998; Brown and Kotler 2004; Caro 2005), and our own results (discussed below), that would lead one to expect that the experience of nest predation likely does affect food use.

That the treatment effect on blood $\delta^{13}C$ values (Fig. 1) was a function of supplemental food use is corroborated by both the comparison to non-food-supplemented sparrows and the relationship to fat accumulation (Fig. 2a; Gloutney et al. 1999; Féret et al. 2003). Similarly, the ^{13}C-enrichment of the eggs of experimental (i.e. food-supplemented) females (Fig. 3a; overall mean ± SE = −22.2 ± 0.54 ‰) is evident in comparison to both the blood $\delta^{13}C$ values of non-food-supplemented sparrows (−24.2 ± 0.53 ‰) and egg $\delta^{13}C$ values (maximum = −26) reported in a non-food-supplemented population of another native songbird (American redstart, *Setophaga ruticilla*) studied by Langin et al. (2006). The females in our study were clearly routing their isotopes to their eggs, as shown not only by the overall ^{13}C-enrichment of their eggs but also by the significant correlation between each female's blood $\delta^{15}N$ and the $\delta^{15}N$ in her eggs.

Given the treatment effects on clutch size (Travers et al. 2010) and evident effect on food use (Fig. 1), and that egg $\delta^{13}C$ values appeared to be affected by food use, some additional mechanism must be responsible for the lack of a treatment effect on egg $\delta^{13}C$ values (Fig. 3a). The significant decrease in egg $\delta^{13}C$ values in successive clutches (Fig. 3a) suggests a reason why there was no treatment effect on egg $\delta^{13}C$, since this indicates that the more clutches (and thus the more eggs) a female lays the lower becomes the $\delta^{13}C$ value in her eggs. If $\delta^{13}C$ decreases the more eggs are laid, and this is true both within and between clutches, then this may be expected to mask a positive effect of food use on both egg $\delta^{13}C$ values and the number of eggs laid (i.e. assuming food use does result in ^{13}C enrichment, because more eggs are laid, there may be no discernible net effect on egg $\delta^{13}C$ values). Langin et al. (2006) found a comparable significant decrease in egg $\delta^{13}C$ values in successive clutches in American redstarts. The birds Langin et al. (2006) studied were not food-supplemented, and the decrease in egg $\delta^{13}C$ values in successive clutches was attributed to a change in $\delta^{13}C$ in the food available. Our results (Fig. 3a) demonstrate that egg $\delta^{13}C$ values decrease in successive clutches even when the $\delta^{13}C$ signature in the food available is constant. Consequently, the decrease in $\delta^{13}C$ in successive clutches appears to be a result of the process of egg production affecting the routing of ^{13}C to eggs. Hobson (2011) recently observed that "we have only just scratched the surface" with respect to understanding the source of nutrients to eggs, and this is corroborated by our study and Langin et al.'s (2006) study apparently being the only two to date to have examined stable isotopes in successive clutches.

We have previously reported that, compared to food-supplemented sparrows at sites with low levels of nest predation, food-supplemented sparrows at sites with high levels of nest predation lay smaller clutches (Zanette et al. 2006b), have lower hatching success and suffer more partial brood loss (Zanette et al. 2006a), and are in poorer physiological condition (Clinchy et al. 2004). All these results are consistent with the experience of nest predation affecting supplemental food use and thereby affecting demography, and our current results corroborate this experimentally. Differences in demography between food-supplemented animals subject to different levels of predation risk have similarly been reported in snowshoe hares (*Lepus americanus*; Krebs et al. 1995) and arctic ground squirrels (*Spermophilus parryii*; Karels et al. 2000), and such results have been interpreted as indicating that the perception of predation risk can cause prey to chronically alter their food intake to an extent that affects their demography (Creel and Christianson 2008). Zanette et al.'s (2011) experiment demonstrates that this can be the case with respect to the death rate (Martin 2011), and our results strongly indicate that this can also be the case with respect to the birth rate. Consequently, it is now becoming clear that, by chronically altering prey food intake, perceived predation risk may be imposing food limitation on prey even in environments where the food supply is superabundant. Such predator-induced food limitation ought thus to be added to direct killing when considering the total effect that predators have on prey numbers (Creel and Christianson 2008).

Acknowledgments R. DeCaire, K. Pagnucco and R. Rincent provided assistance in the field, and S. Bourgeon, O. Love, and E. Wagner helped in the laboratory. Thanks to Parks Canada and the Bateman family for access to the sites, T. Golumbia and B. Clinchy for their support, and S. Bearhop for providing many helpful suggestions that improved the manuscript. Stable isotopes were assayed by G. Koehler at the Stable Isotope Laboratory of Environment Canada in Saskatoon, Canada. B.X. Mora Alvarez assisted in preparing samples for isotopic analysis.. Isotopic analyses were funded by an Environment Canada operating grant to K.A.H. All other aspects of the work were funded by NSERC grants to T.D.W. and L.Y.Z. in addition to a Premier's Research Excellence Award and Canada Foundation for Innovation grant to L.Y.Z. This work was approved by the Animal Care Committee of the University of Western Ontario and conforms to the legal requirements of Canada.

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