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ORIGINAL PAPER

Nest predation, food, and female age explain seasonal declines in clutch size

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Abstract The selection pressures responsible for intra- and interspecific variation in avian clutch size have been debated for over half a century. Seasonal declines in clutch size represent one of the most robust patterns in avian systems, yet despite extensive research on the subject, the mechanisms underlying this pattern remain largely unknown. We tested a combination of experimental and observational predictions to evaluate ten hypotheses, representing both evolutionary and proximate mechanisms proposed to explain seasonal declines in avian clutch size. In line with long held life-history theory, we found strong support for both an evolved and proximate response to food availability for young. We also found evidence consistent with predictions that proximate level experiential nest predation influences seasonal declines in clutch size. Finally, older females appear to invest more in reproduction (initiate nests earlier and lay larger clutches) and choose better territories than younger females. Our results highlight the importance of examining multiple hypotheses in a theoretical context to elucidate the ecological processes underlying commonly observed patterns in life history.

Keywords Clutch size · Seasonality · Food abundance · Nest predation · Female age

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Introduction

A critical issue in understanding life history evolution is identifying sources of selection that shape patterns and trends across diverse taxa (Roff 1992). Clutch size varies markedly both within and among species and has implications to individual fitness (Pettifor et al. 1988; Martin et al. 2000). Hence, studies designed to identify processes that explain variation in clutch size play a key role in our understanding of life history evolution (Roff 1992, 2002; Stearns 1992). Theoretical and empirical studies demonstrate the importance of food availability (Perrins and McCleery 1989; Aparicio 1994) and nest predation (Smith 1993; Wheelwright and Schultz 1994; Martin 2004; Travers et al. 2010) in shaping clutch size expression. However, it is likely that multiple sources of selection (numerous intrinsic and extrinsic factors) operate synergistically in shaping clutch size expression (Jetz et al. 2008).

The pattern of a seasonal decline in clutch size is among the most ubiquitous and well-studied patterns in avian ecology (Murphy 1986; Perrins and McCleery 1989; Hochachka 1990), but the underlying causes of this pattern remain debated. Numerous hypotheses are proposed to explain why avian clutch size declines seasonally, including food availability (Perrins and McCleery 1989; Aparicio 1994), adult mortality (Martin 2004), juvenile mortality (Smith 1993; Wheelwright and Schultz 1994), and even mechanisms related to age of breeding females (Saether 1990; Martin 1995a, b), among others. However, results of past studies show almost equivocal support for, and rejection of, competing hypotheses (e.g., female age Slagsvold and Lifjeld 1988; Wheelwright and Schultz 1994; Winkler and Allen 1996 versus Hamann and Cooke 1989; Sjöberg 1994; Blums et al. 1997). The ambiguity is in part because most studies are limited to testing one or two hypotheses, which can be misleading as competing hypotheses in evolutionary ecology are not typically mutually exclusive (Platt 1964). Moreover, there is inherent complexity in isolating the degree to which variation in clutch size expression is attributable to proximate versus evolutionary responses (Martin 1995a, b). Like the majority of life history traits, clutch size expression is the result of phenotypic adjustment within a broader life history strategy. The evolutionary factors shaping clutch size place bounds upon which proximate ecological conditions lead to phenotypic variation. Understanding the degree to which the seasonal decline in clutch size is due to proximate constraints or an evolved strategy will aid significantly in our understanding of life history evolution. Here, we summarize five selection pressures represented by ten of the most common hypotheses proposed to explain seasonal declines in clutch size and simultaneously test 15 predictions (Table 1) within a framework that considers both proximate and ultimate causation.

Food (evolutionary and proximate)

Egg production and nestling-feeding are costly activities which require substantial food resources (Williams 1966). Thus, it is not surprising that variation in clutch size is commonly attributed to variation in food abundance (Perrins and McCleery 1989; Aparicio 1994). However, food availability may act through both ultimate and proximate processes. If food availability consistently declines (i.e., across years) throughout the breeding season, we would expect an adaptive response among females whereby females lay smaller clutches late in the season because food is predictably less abundant. This hypothesis suggests an adaptive evolutionary strategy to a predictable seasonal reduction in food availability for egg-production or nestling rearing (Lack 1954). In contrast, food may act proximately if food availability during egg-production or even nestling feeding constrains

Table 1 Predictions of ten hypotheses to explain seasonal declines in clutch size

Sources of selection	Hypotheses	Predictions
Food	Food for egg-production	1) *Evolutionary*: Food abundance will decline during the time-frame when females in the population are producing eggs
	Food for nestling-feeding	2) *Evolutionary*: Food abundance will decline during the time-frame when parents in the population are feeding young
	Food for egg-production	3) *Proximate*: Food available in an individual territory during egg production will be positively associated with clutch size
	Food for nestling-feeding	4) *Proximate*: Food available in an individual territory during the nestling period will be positively associated with clutch size
Nest predation	Seasonal nest-predation	5) *Evolutionary*: Probability of daily nest predation will increase seasonally
	Experiential nest-predation	6) *Proximate*: If nests of banded females are force-failed, then females will reduce clutch size in subsequent nesting attempts more so than predicted by the population-wide seasonal decline in clutch size
Female age	Age-dependent reproductive investment	7) *Evolutionary*: Older females will initiate nests earlier than younger females 8) *Evolutionary*: Older females will lay larger clutches than younger females
	Age-dependent territory quality	9) *Proximate*: Older females will nest on territories with higher food abundance 10) *Proximate*: Older females will nest on territories with lower nest predation risk
Trade-offs with future life stages	Time-limitation	11) *Evolutionary*: Nestling mass at pin break will increase with date 12) *Evolutionary*: Clutch size of late season nests will be negatively correlated with nestling mass
Intra-seasonal cost of reproduction	Cost-of-reproduction	13) *Proximate*: Body condition of breeding females will decline with date 14) *Proximate*: Average egg mass of experimentally depredated females will decline from first to second nesting attempts 15) *Proximate*: The number of days needed for females to renest after experimental depredation will be greater than the physiological minimum (5 days)

clutch size. In this case, the seasonal decline in clutch size is a proximate response by individual females nestling later in the season to less food for egg production (Aparicio 1994), or given a proximate indicator of future food conditions (i.e. leaf phenology; Nilsson and Källander 2006), for rearing dependent young.

Nest predation (evolutionary and proximate)

In most avian systems, nest predation is the primary cause of reproductive failure (Ricklefs 1969; Martin 1995a, b) and therefore an important selection agent that can clearly shape avian reproductive strategies (e.g. Martin et al. 2000). Like food availability, nest predation

has the potential to act through both ultimate and proximate mechanisms. In many locations, nest predation increases seasonally (Price et al. 1988; Perrins 1996). If nest predation risk predictably increases throughout the breeding season (and across years), then we would expect an adaptive response whereby females lay smaller clutches late in the season to avoid the costs associated with nest loss (Lepage et al. 2000). However, individual birds also appear capable of assessing nest predation risk and adjusting reproductive strategies to minimize the costs of nest failure (Fontaine and Martin 2006a; Lima 2009; Travers et al. 2010). While the proximate cues underlying these decisions remain largely unknown, failure of an entire clutch would seem to be a highly reliable cue indicating a high-risk environment. This proximate hypothesis implies that a reduction in clutch size is a plastic response to a perceived increase in risk whereby individual females employ a bet-hedging strategy to reduce the costs of nest failure (Siikamäki 1998; Nilsson 2000). Hence, this hypothesis posits that the seasonal decline in clutch size that we detect at a population level is a manifestation of the increasing proportion of second nesting attempts with smaller clutch sizes (after experiencing nest predation). This hypothesis cannot explain seasonal declines in clutch size among individual birds initiating a second brood after a successful first nesting attempt or a reduced clutch size for individuals initiating their first clutch late in the season. Hence, this proximate hypothesis assumes the pattern is caused by differences between successive breeding attempts by the same birds (rather than any variation among individuals).

Female age (evolutionary and proximate)

Life history theory predicts that reproductive investment should increase with age as a result of diminishing reproductive potential (Stearns 1992; Roff 2002). Thus, one explanation for the seasonal decline in clutch size, is an adaptive evolutionary strategy such that older individuals breed earlier and lay larger clutches (Hochachka 1990; Smith 1993). This evolutionary hypothesis assumes that earlier breeding and increased clutch size both indicate increased reproductive effort. Although there are costs to females that arrive and breed earlier (e.g. Decker and Conway 2009), there are also potential benefits (i.e., higher probability of young surviving to reproduce, Drent and Daan 1980). The female-age hypothesis is one of the most commonly tested hypotheses to explain the seasonal decline in avian clutch size; however, the proximate mechanisms behind why older females lay larger clutches earlier in the season often go untested. There is evidence to suggest that birds are capable of assessing territory-level quality and differentially selecting breeding sites either to maximize their energetic needs (e.g. Johnson and Sherry 2001; Borgmann et al. 2004) or reduce nest predation risk (Fontaine and Martin 2006b; Lima 2009; Travers et al. 2010). Given the importance of food and nest predation, it is reasonable that females that arrive and initiate nests earlier (older females) are choosing nesting territories with greater food resources and/or territories that are safer (indicating high quality territories). This proximate hypothesis suggests that the seasonal decline in clutch size is a result of a proximate response by individual females that arrive earlier having more resources available for egg production and/or safer nesting sites, either of which facilitate increased reproductive effort (e.g., Fontaine and Martin 2006a, b).

Time-limitation (evolutionary)

In temperate regions, breeding seasons are highly constrained by seasonality and the need to progress to future life stages (e.g. molt, migration, food stashing). As the breeding

season progresses, the trade-off between reproductive investment and investment in future life stages can be substantial (Siikamäki et al. 1994). Thus, the seasonal decline in clutch size may reflect an adaptive response, whereby females invest less in current reproduction later in the season in order to save time and energy costs for future stages (molt, migration, etc.). The advantage of laying a smaller clutch late in the season is that it shortens the entire nesting period and reduces investment, allowing parents to trade-off reproductive investment with investment in future life stages. If individuals do not trade-off reproductive investment by reducing clutch size (i.e., those that did not follow the population-wide pattern of laying fewer eggs late in the season), we might expect trade-offs in other aspects of reproduction (i.e., nestling feeding rates), which could result in reduced offspring quality.

Costs-of-reproduction (proximate)

Cost of reproduction is a central concept in life history theory, whereby investment in current reproduction constrains investment in future reproductive attempts, even within the same breeding season (Williams 1966; Charnow and Krebs 1974). Nest failure represents a significant loss of time and energy that is exacerbated by the limited opportunity to mitigate these energetic costs before attempting to renest. This hypothesis implies that the seasonal decline in clutch size reflects a seasonal reduction in female condition following nest failure (Price et al. 1988; the "condition hypothesis" in Winkler and Allen 1996). Indeed, both initiation date and clutch size are associated with body condition in waterfowl (Cooke et al. 1984; Blums et al. 1997), but the relationship is less clear in songbirds (Slagsvold and Lifjeld 1988; Winkler and Allen 1996). This hypothesis relies on a proximate mechanism (decline in body condition as a direct result of the energy expended on a prior nesting attempt) and assumes that the seasonal decline in clutch size reflects a constraint rather than an adaptive response to selective pressures. The hypothesis posits that the pattern is caused by differences between successive breeding attempts by the same birds (rather than any variation among individuals). However, support for this hypothesis may reflect the alternative that individuals reproducing early are in better condition and those reproducing later in poorer condition and may not indicate an actual decline in body condition. Studies assessing seasonal condition changes within individuals are required to separate these two mechanisms.

Materials and methods

Study system

From April through July of 2008 and 2009, we studied clutch size expression in red-faced warblers (*Cardellina rubrifrons*) nesting in the Coronado National Forest, Santa Catalina Mountains, Pima County, Arizona (32°43′ N, 110°76′ W). Our site consisted of four, 16- to 20-ha study plots in riparian drainages within mixed-conifer forest (2,300–2,800 m elevation); however, since study plot did not influence our results, data were lumped for analysis. Red-faced warblers are a small (9.8 g; Martin and Barber 1995), single-brooded, short-distance migratory songbirds that show a seasonal decline in clutch size at this site (based on an ordinal logistic regression with clutch size as the response variable and nest initiation date and year (random factor) as explanatory variables).

General sampling procedures

We located and monitored nests using long-standing techniques (Martin and Geupel 1993) by visiting each nest a minimum of every 2 days. Most nests were located during the building stage enabling precise identification of nesting stages, beginning with nest initiation (the day that the first egg was laid) which is typically 5–6 days after building begins. At a subset of nests (approximately 50%) we captured adult females by placing a 6-m mist-net 2–4 m from their nest and either flushing the female from the nest or waiting for her to return following an off-bout. All captured females were given a unique color-band combination, aged as second-year or after-second-year (see below), and weighed. For nests found prior to clutch completion, eggs were weighed within 2 days of clutch completion and when possible we also weighed nestlings on day six of the nestling period (pin-break). We used a digital scale (±0.001 g) for all measurements. To limit the influence of missing data on nest predation estimates we only calculated nest predation rates for nests found prior to clutch completion (following Fontaine et al. 2007) and calculated nest predation rate as 1-number of days survived/number of days in the nesting period. Due to differences in data collection we use a different subset of nests to test our 15 predictions (see Table 1), which precluded us from evaluating all predictions simultaneously in one analytical model (or in a single information-theoretic framework). However, this study is the first effort to test predictions of ten of these hypotheses within the same study system.

Hypotheses examined

Food for egg-production/nestling-feeding (evolutionary)

We examined if an adaptive evolutionary strategy to a predictable seasonal reduction in food availability (for egg-production or nestling-feeding) explains seasonal declines in clutch size. To do this, we measured food abundance weekly beginning 2 weeks prior to when the first egg is typically laid (20 April) until the last nestling typically fledges (9 July) in our study area. Lepidoptera larvae are the primary food for adult and nestling red-faced warblers during the breeding season, comprising nearly 80% of their diet (Martin and Barber 1995; K. Decker, unpublished data). We estimated food abundance by establishing eight sampling locations within each study plot from which we collected 25 cm branch clippings from typical foraging sites (branch ends from 0.25 to 4 m in height; Martin and Barber 1995; K. Decker,unpublished data) on fir (*Pseudotsuga menziessi*) and maple trees (*Acer grandidentatum*) for a total of 128 branch clippings/week. We counted the number of larvae on each branch clipping and corrected for surface area by placing each branch clipping on a grid board (30 cm × 30 cm) and recording the number of squares (out of 144) obstructed by leaves or branches (Decker and Fontaine, unpublished data). Prior to analysis, we normalized the data by applying a square-root-transformation.

We conducted two separate linear regression analyses to examine seasonal trends in food abundance during the egg-production period (approx. 26 April–8 June) and the nestling-feeding period (approx. 13 May–13 July) with food abundance (during the egg-production or nestling-feeding period) as the response variable and date and year as explanatory variables. We tested the prediction that if food abundance for egg-production drives seasonal declines in clutch size, then food abundance would decline seasonally during the time-frame when females in the population were producing eggs (Table 1, prediction 1). Alternatively, if food abundance for nestlings drives seasonal declines in

clutch size then food abundance would decline during the time-frame when parents in the population were feeding nestlings (Table 1, prediction 2).

Food for egg-production/nestling-feeding (proximate)

We examined if the seasonal decline in clutch size is a proximate response by individual females nesting later in the season because less food is available for egg production, or for nestling feeding (given a proximate indicator of future food conditions). We sampled larvae from four maple trees and four fir trees within known nesting territories 1 week prior to nest initiation and on day 6 of the nestling period. We used known nesting territories based on the location of nest sites from the previous 7 years on these same study plots to identify larvae sampling areas for examining food available for egg-production because samples were collected prior to nest initiation (to represent the time when females were gaining energy for egg production). We used two separate ordinal logistic regression analyses with clutch size as the response variable and food abundance during egg-production or nestling-feeding (at the territory level), year, and female age as explanatory variables to test whether food available for egg production was associated with clutch size; predicting a positive association between food abundance during egg-production and clutch size (Table 1, prediction 3). We also predicted that if food available for nestlings explains the seasonal decline in clutch size, then food abundance during the nestling period will be positively associated with clutch size (Table 1, prediction 4).

Seasonal nest predation (evolutionary)

We examined if seasonal declines in clutch size are due to an adaptive response among females to consistent seasonal increases in nest predation risk. We used a linear regression analysis with nest predation rate as the response variable and nest initiation date as the explanatory variable. If clutch size declines in response to seasonal changes in nest predation risk, we predicted that the probability of daily nest predation would increase seasonally and that clutch size would be negatively associated with the risk of nest predation (Table 1, prediction 5).

Experiential nest predation (proximate)

We examined if individual females respond to experimental nest predation by reducing clutch size. We mimicked nest predation by experimentally depredating the first attempt of a subset of nests of color banded females to compare clutch size between nesting attempts. We also recorded clutch size of five renests in 2009 for females banded the previous year that experienced natural nest predation on first nesting attempts. Only nests that failed within 2 days of clutch completion were considered to control for potential costs of reproduction (see *Cost-of-reproduction hypothesis* below; Stearns 1992; Roff 2002). We used a paired *t*-test to examine differences in clutch size between nesting attempts of individual females, predicting a decrease in clutch size when renesting. We also calculated the predicted change in clutch size (per day) for females involved in the force-fail experiment and compared the average with the calculated change in clutch size among females in the population (including all nesting attempts) during the same time period (i.e., the overall rate of seasonal decline in clutch size for the population). We used a paired *t*-test to test the prediction that if nests are force-failed, then females will reduce clutch size in the subsequent nesting attempt more than predicted by the average rate of decline in clutch size of the population (Table 1, prediction 6).

Female age–reproductive investment (evolutionary)

We examined if seasonal declines in clutch size are an adaptive evolutionary strategy such that older individuals breed earlier and lay larger clutches (increased reproductive investment with age due to diminishing reproductive potential). We classified females as after-second-year (ASY) or second-year (SY) birds based on plumage characteristics (Pyle 1997), and tested the prediction that older females will initiate nests earlier and lay larger clutches than younger females using two separate ANOVA analyses with female age and year as explanatory variables (Table 1, prediction 7 and 8).

Female age–territory quality (proximate)

We examined if older females nest on higher quality territories (in response to a proximate cue) and thus are able to lay larger clutches than younger females. As indices of territory quality, we tested the prediction that older females nest on territories with higher food abundance and/or lower nest predation risk using two ANOVA analyses with food abundance/nest predation risk as the response variable and female age and year as explanatory variables (Table 1, prediction 9 and 10).

Time-limitation (evolutionary)

We examined whether females trade-off between reproductive investment and investment in future life stages due to limited time available during the breeding season. We used linear regression analysis with per-nestling mass (as a proxy for nestling quality) as the response variable and date, year, and female age, as explanatory variables to test whether nestling mass was positively associated with date (Table 1, prediction 11). We predicted that nestling mass would increase throughout the breeding season because females trade-off the number of offspring for quality of offspring. A reduction in number of offspring (but not quality) allows females to reduce investment in rearing young while investing more in themselves to prepare for other life stages. We then used Pearson's correlation analysis to test for a correlation between nestling mass and clutch size for nests initiated during the second half of the breeding season only (controlling for year and female age; Table 1, prediction 12). We predicted a negative correlation between clutch size and nestling mass for nests initiated late in the season (i.e., those that did not follow the population-wide pattern of laying fewer eggs late in the season) which would result in reduced quality (mass of) offspring.

Cost-of-reproduction (proximate)

We examined whether costs associated with nest failure early in the season constrain females to laying smaller clutches later in the season. We used linear regression analysis with female mass as the response variable and date and year as explanatory variables to test the prediction that female body condition (mass) at the time of clutch completion declined seasonally (Table 1, prediction 13). In addition, we used a paired *t*-test to test the prediction that in experimentally depredated nests (see methods within the *Nest Predation Hypothesis*), egg mass would decline between first and second nesting attempts of the same female (Table 1, prediction 14). Finally, we calculated the average number of days between the first and second nesting attempts of experimental females and compared this to the physiological minimum number of days (five) required for songbird oocytes to reach

maturity (von Haartmann 1990). We examined the prediction that the number of days needed for experimental females to renest will be greater than the physiological minimum number of days (Table 1, prediction 15).

Results

In 2008 and 2009, we monitored 123 red-faced warbler nests and found a seasonal decline in clutch size (Ordinal logistic regression: Wald's $\chi^2 = 14.81$, $P < 0.001$). We examined a total of ten hypotheses (five evolutionary and five proximate explanations) and tested 15 predictions to explain this seasonal clutch size pattern (Table 1). We found support for both nestling-feeding hypotheses (evolutionary and proximate), and both female-age hypotheses (evolutionary and proximate). We also found support for the nest-predation hypothesis at the proximate level.

Food abundance decreased during the time-frame when parents in the population were feeding young (Linear regression: $t_{60} = -3.76$, $P < 0.001$; Fig. 1) and we found a positive association between clutch size and food availability when parents were feeding nestlings (Ordinal logistic regression: Wald's $\chi^2 = 4.28$, $P = 0.039$), thus supporting the *Nestling-feeding hypotheses* at both the evolutionary and proximate levels, respectively. However, we found patterns opposite to both predictions of the *Egg-production hypotheses*; food abundance increased when females in the population were laying eggs (Linear regression: $t_{55} = 3.54$, $P = 0.001$; Fig. 1) and was negatively (not positively) associated with clutch size (Ordinal logistic regression: Wald's $\chi^2 = 4.77$, $P = 0.029$).

Opposite to the prediction of the *Seasonal-nest-predation hypothesis,* the risk of nest predation decreased, rather than increased, seasonally (Linear regression $t_{64} = 2.45$, $P = 0.017$; Fig. 2a). However, clutch size of forced renests (3.8 ± 0.13 eggs; $N = 10$) was smaller (Paired t test: $t_9 = 3.28$, $P = 0.010$) than initial nesting attempts (4.5 ± 0.17 eggs, $N = 10$; Fig. 2b), as was predicted under the *Experiential-nest-predation hypothesis.*

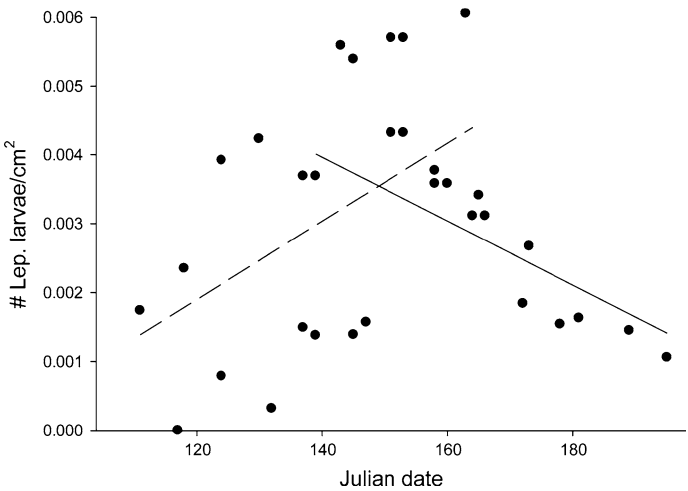

Fig. 1 *Egg-production hypotheses rejected, Nestling-feeding hypotheses* supported. Lepidoptera larvae abundance increased during egg production (1 week prior to egg laying; approx. 26 April–8 June; *dashed line*), but decreased during nestling feeding (day 6 of the nestling stage; approx. 13 May–13 July; *solid line*)

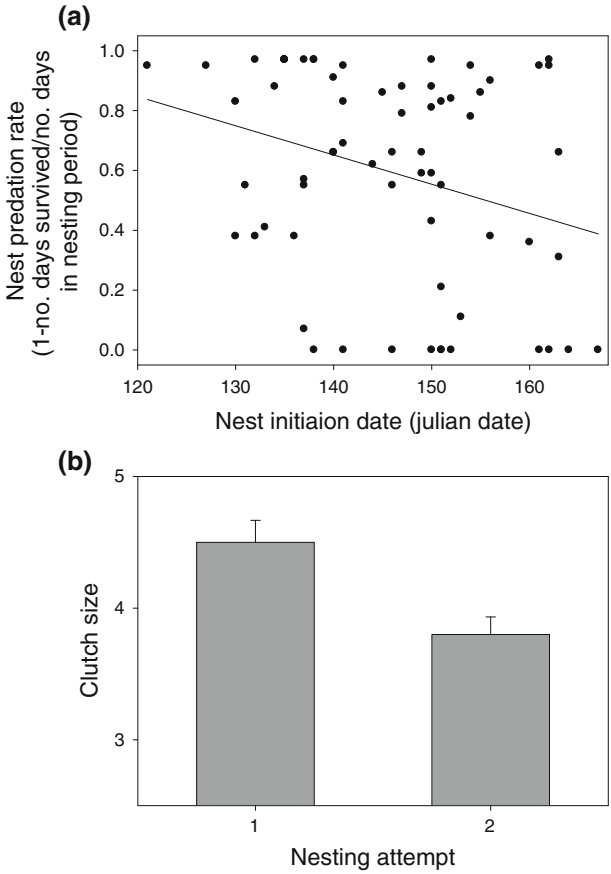

Fig. 2 *Seasonal-nest-predation hypothesis* rejected, *Experiential-nest-predation hypothesis* supported. **a** Nest predation risk decreased with date; and **b** Clutch size decreased in experimentally depredated nests ($N = 10$)

Also, the per-day change in clutch size between initial nesting attempts and the experimentally induced renests (-0.07 ± 0.02 eggs/day, $N = 10$) was greater (Paired t-test: $t_9 = -2.37$, $P = 0.042$) than the per-day change in clutch size of the entire population (among all females) during the same window of time (-0.01 ± 0.04 eggs/day, $N = 46$).

Older females did initiate nests earlier (ANOVA: $F_{1,37} = 11.91$, $P = 0.001$) and laid larger clutches (ANOVA: $F_{1,36} = 8.07$, $P = 0.007$) than younger females (Fig. 3a), supporting the *Female-age hypothesis* (evolutionary). In addition, older females nested on territories with higher relative food abundance (0.012 ± 0.01 Lepidoptera larvae/cm^2) than younger females (0.005 ± 0.004 Lepidoptera larvae/cm^2) as predicted (ANOVA: $F_{1,37} = 6.03$, $P = 0.019$; Fig. 3b). However, older females did not nest on territories with lower nest predation risk (ANOVA: $F_{1,31} = 0.18$, $P = 0.676$), so we provide some support for the *Female-age hypothesis* at the proximate level.

We did not detect an association between nestling mass and date (Linear regression: $t_{43} = 0.75$, $P = 0.460$) as would be predicted under the *Time-limitation hypothesis*. Nor did we find a correlation between nestling mass (at pin-break) and clutch size for nests

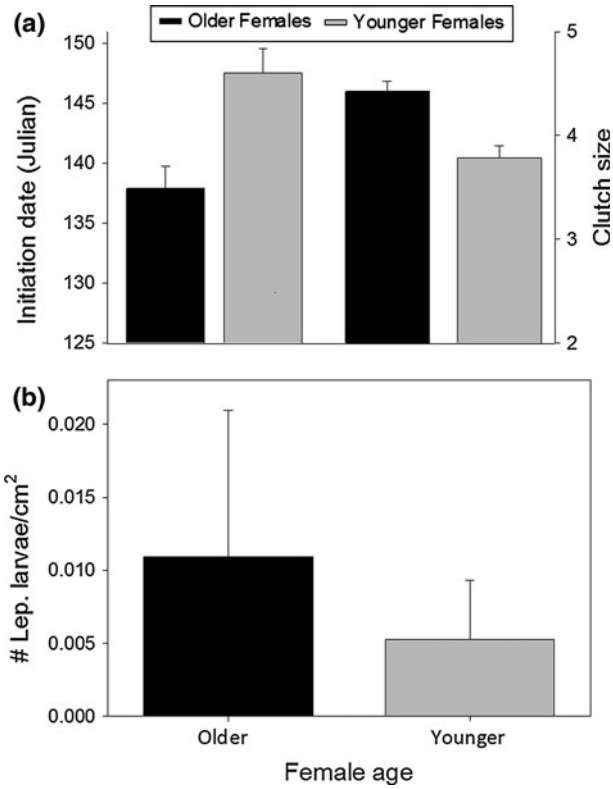

Fig. 3 *Female-age hypotheses* supported. **a** Older (after-second-year) females (*dark bars*, $N = 26$) initiated nests earlier and laid larger clutches than younger (second-year) females (*grey bars*, $N = 14$); **b** Older females (*dark bars*, $N = 26$) nested in territories with higher food abundance than younger females (*grey bars*, $N = 14$)

initiated during the second half of the season (Pearson's correlation: $t_{35} = -0.15$, $P = 0.424$). We predicted that those individuals with a larger clutch size late in the season (i.e., those that did not follow the population-wide pattern of laying fewer eggs late in the season) would face a trade-off with other forms of reproductive investment (nestling feeding rates) and have reduced quality offspring (as measured by mass).

In line with this finding, there was no evidence to support the *Cost-of-reproduction hypothesis*. Female mass did not decline throughout the breeding season (Linear regression: $t_{31} = 0.551$, $P = 0.586$) and although *clutch mass* was less for second nests of the experimental individuals due to smaller clutch sizes (Paired t test: $t_8 = 3.16$, $P = 0.013$), egg mass was greater (1st = 1.44 g ± 0.20, 2nd = 1.50 g ± 0.02; Paired t test: $t_8 = 4.51$, $P = 0.002$), and the renesting interval (8.0 days ± 1.21 SE) was only 3 days longer than the physiological minimum.

Discussion

Clutch size of red-faced warblers declined as the breeding season progressed, a commonly observed pattern in birds. Our test of ten alternative hypotheses proposed to explain this pattern provides one of the most thorough empirical studies to evaluate the underlying causes of seasonal declines in clutch size. Our data failed to support five hypotheses (three at the evolutionary level and two at the proximate level), that were previously proposed to

explain this ubiquitous pattern: *Food for egg-production* (evolutionary and proximate), *Seasonal-nest-predation, Time-limitation,* and *Cost-of-reproduction*. We did, however, find support for the *Nestling-feeding* (both evolutionary and proximate), *Experiential-nest-predation* and *Female-age* (both evolutionary and proximate) hypotheses.

Our understanding of avian life history evolution has been greatly influenced by a historical focus on the importance of food in shaping clutch size (Lack 1947, 1954; Perrins 1970), with the underlying assumption that investment in offspring is limited by access to adequate food resources either during egg production or during the nestling period. Following that tradition, we measured food abundance during both of these developmental stages to test whether changes in food abundance predicted an adaptive change in clutch size. Our hypotheses addressed both evolutionary and proximate mechanisms for the *Nestling-feeding* and *Egg-production hypotheses*. Our assessment of the *Nestling-feeding hypothesis* suggests that food may indeed play an important role in shaping clutch size evolution. As predicted, food available to nestlings declined as the season progressed (Fig. 1). Hence, our results support the idea, originally proposed by Lack (1954), that food available to nestlings constrains clutch size in birds. Female red-faced warblers appear to time egg-laying so that they are feeding nestlings during the peak in food abundance, and those doing so, are laying the largest clutches. Although we present this pattern as a fixed response to predictable levels of food abundance, a parsimonious explanation (given the repeatability of this pattern at this site even in poor nesting conditions; Decker and Conway 2009), it is possible that females may also use a proximate cue available at nest initiation that predicts food abundance when young are in the nest. Indeed, females on territories with greater food resources had a larger clutch size, which suggests that females may express adaptive clutch size plasticity. Given the close relationship between clutch size, nest initiation date, and proximate cues regulated by climatic conditions (e.g., Bourgault et al. 2010), this area warrants further study not only for understanding clutch size evolution but also avian conservation.

While we found support for the *Nestling-feeding hypothesis,* there is a clear lack of support for the *Egg-production hypothesis* (at either the evolutionary or proximate level), as there was no evidence that seasonal changes in food abundance during egg production would result in decreased clutch size. Food abundance actually increased during the period when females in the population were laying eggs (Fig. 1). Hence, energetic constraints on egg production were reduced for females that laid eggs later in the season. Indeed, some supplemental-food experiments suggest that clutch size in most birds is not constrained by food availability (reviews in Arcese and Smith 1988; Meijer et al. 1990, but see Nilsson 1991; Nager et al. 1997). Alternatively the *quality* of food resources may have decreased seasonally, via increases in secondary chemical compounds of plants eaten by Lepidoptera or because the size of Lepidoptera decreases seasonally as the largest are consumed first. In addition, females may suffer from calcium depletion (Reynolds and Perrins 2010) later in the season which could constrain clutch size. Both scenarios present additional challenges and further research is required to separate these potential effects.

Many prior studies have examined how food for egg production (Nilsson 1991; Aparicio 1994) *or* food for young (Smith 1993) influence seasonal declines in clutch size. To our knowledge, ours is the first study to differentiate between, and explicitly test, both of these possible mechanisms by which food limitation can influence breeding date and constrain clutch size, and thus the first to illustrate the interaction between the proximate and ultimate causations of food resources on seasonal patterns of clutch size.

Several studies have suggested that seasonal changes in nest predation may ultimately drive seasonal declines in clutch size (Perrins and McCleery 1989; Smith 1993; Lepage

et al. 2000). The *Seasonal-nest-predation hypothesis* assumes a predictable seasonal increase in the probability of nest predation which would then favor a predictable seasonal decline in clutch size. Instead, we and others (e.g., Roos 2002) found a seasonal decrease in nest predation (Fig. 2a). At our site, increasing foliage cover associated with advancing deciduous plant phenology may lead to reduced nest predation later in the season, independent of other ecological conditions such as numerical or functional responses of predators (Fontaine et al. 2007; Chalfoun and Martin 2009).

While clutch size does not appear to covary predictably with seasonal changes in the probability of nest predation, our results do suggest that females respond to nest predation risk at the proximate level after experiencing a depredation event (supporting the *Experiential-nest-predation hypothesis*), but do not assess predation risk a priori as has been shown by others (e.g., Julliard et al. 1997, but see Fontaine and Martin 2006a). Females whose nests were experimentally depredated reduced their clutch size by an average of 0.7 eggs in their subsequent nesting attempt (Fig. 2b). Given that most bird species experience high rates of nest failure (Ricklefs 1969), the experience of nest predation itself may lead to seasonal declines in clutch size at the population level as the proportion of laying females that experienced a prior nest predation event increases as the breeding season progresses. Moreover, experimental evidence suggests that some nest sites are more risky than others; artificial eggs were more likely to be depredated when placed at sites that were depredated (compared to a successful site) in a prior year (Martin et al. 2000; C. Conway, unpubl. data). Hence, in some cases, the strength of nest predation risk as a source of selection may override other sources that would support a larger clutch. This hypothesis cannot explain, however, a decline in clutch size in subsequent nesting attempts for those individuals rearing a second brood after a successful first nest, or a reduced clutch size in individuals rearing a late first brood. Future studies should assess the relative rate of clutch size decline in double-brooded species following successful and failed first attempts, a comparison we were not able to make in our system because red-faced warblers are single-brooded.

Our results indicate that older females initiated nests earlier and laid larger clutches than younger females (Fig. 3a), corroborating several past studies (Murphy 1986; Hochachka 1990; Smith 1993). The relationship between female age and clutch size may simply reflect life history trade-offs driven by reduced reproductive opportunities; however, based on our results, the more parsimonious cause may be that the age-clutch size relationship is a reflection of proximate condition dependent responses that covary with age. Although measures of territory quality are debated (see Ens et al. 1992), we found that older females nested on territories with overall higher food abundance (Fig. 3b), and were thus energetically able to produce and support a larger clutch. Older females did not however, nest on territories with lower nest predation risk. Older, more experienced individuals may occupy higher quality winter territories (Marra et al. 1993) which facilitates earlier arrival at breeding locations, and thus procurement of higher quality breeding territories (Saether 1990; Martin 1995a, b), and greater investment in offspring. These findings suggest that those individuals arriving first are selecting the highest quality territories as measured by food (see Johnson and Sherry 2001; Borgmann et al. 2004) but not by risk of nest predation (as shown by Fontaine and Martin 2006b; Lima 2009, Travers et al. 2010). Perhaps individuals are unable to assess cues that indicate qualitative differences in predation risk among territories, or there may be trade-offs between food abundance and nest predation and potentially other measures of territory quality that we have not examined (e.g., temperature; Ligon and Ligon 1988).

The reduction in clutch size following experimental depredation may be due to a time constraint rather than an energy constraint. However, this seems unlikely as we failed to

find support for the *Time-limitation* hypothesis which suggests that clutch size declines seasonally due to time and energy constraints required by the need to switch to subsequent life stages. For example, because molt and reproduction are energetically demanding (e.g. Drent and Daan 1980), we might expect a trade-off between the time and energy needed for both processes (Siikamäki et al. 1994) which may ultimately result in adult molt timing that is dependent on the size of brood reared (Lessells 1986). As such, reproductive investment should have similar trade-offs with migratory timing, winter territory establishment, or winter food stashing (for residents), all of which may be delayed by late breeding events. Our results do not support such a trade-off in red-faced warblers as nestling mass did not increase seasonally, and clutch size of late-reared nests was unrelated to nestling mass.

Finally, seasonal declines in clutch size may be a response to reduced energetic condition after a nest predation event (*Cost-of-reproduction hypothesis*). Birds can clearly assess nest predation risk and adjust reproductive strategies to minimize costs (active bet-hedging; reviewed in Lima 2009), and experiencing a nest predation event likely represents the most accurate source of information regarding future risk available to a laying female. Females producing smaller replacement clutches did not appear to do so because of lower body condition (Norris 1993) or reduced food availability because female mass did not decrease seasonally. Also, experimental females renested within 3 days of the physiological minimum and produced heavier eggs in subsequent attempts (rejecting the *Cost-of-reproduction hypothesis*). Although mass has been challenged as an accurate measure of condition (see Hillstrom 1995), females were able to assess nest predation, build a new nest at a different location within the territory, and produce an egg in a mere 8 days. Together, this evidence suggests that females were not energetically limited, at least in terms of egg production although they may suffer from calcium depletion (Reynolds and Perrins 2010), which would require further assessment of available resources. However, support for this hypothesis may reflect an alternative scenario where individuals reproducing early are in better condition and may not indicate an actual decline in body condition. Studies assessing changes in body condition within individuals are required to separate these two mechanisms.

Conclusion

Many alternative hypotheses may explain the seasonal declines in clutch size (both at the proximate and evolutionary levels). Potential alternatives not considered in this paper which warrant more attention include: seasonal variation in parasites or disease (Gustafsson et al. 1994), seasonal changes in temperature (Cooper et al. 2005), and temporal heritability (clutch size as an adaptive tactic of individuals where breeding time exhibits a heritable component; Sheldon et al. 2003; Hendry and Day 2005). In addition, better knowledge regarding variation in the timing of nest initiation (i.e., based on expected clutch size, extrinsic factors, or genetically determined breeding times) will ultimately help assess which hypotheses are more likely to explain seasonal declines in clutch size (and which predictions are most viable). We examined the hypotheses that represent a fair subset of those considered frequently in past and current literature. In the end, by examining predictions of multiple alternative hypotheses in an a priori theoretical framework that considered both proximate and ultimate causation, we were able to isolate several important selection pressures shaping a ubiquitous pattern whose underlying cause has been pondered for over 60 years. In doing so, our findings suggest that seasonal patterns of

clutch size expression result from the combination of ultimate and proximate sources of selection such that seasonal patterns of food abundance for young bounds clutch size evolution, while proximate responses to nest predation and age- related territory decisions fine tune an individual's clutch size expression.

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