

Stable isotopes predict reproductive performance of European starlings breeding in anthropogenic environments

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Abstract. Understanding reproductive performance in ecologically impoverished vs. more sustained anthropogenic habitats is critical to assess population health status and to develop land use and conservation management strategies. We compared resource-based maternal effects, reproductive performance, and offspring quality in a model migratory passerine bird, the European starling (*Sturnus vulgaris*). We assessed female condition, quantity of egg constituents, quality of diet consumed during egg formation, and nestling growth and survival in two habitats: cultivated farmland and meadows. Egg, albumin, and shell mass were greater at the meadow site, while yolk mass did not differ significantly between sites; albumin mass most strongly predicted egg mass. Stable isotope enrichment ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) in yolk but not albumin suggests a broader range of diet for yolk formation at the meadow site but could also reflect different hydric conditions between sites. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ enrichment did not predict yolk, albumin, or egg mass. Concentration of yolk testosterone was higher at the meadow site and correlated with $\delta^{13}\text{C}$ enrichment in yolk. Nestling survival was higher in the meadow than in the farmland site and corresponded to egg mass and $\delta^{13}\text{C}$ enrichment in lipid-free yolk. Surviving nestlings were larger in the meadow than in the farmland site. Results indicate that agricultural practice influences reproductive output through resource-based maternal effects. The analyses of isotopic and biochemical composition of small samples of yolk and albumin may provide a minimally invasive tool to assess individual reproductive performance and predict impacts of habitat quality on population health.

Key words: anthropogenic environment; carbon stable isotopes; egg quality; European starling; nitrogen stable isotopes; reproductive performance; *Sturnus vulgaris*; survival; yolk testosterone.

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INTRODUCTION

The health and status of wildlife populations depends on abiotic and biotic environmental conditions that influence reproductive performance and offspring quantity, quality, and survival. Life history theory and models of optimal reproduction have identified two key trade-off functions that operate to maximize fitness in spatially and temporally heterogeneous environments: investment

into current vs. future reproduction and offspring quantity vs. quality (Stearns 1992). The dietary quality of the breeding environment influences the trade-offs between reproductive investment and performance, and the consequent resource-based maternal effects act at different stages and by different mechanisms to influence quantity and quality of progeny (e.g., Martin 1987, Rossiter 1996, Norris et al. 2004, Boonstra 2013). Avian resource-based maternal effects include clutch

size (Mousseau and Fox 1998) and allocation patterns within a clutch that can facilitate efficient management of offspring number (i.e., brood reduction) in response to food abundance. These include within-clutch variation in egg mass, egg composition, and yolk hormone concentrations, all of which can influence offspring growth and quality (e.g., Schwabl et al. 1997, Eising et al. 2001, Groothuis and Schwabl 2008).

Agricultural intensification has strikingly modified landscapes and the abundance and distribution of many species, some seemingly successfully adapting to and thriving in agricultural landscapes, others declining and becoming lost from certain landscapes (Chace and Walsh 2006, McKinney 2008). Consequences of habitat alteration by agriculture and land use range from loss of foraging, shelter, and nesting sites to changes in predation pressure and abundance and diversity of food necessary to meet the energetic costs of reproduction. Egg formation is an energetically challenging process (Nilsson and Raberg 2001, Vézina et al. 2003), and metabolic costs are compounded with caloric investment in eggs (King 1973, Ricklefs 1974, Walsberg 1983, Rahn et al. 1985). How variation in diet caused by agricultural practice impacts reproductive investment strategies, maternal effects, and performance is little understood but critical to assess impacts on populations.

Previous studies applied a stable isotope approach to investigate sources of nutrients for egg formation by analyzing stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) values in bird eggs (e.g., Klaassen et al. 2001, Morrison and Hobson 2004, Yohannes et al. 2010). We used $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ enrichment to examine how agricultural land use influences reproductive investment and performance of European starlings. We hypothesized that cultivated “farmland,” managed to produce a single crop (i.e., wheat or corn), provides sub-optimal dietary conditions for egg formation and nestling rearing compared to “meadows” (relatively rich in insect and plant abundance, partially under conservation program “Wiesenbrüter,” used for the harvesting of hay). To assess:

1. the trophic quality of diet from which egg constituents were formed, we compared the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of whole yolk, lipid-extracted yolk, and albumin.
2. whether females respond to dietary quality by modifying resource allocation strategies within a clutch, we compared these measures and yolk testosterone concentrations between the first and fifth eggs of clutches.
3. the quality of diet fed to nestlings, we determined the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of nestling feathers (grown after hatching). Finally, we assessed reproductive performance using nestling mass, size, and survival.

METHODS

Study species

We selected the European starling as our model species to compare reproductive performance between habitats for the following reasons. The starling occurs throughout Europe in landscapes with diverse human use and thrives in many habitats, but also experienced serious population declines in certain areas. Although it is a non-threatened, relatively common migratory passerine bird, population sizes have declined in certain parts of Europe and in the UK (Snow and Perrins 1998). The species has been adversely affected by agricultural intensification. For example, in England populations declined by more than 80% between 1966 and 2004, presumably due to low survival rates of young birds (Granbom and Smith 2006, Baillie et al. 2012). It breeds in nest boxes, facilitating finding nests and monitoring nestling development. Similar to many other passerine birds, as an income breeder (Perrins 1996, Meijer and Drent 1999) female starlings rely on food resources gathered at the breeding grounds for egg formation. Food resources for egg formation and raising nestlings are invertebrates (Rhymer et al. 2012). It breeds in colonies allowing easy spot sampling in select environments of interest. Onset of breeding occurs synchronously in these colonies, avoiding confounding effects of temporal variation in diet. Therefore, the European starling may be a useful sentinel to monitor individual reproductive performance in different habitats.

Study sites

The study was conducted with two nest-box colonies in Southern Germany, 10 km apart from each other. Site 1 (Frieding, 48°0' N, 11°12' E,

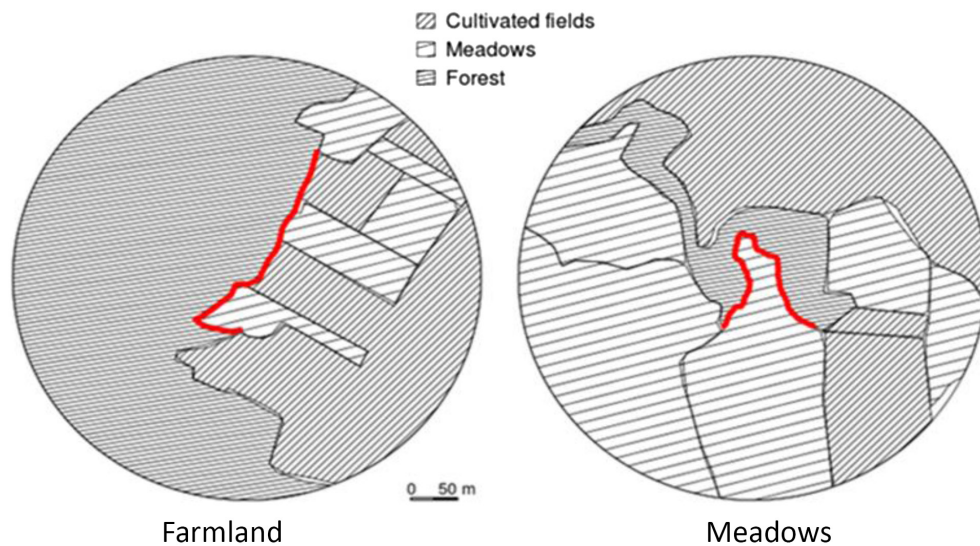


Fig. 1. Polygons delineating each of the two study sites. Left: farmland site; right: meadow site. Cultivated agriculture fields, meadows, and forest are depicted by different cross-hatching. A circle with a radius of 500 m (78 ha) was created around the centroid of each study area where nest boxes were hung (thick red line), and the surface of each habitat type was then computed. The projection shows that while in the farmland site only 10.26 ha (13%) of the area was covered by meadows (57% forest and 30% cultivation), in the meadow site about 42 ha (53.5%) of the area was covered by meadows (10% forest and 36.5% cultivation). Note that forested areas are unsuitable foraging habitat for the starling.

590 m asl) consisted of farmland dominated by monoculture of corn and/or wheat (hereafter referred to as farmland); site 2 (Fischen near the river Ammer, 47°58' N, 11°11' E, 700 m asl) consisted predominantly of meadows and grassland with hardly any agricultural practices but for the harvesting of grasses for hay (hereafter referred to as meadows or meadow site). Nest boxes were mounted on trees of bordering woodland: 24 boxes on spruce trees in farmland and 54 boxes on riparian deciduous trees close to the Ammer River in the meadow site.

We estimated the fraction of cultivated fields, meadows, and forest at the two study sites using Google Earth photographs that were imported in an R-based GIS and projected to a Mercator projection with Potsdam datum. We drew a circle with a radius of 500 m (78 ha) around the centroid of each study area where nest boxes were hung and computed the surface of each habitat type. The projection shows that in the farmland site only 10.26 ha (13%) of the area was covered by meadows, while in the meadow site about 42 ha (53.5%) of the area was covered by meadows (Fig. 1). We do not have information on

abundance and diversity of invertebrates in these habitats, but studies have shown that such habitats can differ dramatically in invertebrate communities (e.g., Tschardt et al. 2005, Hendrickx et al. 2007, Littlewood et al. 2012).

Field methods

From April to early May, we checked nest boxes daily, marked eggs, and collected the first and fifth egg (0–1 d after laying) from nests with five eggs (almost all nests had a clutch size of 5). Collected eggs were not replaced with dummies. We weighed the eggs and their constituents (shell, yolk, and albumin) to the nearest 0.01 g and stored the material at -80°C until hormone and stable isotope analyses were performed. On day 14 after the first egg hatched, we caught the female, measured body mass and tarsus length, and collected body and tail feathers for stable isotope analysis. Female age could not be determined. At this time, we also obtained body mass and tarsus length of nestlings. We collected a tail feather from a randomly selected nestling of each brood (including dead nestlings) for stable isotope analysis.

Stable isotopes

Feather samples were rinsed with methanol and air-dried in a fume hood. We extracted lipids from each homogenized yolk sample with 2:1 chloroform–methanol solution (De Niro and Epstein 1978). Dried and powdered subsamples of approximately 0.5 g whole and lipid-extracted yolk, approximately 0.45 mg albumin, and approximately 0.5 mg feathers were weighed into small tin cups to the nearest 0.001 mg, using a microanalytical balance. Samples were then combusted in a Eurovector (Milan, Italy) elemental analyzer. The resulting N_2 and CO_2 gases were separated by gas chromatography and admitted into the inlet of a Micromass (Manchester, UK) Isoprime isotope ratio mass spectrometer (IRMS) for determination of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios. Measurements are reported in δ -notation relative to the international standards in parts per thousand deviations (‰). The standard for $\delta^{15}N$ is atmospheric nitrogen and for $\delta^{13}C$ Peedee belemnite (PDB). Egg albumin was used as a daily reference material. Typical precision of analyses was $\pm 0.2\%$ for $\delta^{15}N$ and $\pm 0.1\%$ for $\delta^{13}C$.

Yolk testosterone

The procedures for extraction and radioimmunoassay of yolk testosterone (T) were performed following the protocol described previously (Schwabl 1993, Gwinner et al. 2013). Intra-assay coefficient of variation in the single assay that was performed was 5.4%, and the mean recovery of tritiated T added to each sample was 75% after extraction and purification. T and stable isotope analyses were performed at the School of Biological Sciences, Washington State University.

Statistical analysis

Except for yolk T, all variables were normally distributed (Kolmogorov–Smirnov test $P > 0.05$). To achieve normality, yolk T values were ln-transformed. We calculated body condition as the ratio of mass/tarsus. We used lay date of the first egg of first clutches for comparison of laying onset in the two colonies. We compared egg characteristics using multivariate general linear models (with site and laying sequence in a clutch (eggs 1 and 5) and their interactions as fixed factors). Table 2 summarizes statistical analyses with and without these interactions (the

interaction between site and egg order was only significant for albumin and lipid-free yolk $\delta^{15}N$). The relationships between isotope values and egg, yolk, and albumin mass and chick survival were explored using Pearson correlation coefficients. Statistical analyses were performed in SPSS 23 and R (R Development Core Team 2014).

Isotopic niche analysis

We applied standard Bayesian ellipses and coupled information from $\delta^{13}C$ and $\delta^{15}N$ variances, produced figures for standard ellipse (SEA), and corrected using the metrics SEAc (Layman et al. 2007, Jackson et al. 2011) and generated total isotopic niche area (TA). These calculations were conducted using Stable Isotope Bayesian Ellipse in R (SIBER). All metrics were calculated in R statistical computing package (R Development Core Team 2014), using the SIAR package (Jackson et al. 2011).

RESULTS

Female characteristics and onset of egg laying

Females of the two sites did not differ in body mass, tarsus length, body condition index (mass/tarsus), and feather $\delta^{13}C$ and $\delta^{15}N$ enrichment ($P > 0.05$ in all cases; Table 1). First clutches were laid at the same time (mean \pm SD: farmland: 14 April ± 2.3 d; meadow site: 14 April ± 2.5 d), but only 50% of 24 nest boxes offered at the farmland site were occupied compared to 78% of 54 boxes at the meadow site.

Clutch size, egg mass, and egg composition

Average clutch size was 5 eggs (range: 4–6 eggs) at both sites. Egg, albumin, and shell mass were significantly greater at the meadow site,

Table 1. Summary of multivariate GLM analysis for comparison of body mass, tarsus length, condition (mass/tarsus), and $\delta^{13}C$ and $\delta^{15}N$ values of tail feathers of female starlings breeding at the farmland and the meadow study sites.

Variable	$F_{1,27}$	P
Mass	1.677	0.207
Tarsus length	0.58	0.454
Condition	0.29	0.595
Feather $\delta^{13}C$	2.477	0.128
Feather $\delta^{15}N$	2.765	0.109

Table 2. Summary of multivariate GLM analysis of egg composition in the farmland vs. rich meadow habitat and in relation to egg order (egg 1 vs. egg 5).

Variable	Site	Egg order	Site × Egg order
	$F_{1,37}, P$	$F_{1,37}, P$	$F_{1,37}, P$
Egg mass (g)	10.76, 0.002	0.364, 0.550	0.01, 0.925
	12.29, 0.001	0.366, 0.549	
Yolk mass (g)	3.07, 0.089	2.61, 0.115	0.120, 0.731
	3.06, 0.089	2.58, 0.117	
Albumin mass (g)	6.83, 0.013	0.60, 0.443	0.05, 0.831
	8.03, 0.008	0.58, 0.449	
Shell mass (g)	4.38, 0.044	1.57, 0.219	0.02, 0.892
	4.73, 0.037	1.68, 0.203	
Whole yolk $\delta^{13}\text{C}$	143.30, <0.001	1.18, 0.286	0.604, 0.443
	164.19, <0.001	1.00, 0.324	
Whole yolk $\delta^{15}\text{N}$	11.97, 0.001	0.02, 0.882	0.01, 0.920
	13.22, 0.001	0.02, 0.890	
Lipid-free yolk $\delta^{13}\text{C}$	44.35, <0.001	0.02, 0.880	0.003, 0.959
	31.23, <0.001	2.26, 0.147	
Lipid-free yolk $\delta^{15}\text{N}$	20.14, <0.001	9.81, 0.004	10.71, 0.003
	49.79, <0.001	5.65, 0.027	
Albumin $\delta^{13}\text{C}$	0.53, 0.469	1.71, 0.200	0.28, 0.602
	0.37, 0.454	1.60, 0.215	
Albumin $\delta^{15}\text{N}$	0.06, 0.813	0.82, 0.372	5.432, 0.026
	0.81, 0.375	1.29, 0.236	
Yolk testosterone	3.87, 0.057	1.11, 0.298	0.08, 0.774
	4.72, 0.037	1.24, 0.272	

Note: Upper rows show results of models that include interactions between breeding site and egg order (egg 1 vs. egg 5) for all variables; lower rows show results of models without interactions when the interaction was not significant and removed from the model.

while yolk mass was not significantly different (Table 2, Fig. 2a–c). Egg, yolk, albumin, and shell mass were not related to laying order (eggs 1 and 5) or the interaction between site and laying order (Table 2). Egg mass was strongly predicted by albumin mass (eggs 1 and 5 = Pearson's $r = 0.94$, $P < 0.0001$, $N = 54$) and less so by yolk mass (egg 1 and 5 = Pearson's $r = 0.39$, $P = 0.004$, $N = 54$). Yolk mass did not predict albumin mass (Pearson's $r = 0.12$, $P > 0.05$, $N = 54$).

Stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) values

Yolk.— $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of whole yolk differed significantly between sites with more enriched $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at the meadow than at the farmland site. Laying order (egg 1 or 5) and its interaction with site had no effect (Table 2, Fig. 3d, c). The values of lipid-free yolk differed between sites. In contrast to whole yolk, egg order had a main effect on $\delta^{15}\text{N}$ values of lipid-free yolk, and there was significant interaction between egg order and site (Table 2, Fig. 3e). While $\delta^{15}\text{N}$ was similarly low in eggs 1 and 5 of

farmland clutches, it decreased from high values in egg 1 to lower values in egg 5 in meadow clutches. These lower levels were comparable to those in eggs 1 and 5 of farmland clutches.

Albumin.—Site, laying order, and their interactions were not related to $\delta^{13}\text{C}$ values of albumin (Fig. 3b, Table 2). However, albumin $\delta^{15}\text{N}$ values were related to the interaction between site and laying order (Table 2). The $\delta^{15}\text{N}$ values of albumin were similarly high in eggs 1 and 5 in the meadow population, while they increased from low in egg 1 to high in egg 5 in the farmland population (Fig. 3a). Values of egg 5 in farmland clutches were similar to those of eggs 1 and 5 in meadow clutches.

Correlation of egg, yolk, and albumin mass with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values

Egg mass was not predicted by the $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values of any of the egg constituents (whole yolk, lipid-free yolk, albumin) (all $P > 0.05$ when controlling for site and egg order). Similarly, yolk mass was not predicted by $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values in

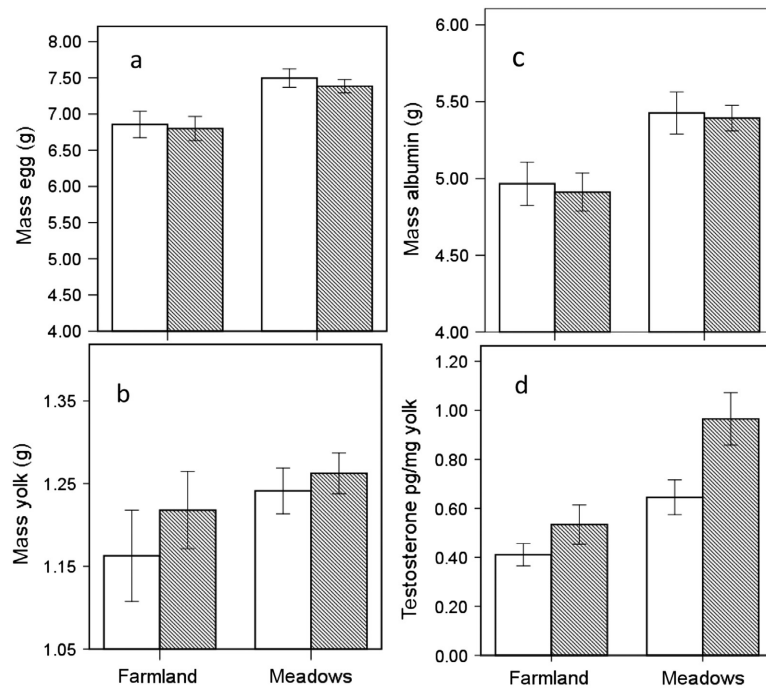


Fig. 2. Mean (± 1 SE) egg mass (a), yolk mass (b), albumin mass (c), and yolk testosterone concentrations (d) of the first (open bar) and fifth (hatched bar) egg in farmland and meadow site clutches. Dispersion lines are representing ± 1 SE. Numbers above bars indicate sample size.

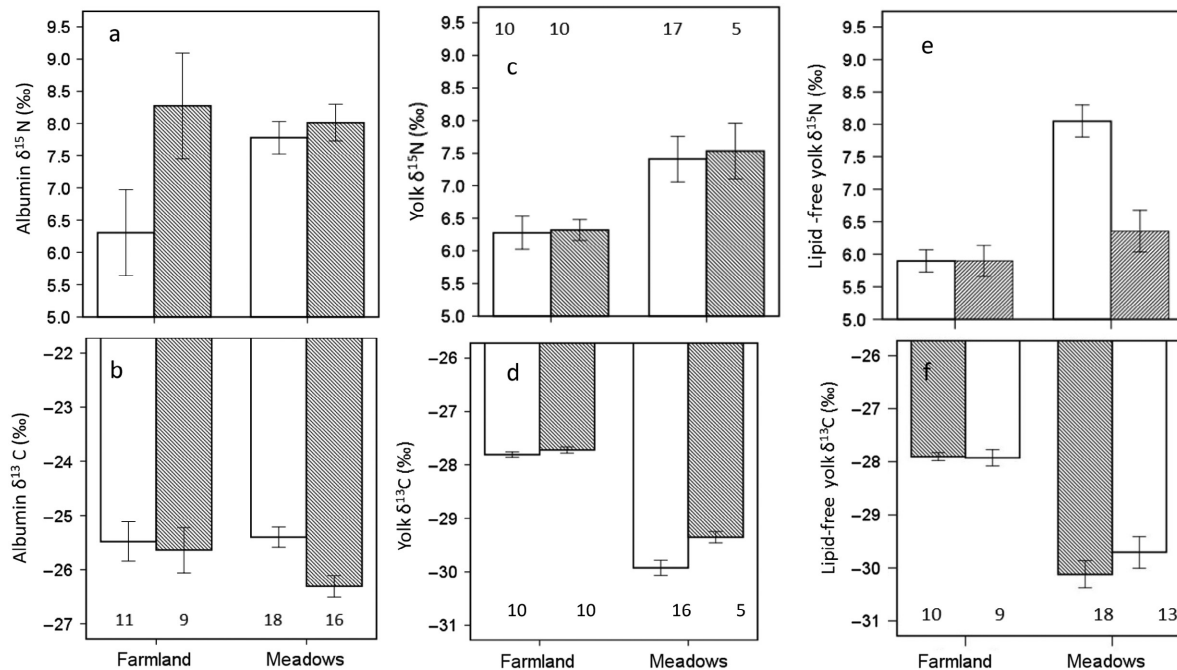


Fig. 3. Mean ± 1 SE values of $\delta^{15}\text{N}$ (a, c, e) and $\delta^{13}\text{C}$ (b, d, f) measured in albumin, whole yolk, and lipid-free yolk of the first (open bar) and fifth (hatched bar) egg of farmland and meadow site clutches. Sample sizes are reported above or below bars.

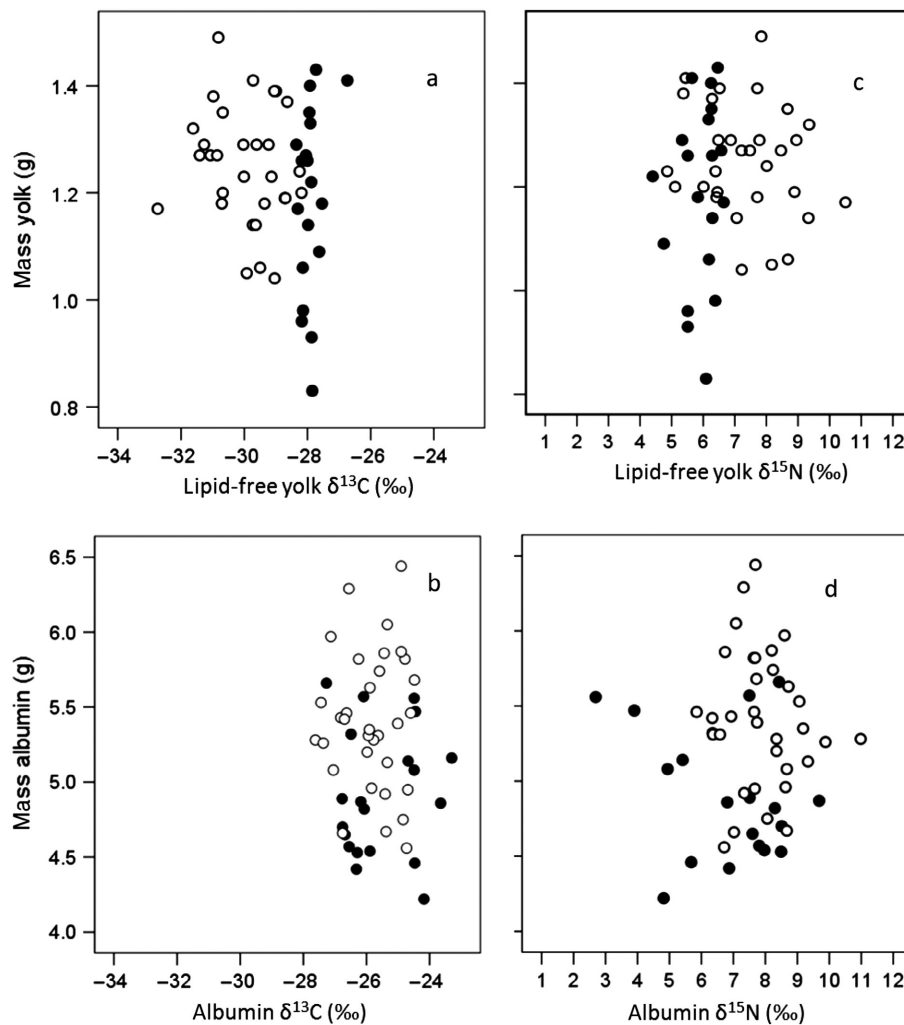


Fig. 4. Relationship of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in lipid-free yolk and albumin with yolk mass (a, c) and albumin mass (b, d), respectively, in eggs from the farmland (filled circles) and the meadow site (open circles).

lipid-free (Fig. 4a, c) or whole yolk (data not shown). Albumin mass was also not related to albumin $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values (Fig. 4b, d). Variation in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ enrichment of whole and lipid-free yolk was larger at the meadow than at the farmland site where values were restricted to a narrow range (Fig. 4a, c, Table 3 for coefficients of variation: SD/mean). Despite the narrow range in yolk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, yolk mass varied dramatically at the farmland site. Variation in albumin $\delta^{13}\text{C}$ was comparable in farmland and meadow eggs (Fig. 4b), while variation in albumin $\delta^{15}\text{N}$ tended to be larger in the farmland than in the meadow-breeding colony (Fig. 4d, Table 3).

Correlation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values across egg constituents

Whole and lipid-free yolk $\delta^{13}\text{C}$ values were not correlated with albumin $\delta^{13}\text{C}$ values (both $P > 0.05$; data not shown). Whole and lipid-free yolk $\delta^{15}\text{N}$ values were also not correlated with albumin $\delta^{15}\text{N}$ values (both $P > 0.05$). $\delta^{13}\text{C}$ but not $\delta^{15}\text{N}$ shifted toward less enrichment from yolk to albumin in both populations (Fig. 3).

Yolk testosterone

Yolk T concentrations were higher (eggs 1 and 5) in meadow than in farmland site eggs. Laying order and interaction between site and laying order had no effect (Fig. 2d, Table 2). Yolk T

Table 3. Population-level stable isotope metrics derived from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of samples of starlings at the farmland and meadow breeding sites: coefficients of variation (SD/mean in %) in mean clutch (eggs 1 and 5) $\delta^{13}\text{C}$ (CVc) and $\delta^{15}\text{N}$ (CVn); standard ellipse corrected for sample size (SEAc), isotopic niche area (TA) and p for TA (P_{TA}).

Variable	CVc	CVn	SEAc	TA	N	P_{TA}
Farmland site						
Whole yolk	0.61	8.45	0.36	1.06	20	
Lipid-free yolk	1.27	10.36	0.71	2.02	20	
Albumin	4.68	34.39	9.96	23.10	20	
Nestling feather	1.42	20.87	0.24	0.13	5	
Female feather	3.07	13.03	2.91	5	9	
Meadow site						
Whole yolk	2.50	17.72	2.69	6.37	20	0.01
Lipid-free yolk	3.63	18.69	4.52	15.30	30	<0.001
Albumin	3.50	13.82	2.91	9.37	33	<0.001
Nestling feather	1.29	11.96	0.94	2.33	15	0.28
Female feather	1.94	7.39	0.99	2.36	15	0.02

concentrations were unrelated to egg mass, yolk mass, and whole and lipid-free yolk $\delta^{15}\text{N}$ values (all $P > 0.05$). Yolk T concentrations were, however, associated with $\delta^{13}\text{C}$ values in whole yolk (pooled data Pearson's $r = -0.30$; $P < 0.05$).

Nestling growth, survival, and stable isotope values

Only 24.2% of the farmland broods survived to nestling day 14 (at least one nestling), while in the meadow site 75.8% of broods survived ($\chi^2 = 13.99$ $P < 0.001$). Farmland broods had 1.2 surviving nestlings ($N = 10$), while meadow site broods contained 2.68 nestlings ($N = 22$) at this age ($F_{1,31} = 10.90$, $P = 0.003$). Brood survival was positively correlated with egg mass (Pearson's $r = 0.50$; $P = 0.007$; Fig. 5). Surviving farmland nestlings had significantly shorter tarsi ($F_{1,19} = 15.71$, $P = 0.001$) and tended to have lower body mass (mean \pm SE = 77.0 ± 0.02 g) than meadow nestlings (mean \pm SE = 80.4 ± 0.02 g).

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in nestling feathers were more enriched in the meadow site than in the farmland site ($\delta^{15}\text{N}$: $F_{1,51} = 57.12$, $P < 0.001$; $\delta^{13}\text{C}$: $F_{1,51} = 3.94$, $P = 0.053$). Broods with higher survival rate (3–4 nestlings) came from clutches with significantly more enriched $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in lipid-free yolk than broods which had only 0–2 nestlings (Fig. 6; t -test: $\delta^{13}\text{C}$: $t = 2.22$, $N = 35$, $P = 0.03$; $\delta^{15}\text{N}$: $t = -2.44$, $N = 35$, $P = 0.019$).

Isotopic niche analysis

Fig. 7 provides isotopic ellipses and total isotopic niche area (TA) for tissues analyzed in

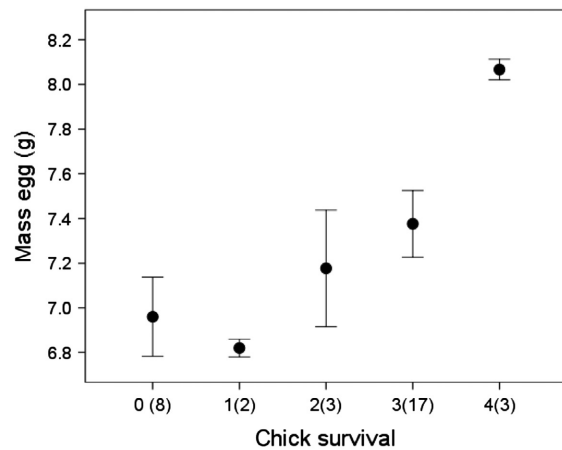


Fig. 5. Relationship between nestling survival rate (number of surviving nestlings per brood) and egg mass, number of broods in parentheses. Error bars represent standard error of the mean.

this study in farmland- and meadow-site-breeding starlings. Bayesian statistics indicate a significant probability that the SEAc of whole yolk and lipid-free yolk was larger in meadow than in farmland eggs (Table 3). However, albumin SEAc was smaller in meadow than in farmland eggs. We note a considerable within-site variation in the farmland breeding site (Table 3). For example, for eggs and nestlings (female feather not considered), the nitrogen isotope coefficient of variation at the farmland site ranged from 8.45% in whole yolk to 34.39% in albumin, while in the meadow site it ranged

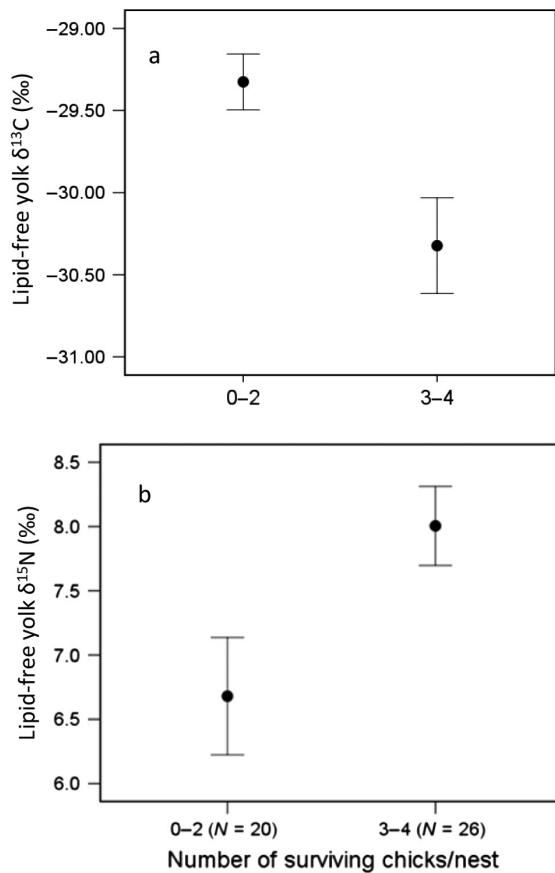


Fig. 6. Relationship between nestling survival per nest and $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) values in lipid-free yolk. Error bars represent standard error of the mean.

from 11.96% in nestling feather to 18.60% in lipid-free yolk.

DISCUSSION

Our comparison of reproductive performance of European starlings shows (1) females apparently preferred meadow site over farmland but were in comparable body condition, started to lay at the same time, and produced similar clutch sizes; (2) lower egg mass in the farmland than in the meadow site which is best explained by a significantly reduced albumin complement; (3) higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ enrichment and higher isotopic niche width in yolk and lipid-free yolk, but not albumin, in the meadow site than in the farmland site; (4) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ enrichment does not predict yolk, albumin, and egg mass; and

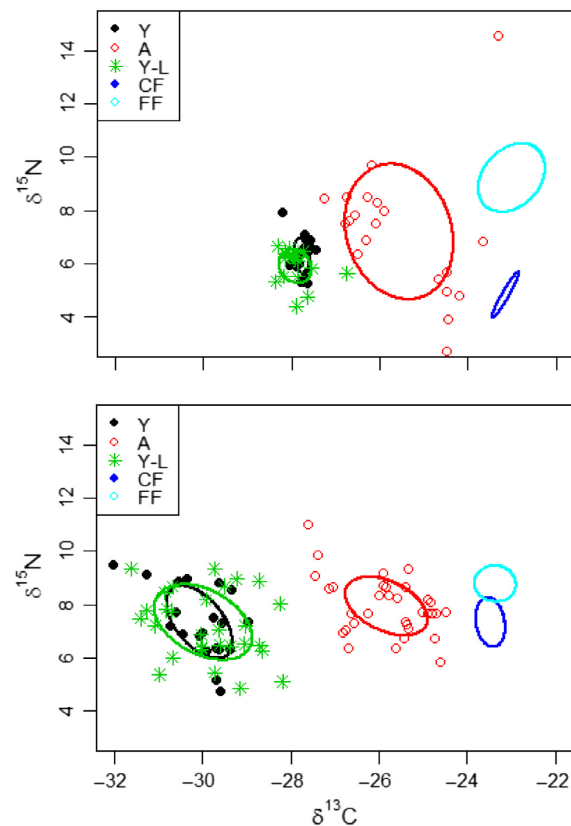


Fig. 7. Isotopic ellipses ($\delta^{13}\text{C}$ [‰] and $\delta^{15}\text{N}$ [‰]) for tissues analyzed in this study in farmland (top)- and meadow (bottom)-breeding starlings: Y, whole yolk; A, albumin; Y-L, lipid-free yolk; CF, chick (nestling) feather; FF, female feather.

(5) clutches of the meadow site produced more and larger offspring than those of the farmland site. The available diet in the meadow site apparently better supported reproductive investment resulting in greater reproductive output. The agricultural practices used in the farmland likely limit the abundance and diversity of invertebrates needed for egg formation (yolk and albumin) and nestling feeding and impact egg composition and quality, embryo and nestling development, and eventually nestling survival.

The similar body condition and feather $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of females breeding in the two habitats indicate comparable dietary conditions at their nonbreeding sites where they had molted. Therefore, a carryover effect from the nonbreeding period in this income breeder, as suggested for some capital breeders (e.g., Varpe et al. 2009), is

unlikely. Yet, females breeding in farmland exhibited a larger SEAc indicating a wider winter isotopic niche. As females started to lay at the same time at the two sites and laid similar-sized clutches, the ecological and/or genetic conditions and food availability were apparently adequate at both sites to allow for a timely laying initiation and the production of similar clutch sizes. However, egg mass was greater at the meadow than at the farmland site. Egg mass responds to food abundance and quality (e.g., Williams 1994, 2012) with larger eggs generally producing larger and heavier nestlings with higher fledging success (e.g., Bolton 1991, Christians 2002, Vergauwen et al. 2012) and survival after fledging (Krementz et al. 1989).

Egg mass

Egg mass was strongly predicted by albumin mass, and albumin mass differed between sites, while yolk mass did not. Both albumin and yolk are crucial for development (Romanoff and Romanoff 1949, Sotherland and Rahn 1987, Carey et al. 1989). Albumin is the major reservoir of protein and water for the embryo (Romanoff and Romanoff 1949, Sotherland and Rahn 1987, Carey et al. 1989), and experimental reduction in albumin delayed hatching, reduced nestling growth and survival, and had consequences for immune competence (Finkler et al. 1998, Bonisoli-Alquati et al. 2008). In the European starling, the albumin complement represents 70% of total egg mass with 89% of it being water (Ricklefs 1977). The lower albumin mass of eggs of farmland starlings may reflect less albumin water due to drier conditions in farmland compared to meadow habitat, but albumin protein synthesis and deposition may also have been constrained.

Stable carbon and nitrogen isotopes

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were more enriched and variable in whole and lipid-free yolk at the meadow site than at the farmland site. Females of the meadow site therefore could rely on a more diverse diet of higher trophic level than farmland females during yolk formation. The more enriched $\delta^{13}\text{C}$ in whole yolk of meadow site eggs may also indicate higher lipid content (McConnaughey and McRoy 1979, Tieszen and Boutton 1988). Yolk lipid content may impact embryonic energy reserves, possibly contributing to offspring quality and survival (e.g., Noble

and Cocchi 1990, Noble et al. 1996, Speake et al. 1996, Surai et al. 2000).

The most salient result of our study is the difference in degree of variation in yolk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ enrichment at each site. The coefficient of variation in $\delta^{13}\text{C}$ enrichment was 4.1 and 2.8 times higher at the meadow site compared to the farmland site for whole yolk and lipid-free yolk, respectively. For $\delta^{15}\text{N}$, the variation was 2.1 and 1.8 times larger in the meadow site than in the farmland site for whole yolk and lipid-free yolk, respectively. Despite this restriction to a narrow range, many farmland females were able to produce yolks as large as those females of the meadow site. Factors other than diet apparently influenced egg formation, for example, female age and/or experience (Christians 2002). We do not have information on the age of laying females in the present study, but almost all breeding females in other study years (in our two study sites) were older than 1 year (S. Laucht, *personal communication*, E. Yohannes and H. Gwinner, *unpublished data*). Age differences therefore are an unlikely explanation for variation in egg formation at the two study sites. Moreover, egg mass was only very weakly related to age in another study of starlings (Smith et al. 1993).

$\delta^{13}\text{C}$ enrichment of albumin, in contrast to yolk, did not significantly differ between sites, and both populations showed a shift toward less enriched $\delta^{13}\text{C}$ in albumin compared to whole and lipid-free yolk. Such a clear shift was not apparent for $\delta^{15}\text{N}$ values, females at the farmland site apparently relying on a large range of sources to form albumin compared to yolk. Here, we detected a difference between egg 1 and egg 5, namely a shift toward higher $\delta^{15}\text{N}$ enrichment in albumin from egg 1 to egg 5 in farmland eggs, while levels remained the same in meadow eggs. Females of farmland may have changed their foraging strategy (or the composition of invertebrate diet changed) and consumed available invertebrates that contain enough protein but relatively little lipid. Alternatively, the higher $\delta^{15}\text{N}$ values of albumin in the fifth egg in farmland clutches could have resulted from females tapping into stored protein or protein catabolism to form the albumin of the last egg (e.g., Ramirez et al. 2011).

Isotopic niche

The SEAc of albumin in the farmland site was most different from other tissues collected at the

breeding areas due to high $\delta^{15}\text{N}$ values. In contrast, at the meadow site, lipid-free yolk showed the highest SEAc values due to higher $\delta^{15}\text{N}$ values. Total isotopic niche areas (TA) of yolk and albumin between the two sites were different. These results support the contention that birds at the two breeding sites undertake different nutrient investment for egg reproduction and imply that females might modulate their reproductive strategy based on breeding site condition. No such differences were detected for nestling feather in this study, but there was a very high intraspecific site variation in chick nitrogen isotopes at the farmland site.

Testosterone concentrations

Yolks of the meadow site had higher testosterone concentrations. This could have been a consequence of differences in population composition and degree of female competition that are well documented social conditions that relate to yolk testosterone in the European starling (e.g., Pilz and Smith 2004) and other species (e.g., Schwabl 1996, Reed and Vleck 2001, Whittingham and Schwabl 2002). Alternatively, among other factors food availability and quality could have influenced yolk testosterone concentrations (Verboven et al. 2003, Vergauwen et al. 2012, but see Ruuskanen et al. 2015). Indeed, we detected an association between yolk testosterone concentrations and $\delta^{13}\text{C}$ enrichment in whole and lipid-free yolk. Given that yolk testosterone can enhance nestling growth (e.g., Schwabl 1996), the higher testosterone concentrations in meadow eggs could have contributed to the faster growth of nestlings in this habitat.

Nestling growth and survival

Nestling were heavier and more of them survived at the meadow site, where increased egg size and quality, yolk testosterone concentrations, or nestling feeding provided better conditions for nestling development. Higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ enrichment in nestling feathers at the meadow site potentially suggests a better diet for nestlings. Similar to other studies (Williams 1994, 2012), egg mass predicted survival (across sites, as well as within the meadow site), and nestling survival rate within broods was related to the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ enrichment level in lipid-free yolk. This suggests that the diet of the mothers during forming yolk

influenced nestling growth and survival. Theoretically, females that are able to form their eggs from a high-quality diet may also feed their nestlings with a high-quality diet. We propose that the nutritional quality of the breeding environment impacts reproductive success via resource-based prenatal maternal and postnatal parental effects that influence nestling growth and survival.

FINAL REMARKS AND CONCLUSIONS

Our comparison revealed significant habitat differences in female reproductive investment and reproductive success. The lower egg mass in the farmland than meadow habitat was mainly explained by reduced albumin mass. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ enrichment in yolk, but not albumin, indicated differences in spectrum and possibly trophic level of food items available for females to form eggs. Broods of the meadow site had higher survival rates than those of the farmland site, and surviving nestlings were larger. The practices used in crop farming likely limit the abundance and diversity of invertebrates to support the formation of high-quality eggs and nestling growth. Such environment-based prenatal and postnatal maternal effects may have critical impact on population structure and health. We propose that the compositional analysis of a single egg per female (or a nondestructively obtained sample of yolk and albumin) could be a minimally destructive diagnostic tool to gauge reproductive performance and predict consequences of habitat on food webs and the dynamics of consumer populations before they start to decline.

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