Short communication

Corticosterone and stable isotopes in feathers predict egg size in Atlantic Puffins *Fratercula arctica*

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Examining factors that operate outside the breeding season may provide new insights into life-history traits such as egg size, in which individual variation has not been fully explained. We measured corticosterone (CORT) levels and δ^{15}N values (trophic level) in feathers grown several months before egg-laying to test the prediction that a female's physiological state and feeding behaviour prior to the breeding season can influence egg mass in Atlantic Puffins *Fratercula arctica*. As predicted, egg mass increased with both CORT and δ^{15}N values in feathers, suggesting that the ability of female Puffins to meet the nutritional costs of egg production is related to CORT promoting increased foraging effort during moult and to consumption of a higher trophic-level diet.

Keywords: carry-over effects, feather corticosterone, nitrogen stable isotopes.

The fitness consequences of egg size have been documented for mothers and offspring in many taxa (Sinervo *et al.* 1992, Einum & Fleming 1999, Kaplan & Phillips 2006). In birds, there is an advantage to offspring that hatch from a larger egg (Krist 2011), yet egg size varies greatly among individual females with no satisfactory

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explanation (Christians 2002). In his meta-analysis, Christians (2002) found that repeatability of avian egg size generally exceeds 0.6, higher than for clutch size or laying date, and that single factors such as female age, body size and body mass generally account for less than 20% of the intraspecific variation in egg size. Recent studies support this conclusion, with female age and body condition either explaining a small to moderate amount of variation in egg size (Johnson *et al.* 2006, Beamonte-Barrientos *et al.* 2010) or having no apparent effect (Potti 2008, Svagelj & Quintana 2011). Within-season factors such as food availability and temperature also generally account for less than 15% of egg size variation in birds (Christians 2002).

Bernardo (1996) suggested that egg size may be determined by many interacting factors, and might largely depend on the ecological context in which the egg is produced. Given the longstanding inability to account adequately for intraspecific variation in avian egg size, researchers have recently begun to consider how behavioural factors operating outside the breeding season might be involved (Sorensen *et al.* 2009), and Williams (2005) has called for more rigorous studies of the physiological basis of the intraspecific variation. In Macaroni Penguins *Eudyptes chrysolophus*, for example, physiological processes underlying egg formation, which determine 'reproductive readiness', begin while females are migrating to nesting areas, such that females that lay shortly after returning to the colony produce clutches with greater size variance than clutches from females that spend more time at the colony before laying (Crossin *et al.* 2010).

Here, we test the prediction that physiological and behavioural factors operating prior to the breeding season influence the size of the single egg laid by a common North Atlantic seabird, the Atlantic Puffin *Fratercula arctica*. To do this, we measured δ^{15}N values, which gauge the relative trophic level of feeding, and levels of the steroid hormone corticosterone (CORT) in wing feathers grown several months prior to breeding. CORT levels in avian blood fluctuate in response to environmental challenges (Wingfield & Kitaysky 2002), food availability (Kitaysky *et al.* 1999, 2007) and reproduction (Wingfield & Sapolsky 2003, Goutte *et al.* 2010). However, it is not possible to collect blood from Puffins outside the breeding season. Fortunately, CORT circulating in the blood is incorporated into growing feathers, such that CORT levels in feathers are correlated with circulating levels during moult (Bortolotti *et al.* 2008, 2009). We therefore predicted that δ^{15}N values and CORT levels in primary feathers would be correlated with egg mass. We also included laying date as a covariate because egg size declines with laying date in Puffins (Harris 1980) and other seabirds that lay single-egg clutches (Birkhead & Nettleship 1982). In 2010, the abundance of Capelin *Mallotus villosus*, an

important prey species for Puffins in the northwest Atlantic (Nettleship 1972), was extremely low during the winter and early spring (Department of Fisheries and Oceans 2011); at this time of year, Puffins are distributed from the Newfoundland Grand Bank to the Scotian Shelf in the Atlantic Ocean (Hedd et al. 2010).

METHODS

We worked on Gull Island, Newfoundland, Canada (47°16′N, 52°46′W), in 2010. Puffins were monitored from early May to determine date of egg-laying and fresh egg mass. After hatching, the female parent in 12 nesting burrows was caught by hand or with a burrow noose. Primary feather six (p6), which is grown between January and April during the end of the prebasic moult (Pyle 2008), was collected from each adult, as was a small blood sample for genetic sexing. A DNeasy Blood and Tissue kit was used for DNA extraction (QIAGEN, Hilden, Germany). Individuals were sexed using highly conserved primers (2550F and 2718R) and a Chromodomain Helicase DNA-based method (Fridolfsson & Ellegren 1999). Capelin ($n = 7$) were also collected at this time.

The top 2–3 mm of each p6 feather and a small piece of muscle from each fish were used for stable nitrogen isotope analysis. Feather tips and muscle pieces were placed in individual vials and soaked in 2 : 1 chloroform/methanol solution for 24 h and then decanted. Feather tips were air-dried and minced with scissors. Muscle samples were dried in an oven and ground with a mortar and pestle. Approximately 1 mg of each sample was weighed and placed in an individual tin capsule. Relative abundance of $^{15}N/^{14}N$ was measured at the Stable Isotope Facility of the University of California, Davis. Stable isotope values are presented in delta notation (δ) as parts per thousand (‰) using the equation:

$$\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R is the ratio of $^{15}N/^{14}N$ and $R_{standard}$ for ^{15}N is atmospheric N_2 (AIR). Measurement error was estimated to be ± 0.12‰ based on within-run replicate measurements of nylon (mean = -9.77‰) and glutamic acid (mean = -4.26‰) laboratory standards (two standards for 12 unknowns). As whole fish are depleted in $\delta^{15}N$ compared with fish muscle alone (Cherel et al. 2005), Capelin values were corrected by -0.9 ± 0.1‰. The discrimination factor of Cherel et al. (2005) for fish-eating seabirds ($+4.2 \pm 0.7$‰) was applied to correct for diet-to-feather fractionation.

The remaining part of each feather was used for determination of CORT levels. The calamus was removed and feather length was measured to the nearest millimetre. The feather was minced with scissors into a vial and immersed in 5 mL methanol for 15 h (modified from Bortolotti et al. 2008). The solution was vacuum-filtered through filter paper (Whatman GF/B, 2.4-cm circles) and the resulting filtrate was evaporated under nitrogen gas. To reduce interference from lipids, each sample was subjected to a series of acetonitrile-hexane extractions, where CORT is partitioned into the acetonitrile (Mansour et al. 2002). Previously, we had found that extracting lipids from samples increased the consistency of CORT measurements (A. Kouwenberg unpubl. data). The purified residue was dissolved in 200 mL of EIA buffer solution (Cayman Chemical Company, Ann Arbor, MI, USA). CORT levels were measured in duplicate using an enzyme immunoassay kit (EIA, Cayman Chemical Company, Ann Arbor, MI, USA), which is highly sensitive (detection limit: 35 pg/mL) and has low cross-reactivity with non-CORT compounds. As well, the standard curve produced by this EIA was found to run parallel to a curve formed by CORT values of serially diluted Puffin feather samples (A. Kouwenberg unpubl. data). Before being assayed, all samples were diluted with EIA buffer, resulting in values that were between 20 and 80% binding, the optimal detection range for the EIA. The intra-assay coefficient of variation (CV) calculated from duplicate absorbance values was 3.13%. There is no inter-assay CV to report because all samples were run within the same assay. CORT values were converted to pg/mm using feather length measurements (see Bortolotti et al. 2009). All assayed feathers were of similar length (mean = 74.83 mm, range = 68–83 mm) and mass (mean = 53.74 mg, range = 46.89–61.85 mg) such that analyses were not compromised by feather mass differences (Lattin et al. 2011). Because CORT extraction and assay techniques reflect the extractable, immunoreactive CORT in feathers, rather than absolute biological levels, we limited our analysis to identifying relative differences in CORT levels among feathers extracted identically and measured on a single assay plate.

We expected egg size to decline with laying date (Harris 1980), so were interested in whether effects of $\delta^{15}N$ and CORT, alone or in combination with laying date, would be additive. Five candidate models were developed to explain variation in egg size: (1) null model (intercept-only model), (2) laying date, (3) laying date + $\delta^{15}N$, (4) laying date + CORT and (5) laying date + $\delta^{15}N$ + CORT. All models within each candidate set were ranked using Akaike's information criterion corrected for small sample size (AICc), based on the difference between each model's AICc and the lowest AICc from among the candidate set. These methods identify the single most parsimonious model ($\Delta AIC_c = 0.0$), plus others receiving strong support (ΔAIC_c scores ≤ 4.0; Burnham & Anderson 2002). The AICw measures the weight of evidence in favour of a particular model on a scale from 0 to 1, given the data and candidate model set.

RESULTS

The full model including laying date, δ^{15}N and CORT offered the most parsimonious explanation for intraspecific variation in egg mass (mean mass ± sem = 68.75 ± 3.72 g, range = 63–75 g) in Puffins (Table 1). This model received 95% of the model weight and had very strong explanatory power ($R^2 = 0.82$); no other model received strong support. Egg mass declined with laying date (parameter estimate in the full model = −0.67 g per day, 95% confidence limits: −0.95 to −0.39) but increased with both δ^{15}N values (1.26 g egg mass per ‰ of δ^{15}N, 95% confidence limits: 0.49–2.03) and CORT levels (0.11 g egg mass/pg/mm of CORT, 95% confidence limits: 0.04–0.18) measured in primary feathers grown during the pre-breeding period (Fig. 1). Based on the regression line (Fig. 1b), egg mass peaked as δ^{15}N values approached that for a diet consisting of 100% Capelin (mean ± sd = 11.96 ± 0.22‰).
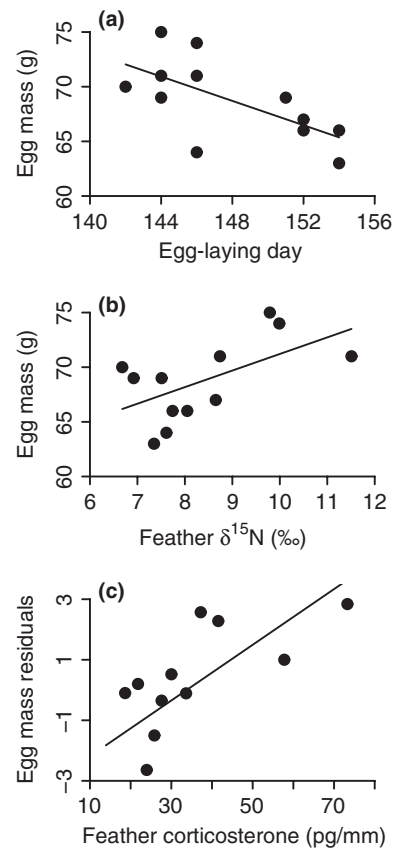
DISCUSSION

As expected (Harris 1980), egg mass declined with laying date in Atlantic Puffins but increased with both δ^{15}N values (trophic level) and CORT levels measured in primary feathers grown several months before eggs were laid. These results support the hypothesis that behavioural and physiological factors operating prior to the breeding season influence egg size in this species. It appears therefore that a Puffin's ability to meet the considerable nutritional demands of egg production (Williams 2005) may be heightened both by elevating total CORT levels and by consuming a high trophic-level diet prior to breeding. In birds, larger eggs produce larger hatchlings, which are more likely to survive periods of low food availability (Parsons 1970), and in auks, nestlings from larger eggs develop wing feathers more quickly (Hipfner & Gaston 1999, Hipfner 2000). Thus, all else being equal, females should benefit by producing large eggs.

Figure 1. (a) Relationship between egg mass (g) and egg-laying date (day of year). (b) Relationship between egg mass (g) and stable nitrogen isotopes (corrected by a discrimination factor for fish-eating seabirds) measured in female Atlantic Puffin p6 feathers (δ^{15}N, ‰). (c) Relationship between corticosterone in female Atlantic Puffin p6 feathers (CORT, pg/mm) and the residual variation arising from the multiple linear regression of egg mass (g) against egg-laying date and feather stable nitrogen isotopes measured in female Atlantic Puffin p6 feathers (δ^{15}N, ‰). Solid lines in (a), (b) and (c) depict linear regressions.

Table 1. Regression models considered for predicting variation in egg mass of Atlantic Puffins. Models were assessed with Akaike's information criterion for small sample size (AIC$_c$; Burnham & Anderson 2002).

Model	R^2	K	LIKAIC	ΔAIC$_c$	AIC$_w$
Lay date + δ^{15}N + CORT	0.82	5	1	0	0.95
Lay date + CORT	0.57	4	0.02	7.7	0.02
Lay date + δ^{15}N	0.58	4	0.03	7.35	0.02
Lay date	0.37	3	0.01	9.61	0.01
Null		2	0	13.31	0

K, number of estimable parameters; AIC$_w$, model weight.

Egg mass increased with trophic level in Puffins, peaking amongst females whose δ^{15}N values approached those expected from a diet consisting exclusively of Capelin. In contrast, egg mass declined with trophic level in Cassin's Auklets *Ptychoramphus aleuticus* (Sorensen *et al.* 2009), but whereas the mouthparts of Auklets are adapted for feeding on zooplankton, the mouthparts of Puffins are adapted for feeding on both zooplankton and fish (Bédard 1969). However, we caution that δ^{15}N values measured in feathers can also be affected by the degree to which a diet meets a consumer's amino acid needs, by the efficiency of protein deposition and by the amount of time spent fasting (Wolf *et al.* 2009). Hence, available discrimination factors may not fully reflect the

dynamics of fractionation between the tissues of predator and prey (Hobson 2011). Determining trophic level on a finer scale may require analysis of individual amino acids that show constant isotopic variation with trophic level (Hobson 2011).

Moult is known to be a nutritionally demanding process for birds, requiring large amounts of protein (Murphy & King 1992) and an increase of up to 111% of basal metabolic rate (Lindström *et al.* 1993, Hoye & Buttemer 2011). Birds that experience food restriction during moult tend to grow weaker feathers and show abnormal patterns of feather regrowth (Strochlic & Romero 2008, DesRochers *et al.* 2009). Elevated plasma CORT levels are often associated with increased foraging effort in birds, and may help individuals to meet environmental challenges (Astheimer *et al.* 1992, Kitaysky *et al.* 2001, Angelier *et al.* 2007, Doody *et al.* 2008). With feeding conditions so poor (Department of Fisheries & Oceans 2011), a female Puffin that increased CORT levels might have worked harder to obtain the nutrients required by wing-propelled divers to grow high-quality feathers. These high-quality feathers would facilitate maximum foraging efficiency later, during breeding. Although artificially elevating circulating CORT using implants has been found to reduce feather quality, CORT elevated through natural sources (psychological stress) affects feather quality only if birds are also food-restricted (Strochlic & Romero 2008, DesRochers *et al.* 2009). Therefore, greater nutrient intake due to increased CORT levels may have had a net positive effect on feather quality. In addition, the production of both eggs and feathers can be limited by the availability of sulphur-containing amino acids (Murphy & King 1992, Murphy 1994), so efficient foraging during moult might create a store of these and other amino acids that might later be used in egg production (Kendall *et al.* 1973, Houston *et al.* 1995a,b).

Based on the definition of Harrison *et al.* (2011), our results are consistent with a role for carry-over effects in influencing egg size in Puffins: under poor feeding conditions, differences among individuals in the trophic level at which they fed and in their CORT levels while they moulted their primary feathers (a clearly defined transition period) closely predicted inter-individual variation in egg size in the following breeding season. Although our study spanned just one transition period, and was correlational in nature, our results suggest that it is necessary to consider the entire annual cycle to understand variation during each stage of the cycle. Harrison *et al.* (2011) have called for experimental approaches that might better determine whether relationships such as we found are causal.

We conclude that measurement of CORT in feathers may provide valuable information about birds during stages of the annual cycle when they are inaccessible for sampling. Feathers provide an integrated value of CORT over a longer term, as opposed to the shorter-term 'snapshot' CORT value attained by assaying blood. Furthermore, feathers do not require sampling to be completed in less than 3 min, as does blood (Romero & Reed 2005), and are better suited than plasma for storage in remote field settings. However, like Lattin *et al.* (2011), we believe there is a need for further study of the physiology of integration of CORT into feathers if the technique is to provide results that can be fully interpreted.

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