Research paper

Egg size is independent of variation in pre-breeding feather corticosterone in Cassin's auklets during favorable oceanographic conditions

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A B S T R A C T

The measurement of corticosterone levels in feathers (fCort) is gaining recognition as an effective means for describing links between stages of the annual cycle in birds. Many seabirds are especially good models for exploring these links, or carryover effects, due to their migratory behavior and reproductive investment in a single-egg clutch. Here, we measure fCort in Cassin's auklet (*Ptychoramphus aleuticus*) breast feathers at two colonies in British Columbia during 2011, a year of favorable oceanographic conditions, and examine its relationship with egg size. These feathers are grown at sea during the late winter period, 1–2 months prior to egg laying. Assuming that fCort provides some measure of nutritional stress and hypothalamic-pituitary-adrenal axis activity during feather growth, we predicted that fCort would be positively correlated with egg size via increased support for foraging activity during the nutritionally demanding molt stage. We also analyzed the $\delta^{13}C$ and $\delta^{15}N$ stable isotope content of breast feathers, measures commonly used to characterize seabird diet composition. Contrary to prediction, neither fCort nor stable isotope ratios were good predictors of egg size. Our results appear to conflict with two previous studies on alcids in which fCort and stable isotopes showed clear links with egg size; however, both studies were conducted in years when oceanographic and foraging conditions were poor. Under these conditions, upregulation of corticosterone may be needed to mediate the energetic tradeoffs between self-maintenance and reproduction, supporting increased foraging effort and thus increasing both the likelihood of reproduction and large egg size. However, when foraging conditions are favorable, we suggest that such tradeoffs and associated physiological constraints are minimized and regulation of egg size may be effectively independent of circulating corticosterone levels and diet type.

1. Introduction

Many contributors to lifetime fitness and population level processes, including variation in breeding propensity, reproductive success, and survivorship, can be influenced by carryover effects: features of an organism's current performance that are explained by its past experience (O'Connor et al., 2014). These effects can act across a variety of temporal and spatial scales and have been documented in many marine species, including seabirds (e.g. Harrison et al., 2011). The most commonly identified carryover effects on reproduction stem from non-breeding or pre-breeding body condition (Crossin et al., 2013b, 2012a; Harrison et al., 2011; Salton et al., 2015), winter habitat quality (Norris et al., 2004; Sedinger et al., 2011), diet (Inger et al., 2010; Sorensen et al., 2009), and physiologic state (Crossin et al., 2013b, 2012a, 2010;

Kouwenberg et al., 2013). Each of these factors has been implicated in effects on breeding, including the decision to breed, reproductive phenology, and success, as well as levels of investment such as egg size and chick provisioning.

Since many carryover effects from the non-breeding period appear to be driven by environmental experience and nutritional stressors, physiologic state and diet are likely to be important indicators of these effects. Glucocorticoid hormones, including corticosterone (Cort), act as metabolic regulators of physiological and behavioral responses to unpredictable stressors (Hau et al., 2010; Wingfield et al., 1998), helping to regain positive energy balance in situations of allostatic overload (McEwen and Wingfield, 2003). These hormones are thus important components of pre-breeding physiologic state and have been identified as key mediators of many carryover effects.
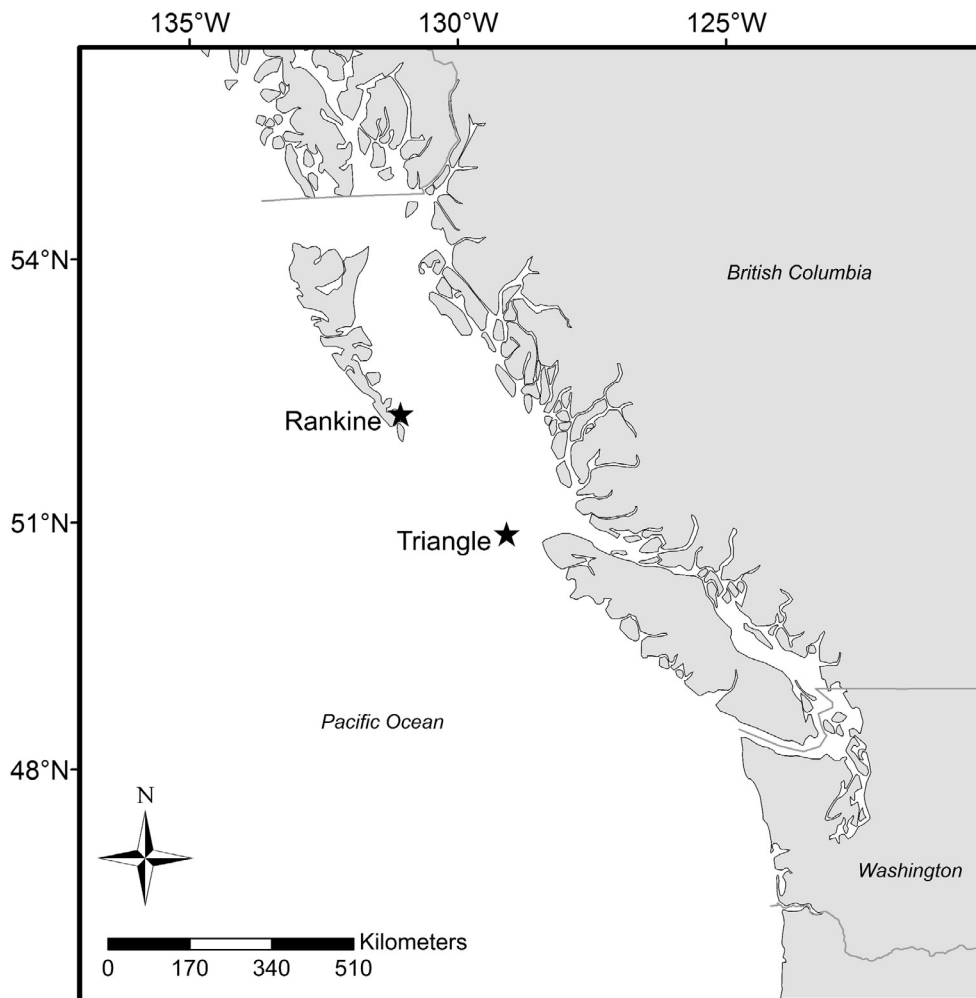
Fig. 1. Geographic location of the Triangle and Rankine Island study sites in British Columbia. Map generated using ArcMap 10.3 (ESRI, Redlands, CA, USA).

For seabirds, the measurement of Cort in feathers (fCort) has been a particularly useful way to examine longer term, cumulative Cort dynamics during the non-breeding period when most birds molt and grow new feathers (Bortolotti et al., 2009, 2008; Fairhurst et al., 2013). Similarly, feathers can be used to obtain integrated information on diet during the non-breeding period via stable isotope composition (Inger and Bearhop, 2008); 15-nitrogen is enriched in consumer tissues as trophic level increases (increasing $\delta^{15}N$) and 13-carbon exhibits an enrichment gradient from offshore to inshore prey sources and from pelagic to benthic prey sources (increasing $\delta^{13}C$) (Bond and Jones, 2009; Hobson et al., 1994; Kelly, 2000). Increases in fCort can be regarded as a direct physiological indicator of increased nutritional stress during periods of low food availability (Kitaysky et al., 2007; Will et al., 2015, 2014; but see opposite relationship in Patterson et al., 2015), and have also been linked to enhanced foraging effort, with positive effects on foraging success and reproductive investment (Crossin et al., 2012b; Kouwenberg et al., 2013). Feather Cort, $\delta^{15}N$, and $\delta^{13}C$ each provide unique information about non-breeding foraging experience and are useful tools for assessing the potential mechanisms governing carryover effects, particularly when examined together.

Seabirds share a suite of life-history traits across species, including high survival rates and low reproductive rates (Lack, 1968). Recent studies of these species have linked elevated pre-breeding fCort and diet quality to key fitness traits such as earlier laying and larger egg sizes (Kouwenberg et al., 2013; Sorensen et al., 2009), and have also demonstrated that elevated fCort can be associated with increases in foraging activity required to support successful reproduction (Angelier

et al., 2008; Crossin et al., 2013a, 2012b). If Cort is elevated to stress-induced levels, however, anti-gonadotropic effects can ensue (Salvante and Williams, 2003; Sapolsky et al., 2000), possibly by altering protein and lipid metabolism to favor self-maintenance over reproduction (Wingfield et al., 1998) and by suppressing the release of luteinizing hormone (LH) and subsequent estrogenic pathways (Brann and Mahesh, 1991; Etches et al., 1984; Goutte et al., 2010). Elevated fCort has thus been linked to negative reproductive effects, including later arrival at the breeding colony, poorer body condition at arrival, and reduced reproductive success (Harms et al., 2015), as well as breeding deferral (Crossin et al., 2013a; Hansen et al., 2016). Although such effects are detrimental to current reproduction, they may benefit lifetime fitness by helping balance reproductive demands with maternal quality and nutritional state (similar to Love and Williams, 2008).

Here, we test the hypothesis that the pre-breeding physiologic state and diet of a sentinel seabird, the Cassin's auklet (*Ptychoramphus aleuticus*), as represented by feather Cort and stable isotope content, will carry over to affect breeding investment and performance in the form of egg size. Our study took place in a year with favorable oceanographic conditions, thus we did not anticipate any chronic elevations in Cort sufficient to inhibit reproduction. Using the single-egg clutch of the Cassin's auklet as a model, we predicted (1) that higher fCort levels and (2) lower $\delta^{15}N$ would be linked to larger egg size, due to increased physiological support for foraging activities during the nutritionally demanding molt stage and preferred, lower trophic level diet, respectively. Cassin's auklet mouth parts are adapted for zooplanktivory (Bédard, 1969) and, though non-breeding diet is not well understood,

known prey items include copepods and euphausiids, as well as larval fish, crab, and squid (Ainley et al., 2011). We assume a lower trophic level preference partly because nestling growth is higher with lower trophic level, higher energy density diets (Hedd et al., 2002; Vermeer and Cullen, 1982). Shifts in adult diet from higher to lower trophic levels have also been documented between the pre- and post-laying period when zooplankton biomass is increasing off the southern coast of British Columbia (Hipfner et al., 2014; Mackas, 1992), suggesting that adult auklets may preferentially select energy dense, lower trophic level prey when it is available. In addition, we predicted (3) that higher $\delta^{13}C$ would be linked to larger egg size, reflecting easily accessed nearshore prey rather than potentially more energetically demanding offshore prey types. Potential effects of colony of origin were also considered.

2. Materials and methods

All research protocols were approved by Simon Fraser University Animal Care Services (974B-94), and carried out under additional permits from Environment Canada (banding permit 10667F, and Migratory Birds scientific permit BC-11-0016).

2.1. Field methods

Cassin's auklets nest in burrows on offshore islands along the west coast of North America (Ainley et al., 2011), and typically lay their single egg from late March to early May at British Columbian colonies (Hipfner et al., 2010). Breast feathers were collected from breeding birds at two colonies, Triangle Island (n = 14, 50° 52′ N, 129° 05′ W) and Rankine Island (n = 16, 52° 15′ N, 131° 03′ W) (Fig. 1), in April of 2011. Cassin's auklets undergo body molt to replace these feathers during the late wintering, spring migration, and pre-laying (colony region) residency periods, with peak replacement occurring in February and March (Ainley et al., 2011; Pyle, 2009). During February, most birds are either already resident in the colony region or are returning from southern wintering grounds, with the majority of birds resident in the colony region during the entire month of March prior to laying (Studholme, 2018), thus our samples represent reasonably comparable, integrated measures of circulating Cort levels and diet at that time. Adult auklets were removed from their incubation chambers and up to eight feathers were sampled by putting pressure at the base of the calamus and giving a quick tug in the natural direction of the feather. Samples were stored in envelopes in a dark dry place until processing and analysis. Egg length and breadth, adult morphometrics, and sex based on bill depth (Knechtel, 1998) were also recorded (measurements within 0.05 mm and 0.5 g). Only female birds were used in this study.

2.2. Hormone analysis

Feather corticosterone was determined using an established radioimmunoassay protocol (see Crossin et al., 2013a). Prior to analysis, each sample was prepared according to a protocol adapted from Bortolotti et al. (2008) and modified by Lattin et al. (2011). In short, the calamus was removed from each feather and the samples were standardized by mass to 0.0090 ± 0.0002 g (Mettler Toledo, New Classic MF, model ML204/03, d = 0.1 mg) to minimize any potential mass bias. The total length of feathers in each sample was then recorded for use in standardizing the fCort measurement (pg/mm). Final samples included material from 4 to 7 feathers. These samples were placed into vials, minced, and heat treated at 74 °C for at least 30 min, as per United States of America import regulations, before being sent to Tufts University (Medford, MA) for fCort extraction and analysis.

The minced feather samples were mixed with 7 mL of methanol, placed in a sonicating bath for 30 min, and incubated overnight in a 50 °C shaking water bath. A vacuum filtration system was used to separate out feather fragments and the methanol extracts were dried under nitrogen gas in a 50 °C water bath. Samples were reconstituted

with Tris-HCl buffer (0.05 M, pH 8.0) and run in duplicate through a standard radioimmunoassay (Wingfield et al., 1992) using a Sigma-Aldrich anti-corticosterone antibody (C8784, Saint Loui, MO, USA). Samples were processed in two assays with a mean intra-assay coefficient of variation (CV) of 2.77% and an inter-assay CV of 8.56%. Finally, these fCort data were plotted against sample mass to test for any potential bias; no relationship was found between these variables (linear regression: $F_{1,89} = 1.52$, $p = 0.22$, adjusted $R^2 = 0.01$).

2.3. Stable isotope analysis

Stable isotope composition was determined using one feather selected at random from each individual. Each feather was soaked in 2:1 chloroform:methanol solution for 24 h to remove surface oils, rinsed twice with fresh solution, and air dried in a fume hood for at least another 24 h. These feathers were then packaged in individual envelopes and sent to the University of Windsor Great Lakes Institute for Environmental Research (Windsor, ON) for analyses. There, the calami were removed and the remaining feather material was freeze-dried, minced to a fine consistency, subsampled, weighed, and run through an Elemental Analyzer-Isotope Ratio Mass Spectrometer (EA-IRMS) to determine $\delta^{15}N$ and $\delta^{13}C$ using standard procedures. Results are reported in parts per thousand (‰) using delta notation (δ) according to the following equation:

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

where X denotes the heavy isotope of the element of interest (e.g. ^{15}N) and R is the ratio of the heavy isotope to the lighter isotope of that element (e.g. $^{15}N/^{14}N$). $R_{standard}$ for ^{15}N is that of atmospheric N_2 air and for ^{13}C is that of Pee Dee Belemnite (PDB). Precision was determined using standards with n = 19. For $\delta^{15}N$, standard deviation was ± 0.10‰ for both internal standard tilapia and NIST standard bovine liver and for $\delta^{13}C$, standard deviation was ± 0.13‰ for tilapia and ± 0.20‰ for bovine liver. Additionally, 17 samples were run in duplicate. The two-way intraclass correlation coefficients ('icc', R package 'irr') for these duplicates were 0.951 for $\delta^{15}N$ and 0.997 for $\delta^{13}C$. Duplicate samples were averaged to yield single values prior to analysis.

2.4. Statistical analysis

All statistics were run in R version 3.4.1 (R Core Team, 2016). To assess the potential effects of fCort, $\delta^{15}N$, and $\delta^{13}C$ on egg size in Cassin's auklets, we built a candidate set of *a priori* models including all possible combinations of these variables and the two-way interactions among them (Table 1), representing all biologically plausible parameters while omitting more complex interactions with unclear biological implications. This candidate set was assessed separately for each colony since many seabirds, including auks, exhibit local adaptation in morphology and behavior between colonies of the same or closely related species (e.g., Catry et al., 2011; Friesen et al., 2007; Ibarguchi et al., 2011; Sausner et al., 2016; Wiley et al., 2012). This approach was favored over assessing a single candidate set with additional parameters for colony and all biologically plausible interactions, which likely would result in over-parameterization – especially given limited sample size. There was no multicollinearity in these variables at either colony as indicated by variance-inflation factors below a threshold of 3 (Fox and Monette, 1992). Egg volume index (EVI) was used as a standardized response variable, calculated as length × breadth^2 × 0.51 (cm^3) (Hoyt, 1979). The distribution of the dependent variable (EVI) at both colonies most closely resembled a Gamma distribution, therefore we used Gamma family generalized linear models with inverse link functions ('glm', package 'car').

To allow meaningful comparisons between effects in the presence of interactions and to improve interpretability, we centered (mean = 0) and scaled (SD = 1) all variables prior to model selection

Table 1

Candidate models used to assess potential predictors of egg volume in Cassin's auklets at each colony.

Parameters
~1 (null model)
fCort
$\delta^{15}N$
$\delta^{13}C$
fCort + $\delta^{15}N$
fCort + $\delta^{13}C$
$\delta^{15}N$ + $\delta^{13}C$
fCort + $\delta^{15}N$ + $\delta^{13}C$
fCort * $\delta^{15}N$
fCort * $\delta^{13}C$
$\delta^{15}N$ * $\delta^{13}C$
fCort * $\delta^{15}N$ + $\delta^{13}C$
fCort * $\delta^{13}C$ + $\delta^{15}N$
fCort + $\delta^{15}N$ * $\delta^{13}C$
fCort * $\delta^{15}N$ + fCort * $\delta^{13}C$
fCort * $\delta^{15}N$ + $\delta^{15}N$ * $\delta^{13}C$
fCort * $\delta^{13}C$ + $\delta^{15}N$ * $\delta^{13}C$
fCort * $\delta^{15}N$ + fCort * $\delta^{13}C$ + $\delta^{15}N$ * $\delta^{13}C$

(Schielzeth, 2010). Comparisons of models in each candidate set were performed using Akaike Information Criteria corrected for finite sample size (AIC$_c$; Burnham and Anderson, 2004; Sugiura, 1978; 'AICc', package 'AICcmodav') and normalized evidence weights (AIC$_w$; 'aicw', package 'geiger'). Models with $\Delta AIC_c < 2$ were considered to have the most substantial support. In addition, we calculated percent increase in explained deviance relative to the null model ($100 \times [(deviance_{null} - deviance_{model})/deviance_{null}]$) and McFadden's pseudo-$R^2$ for each candidate model to further assess goodness-of-fit.

For the most complex initial model and the best supported models ($\Delta AIC_c < 2$) at each colony, assumptions of homogeneity, normality of residuals, and independence were examined and found to be reasonable. Explanatory variables were assumed to be functionally deterministic. There was no indication that the relationship between the response and the predictors was non-linear. Multi-collinearity was also assessed for the best candidate models and confirmed absent.

3. Results

Average values of all variables were similar between Triangle Island and Rankine Island (Table 2). No variables were correlated at either colony (Pearson's correlations: $p > 0.05$; Supplementary Table A). Model comparison by AIC$_c$ indicated that the best model to explain variation in EVI at Triangle Island included only the parameter $\delta^{13}C$; however, three other models received substantial support ($\Delta AIC_c < 2$), including the null model, the model including $\delta^{15}N$ and $\delta^{13}C$, and the model including only $\delta^{15}N$ (Table 3). The normalized evidence weight (AIC$_w$) was also highest for the model including only $\delta^{13}C$ (0.342) but that of the null model (0.188) was similar relative to differences in the rest of the candidate set, suggesting like the several small ΔAIC_c values that the top model was not distinctly the best (Table 3). Similarly, percent deviance explained (38.10%) and pseudo-R^2 (still fairly low, 0.11) indicated that the third-best supported model by AIC$_c$ ($\delta^{15}N$ + $\delta^{13}C$) had the best fit among the candidate set. Several other

Table 3

Best supported (AIC$_c$ < 2) generalized linear models (Gamma distribution) predicting variation in egg volume index for Cassin's auklets at Triangle Island and Rankine Island in 2011. All combinations of the variables fCort, $\delta^{15}N$, $\delta^{13}C$, and their pairwise interactions were represented in the original candidate set. For each model below, K is the number of estimable parameters, AIC$_w$ is Akaike model weight, % Deviance Explained is the percent increase in explained deviance relative to the null model, and McFadden's pseudo-R^2 is a goodness-of-fit measure appropriate for generalized linear models.

Parameters	K	AIC$_c$	ΔAIC_c	AIC$_w$	% Deviance Explained	McFadden's pseudo-R^2
Triangle Island						
$\delta^{13}C$	3	63.40	0.00	0.342	27.53	0.08
~1 (null model)	2	64.61	1.20	0.188	0.00	0.00
$\delta^{15}N$ + $\delta^{13}C$	4	65.24	1.84	0.137	38.10	0.11
$\delta^{15}N$	3	65.36	1.96	0.128	16.65	0.04
Rankine Island						
~1 (null model)	2	74.70	0.00	0.315	0.00	0.00
$\delta^{15}N$	3	74.98	0.28	0.274	16.03	0.04

models received some support, with 12 of the 18 models having $\Delta AIC_c < 10$ (Supplementary Table B). For Rankine Island, model comparison by AIC$_c$ indicated that the best model to explain variation in EVI was the null model. One other model, including only the variable $\delta^{15}N$, received substantial support ($\Delta AIC_c < 2$) and had a similar AIC$_w$ to the null model (0.274 and 0.315, respectively; Table 3). Percent deviance explained by the second-best model over the null model was 16.03% and pseudo-R^2 was very low at 0.04. Thirteen of the 18 Rankine Island models had $\Delta AIC_c < 10$ (Supplementary Table C).

Standardized effect sizes (Schielzeth, 2010) for parameters in the best supported models were on the order of 10^{-2} or smaller at both Triangle Island and Rankine Island (Table 4). These scaled estimates indicate that an increase in one population standard deviation of the independent variable results in a change of X population standard deviations in the dependent variable, EVI, where X is the estimate shown in Table 4. For example, an increase of one population standard deviation in $\delta^{13}C$ at Triangle Island (1.11‰, see Table 2) is estimated to decrease EVI by 1.35×10^{-3} population standard deviations, or 2.96×10^{-3} cm^3 ($1.35 \times 10^{-3} \times 2.2$ cm^3). Similarly, an increase of 0.93‰ in $\delta^{15}N$ at Rankine Island corresponds to an increase of 2.70×10^{-3} cm^3 in EVI. In addition to the small magnitude of these effects, the 95% confidence intervals (CIs) overlap zero for all variable estimates except for that of $\delta^{13}C$ in the best supported model at Triangle Island (Table 4). This was also the only variable to show significance at the $\alpha = 0.05$ level when each best supported model was examined (ANOVA, type III sum of squares, LR X^2 (1, n = 14) = 4.42, p = 0.035).

Plots of each single-term variable against EVI revealed no clear relationships overall, consistent with small effect sizes and strong support for the null model at both colonies (Fig. 2). However, Fig. 2c suggests a slight positive relationship between $\delta^{15}N$ and EVI at Triangle Island and a slight negative relationship at Rankine Island, consistent with the presence of the variable among the top models at both colonies and the signs of the estimates reported in Table 4. We further assessed this relationship at each colony using the original, unscaled datasets and simple linear regression (Gaussian) to enable direct comparison of these effects with previous work at Triangle Island, dividing our EVI values

Table 2

Unscaled mean fCort, $\delta^{13}C$, $\delta^{15}N$, egg volume index (EVI), and egg measurements ± SD for Cassin's auklets at each study site and overall in 2011 (Triangle n = 14; Rankine n = 16).

	fCort (pg/mm)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	EVI	Length (mm)	Breadth (mm)
Triangle Island	2.4 ± 0.7	−18.50 ± 1.11	15.45 ± 0.80	28.3 ± 2.2	47.1 ± 1.3	34.3 ± 1.1
Rankine Island	2.6 ± 0.7	−18.50 ± 1.22	15.01 ± 0.93	27.8 ± 2.3	46.7 ± 1.4	34.1 ± 1.2
Overall	2.5 ± 0.7	−18.50 ± 1.15	15.22 ± 0.89	28.0 ± 2.2	46.9 ± 1.4	34.2 ± 1.1

Table 4

Model parameter estimates, errors, and confidence intervals (95% CI) for the best supported (AIC$_c$ < 2) generalized linear models (Gamma distribution) predicting variation in egg volume index for Cassin's auklets at Triangle Island and Rankine Island in 2011. The effect of δ^{13}C on EVI in Triangle Island Model 1 is significant at the α = 0.05 level ([*]).

Rank (AIC$_c$)	Parameter	Estimate	Std. Error	95% CI	
Triangle Island					
Model 1	δ^{13}C[*]	-1.35×10^{-3}	6.32×10^{-4}	-2.57×10^{-3}	-9.26×10^{-5}
	Intercept	3.54×10^{-2}	6.36×10^{-4}	3.42×10^{-2}	3.67×10^{-2}
Model 2	Intercept (null model)	3.54×10^{-2}	7.28×10^{-4}	3.40×10^{-2}	3.68×10^{-2}
Model 3	δ^{15}N	-8.89×10^{-4}	6.60×10^{-4}	-2.19×10^{-3}	4.03×10^{-4}
	δ^{13}C	-1.22×10^{-3}	6.27×10^{-4}	-2.43×10^{-3}	2.57×10^{-5}
	Intercept	3.55×10^{-2}	6.15×10^{-4}	3.43×10^{-2}	3.67×10^{-2}
Model 4	δ^{15}N	-1.09×10^{-3}	7.28×10^{-4}	-2.52×10^{-3}	3.34×10^{-4}
	Intercept	3.54×10^{-2}	6.94×10^{-4}	3.41×10^{-2}	3.68×10^{-2}
Rankine Island					
Model 1	Intercept (null model)	3.60×10^{-2}	7.39×10^{-4}	3.46×10^{-2}	3.75×10^{-2}
Model 2	δ^{15}N	1.17×10^{-3}	7.43×10^{-4}	-2.69×10^{-4}	2.64×10^{-3}
	Intercept	3.61×10^{-2}	7.03×10^{-4}	3.47×10^{-2}	3.75×10^{-2}

by 0.51 to match the EVI format in Sorensen et al. (2009). While Sorensen et al. (2009) reported a negative and significant effect of δ^{15}N on EVI at this colony in 2007 ($\beta = -2.81$, $p = 0.03$, $R^2 = 0.36$, $n = 13$), the equivalent regression using 2011 data yielded a slope which was instead positive and nonsignificant ($\beta = 2.10$, SE = ± 1.41, $F_{1,12} = 2.20$, $p = 0.16$, $R^2 = 0.16$, $n = 14$). The power of our dataset to detect the same effect as Sorensen et al. (2009) at Triangle Island was higher than in their study and the relationship between δ^{15}N and EVI was significantly different between these two studies (t-test, $t_{23} = 2.69$, $p < 0.05$; Supplementary Data S1). The effect of δ^{15}N on EVI at Rankine Island in 2011 was approximately opposite that at Triangle Island in the same year but was also nonsignificant ($\beta = -1.86$, SE = ± 1.18, $F_{1,14} = 2.46$, $p = 0.14$, $R^2 = 0.15$, $n = 16$).

4. Discussion

Based on previous studies of carryover effects in seabirds, we predicted that fCort and isotopes would explain variation in egg size, supporting a mechanistic link between overwinter experience and reproductive investment. However, independent of colony, there was very little support for effects of pre-breeding fCort, δ^{15}N, or δ^{13}C on Cassin's auklet egg size during our study and, if these effects were indeed present, they were remarkably small. Almost all the 95% confidence intervals (CIs) for parameter estimates overlapped zero, with the only exception being that for δ^{13}C in the best supported model at Triangle Island (Table 4), suggesting that diets composed of more inshore prey types (higher δ^{13}C) corresponded to smaller EVI; however,

this effect was extremely small with a 1.11‰ increase in δ^{13}C corresponding to a 2.96×10^{-3} cm^3 decrease in EVI.

Failure to reject the null hypothesis in this study does not necessarily contradict evidence that pre-breeding physiologic state and diet can carry over to affect reproductive investment. Inherent uncertainty in the timing of feather growth, the complex role of Cort in mediating nutritional stress and reproductive investment, and the potential for variation in isotopic baselines among individuals due to distributional differences during molt could have influenced the results of this study, as well as those of previous works (although the majority of Cassin's auklets breeding in British Columbia return to the colony region before March; Studholme, 2018). If we assume these factors had minimal effects, failure to reject the null hypothesis in this study could suggest that the relationship between pre-breeding Cort, diet, and reproductive investment may differ greatly based on the oceanographic conditions and food resources present in any given year. Many studies that identified physiologic or dietary carryover effects on egg size were conducted in resource-limited years where unfavorable oceanographic and/or foraging conditions prevailed. However, our study took place during a favorable year for Cassin's auklets, which may explain the apparent incongruity between our results and those of similar studies.

Cassin's auklets breeding in British Columbia in 2011 experienced moderate La Niña conditions, persisting since June of the previous year, and a negative (cool) phase of the North Pacific Oscillation (NPO) (minimum Oceanographic Niño Index = −1.4; National Oceanographic and Atmospheric Administration, 2016a,b). Such conditions are highly favorable, marked by cold sea surface temperatures,
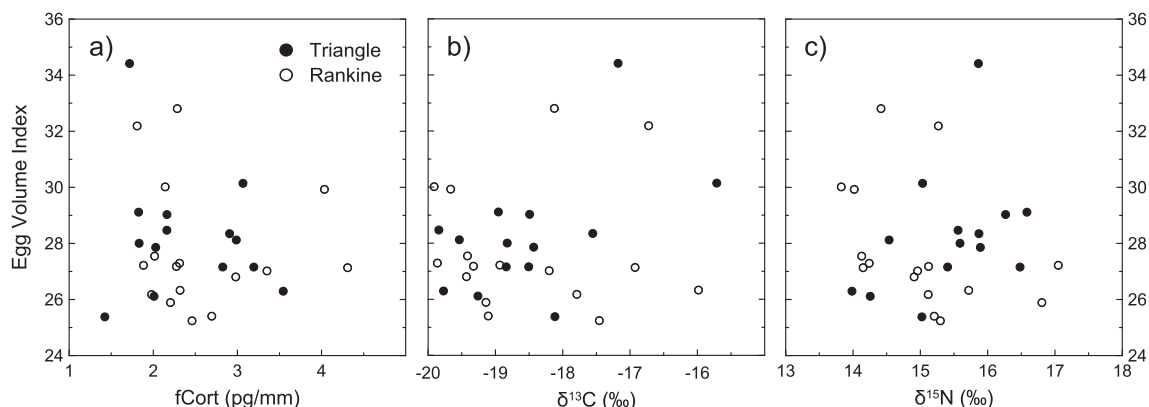
Fig. 2. Plots of each single term variable (fCort, δ^{13}C, δ^{15}N) against Egg Volume Index (EVI). No clear relationships are evident, with the possible exception of a slight positive association between EVI and δ^{15}N at Triangle Island and a slight negative association between the same variables at Rankine Island.

high ocean productivity (Ainley and Hyrenbach, 2010; Mackas et al., 2007), and high reproductive success. Thus, the lack of support for relationships between fCort, diet, and egg size (absent or very small effect sizes, presence of the null model among the best supported models at each colony) suggests that these auklets had little trouble acquiring the resources needed to produce eggs at either Triangle or Rankine Island. In contrast, a similar study with Atlantic puffins (*Fratercula arctica*) found positive relationships between these variables (95% CIs for effects did not overlap zero, strong top model support by AIC_c), but took place when birds were experiencing generally poor environmental conditions and low abundance of their preferred prey (capelin, *Mallotus villosus*) during the pre-breeding period (Kouwenberg et al., 2013). In that context, up-regulation of Cort may have supported increased foraging effort (i.e., the Cort-adaptation hypothesis – Bonier et al., 2009; see also Angelier et al., 2008; Crossin et al., 2012b; Doody et al., 2008), enabling puffins to intensify searching behaviors for preferred prey (thus also elevating $\delta^{15}N$) and/or alternate prey types (Kouwenberg et al., 2013). The positive relationship between pre-breeding fCort and egg size in this potentially food limited context points to the role of Cort in mediating foraging success and reproductive investment. However, as we show here – when conditions are favorable, this relationship between fCort, diet quality, and egg size appears to decouple, or be reduced to the extent that the null model was among the best supported models at each colony and non-zero effect sizes could not be supported for most variables using our dataset, possibly because these birds were under little nutritional constraint.

To illustrate this point further, Sorensen et al. (2009) studied carryover effects in Cassin's auklets at Triangle Island during poor oceanographic conditions and, like Kouwenberg et al. (2013), identified links between diet quality (feather $\delta^{15}N$) and egg size. The pre-breeding period represented in Sorensen et al. (2009) followed six months of unfavorable, weak El Niño conditions, and was characterized by a fairly neutral phase of the NPO (maximum Oceanographic Niño Index = 0.9; National Oceanographic and Atmospheric Administration, 2016a,b). The top models at both colonies in our study did include the variable $\delta^{15}N$, but the CIs for each estimate overlapped zero and no effects were significant (Table 4). For comparative purposes we also replicated the regression analysis in Sorensen et al. (2009) at Triangle Island and Rankine Island. Neither showed a significant effect, again suggesting that a detectable effect of diet quality on EVI may only exist under unfavorable conditions, notwithstanding possibility of Type I errors in previous work or Type II errors in this study due to limited sample sizes or variation in the timing and location of feather growth within or among individuals.

Despite very different oceanographic conditions between our study and Sorensen et al. (2009), egg sizes at Triangle Island were similar in 2007 and 2011 (mean EVI ± SD: 28.8 ± 2.2 cm³ and 28.3 ± 2.2 cm³, respectively; two-tailed *t*-test: t_{25} = 0.63, *p* = 0.53). This suggests that, for Cassin's auklets, egg size may be somewhat buffered from environmental variation. While one might expect mean egg size to be greater and variability to be lower in years when resources are plentiful and egg size is not related to variation in Cort or diet quality, these effects could be masked by the presence of many more, potentially less experienced birds in the laying population that could reduce mean egg size and increase variance to levels similar to those found under unfavorable conditions when fewer, more experienced breeders lay. In poor years, only these 'higher quality' females with superior ability to mediate the inhibitory effects of chronic Cort elevation may initiate breeding, even though breeding may be delayed in order to do so (Salvante and Williams, 2003). In fact, variation in oceanographic and foraging conditions might have their greatest impact at this breeding decision stage, via a body condition threshold for the initiation of laying (Crossin et al., 2016, 2013b, 2012a; Salvante and Williams, 2003) and/or via laying date (Hipfner et al., 2010, 2008). Indeed, despite substantial variation among individuals, egg size has been found to be a relatively fixed individual trait in many bird species.

That is, egg size is generally heritable with a high degree of consistency between breeding attempts in adult birds and often little variation attributable to pre-breeding environmental conditions (Christians, 2002); however, this has not yet been assessed for Cassin's auklets.

In summary, this work highlights several cautions for the study and interpretation of carryover effects acting on reproduction, particularly in relation to egg size. First, it illustrates the potential importance of understanding the specific environmental contexts during pre-breeding and how these can influence the endocrinological and/or physiological processes governing reproductive investment. Our study suggests that during favorable oceanographic conditions, up-regulation of Cort to facilitate increased foraging behavior during the pre-breeding period and the acquisition of preferred prey types may not be necessary for egg production in Cassin's auklets and has very little, if any, effect on egg size. Second, the similarity in egg volume (both mean and variation) between this study and Sorensen et al. (2009) suggests the possibility that egg size could be a relatively fixed individual trait in this species. Additional studies of this population using larger sample sizes, standardized approaches, and broader coverage of environmental conditions across years would help determine whether carryover effects on egg size are present and how they may vary, or whether Type I or Type II errors may be present in the literature. Future studies may also consider sampling back or head feathers (which may be more extensively replaced than breast feathers; Pyle, 2009), adding lay date as a fitness proxy, and combining physiological sampling with year-round tracking in order to control for potential differences in isotopic baselines due to variation in pre-breeding distributions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ygcen.2018.07.019.

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