IBIS
BOU international journal of avian science
Ibis (2015), **157**, 273–283

Feather-based measures of stable isotopes and corticosterone reveal a relationship between trophic position and physiology in a pelagic seabird over a 153-year period

GRAHAM D. FAIRHURST,[1]* ALEXANDER L. BOND,[1,2†] KEITH A. HOBSON[2] & ROBERT A. RONCONI[3,4]
[1]*Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, Saskatchewan, S7N 5E2 Canada*
[2]*Environment Canada, 11 Innovation Boulevard, Saskatoon, Saskatchewan, S7N 3H5 Canada*
[3]*Department of Biology, Acadia University, Wolfville, Nova Scotia, B4P 2R6 Canada*
[4]*Grand Manan Whale and Seabird Research Station, 24 Rte. 776, Grand Manan, New Brunswick, E5G 1A1 Canada*

Diet during the non-breeding period influences condition and subsequent reproduction. Physiological mechanisms underlying such carry-over effects are poorly understood but could be clarified by studying physiological responses to variation in diet during non-breeding. The hormone corticosterone provides a functional link between diet and survival and reproduction, but methodological limitations have prevented previous studies from testing the hypothesis that, on an individual level, avian corticosterone levels during the non-breeding period reflect broader patterns in feeding ecology during that time. Using museum specimens (1859–2002) and live birds (2012), we found that corticosterone from feathers ($\mathrm{CORT_f}$) is negatively related to trophic position (TP) inferred from feather stable-nitrogen isotope values (δ^{15}N) in Leach's Storm-petrels *Oceanodroma leucorhoa*. $\mathrm{CORT_f}$ was not related to stable-carbon isotope values (δ^{13}C). We detected no temporal trends in $\mathrm{CORT_f}$ or δ^{15}N, and neither was related to a large-scale index of winter climate, suggesting a general ecological phenomenon rather than a reflection of historical environmental changes. However, we detected a temporal trend in feather δ^{13}C, and δ^{13}C was related to δ^{15}N. Our findings suggest a physiological benefit of feeding at higher TPs, either through increased nutritional value or reduced foraging costs associated with higher TP prey, and future research should aim to distinguish between these two explanations. Nevertheless, ours is the first evidence of a correlation between individual endocrine levels and foraging ecology, and demonstrates non-lethal variation in a physiological mediator in turn related to variation in resource use.

Keywords: carbon-13, carry-over effects, environmental stressors, foraging ecology, glucocorticoids, North Atlantic Oscillation, nitrogen-15, non-breeding period, non-invasive biomarkers, seabird diet.

For many bird species, the non-breeding period is longer than the breeding period, and studies have increasingly highlighted the importance of non-breeding ecology to subsequent life-history processes (Chastel *et al.* 1995, Norris & Marra 2007, Sorensen *et al.* 2009, Harrison *et al.* 2011). The role of physiology in driving such carry-over effects is poorly understood, partly due to the relative inaccessibility of birds during the non-breeding season, and best exemplified by pelagic seabirds that spend these periods at sea. Diet during the non-breeding season can influence condition (Ronconi *et al.* 2010) and subsequent reproductive

---

†Current address: RSPB Centre for Conservation Science, Royal Society for the Protection of Birds, The Lodge, Sandy, Bedfordshire SG19 2DL, UK.

*Corresponding author.
Email: graham.fairhurst@usask.ca

performance (Chastel *et al.* 1995, Sorensen *et al.* 2009, Kouwenberg *et al.* 2013), suggesting a role for diet in generating carry-over effects. Thus, investigations of physiological responses to diet during the non-breeding period could help identify a potential mechanism underlying such effects.

The glucocorticoid hormone corticosterone (CORT) has been used to study the physiology of feeding because CORT helps maintain energy balance and is released in abundance in response to unpredictable environmental variation (Sapolsky *et al.* 2000, Dallman *et al.* 2001, Romero 2004). CORT levels in seabirds correlate negatively with food availability (Kitaysky *et al.* 1999b, 2010) and diet quality (Kitaysky *et al.* 2001). CORT can also affect foraging behaviour (Angelier *et al.* 2007a, Crossin *et al.* 2012, Cottin *et al.* 2014) and success (Angelier *et al.* 2007a), as well as activity and energy budgets (Angelier *et al.* 2007a,b). Importantly, CORT provides a functional link between diet and survival and reproduction (Angelier *et al.* 2007a, Kitaysky *et al.* 2007, 2010, Kouwenberg *et al.* 2013), making it particularly relevant to carry-over effects (Harrison *et al.* 2011).

Nearly all work with CORT in free-living seabirds has been conducted within the breeding period. Measuring CORT in seabirds during non-breeding has been difficult because it typically required blood sampling. There also has been a focus on CORT responses to specific aspects of seabird diet (e.g. quality or quantity), despite the importance of broader ecological patterns of feeding (e.g. trophic position and foraging location), particularly during non-breeding (Cherel *et al.* 2006, Ronconi *et al.* 2010, Kouwenberg *et al.* 2013). Moreover, previous studies of CORT responses to seabird diet have only addressed short time periods (1–2 years), so historical trends in this relationship are unstudied, hindering our ability to predict future trends. Measuring CORT in feathers (CORT$_f$) overcomes these deficiencies by providing data on physiological responses retrospectively. Many seabird species replace their feathers during the non-breeding period (Pyle 2008), and CORT deposited in such feathers reflects a long-term (i.e. days-to-weeks) integrated measure of CORT physiology (Bortolotti *et al.* 2008, Fairhurst *et al.* 2013a).

No previous study has tested, on an individual level, the hypothesis that birds during the non-breeding period respond physiologically to trophic position (TP) and foraging location. Experimentally manipulating TP and foraging location of most seabirds during non-breeding is impossible, so we addressed this hypothesis by correlating CORT$_f$ with feather-based measures of stable isotopes of nitrogen (δ^{15}N) and carbon (δ^{13}C) from Leach's Storm-petrel *Oceanodroma leucorhoa*, a widely distributed pelagic seabird. Combining CORT$_f$ with feather-based measures of stable isotopes provides a way to study relationships between physiology and foraging within the same individual and over a similar timeframe (Fairhurst *et al.* 2013b). Moreover, CORT$_f$ and feather stable isotope values remain unchanged over time (Kelly 2000, Bortolotti *et al.* 2009 and see below), allowing museum specimens to be used for analyses of both. Values of δ^{15}N and δ^{13}C are indicators of TP and foraging location, respectively, and reveal broad patterns in diet (Peterson & Fry 1987, Kelly 2000).

Leach's Storm-petrels feed predominantly on small crustaceans (e.g. amphipods; δ^{15}N = ~9‰) and fish (e.g. myctophids; δ^{15}N = ~10‰; Huntington *et al.* 1996, Hedd & Montevecchi 2006, Hedd *et al.* 2009), and increasing dietary prevalence of the latter is reflected by larger δ^{15}N values in feathers, making δ^{15}N an estimator of TP (Hedd & Montevecchi 2006, Pollet *et al.* 2014b). Foraging at a higher TP provides more nutritious prey and results in better body condition (Hedd & Montevecchi 2006, Ronconi *et al.* 2010, but see Morrison *et al.* 2014), so we predicted that Leach's Storm-petrels with higher δ^{15}N signatures would have relatively lower CORT$_f$ (Kitaysky *et al.* 1999a, Angelier *et al.* 2007a). Values of δ^{13}C increase toward the equator (Rau *et al.* 1982, Cherel *et al.* 2000) and with increased use of benthic or inshore habitats (Hobson *et al.* 1994, Hobson 1999, Kelly 2000), so Leach's Storm-petrel foraging location can be inferred from feather δ^{13}C values (Hedd & Montevecchi 2006, Pollet *et al.* 2014b). We also tested whether CORT$_f$ was related to foraging location (through feather δ^{13}C values) because the relationship between physiology and spatial segregation of wintering seabirds is poorly understood (Cherel *et al.* 2006). We additionally correlated CORT$_f$ and feather isotope values with large-scale climatic patterns that influence seabird foraging (North Atlantic Oscillation, NAO, December–February; Stenseth *et al.* 2003). We predicted that both CORT$_f$ and feather isotope values could be influenced by winter NAO via alterations to the food web (Reid *et al.* 1998), but such effects are probably spatially heterogeneous (Rose 2005, Harris *et al.* 2014). Therefore, although we could not derive specific predictions regarding the effects of NAO on Leach's Storm-petrels or their prey during the non-breeding season, we included NAO in the final model because it is an important driver of broad-scale climate and ecosystem functioning in the North Atlantic (Philippart *et al.* 2011, Harris *et al.* 2014). To increase the potential range of stable isotope values in our dataset, we used a time series of feathers from museum and live specimens collected in Atlantic Canada from 1859 to 2012, allowing us to test for historical (i.e. 153-year) trends in CORT_f, δ^{15}N, δ^{13}C and their inter-relationships.

METHODS

Sample collection and determination of sex

We sampled breast feathers grown during non-breeding (Huntington *et al.* 1996) from Leach's Storm-petrels collected on their breeding grounds in New Brunswick, Nova Scotia, Quebec and Newfoundland. We were only interested in individuals from the western North Atlantic Ocean and included only those museum specimens that had an exact year of collection (1859–2002; $n = 62$). Additionally, we sampled live birds during breeding on Gull Island, Newfoundland and Labrador (2012; $n = 5$). We sampled feathers from at least one Leach's Storm-petrel in every decade from 1859 to 2012, except the 1860s. Feathers from museum specimens were sampled at the Canadian Museum of Nature, Ottawa, Ontario ($n = 17$); New Brunswick Museum, Saint John, New Brunswick ($n = 24$); Royal Ontario Museum, Toronto, Ontario ($n = 12$); and National Museum of Natural History, Smithsonian Institution, Washington, DC ($n = 9$). Sex was determined for 56 of the 67 individuals using museum specimen labels or for live birds amplification of the CHD1 gene (Fridolfsson & Ellegren 1999).

CORT_f analysis

Because we were using museum specimens and had limited feather material, we needed to ensure that CORT measurements were well within the limits of detection of the radioimmunoassay (RIA). Therefore, we extracted CORT_f from two feathers per individual following procedures in Bortolotti *et al.* (2008). The length of each feather (proximal end of the calamus to distal tip of vane) was measured flat against the edge of a ruler, the calamus removed and discarded, and the length of the remaining feather was re-measured. Feather samples were then placed in glass vials and cut into pieces (*c.* 5 mm^2). We added 10 mL of methanol (HPLC grade; Fisher Scientific, Fair Lawn, NJ, USA) to each vial and placed samples in a sonicating water bath at room temperature for 30 min, followed by incubation overnight at 50 °C. We used a glass funnel fitted with polyester fibre and vacuum filtration to separate the methanol extract from the feather pieces. Collected methanol extract was then evaporated and extract residues were reconstituted in a small volume of phosphate-buffered saline (0.05 M, pH 7.6) and frozen at −20 °C until analysed by RIA. All samples were extracted in a single batch. We assessed the recovery efficiency of the extraction by spiking three extracts with approximately 5000 c.p.m. of ^{3}H-labelled CORT, and found the average recovery was 100%. Serial dilutions of feather extracts were parallel to the CORT standard curve.

Samples were analysed in duplicate in two RIAs. The variability of each assay was assessed using six replicates of an internal standard. Intra-assay coefficient of variation (CV) was 5.6% and inter-assay CV was 4.16%. Average detection limit ($\%B/B_0$) was 11.39 pg/100 μL of extract and all samples were above this limit. CORT_f measurements were corrected for total feather length, expressed as pg CORT/mm (Bortolotti *et al.* 2008). Extraction of CORT from feathers and subsequent analysis by RIA (Bortolotti *et al.* 2008) were performed at the University of Saskatchewan.

Stable isotope analysis

Feathers different from those used in CORT assays were analysed for stable isotopes. We acknowledge that this could introduce a temporal asynchrony between information about stable isotopes in feathers and CORT_f. To attempt to minimize this effect, we selected feathers that were spatially close to each other within the same feather tract. Feathers were washed in a 2 : 1 chloroform/methanol solution to remove surface oils and air-dried, and 1.0 ± 0.05 mg was placed in tin cups for isotopic analyses using continuous-flow isotope-ratio mass spectrometry. Values were corrected using BWB (bowhead whale baleen; δ^{13}C: −20.0‰, δ^{15}N: +14.4‰) and PRC (porcine) gelatin (δ^{13}C: −13.5‰, δ^{15}N: +4.7‰) as isotopic reference materials that were tested after every 24 unknowns. To account for the known temporal decline in δ^{13}C values due to anthropogenic increases in carbon emissions (the 'Suess effect'; Suess 1955, Gruber *et al.* 1999), which could bias our assessment of trends in δ^{13}C, we adjusted δ^{13}C values following eqn 2 in Farmer and Leonard (2011), which concerns the same geographical area and approximate time period. The maximum adjustment to δ^{13}C values was 1.7‰. We did not adjust δ^{13}C values for the increase in $CO_{2(aq)}$, as the magnitude of this change (0.16‰; Hilton *et al.* 2006, Jaeger & Cherel 2011) is within the analytical error of our instruments. Analytical precision among all samples was ±0.2‰ for δ^{13}C and δ^{15}N. Stable isotope analyses were conducted at the National Hydrology Research Centre (Environment Canada, Saskatoon, Saskatchewan).

Statistical analysis

To improve normality, we log-transformed $CORT_f$ values and excluded an individual with a δ^{13}C value > 3 interquartile ranges lower than the mean. Using known-sex birds ($n = 55$), we first tested for sex differences in $CORT_f$, δ^{15}N and δ^{13}C by including sex as the only main effect in separate general linear mixed models (GLMMs) for each of these response variables. Year was included as a random effect to account for clustered data. We then modelled temporal trends in $CORT_f$, δ^{15}N and δ^{13}C in all birds ($n = 66$) by including year as only the main effect in separate linear models for each of these response variables. We modelled sex-specific relationships between $CORT_f$ and stable isotopes using a GLMM that included sex, δ^{15}N, δ^{13}C and isotope-by-sex interactions as main effects, and year as a random effect. Non-significant terms ($P > 0.05$) were removed from final models. We then added winter (average of December, January and February) NAO (Hurrell 1995; downloaded from https://climatedataguide.ucar.edu/climate-data/hurrell-north-atlantic-oscillation-nao-index-station-based) as a covariate to the final GLMM of $CORT_f$. We also related winter NAO to δ^{15}N and δ^{13}C in two separate GLMMs, and correlated δ^{15}N with δ^{13}C in a third GLMM, including year as a random term in each of these three models. Statistical analyses were conducted using SAS v. 9.2 (SAS Institute, Cary, NC, USA).

RESULTS

There were no sex differences in $CORT_f$, δ^{15}N or δ^{13}C (Table 1). We did not detect temporal trends in $CORT_f$ ($F_{1,64} = 1.18$, $P = 0.28$; Fig. 1a) or δ^{15}N ($F_{1,64} = 0.20$, $P = 0.66$; Fig. 1b), but detected a positive trend in δ^{13}C ($F_{1,64} = 4.38$, $P = 0.04$; Fig. 1c). Non-significant sex-by-isotope interactions (δ^{15}N: $F_{1,48.03} = 0.43$, $P = 0.52$; δ^{13}C: $F_{1,48.22} = 1.56$, $P = 0.22$) and effect of sex ($F_{1,51.2} = 0.14$, $P = 0.71$) were removed from the model of $CORT_f$, so the final model of $CORT_f$ included only the significant negative effect of δ^{15}N ($r_{\text{adjusted}} = -0.40$; $F_{1,52.2} = 12.67$, $P = 0.0008$), which persisted when birds of unknown sex were added to the model ($r_{\text{adjusted}} = -0.33$; $F_{1,63.21} = 9.28$, $P = 0.003$; Fig. 2). NAO was not a significant term in either model of $CORT_f$ (all birds: $F_{1,53} = 0.32$, $P = 0.58$; known-sex birds only: $F_{1,53} = 2.75$, $P = 0.10$; Fig. 3a), nor was it related to δ^{15}N ($F_{1,54} = 0.75$, $P = 0.39$; Fig. 3b) or δ^{13}C ($F_{1,53.6} = 0.58$, $P = 0.45$; Fig. 3c). Values of δ^{13}C were negatively related to δ^{15}N values ($F_{1,63.09} = 14.67$, $P = 0.003$; Fig. 4).

DISCUSSION

By correlating $CORT_f$ with δ^{15}N from feathers of the same individuals, we provide the first evidence from the non-breeding season of a relationship between a physiological response and individual variation in a broad pattern of foraging ecology (i.e. TP). In line with our prediction, we detected a negative relationship between $CORT_f$ and TP, suggesting that Leach's Storm-petrels that fed at higher TPs secreted less CORT over the period of feather growth. CORT is released in response to stressors in general (Sapolsky *et al.* 2000, Romero 2004), and $CORT_f$ has been shown experimentally to reflect plasma CORT levels (Fairhurst *et al.* 2013a) and nutritional deficits (Will *et al.* 2014). Although we cannot conclude that variation in diet alone caused CORT responses in our study, whatever factor(s) CORT responded to were apparently linked to the TP at which birds fed. The idea that $CORT_f$ reflects responses to multiple ecological factors may explain why the relationship between $CORT_f$ and TP was not strong, and we cannot rule out the possibility that temporal asynchrony in

Table 1. Descriptive statistics of feather-based measures of corticosterone (CORT$_f$) and stable isotopes of nitrogen (δ^{15}N) and carbon (δ^{13}C) in relation to sex of Leach's Storm-petrels.

	All birds (*n* = 66)			Males (*n* = 27)			Females (*n* = 28)			Effect of sex (*n* = 55)	
Variable	Mean	sd	Range	Mean	sd	Range	Mean	sd	Range	*F* (df)	*P*
CORT$_f$ (pg/mm)	3.90	0.98	1.97–6.94	3.86	0.84	2.43–6.49	3.84	0.95	2.54–6.67	0.02 (1, 52.28)	0.89
δ^{15}N (‰)	+13.3	1.3	10.5–17.1	+13.1	1.2	10.5–15.9	13.5	1.5	10.6–17.1	0.95 (1, 53)	0.33
δ^{13}C (‰)	−16.9	1.1	−19.3 to −14.5	−17.0	1.1	−19.0 to −14.9	−16.7	1.1	−19.3 to −15.3	0.93 (1, 52.2)	0.34

when feathers were grown also could have weakened this relationship. Nevertheless, food supply is a key environmental determinant of CORT levels in seabirds (Kitaysky *et al.* 1999b, 2007, Benowitz-Fredericks *et al.* 2008) and one of only a few environmental stressors believed to face birds at sea (e.g. during the non-breeding period; Huntington *et al.* 1996). Therefore, we can suggest that the energetic benefits of foraging for, or greater fat or protein content of, higher TP prey contributed to lower CORT levels (Kitaysky *et al.* 2001, Forero *et al.* 2002, Angelier *et al.* 2007a, Hedd *et al.* 2009, Ronconi *et al.* 2010), although this may not always be the case for species specializing in lower trophic prey (e.g. Morrison *et al.* 2014).

Our data indicate that Leach's Storm-petrels fed across a range of TPs characteristic of seabird foraging behaviour during the non-breeding period (Hedd & Montevecchi 2006), and that higher δ^{15}N values probably reflected greater proportions of higher TP prey (i.e. fish) in the diet (Hedd & Montevecchi 2006, Ronconi *et al.* 2010, Bicknell *et al.* 2014, Pollet *et al.* 2014b). Foraging for higher TP prey can result in net energetic gains in Leach's Storm-petrels, even when longer-distance foraging trips are required, because of the greater energy content of higher TP prey (Hedd *et al.* 2009, Pollet *et al.* 2014b). Moreover, the essential fatty acid (EFA) profile of prey items can change with increases in TP, resulting in higher δ^{15}N values in tissues of consumers feeding on prey items with relatively greater percentages of some EFAs (Connelly *et al.* 2014). This lends some support to the idea that Leach's Storm-petrels feeding at higher TPs consumed a higher quality diet and may have benefited physiologically from doing so (Kitaysky *et al.* 2001, Ronconi *et al.* 2010, Pollet *et al.* 2014b). The three-fold variation in CORT$_f$ we observed among individuals suggests a correspondingly large variation in plasma CORT (Fairhurst *et al.* 2013a), yet all birds survived to be sampled on breeding grounds the summer following feather growth. This is consistent with the idea that birds feeding at low TPs were not starving (i.e. plasma CORT levels were not continuously high). Instead, CORT$_f$ data are likely to reflect variation in baseline CORT to match foraging conditions (Angelier *et al.* 2007a).

The lack of effects of year and winter NAO on CORT$_f$ in our 153-year time-series suggests that the ecophysiological relationship we report is a general phenomenon and not a reflection of broad historical changes in climate during non-breeding. The δ^{13}C data, from which we can infer general individual foraging location, provide additional insight into the influence of the non-breeding environment. The range of δ^{13}C values in our study is similar to that reported by Hedd and Montevecchi (2006), suggesting that Leach's Storm-petrels moulted in the tropics, as would be expected (Huntington *et al.* 1996, Pollet *et al.* 2014a), but the δ^{13}C data were also quite variable. We did not detect a relationship between CORT$_f$ and δ^{13}C, suggesting that foraging location during the feather growth period (δ^{13}C) apparently did not influence CORT physiology. This may have been due to the relatively weak latitudinal gradient in δ^{13}C values in the North Atlantic, or to annual variation masking effects of foraging location. Despite this, δ^{15}N and δ^{13}C covaried negatively, consistent with the idea of latitudinal (Cherel *et al.* 2014) and/or inshore–offshore (Pollet *et al.* 2014b) spatial variation in Leach's Storm-petrel diet. Although beyond the scope of our study, more complex approaches to the spatial variability in marine climate (e.g. Hemery *et al.* 2008, Harris *et al.* 2014) could improve the ability

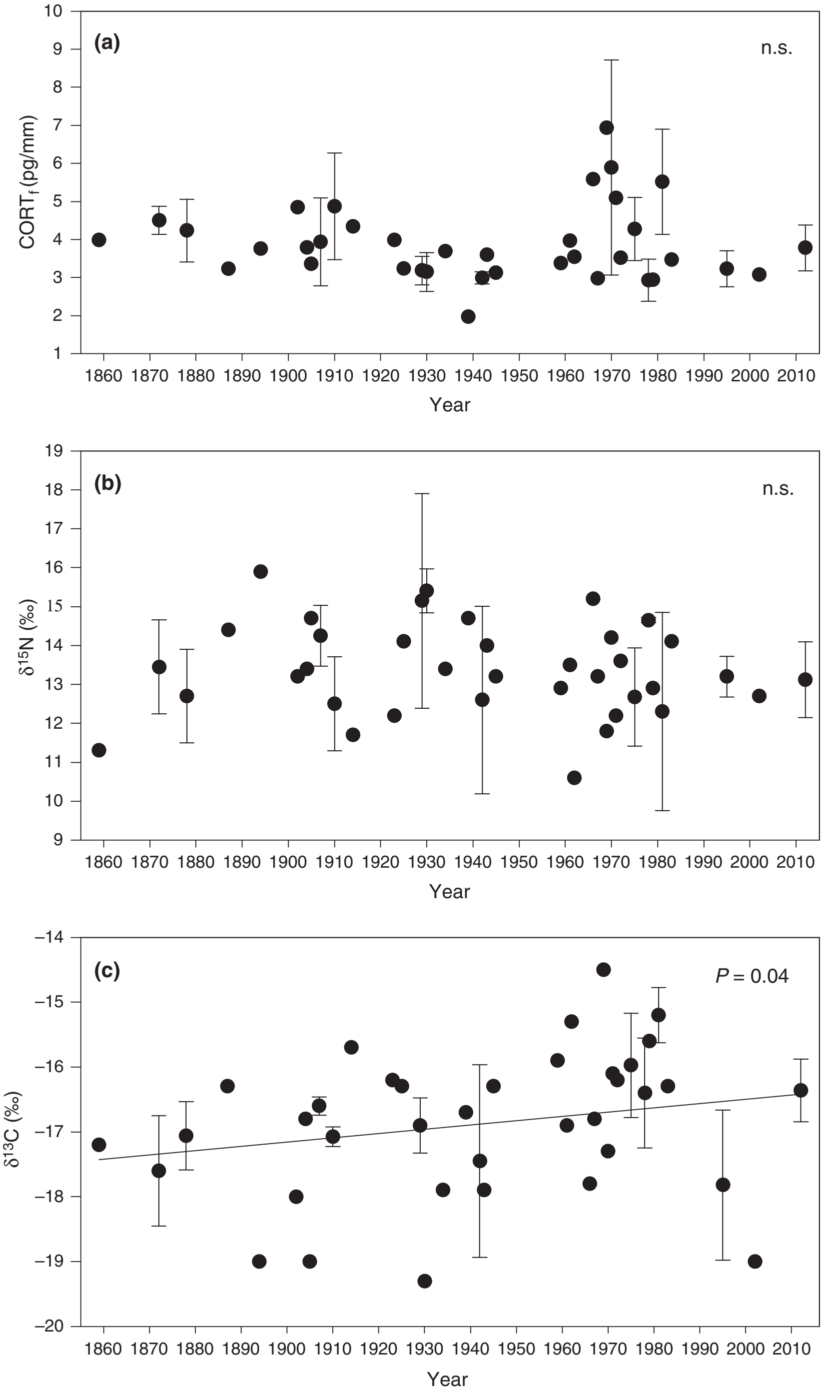

Figure 1. Temporal variation in (a) corticosterone (CORT$_f$), and stable isotopes of (b) nitrogen (δ^{15}N) and (c) carbon (δ^{13}C) from Leach's Storm-petrel feathers over a 153-year period. Feathers were grown during the non-breeding period. Mean ± standard deviations are shown.

of future research to relate large-scale climate patterns to CORT physiology and stable isotopes.

Our set of feathers spanned a 153-year period and thus provided a unique opportunity to assess long-term trends in physiology and stable isotopes. We failed to detect temporal trends in CORT$_f$ or δ^{15}N, but a weak but significant positive trend in δ^{13}C values may provide clues about historical

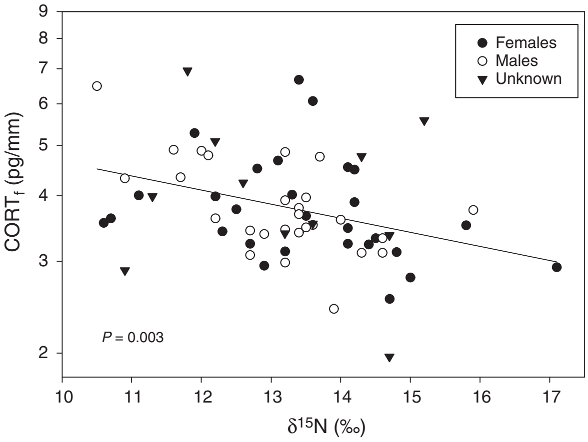

Figure 2. Relationship between nitrogen stable isotopes ($\delta^{15}N$) and corticosterone (CORT$_f$) from Leach's Storm-petrel feathers over a 153-year period. Feathers were grown during the non-breeding period.

spatiotemporal variation in Leach's Storm-petrel foraging. Cherel *et al.* (2014) previously showed that average $\delta^{13}C$ values from Thin-billed Prion *Pachyptila belcheri* feathers decreased significantly from historical (1923–1971) to contemporary (2003) samples from the Kerguelen Islands (southern Indian Ocean), and interpreted this as a temporal shift of moulting grounds to higher latitudes. Hilton *et al.* (2006) similarly detected decreases in $\delta^{13}C$ values from Rockhopper Penguins *Eudyptes chrysocome* (*c.* 1840–2000), but only in some colonies. By contrast, our data indicate that Leach's Storm- petrels breeding in the North Atlantic may have shifted their moulting grounds south to lower latitudes and/or to more benthic or inshore habitats over the past 150 years (i.e. temporal increase in $\delta^{13}C$ values). Rather than being a recent event, our long-term data show a steady enrichment in ^{13}C, indicating a gradual shift from the late 1800s to the present, whereas background variation in both $\delta^{13}C$ and $\delta^{15}N$ in Atlantic marine foodwebs has remained relatively stable over time (MacKenzie *et al.* 2014). Although relatively small, and potentially influenced by anomalous data from the 1960s to 1980s (see below), the temporal shift in $\delta^{13}C$ values was opposite to the Suess effect, suggesting that the shift was stronger than appears at first glance. Determining whether this trend is a response to changing climatic or oceanographic conditions or just a reflection of local variation in $\delta^{13}C$ is a matter for future research.

There is very limited evidence to suggest that the CORT in feathers from historical museum specimens may not be stable and can therefore introduce bias into historical analyses such as those we present here (Bortolotti *et al.* 2009). The single published work addressing this showed that average CORT$_f$ values from Great Horned Owls *Bubo viginianus* were significantly higher in museum specimens from 1931 to 1974 than they were in

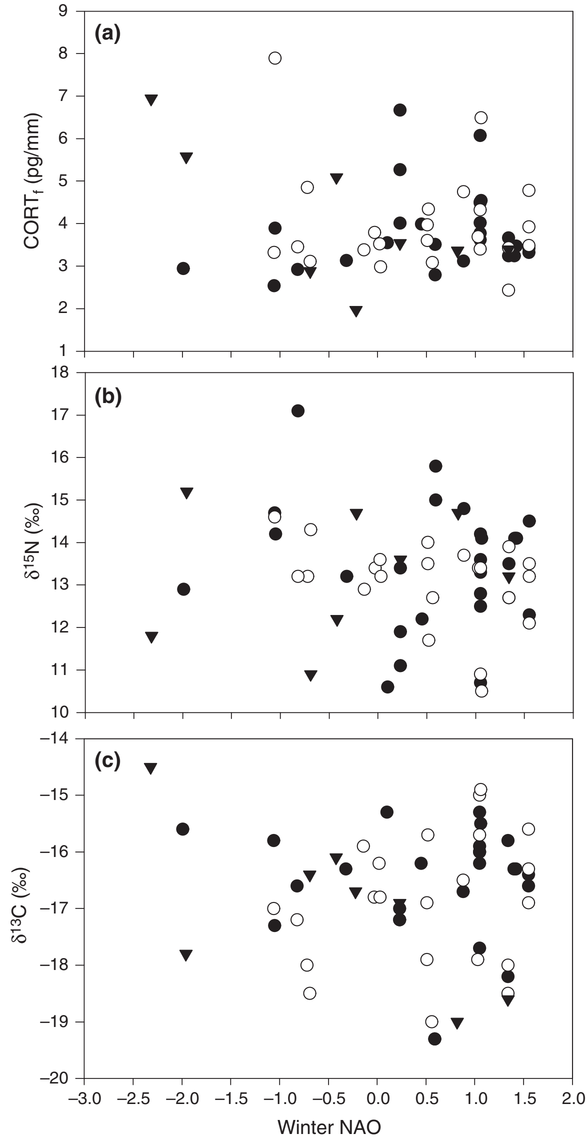

Figure 3. Relationships between the Northern Atlantic Oscillation during December–February from 1902 to 2012 (Winter NAO) and (a) corticosterone (CORT$_f$), and stable isotopes of (b) nitrogen ($\delta^{15}N$) and (c) carbon ($\delta^{13}C$) from Leach's Storm-petrel feathers. Females are represented by filled circles, males are represented by open circles and unknown sex birds are represented by filled triangles. Feathers were grown during the non-breeding period. All relationships are non-significant.

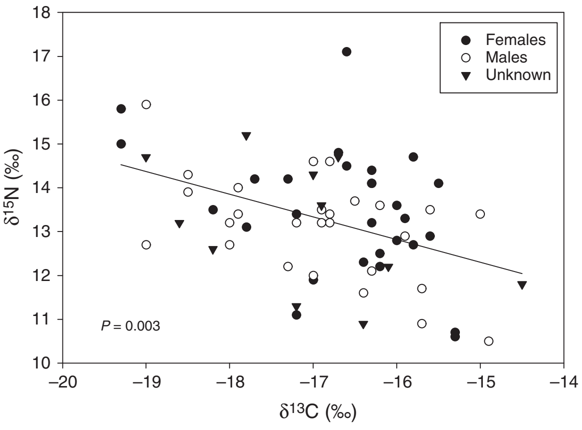

Figure 4. Relationship between feather-based measures of stable isotopes of nitrogen (δ^{15}N) and carbon (δ^{13}C) from Leach's Storm-petrels over a 153-year period. Feathers were grown during the non-breeding period.

carcasses or live birds from 2004 to 2005 (Bortolotti *et al.* 2009). The authors noted that their intent was not to investigate causation, and they provide a valid alternative (ecological) explanation for their results. We add three additional points to this discussion. First, the results from Bortolotti *et al.* (2009) come from a single species from a limited geographical sampling area (Saskatoon, Canada). Secondly, if the CORT in feathers is not stable and older feathers do have higher CORT$_f$ values, then the effect reported by Bortolotti *et al.* (2009) needs to be explained mechanistically. For example, CORT$_f$ values from older feathers could have had a strong bias, but this kind of artificial inflation would require production of a CORT mimic within the feather, which seems unlikely. Thirdly, if age of feathers does influence CORT$_f$ values for reasons other than physiology (e.g. degradation of CORT within the feather), it would be reasonable to predict that a temporal trend in CORT$_f$ would exist in our data. We did not detect a trend in CORT$_f$, although we recognize that ecological effects (e.g. environmental change) could have masked such a trend were it there. Moreover, if CORT in feathers does degrade over time, it seems just as likely (if not more so) that CORT$_f$ would be lower in older feathers, so it may not be fair to criticize higher levels of CORT$_f$ in older feathers as problematic (e.g. Bortolotti *et al.* 2009). Thus, although we cannot eliminate the possibility of a 'degradation bias' in our CORT$_f$ values from museum feathers, currently we lack evidence to suggest that one existed.

Despite not detecting a linear temporal trend in CORT$_f$, it is interesting that the data showed apparent periodic variation. Of note are the late 1960s to early 1980s, when CORT$_f$ values were higher than at any other point in our time series. We did not have demographic data with which to infer possible consequences of this spike in CORT$_f$ values, and the reason for this anomaly remains unidentified. However, there was also an apparent spike in δ^{13}C values during that period. In line with our interpretation of CORT$_f$ reflecting physiological responses to foraging ecology, we can therefore speculate that use of unusually inshore or equatorial foraging areas, perhaps in concert with population dynamics (e.g. a temporary crash) of preferred prey, during those decades could explain this result. Future studies will benefit from identifying potential causes and downstream consequences (e.g. to subsequent breeding) of the variation in CORT$_f$ we show exists during the non-breeding period, including such periods of apparently anomalously high levels.

Our findings demonstrate non-lethal variation in a physiological mediator in relation to variation in resource use. This is a key characteristic of mechanisms underlying carry-over effects (Harrison *et al.* 2011). Among-individual variation in CORT levels is related to foraging behaviour and condition (Kitaysky *et al.* 1999b, Angelier *et al.* 2007a), δ^{15}N is related to body condition (Ronconi *et al.* 2010, 2014), and both δ^{15}N and CORT$_f$ have been shown separately to be positively related to subsequent reproductive performance (Crossin *et al.* 2013, Kouwenberg *et al.* 2013, but see Angelier *et al.* 2009, 2010). The covariation we found between TP and a physiological response during the non-breeding period links these previously independent research lines. Moreover, we demonstrate the utility of combining CORT$_f$ with other feather-based biomarkers (Fairhurst *et al.* 2013b, 2014, Grava *et al.* 2013), and by using museum specimens we gained a historical perspective on physiology–resource use relationships. Given potential associations between CORT and correlates of fitness (Bonier *et al.* 2009), future research should explore a mechanistic role of CORT in mediating how diet affects individual variation in condition and subsequent reproductive performance. Coping with environmental variation is essential to survival and reproduction, and CORT plays a central role in that process, so a CORT-mediated mechanism of carry-over effects could be widespread in birds.

We thank M. Goselin (Canadian Museum of Nature), D. McAlpine (New Brunswick Museum), M. Peck (Royal Ontario Museum) and James Dean (National Museum of Natural History – Smithsonian Institution) for providing access to collections. We gratefully acknowledge T. Marchant for stimulating discussions and the use of her endocrine lab. Funding was provided by the National Science and Engineering Research Council of Canada (Postdoctoral Fellowship to A.L.B.), Environment Canada and the F.M. Christie Research Grants in Botany and Zoology. We kindly thank R. Nager, R. Wanless, R. Dawson, C. Michelson and two anonymous reviewers for helping us improve this manuscript.

REFERENCES

Angelier, F., Shaffer, S.A., Weimerskirch, H., Trouve, C. & Chastel, O. 2007a. Corticosterone and foraging behavior in a pelagic seabird. *Physiol. Biochem. Zool.* **80**: 283–292.

Angelier, F., Clement-Chastel, C., Gabrielsen, G.W. & Chastel, O. 2007b. Corticosterone and time-activity budget: an experiment with Black-legged Kittiwakes. *Horm. Behav.* **52**: 482–491.

Angelier, F., Clement-Chastel, C., Welcker, J., Gabrielsen, G.W. & Chastel, O. 2009. How does corticosterone affect parental behaviour and reproductive success? A study of prolactin in Black-legged Kittiwakes. *Funct. Ecol.* **23**: 784–793.

Angelier, F., Wingfield, J.C., Weimerskirch, H. & Chastel, O. 2010. Hormonal correlates of individual quality in a long-lived bird: a test of the 'corticosterone-fitness hypothesis'. *Biol. Lett.* **6**: 846–849.

Benowitz-Fredericks, Z.M., Shultz, M.T. & Kitaysky, A.S. 2008. Stress hormones suggest opposite trends of food availability for planktivorous and piscivorous seabirds in 2 years. *Deep Sea Res. Part 2 Top. Stud. Oceanogr.* **55**: 1868–1876.

Bicknell, A.W.J., Knight, M.E., Bilton, D.T., Campbell, M., Reid, J.B., Newton, J. & Votier, S.C. 2014. Intercolony movement of pre-breeding seabirds over oceanic scales: implications of cryptic age-classes for conservation and metapopulation dynamics. *Divers. Distrib.* **20**: 160–168.

Bonier, F., Martin, P.R., Moore, I.T. & Wingfield, J.C. 2009. Do baseline glucocorticoids predict fitness? *Trends Ecol. Evol.* **24**: 634–642.

Bortolotti, G.R., Marchant, T.A., Blas, J. & German, T. 2008. Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. *Funct. Ecol.* **22**: 494–500.

Bortolotti, G.R., Marchant, T., Blas, J. & Cabezas, S. 2009. Tracking stress: localisation, deposition and stability of corticosterone in feathers. *J. Exp. Biol.* **212**: 1477–1482.

Chastel, O., Weimerskirch, H. & Jouventin, P. 1995. Body condition and seabird reproductive performance: a study of 3 petrel species. *Ecology* **76**: 2240–2246.

Cherel, Y., Hobson, K.A. & Weimerskirch, H. 2000. Using stable-isotope analysis of feathers to distinguish moulting and breeding origins of seabirds. *Oecologia* **122**: 155–162.

Cherel, Y., Phillips, R.A., Hobson, K.A. & McGill, R. 2006. Stable isotope evidence of diverse species-specific and individual wintering strategies in seabirds. *Biol. Lett.* **2**: 301–303.

Cherel, Y., Connan, M., Jaeger, A. & Richard, P. 2014. Seabird year-round and historical feeding ecology: blood and feather delta C-13 and delta N-15 values document foraging plasticity of small sympatric petrels. *Mar. Ecol. Prog. Ser.* **505**: 267–280.

Connelly, T.L., Deibel, D. & Parrish, C.C. 2014. Trophic interactions in the benthic boundary layer of the Beaufort Sea shelf, Arctic Ocean: combining bulk stable isotope and fatty acid signatures. *Prog. Oceanogr.* **120**: 79–92.

Cottin, M., MacIntosh, A.J.J., Kato, A., Takahashi, A., Debin, M., Raclot, T. & Ropert-Coudert, Y. 2014. Corticosterone administration leads to a transient alteration of foraging behaviour and complexity in a diving seabird. *Mar. Ecol. Prog. Ser.* **496**: 249–262.

Crossin, G.T., Trathan, P.N., Phillips, R.A., Gorman, K.B., Dawson, A., Sakamoto, K.Q. & Williams, T.D. 2012. Corticosterone predicts foraging behavior and parental care in Macaroni Penguins. *Am. Nat.* **180**: E31–E41.

Crossin, G.T., Phillips, R.A., Lattin, C.R., Romero, L.M. & Williams, T.D. 2013. Corticosterone mediated costs of reproduction link current to future breeding. *Gen. Comp. Endocrinol.* **193**: 112–120.

Dallman, M.F. & Bhatnagar, S. 2001. Chronic stress and energy balance: role of the hypothalamo-pituitary-adrenal axis. In McEwen, B.S. (ed.) *Handbook of Physiology, Section 7: The Endocrine System, Vol. IV: Coping with the Environment: Neural and Endocrine Mechanisms*: 179–210. New York: Oxford University Press.

Fairhurst, G.D., Marchant, T.A., Soos, C., Machin, K.L. & Clark, R.G. 2013a. Experimental relationships between plasma- and feather-levels of corticosterone in a free-living bird. *J. Exp. Biol.* **216**: 4071–4081.

Fairhurst, G.D., Vögeli, M., Serrano, D., Delgado, A., Tella, J.L. & Bortolotti, G.R. 2013b. Can synchronizing feather-based measures of corticosterone and stable isotopes help us better understand habitat-physiology relationships? *Oecologia* **173**: 731–743.

Fairhurst, G.D., Dawson, R.D., van Oort, H. & Bortolotti, G.R. 2014. Synchronizing feather-based measures of corticosterone and carotenoid-dependent signals: what relationships do we expect? *Oecologia* **174**: 689–698.

Farmer, R.G. & Leonard, M.L. 2011. Long-term feeding ecology of great Black-backed Gulls (*Larus marinus*) in the northwest Atlantic: 110 years of feather isotope data. *Can. J. Zool.* **89**: 123–133.

Forero, M.G., Hobson, K.A., Bortolotti, G.R., Donázar, J.A., Bertellotti, M. & Blanco, G. 2002. Food resource utilisation by Magellanic Penguin evaluated through stable isotope analysis: segregation by sex and age and influence of offspring quality. *Mar. Ecol. Prog. Ser.* **234**: 289–299.

Fridolfsson, A.-K. & Ellegren, H. 1999. A simple and universal method for molecular sexing of non-ratite birds. *J. Avian Biol.* **30**: 116–121.

Grava, T., Fairhurst, G.D., Avey, M.T., Grava, A., Bradley, J., Avis, J.L., Bortolotti, G.R., Sturdy, C.B. & Otter, K.A. 2013. Habitat quality affects early physiology and subsequent neuromotor development of juvenile Black-capped Chickadees. *PLoS ONE* **8**: e71852.

Gruber, N., Keeling, C.D., Bacastow, R.B., Guenther, P.R., Lueker, T.J., Wahlen, M., Meijer, H.A., Mook, W.G. &

Stocker, T.F. 1999. Spatiotemporal patterns of carbon-13 in the global surface oceans and the oceanic Suess effect. *Global Biogeochem. Cycles* **13**: 307–335.

Harris, V., Edwards, M. & Olhede, S.C. 2014. Multidecadal Atlantic climate variability and its impact on marine pelagic communities. *J. Mar. Syst.* **133**: 55–69.

Harrison, X.A., Blount, J.D., Inger, R., Norris, D.R. & Bearhop, S. 2011. Carry-over effects as drivers of fitness differences in animals. *J. Anim. Ecol.* **80**: 4–18.

Hedd, A. & Montevecchi, W.A. 2006. Diet and trophic position of Leach's storm-petrel *Oceanodroma leucorhoa* during breeding and moult, inferred from stable isotope analysis of feathers. *Mar. Ecol. Prog. Ser.* **322**: 291–301.

Hedd, A., Montevecchi, W.A., Davoren, G.K. & Fifield, D.A. 2009. Diets and distributions of Leach's storm-petrel (*Oceanodroma leucorhoa*) before and after an ecosystem shift in the Northwest Atlantic. *Can. J. Zool.* **87**: 787–801.

Hemery, G., D'Amico, F., Castege, I., Dupont, B., D'Elbee, J., Lalanne, Y. & Mouches, C. 2008. Detecting the impact of oceano-climatic changes on marine ecosystems using a multivariate index: the case of the Bay of Biscay (North Atlantic-European Ocean). *Glob. Change Biol.* **14**: 27–38.

Hilton, G.M., Thompson, D.R., Sagar, P.M., Cuthbert, R.J., Cherel, Y. & Bury, S.J. 2006. A stable isotopic investigation into the causes of decline in a sub-Antarctic predator, the Rockhopper Penguin *Eudyptes chrysocome*. *Glob. Change Biol.* **12**: 611–625.

Hobson, K.A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* **120**: 314–326.

Hobson, K.A., Piatt, J.F. & Pitocchelli, J. 1994. Using stable isotopes to determine seabird trophic relationships. *J. Anim. Ecol.* **63**: 786–798.

Huntington, C.E., Butler, R.G. & Mauck, R.A. 1996. Leach's Storm-Petrel (*Oceanodroma leucorhoa*). In Poole, A. (ed.) *The Birds of North America Online*. Ithaca, NY: Cornell Lab of Ornithology.

Hurrell, J.W. 1995. Decadal trends in the North Atlantic Oscillation: regional temperatures and precipitation. *Science* **269**: 676–679.

Jaeger, A. & Cherel, Y. 2011. Isotopic investigation of contemporary and historic changes in penguin trophic niches and carrying capacity of the southern Indian Ocean. *PLoS ONE* **6**: e16484.

Kelly, J.F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Can. J. Zool.* **78**: 1–27.

Kitaysky, A.S., Piatt, J.F., Wingfield, J.C. & Romano, M. 1999a. The adrenocortical stress-response of Black-legged Kittiwake chicks in relation to dietary restrictions. *J. Comp. Physiol.* **169**: 303–310.

Kitaysky, A.S., Wingfield, J.C. & Piatt, J.F. 1999b. Dynamics of food availability, body condition and physiological stress response in breeding Black-legged Kittiwakes. *Funct. Ecol.* **13**: 577–584.

Kitaysky, A.S., Kitaiskaia, E.V., Wingfield, J.C. & Piatt, J.F. 2001. Dietary restriction causes chronic elevation of corticosterone and enhances stress response in Red-legged Kittiwake chicks. *J. Comp. Physiol.* **171**: 701–709.

Kitaysky, A.S., Piatt, J.F. & Wingfield, J.C. 2007. Stress hormones link food availability and population processes in seabirds. *Mar. Ecol. Prog. Ser.* **352**: 245–258.

Kitaysky, A.S., Piatt, J.F., Hatch, S.A., Kitaiskaia, E.V., Benowitz-Fredericks, Z.M., Shultz, M.T. & Wingfield, J.C. 2010. Food availability and population processes: severity of nutritional stress during reproduction predicts survival of long-lived seabirds. *Funct. Ecol.* **24**: 625–637.

Kouwenberg, A.-L., Hipfner, J.M., McKay, D.W. & Storey, A.E. 2013. Corticosterone and stable isotopes in feathers predict egg size in Atlantic Puffins *Fratercula arctica*. *Ibis* **155**: 413–418.

MacKenzie, K., Longmore, C., Preece, C., Lucas, C. & Trueman, C. 2014. Testing the long-term stability of marine isoscapes in shelf seas using jellyfish tissues. *Biogeochemistry* **121**: 441–454.

Morrison, K.W., Bury, S.J. & Thompson, D.R. 2014. Higher trophic level prey does not represent a higher quality diet in a threatened seabird: implications for relating population dynamics to diet shifts inferred from stable isotopes. *Mar. Biol.* **161**: 2243–2255.

Norris, D.R. & Marra, P.P. 2007. Seasonal interactions, habitat quality, and population dynamics in migratory birds. *Condor* **109**: 535–547.

Peterson, B.J. & Fry, B. 1987. Stable isotopes in ecosystem studies. *Annu. Rev. Ecol. Syst.* **18**: 293–320.

Philippart, C.J.M., Anadon, R., Danovaro, R., Dippner, J.W., Drinkwater, K.F., Hawkins, S.J., Oguz, T., O'Sullivan, G. & Reid, P.C. 2011. Impacts of climate change on European marine ecosystems: observations, expectations and indicators. *J. Exp. Mar. Biol. Ecol.* **400**: 52–69.

Pollet, I.L., Hedd, A., Taylor, P.D., Montevecchi, W.A. & Shutler, D. 2014a. Migratory movements and wintering areas of Leach's Storm-Petrels tracked using geolocators. *J. Field Ornithol.* **85**: 321–328.

Pollet, I.L., Ronconi, R.A., Jonsen, I., Leonard, M.L., Taylor, P.D. & Shutler, D. 2014b. Foraging movements of Leach's storm-petrels *Oceanodroma leucorhoa* during incubation. *J. Avian Biol.* **45**: 305–314.

Pyle, P. 2008. *Identification Guide to North American Birds. Part 2*. Bolinas, CA: Slate Creek Press.

Rau, G.H., Sweeney, R.E. & Kaplan, I.R. 1982. Plankton ^{13}C:^{12}C ratio changes wtih latitude: differences between northern and southern oceans. *Deep Sea Res. Part 2 Top. Stud. Oceanogr.* **29**: 1035–1039.

Reid, P.C., Edwards, M., Hunt, H.G. & Warner, A.J. 1998. Phytoplankton change in the North Atlantic. *Nature* **391**: 546.

Romero, L.M. 2004. Physiological stress in ecology: lessons from biomedical research. *Trends Ecol. Evol.* **19**: 249–255.

Ronconi, R.A., Koopman, H.N., McKinstry, C.A.E., Wong, S.N.P. & Westgate, A.J. 2010. Inter-annual variability in diet of non-breeding pelagic seabirds *Puffinus* spp. at migratory staging areas: evidence from stable isotopes and fatty acids. *Mar. Ecol. Prog. Ser.* **419**: 267–282.

Ronconi, R.A., Steenweg, R.J., Taylor, P.D. & Mallory, M.L. 2014. Gull diets reveal dietary partitioning, influences of isotopic signatures on body condition, and ecosystem changes at a remote colony. *Mar. Ecol. Progr. Ser.* **514**: 247–261.

Rose, G. 2005. On distributional responses of North Atlantic fish to climate change. *ICES J. Mar. Sci.* **62**: 1360–1374.

Sapolsky, R.M., Romero, L.M. & Munck, A.U. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* **21**: 55–89.

Sorensen, M.C., Hipfner, J.M., Kyser, T.K. & Norris, D.R. 2009. Carry-over effects in a Pacific seabird: stable isotope evidence that pre-breeding diet quality influences reproductive success. *J. Anim. Ecol.* **78**: 460–467.

Stenseth, N.C., Ottersen, G., Hurrell, J.W., Mysterud, A., Lima, M., Chan, K.S., Yoccoz, N.G. & Adlandsvik, B. 2003. Studying climate effects on ecology through the use of climate indices: the North Atlantic Oscillation, El Nino Southern Oscillation and beyond. *Proc. R. Soc. B Biol. Sci.* **270**: 2087–2096.

Suess, H.E. 1955. Radiocarbon concentration in modern wood. *Science* **122**: 415–417.

Will, A.P., Suzuki, Y., Elliott, K.H., Hatch, S.A., Watanuki, Y. & Kitaysky, A.S. 2014. Feather corticosterone reveals developmental stress in seabirds. *J. Exp. Biol.* **217**: 2371–2376.

Received 20 December 2013;
revision accepted 29 November 2014.
Associate Editor: Ross Wanless.