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ORIGINAL PAPER

Corticosterone levels in feathers and blood of rhinoceros auklets *Cerorhinca monocerata* are affected by variation in environmental conditions

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Abstract In order to fully understand factors that affect animals during their annual cycle, it is important to measure physiological and behavioral responses to environmental conditions in multiple seasons. We tested the hypothesis that corticosterone levels (CORT) are affected by spatial and temporal variations in marine environmental conditions by measuring levels in the feathers (grown outside of the breeding season) and blood (collected during the breeding season) of an abundant North Pacific seabird, the rhinoceros auklet *Cerorhinca monocerata.* Birds involved in our study bred on three widely dispersed colonies in three years in which oceanographic conditions differed markedly. Combined nitrogen (δ^{15}N) and carbon (δ^{13}C) stable isotope values in blood differed among colonies, while values in feathers did not, suggesting that individuals from the three colonies were segregated during the breeding season (as expected from foraging range around breeding colonies), but not during the nonbreeding period (as expected from genetic homogeneity). CORT showed the same pattern of dichotomy; of particular note, but contrary to our a priori prediction, blood CORT levels were higher in auklets that bred on a colony in which early chlorophyll-*a* bloom in local waters suggested good feeding conditions, than in auklets that bred on a colony with later local chlorophyll-*a* bloom. Also contrary to prediction, feather CORT was higher in a year featuring favorable, cold-water La Niña conditions than in a year of unfavorable, warm-water El Niño conditions; values in a third, moderate year were intermediate. We conclude that CORT levels are affected by spatiotemporal variation in marine environmental conditions, but relationships appear to depend heavily on context and thus require careful interpretation and more study.

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Introduction

Glucocorticoid hormones mediate the physiological and behavioral responses of vertebrates to environmental variation and life-history transitions. They are essential for the maintenance of energy homeostasis during both predictable seasonal activities such as reproduction (McEwen and Wingfield 2003), and unpredictable events such as extreme weather (Wingfield and Kitaysky 2002). However, their specific effects on resource allocation trade-offs depend heavily on the ecological and evolutionary context of the organism in question (Crespi et al. 2013). For example, even within a single avian species (Tree swallow *Tachycineta bicolor*), corticosterone levels (CORT, the main glucocorticoid in birds) were negatively related to reproductive success and adult survival during one breeding stage (incubation), but positively related to these same factors during a later breeding stage (provisioning; Bonier et al. 2009). Interspecifically, CORT was negatively related to

Table 1 Ocean conditions prior to breeding and during the breeding season in 2009, 2010 and 2011 (DFO 2009, 2010, 2011) and predicted CORT levels of adult rhinoceros auklets from Lucy Island, Pine Island and S'Gang Gwaay during the same periods

	Ocean conditions		Predicted adult auklet CORT levels	
	Pre-breeding (Feb–April)	Breeding (May–Aug)	Pre-breeding (feather)	Breeding (blood)
2009	Exceptionally cold ocean, strong La Niña	Warming ocean, start of strong El Niño	Lowest	No data
2010	Warm ocean, strong El Niño	Cooling ocean, start of La Niña	Highest	Higher than 2011
2011	Cold ocean, La Niña	Cold ocean, La Niña	Intermediate	Lower than 2010

Colder water conditions are assumed to indicate better feeding conditions than warmer water conditions

food abundance in one seabird species (Doody et al. 2008; Kitaysky et al. 2007), but not in a closely related species breeding in the same area (Rector et al. 2012). Therefore, in order to understand how glucocorticoids mediate life-history traits, it is essential to examine variation in levels across different seasons and through a range of environmental conditions relevant for the species in question (Crespi et al. 2013).

Marine ecosystems are highly variable, both within and across seasons, and top predators such as seabirds exhibit both physiological and behavioral responses to this variation (Sommerfield et al. 2015). CORT measurements in blood have proven useful for assessing physiological responses in marine birds (Buck et al. 2007; Barger and Kitaysky 2011), and much more limited recent work suggests the potential utility of CORT measurements in feathers for this purpose (Kouwenberg et al. 2013; Legagneux et al. 2013). Blood sampling enables measurements of baseline or stress-induced CORT levels at a point in time, typically during the breeding season, when individuals are accessible on colonies. Because CORT circulating in the blood is integrated into feathers at the time they are grown (Bortolotti et al. 2008, 2009), feathers that are molted outside the breeding season provide a measure of CORT during that period, when individuals are often based at sea and are less accessible.

To further test the hypothesis that CORT levels reflect spatiotemporal variation in marine environmental conditions, we measured CORT levels in the feathers (3 years), and blood (2 years) of rhinoceros auklets *Cerorhinca monocerata* nesting on three colonies. The rhinoceros auklet is an abundant North Pacific seabird, and individuals from our three study colonies are genetically similar (Abbott et al. 2014), suggesting that they are not segregated during their nonbreeding season (Friesen et al. 2007). While breeding, auklets are central-place foragers (Orians and Pearson 1979) and range only within tens of kilometers of their respective colonies (McFarlane-Tranquilla et al. 2005). Therefore, we expected that auklets from the three colonies experience similar conditions outside of the breeding season, but different (local) conditions while breeding. To validate that assumption, we compared δ^{15}N and δ^{13}C stable isotope values in feathers and blood across the three colonies, an analysis that assesses segregation according to habitat use or ecological niche (Jaeger et al. 2009; Cherel et al. 2013). We will consider our assumption validated if auklets from the three colonies cannot be distinguished by δ^{15}N and δ^{13}C values in feathers, but can be by δ^{15}N and δ^{13}C values in blood. Based on our a priori assumptions, we predicted that feather CORT would be similar among colonies, but blood CORT would not.

Our three-year study spanned a period of dramatic variation in environmental conditions in the Northeast Pacific Ocean (Table 1). Rhinoceros auklets feed across a range of trophic levels, from zooplankton to forage fish (Hipfner et al. 2013). As cold-water (higher latitude) zooplankton species tend to be more lipid rich and larger than lower-latitude species (Mackas et al. 2007), and cold-water species are more abundant when ocean waters are cooler across our study region (Crawford and Irvine 2010, 2011, 2012), we assumed that feeding conditions prior to breeding were better for auklets in 2009 than 2010, and intermediate in 2011. In 2009, the auklet pre-breeding period (February–March) featured unusually cold-water ocean conditions associated with a La Niña event that began in 2008. Cold-water conditions persisted until June 2009, but by July ocean conditions were very warm, beginning the strongest El Niño event of the century. Warm-water El Niño conditions persisted until April 2010, when cold-water La Niña conditions returned and persisted through the breeding seasons of 2010 and 2011. In birds, previous studies have linked unfavorable environmental conditions (Romero et al. 2000) and unfavorable feeding conditions (Kitaysky et al. 2007; Doody et al. 2008) with elevated blood CORT, so we predicted that feather CORT levels in auklets would be higher in 2010 than in 2009, and intermediate in 2011.

At a broad scale, our three breeding colonies span the transition zone between the California Current and Alaska Current systems, and zooplankton communities differ across the bifurcation between these systems (Batten and Freeland 2007). To assess the oceanographic conditions to which auklets were exposed during chick-rearing, we downloaded ocean chlorophyll data for April and May for

Table 2 Mean monthly chlorophyll concentrations (mg ml^{-3}) recorded by NASA Earth Observations (http://neo.sci.gsfc.nasa.gov), which indicate the intensity of spring phytoplankton bloom in the ocean waters near Lucy Island, Pine Island and S'Gang Gwaay in April and May of 2010 and 2011

Colony	Chlorophyll concentration (mg ml^{-3})				Predicted auklet CORT levels during chick-rearing	
	2010		2011		2010	2011
	April	May	April	May		
Lucy	5.77	9.86	0.85	4.41	Lower than S'Gang Gwaay	Similar to other colonies
Pine	8.50	18.55	0.94	4.95	Lower than S'Gang Gwaay	Similar to other colonies
S'Gang Gwaay	0.96	1.14	0.54	2.09	Higher than Lucy and Pine	Similar to other colonies

The chlorophyll concentration reached 2 mg ml^{-3} in April 2010 on Lucy and Pine, but never reached 2 mg ml^{-3} on S'Gang Gwaay in 2010. All colonies reached 2 mg ml^{-3} in May in 2011. Auklet CORT levels during chick-rearing are predicted based on previous correlations found between April chlorophyll concentration and Pacific sandlance availability during the subsequent breeding season

waters surrounding each of the three colonies from http://neo.sci.gsfc.nasa.gov/view.php?datasetId=MY1DMM_CHLORA (Table 2). On Triangle Island, British Columbia (BC), rhinoceros auklets bred more successfully in years in which surface chlorophyll-*a* concentrations first exceeded 2 mg ml^{-3} in waters within a 15 km radius of the island in the first 2 weeks of April (Borstad et al. 2011), suggesting that breeding success in this species is strongly controlled by bottom-up processes (Bertram and Kaiser 1993). Increased ocean productivity has similarly been found to increase Pacific sandlance *Ammodytes hexapterus* abundance in auklet diets on two of our study colonies (Bertram and Kaiser 1993; Bertram et al. 2002). At colonies throughout BC, the amount of sandlance in nestling diets has been positively correlated with nestling growth and survival (Bertram and Kaiser 1993; Bertram et al. 2002; Borstad et al. 2011). In 2010, chlorophyll concentration reached 2 mg ml^{-3} by April within 15 km of Lucy Island and Pine Island, but never reached 2 mg ml^{-3} near S'Gang Gwaay (Table 2). In 2011, chlorophyll concentration within 15 km of Lucy, Pine and S'Gang Gwaay reached 2 mg ml^{-3} in May. Therefore, we predicted that auklet CORT levels during chick-rearing would be higher on S'Gang Gwaay than Lucy and Pine in 2010 and that CORT levels would be similar among study colonies in 2011.

Methods

Locations of study colonies

We worked on Lucy Island (54°17′N, 130°37′W), Pine Island (50°35′N, 127°26′W) and S'Gang Gwaay (52°05′N, 131°13′W), three islands off the coast of British Columbia, Canada, in 2009, 2010 and 2011. Lucy and Pine are both situated along the mainland coast, well up on the continental shelf, while S'Gang Gwaay is further offshore, closer to the shelf break, along the west coast of Haida Gwaii (Fig. 1).

Study species

Rhinoceros auklets are nocturnal, monogamous seabirds with biparental care that spend the majority of their lives at sea, coming to land only for a few months each year to lay eggs and raise nestlings. They nest on island colonies, returning to the same island each year to raise a single chick. They are mainly zooplanktivorous through the pre- and early-breeding season, but become more piscivorous after chicks hatch (Hipfner et al. 2013).

Corticosterone analyses

We measured CORT in a single breast feather from 144 adult breeding auklets (8 females and 8 males on all three colonies in all 3 years; Table 3). Birds were captured with nets when landing near their burrow holes to deliver food loads to their nestlings. Feathers were collected during the chick-rearing period on each colony. Although most breast feathers are replaced during a pre-basic molt from August to January, others are replaced during a partial pre-alternate molt in February and March (Pyle 2008; Howell 2010). Therefore, as in other studies (Sorensen et al. 2010), we assumed that breast feathers showing minimal wear were grown in February–March and we chose these least-worn feathers for our analysis. We acknowledge that some feathers analyzed could have been grown in August to January, but to minimize this issue ~5 feathers were collected from each auklet, all by the same person, and only one person chose (based on feather condition) the feathers to be analyzed.

We removed the calamus from each breast feather and measured feather length to the nearest millimeter. Whole feathers (without calamus) were minced, incubated in methanol, filtered, purified using acetonitrile and hexane, and assayed using an Enzyme Immunoassay kit (Cayman Chemical Company, Ann Arbor, USA) as described in Kouwenberg et al. (2013, 2015). The intra-assay coefficient of variation (CV) calculated from duplicate absorbance

Fig. 1 Map of islands with rhinoceros auklet colonies on the coast of British Columbia, Canada. Our study colonies are on Lucy Island (54°17′N, 130°37′W), Pine Island (50°35′N, 127°26′W) and S'Gang Gwaay (52°05′N, 131°13′W)

values was 2.75 %. Samples were counterbalanced by colony and year over four EIA plates, and the inter-assay CV between known quantities of CORT assayed in each plate was 12.57 %. As suggested by Bortolotti et al. (2009), we report CORT values in pg mm^{-1}. Breast feathers used in our study were all of similar length (mean 35 mm, range 29–40 mm) and mass (mean 4.04 mg, range 2.41–5.11 mg) such that analyses were not likely to be compromised by differences in feather mass (Lattin et al. 2011).

We measured CORT in blood taken during chick-rearing from 156 adult auklets breeding on three colonies in 2010 and 2011. Sample sizes for each colony and year are outlined in Table 3. For blood CORT analysis, ~1 ml of blood was taken from the brachial vein within 3 min of capture to ensure baseline levels (Romero and Reed 2005) and dispensed in drops onto a paper blood spot card (Whatman), ensuring that blood soaked through. Blood cards were dried for at least 24 h, and a 3.2-mm punch was used to punch 24 spots from each sample card. Spots were analyzed in duplicate (12 per duplicate) using COAT-A-COUNT Rat Corticosterone radioimmunoassay kits (Cat. # TKRC1, InterMedico, Markham, ON; Doody et al. 2008; Rector et al. 2012). Resulting blood spot CORT values were converted to plasma equivalents as in Rector et al. (2012), and reported as ng CORT ml^{-1} blood. Blood spots from 2010 and 2011 were assayed with different kits, which had intra-assay CVs of 8.5 and 6.3 %, respectively, similar to values reported by Rector et al. (2012). Variation between assays (2010 and 2011) was quantified by including a standardized blood spot sample (the same pooled murre sample described in Rector et al. 2012). In the 2010 assay, CORT level of the standardized sample was very similar to

Table 3 Number of rhinoceros auklets for which feather and blood stable isotope (SIA) and corticosterone (CORT) analyses were carried out on each colony in each year

Colony	Year	Sex	Feather SIA	Blood SIA	Feather CORT	Blood CORT
Lucy	2009	Male	0	0	8	0
		Female	8	0	8	0
		Unknown	0	0	0	0
	2010	Male	0	16	8	16
		Female	8	10	8	10
		Unknown	0	4	0	4
	2011	Male	0	10	8	10
		Female	8	11	8	11
		Unknown	0	9	0	9
Pine	2009	Male	0	0	8	0
		Female	7	0	8	0
		Unknown	0	0	0	0
	2010	Male	0	9	8	9
		Female	8	17	8	17
		Unknown	0	0	0	0
	2011	Male	0	9	8	9
		Female	8	9	8	9
		Unknown	0	12	0	12
S'Gang Gwaay	2009	Male	0	0	8	0
		Female	6	0	8	0
		Unknown	0	0	0	0
	2010	Male	0	11	8	11
		Female	6	10	8	10
		Unknown	0	4	0	4
	2011	Male	0	7	8	7
		Female	7	4	8	4
		Unknown	0	4	0	4

previous CORT levels reported for the same sample in different assays (Rector et al. 2012). However, in 2011, CORT levels of the standardized sample were higher than average, so CORT concentrations for 2011 were reduced by 15 %—as in previous studies (Rector et al. 2012; Doody et al. 2008). The difference between the standardized samples is likely due to kits being manufactured in different lots.

Stable isotope analysis

We measured δ^{15}N and δ^{13}C values in a single breast feather from female auklets in 2009, 2010 and 2011 (same females as for feather CORT; Table 3). We also measured δ^{15}N and δ^{13}C values in whole blood of the same females used for feather δ^{15}N and δ^{13}C analysis, plus additional male and unknown sex birds (same as for blood CORT analysis). Sample sizes for each colony and year are outlined in Table 3. Feather stable isotope data were available for females only, but data from auklets on Triangle Island (A.-L. Kouwenberg, unpublished data) indicated no significant effect of sex on feather stable isotope values: Feather δ^{15}N and δ^{13}C values for 23 female and 17 male auklets from Triangle Island were not significantly different ($F_{1,38} = 1.108$, $P = 0.299$ and $F_{1,38} = 0.729$, $P = 0.399$, respectively).

Fish samples were collected from auklets on all three islands in 2010. Auklets returning to the colony with food for their chicks were startled and/or caught with a large dipnet in order to collect the fish they were carrying in their bills. Fish in each food load were identified to genus or species, but only Pacific sandlance ($n = 12$) were stored in 70 % ethanol in order to be analyzed for δ^{15}N and δ^{13}C. We decided to focus on the sandlance as they are the commonest prey species among all colonies (Bertram and Kaiser 1993; Bertram et al. 2002) and are an important prey species for auklets breeding in British Columbia (Bertram and Kaiser 1993).

Whole breast feathers, dried whole blood samples, and a small piece of muscle from each sandlance were used for δ^{15}N and δ^{13}C analyses. Feathers and muscle pieces were placed in individual vials and soaked in 2:1 chloroform/methanol solution for 24 h and then decanted in order to

remove any surface contaminants from feathers and to extract lipids from muscle. Feathers were air-dried and minced with scissors. Muscle samples and whole blood samples were dried in an oven and ground with mortar and pestle. Approximately one milligram of each sample (blood, feather and muscle) was weighed and placed in an individual tin capsule. Relative abundance of $^{15}N/^{14}N$ and $^{12}C/^{13}C$ 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:

$$\delta^{15}N(\text{or } \delta^{13}C) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where R is the ratio of $^{15}N/^{14}N$ and R_{standard} for ^{15}N is atmospheric N_2 (AIR), or R is the ratio of $^{12}C/^{13}C$ and R_{standard} for ^{13}C is PeeDee Belemnite. Measurement error was estimated to be ±0.09 ‰ for $\delta^{15}N$ and ±0.17 ‰ for $\delta^{13}C$ based on within-run replicate measurements of nylon (mean −9.77 ‰) and glutamic acid (mean −4.26 ‰) laboratory standards (2 standards for 12 unknowns). Feather stable isotope values were corrected by the discrimination factors suggested by Cherel et al. 2005: subtracting 4.2 ‰ from feather $\delta^{15}N$ values and applying no correction for $\delta^{13}C$ values. Blood stable isotope values were corrected by subtracting 3.49 ‰ from blood $\delta^{15}N$ values and applying no correction for $\delta^{13}C$ values based on the discrimination factors of Sears et al. (2009). Prey muscle stable isotope values were converted to whole fish values by subtracting 0.32 ‰ from prey $\delta^{13}C$ values and 0.86 ‰ from prey $\delta^{15}N$ values (Cherel et al. 2005).

Statistical analysis

RStudio 0.97.551 (RStudio Team 2013) and R version 3.0.0 (R Core Team 2013) were used for all data analysis.

Feather CORT

We used the generalized least squares (GLS) function to analyze the following model: Feather CORT ~ Colony + Year + Lot + ε (error term). Since feather CORT was measured on EIA plates that were manufactured in two different lots, we used the 'VarIdent' term to formulate a variance structure that implements different variances for Lot 1 and Lot 2 (Zuur et al. 2009). We used analysis of variance (ANOVA) to first examine full models containing all interaction terms (including sex), but dropped nonsignificant terms one by one. Interaction terms were not significant and thus were not included in the final model in order to improve statistical power.

Blood CORT

To test for differences in blood CORT among colonies and years, we built the following linear model: Blood CORT ~ Colony + Year + Colony × Year + ε. We analyzed this model using ANOVA.

Stable isotope values

We used multivariate analysis of variance (MANOVA, Wilks' Lambda test) to determine whether auklets from the three study colonies could be distinguished by their $\delta^{15}N$ and $\delta^{13}C$ values. We ran separate MANOVAs for each year for feather (2009, 2010 and 2011), blood (2010 and 2011) and sandlance muscle (2010) $\delta^{15}N$ and $\delta^{13}C$ values. Tukey post hoc tests were used to examine differences.

Results

Can colonies be distinguished by their blood or feather $\delta^{15}N$ and $\delta^{13}C$ values?

As expected, female auklets that bred on Pine, Lucy and S'Gang Gwaay could not be distinguished by their feather (pre-breeding) $\delta^{15}N$ and $\delta^{13}C$ values in 2009 (Wilks' lambda = 0.875, $F_{2,18} = 0.589$, $P = 0.673$), 2010 (Wilks' lambda = 0.744, $F_{2,19} = 1.433$, $P = 0.243$) or 2011 (Wilks' lambda = 0.681, $F_{2,20} = 2.010$, $P = 0.113$; Table 4). Also as expected, auklets from the three colonies were easily distinguished by their blood (breeding season) $\delta^{15}N$ and $\delta^{13}C$ values in both 2010 (Wilks' lambda = 0.301, $F_{2,78} = 31.624$, $P < 0.001$) and 2011 (Wilks' lambda = 0.321, $F_{2,73} = 27.526$, $P < 0.001$; Table 4). There was no significant difference in blood stable isotope values between males and females ($\delta^{15}N$: $F_{1,155} = 1.202$, $P = 0.303$; $\delta^{13}C$: $F_{1,155} = 0.072$, $P = 0.931$).

Muscle samples from Pacific sandlance (auklet prey samples collected in 2010) from different colonies could be distinguished by their $\delta^{15}N$ and $\delta^{13}C$ values (Wilks' lambda = 0.303, $F_{2,9} = 4.798$, $P = 0.038$; Table 4), suggesting different isotopic baselines among colonies. Consistent with blood stable isotopes, Pacific sandlance collected on S'Gang Gwaay had lower $\delta^{13}C$ values than those collected on Lucy (Tukey pairwise comparison: $t_9 = 2.968$, $P = 0.038$). $\delta^{13}C$ values of Pacific sandlance collected on Pine were not different from those of Pacific sandlance collected on S'Gang Gwaay (Tukey pairwise comparison: $t_9 = 0.715$, $P = 0.761$) or Lucy (Tukey pairwise comparison: $t_9 = 2.253$, $P = 0.115$).

Table 4 Mean corrected feather and blood nitrogen (δ^{15}N) and carbon (δ^{13}C) stable isotope values of rhinoceros auklets and Pacific sandlance prey samples from Lucy, Pine and S'Gang Gwaay colonies in 2009–2011 (feathers from females), 2010 and 2011 (blood from females and males), and 2010 (Pacific sandlance)

Colony	Year	Feather ‰ ± SEM		Blood ‰ ± SEM		Sandlance ‰ ± SEM	
		δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N
Lucy	2009	−17.84 ± 0.28	12.70 ± 0.54				
	2010	−17.61 ± 0.18	12.55 ± 0.29	−18.28 ± 0.12	11.73 ± 0.21	−17.00 ± 0.19	12.98 ± 0.46
	2011	−17.05 ± 0.33	12.55 ± 0.47	−18.69 ± 0.13	11.68 ± 0.12		
Pine	2009	−17.53 ± 0.36	13.13 ± 0.54				
	2010	−17.76 ± 0.30	13.17 ± 0.26	−17.71 ± 0.07	12.17 ± 0.03	−17.38 ± 0.10	12.05 ± 0.12
	2011	−17.20 ± 0.57	13.18 ± 0.42	−18.28 ± 0.22	11.61 ± 0.06		
S'Gang Gwaay	2009	−17.80 ± 0.18	11.95 ± 0.66				
	2010	−17.80 ± 0.40	12.04 ± 0.49	−19.04 ± 0.25	12.10 ± 0.10	−18.79 ± 0.74	12.50 ± 0.30
	2011	−17.81 ± 0.31	13.74 ± 0.26	−19.03 ± 0.19	11.91 ± 0.04		

Does feather (pre-breeding) CORT vary among colonies and years?

Mirroring the stable isotope results, feather CORT did not vary among the three colonies ($F_{2,138} = 1.685$, $P = 0.189$), but did vary among the 3 years ($F_{2,138} = 3.636$, $P = 0.029$; Fig. 2). However, the direction of difference among years was opposite to our prediction, being higher in the cold-water years of 2009 than in the warm-water El Niño year of 2010 (Tukey pairwise comparison: $t_{138} = 2.680$, $P = 0.022$); but feather CORT in 2011, the year of intermediate conditions, was not different from the other 2 years. There was also the expected effect of lot (EIA plates were manufactured in two different lots; $F_{1,138} = 237.380$, $P < 0.001$), which was accounted for by the addition of the 'VarIdent' term in the gls model.

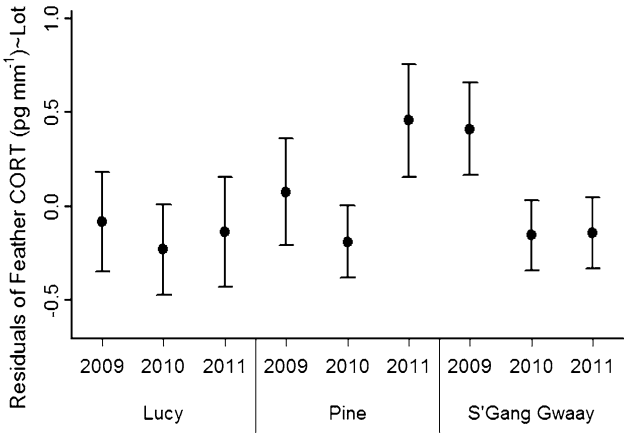

Fig. 2 Feather CORT levels (pg mm^{-1}) for rhinoceros auklets on Lucy Island, Pine Island and S'Gang Gwaay in 2009, 2010 and 2011. In order to account for differences in variance due to Lot, feather CORT values are presented as standardized residuals of the generalized least-squares model: Feather CORT ~Lot. Standard errors are plotted for each mean

Does blood (breeding) CORT vary among colonies and years?

As predicted, there was a significant effect of colony ($F_{2,150} = 5.108$, $P = 0.007$) on auklet blood CORT levels (Fig. 3). Blood CORT was significantly higher for auklets that bred on Lucy than for auklets that bred on S'Gang Gwaay (Tukey pairwise comparison: $t_{153} = 3.150$, $P = 0.006$), but CORT levels of Pine auklets did not differ from auklets on either Lucy (Tukey pairwise comparison: $t_{153} = 1.602$, $P = 0.248$) or S'Gang Gwaay (Tukey pairwise comparison: $t_{153} = 1.667$, $P = 0.221$). Although the lower blood CORT levels of auklets in S'Gang Gwaay appear to be driven mostly by 2010 values (Fig. 3), contrary to prediction, there was no statistically significant effect of year on auklet blood CORT levels ($F_{1,150} = 1.313$, $P = 0.253$) and no interaction between year and colony ($F_{2,150} = 2.267$, $P = 0.073$). There was no significant difference in blood CORT values between known sex males and females ($F_{1,121} = 0.2401$, $P = 0.625$; Fig. 3).

Discussion

Rhinoceros auklets that bred on three colonies in British Columbia, Canada (Pine Island, Lucy Island, S'Gang Gwaay) could not be distinguished by their feather δ^{15}N and δ^{13}C values, but could be distinguished by their blood δ^{15}N and δ^{13}C values. These results validate our assumption that auklets from different colonies were not strictly segregated during the nonbreeding season (feather values), but were segregated while breeding (blood values). The

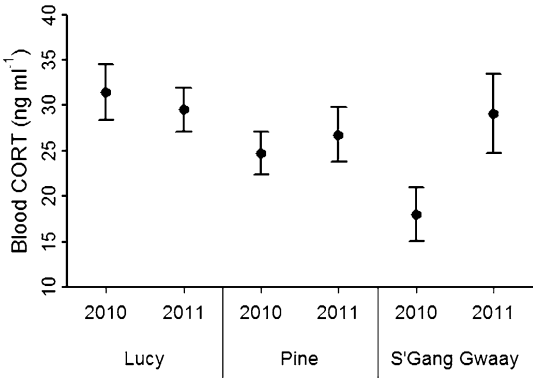

Fig. 3 Blood CORT levels (ng ml^{-1}) for rhinoceros auklets on Lucy Island, Pine Island and S'Gang Gwaay in 2010 and 2011. Blood CORT levels of auklets on S'Gang Gwaay were significantly lower than blood CORT levels of auklets on Lucy. There was no significant year effect or interaction between years. Standard errors are plotted for each mean

nonbreeding season (feather) results are consistent with the genetic similarity of auklets on the three colonies (Abbott et al. 2014) because segregation (or lack thereof) in wintering areas has been shown to be the major driver of population genetic structuring in seabirds (Friesen et al. 2007). The breeding season (blood) results are consistent with the relatively limited foraging range around breeding colonies of these central-place foragers (Orians and Pearson 1979). As well, our stable isotope results are supported by previous studies that link seasonal differences in foraging habitats with patterns in isotopic composition of seabird tissues (Cherel et al. 2000; Cherel and Hobson 2007, Thompson et al. 2015).

Our finding that CORT in rhinoceros auklets showed the same pattern of intercolony variation as the isotopic data (different in blood, but not in feathers) supports our general hypothesis that CORT levels vary with environmental conditions. Although not in all cases (e.g., Rector et al. 2012), studies of seabirds (including rhinoceros auklet chicks; Will et al. 2014) have consistently found negative correlations between blood CORT and measures of food availability (Kitaysky et al. 2007, 2010)—so much so that researchers sometimes consider lower CORT to be a proxy for greater food availability (Kitaysky et al. 2010; Satterthwaite et al. 2012). Based on the general tendency for poor feeding conditions to link to elevated CORT in seabirds, and given the weak phytoplankton bloom near S'Gang Gwaay as compared to Lucy and Pine, we expected CORT to be higher in auklets on S'Gang Gwaay. In fact, we found that auklets breeding on S'Gang Gwaay had lower blood CORT levels than those on Lucy. Although this result was unexpected, it is consistent with a recent finding that great skuas *Stercorarius skua* experiencing better local feeding conditions had relatively higher CORT than skuas experiencing relatively poorer conditions (Bourgeon et al. 2014). It is possible, as some studies have found, that phytoplankton bloom was not a reliable indicator of zooplankton and fish abundance (Gremillet et al. 2008), but this is unlikely as timing of the spring phytoplankton bloom has been directly linked biomass of young-of-the-year sandlance in the waters near to our study colonies (Borstad et al. 2011).

Considering that blood CORT results were opposite to our prediction, it is also possible that other unquantified differences among colonies may have affected blood CORT levels. Our study colonies have similar population density and composition (Rodway and Lemon 2011), so factors such as within-colony competition among birds are unlikely to have influenced CORT levels. In terms of predation danger, another factor that may affect CORT levels (Clinchy et al. 2004; Fontaine et al. 2011), all three colonies lack mammalian predators. However, all three auklet populations do experience predation by bald eagles *Haliaeetus leucocephalus* when they return at night (Kaiser 1989; Harfenist and Ydenberg 1995, J.M. Hipfner, unpublished data).

Our blood CORT results may also be explained by the fact that we did not measure corticosteroid binding globulins (CBG), proteins that bind to CORT as it circulates in plasma and may prevent CORT from binding to receptors to initiate biological action (Mendel 1989; Breuner et al. 2013). We analyzed total CORT levels rather than estimating free CORT levels using CBG measurements. Conclusions drawn using free CORT values may differ from those drawn using total CORT (Breuner et al. 2013), but some argue that total CORT levels are more informative and easier to interpret biologically than free CORT (Schoech et al. 2013).

While CORT in rhinoceros auklet breast feathers did not vary among colonies, it did vary among years. Again, however, the direction of difference was opposite to what we had predicted. Feather CORT was significantly lower in 2010, which featured warm-water El Niño conditions during the time when birds grew feathers, than in 2009, which featured exceptionally cold-water La Niña conditions. Conditions were presumed to be more favorable for food availability in 2009 than 2010, and both feather CORT levels and oceanographic conditions were intermediate in 2011. Lower CORT during less favorable conditions was found previously in 1 year for one colony of adult Atlantic puffins *Fratercula arctica*, a close relative of the rhinoceros auklet, but over many years, puffins showed no relationship between feeding conditions and CORT (Rector et al. 2012).

These differences among studies may reflect species or population differences, or they may reflect differences in the degree of "bad" conditions experienced. Rector et al. (2012) suggested that CORT levels might decrease

in extreme conditions when parents decrease their foraging effort and provisioning rates to the point of decreased reproductive success. In contrast, CORT levels may increase under moderately bad conditions where parents can increase foraging effort enough to successfully fledge their chicks. Although we did not measure reproductive success in our study, the findings of Rector et al. (2012) suggest that the processes that regulate CORT elevation may be different for moderate conditions compared to more extreme conditions (Rich and Romero 2005), possibly resulting in a nonlinear relationship between CORT levels and conditions. The existence of a nonlinear relationship is further supported by studies that show CORT suppression during instances of chronic stress (Cyr and Romero 2007).

CORT levels measured over different seasons (pre-breeding and breeding) and locations (colonies) provide evidence for relationship between CORT levels and the environmental factors that influence seabirds throughout their annual cycle. Our results suggest that auklets experiencing poorer feeding conditions had lower CORT levels, suggesting that the assumption made for kittiwakes (that low CORT levels are a proxy for good feeding conditions) is not necessarily true for all seabirds. These findings further indicate the complex nature of the relationship between glucocorticoids and environmental conditions (Crespi et al. 2013) and highlight the need for further study, and for caution when making interspecific generalizations.

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Compliance with ethical standards

Ethical approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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