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Wingfield, John C
Hau, Michaela
Boersma, P Dee
et al.

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Effects of El Niño and La Niña Southern Oscillation events on the adrenocortical responses to stress in birds of the Galapagos Islands

John C. Wingfield [a,b,*], Michaela Hau [c,d], P. Dee Boersma [a], L. Michael Romero [e], Nigella Hillgarth [f], Marilyn Ramenofsky [a,b], Peter Wrege [g], Robert Scheibling [h], J. Patrick Kelley [b], Brian Walker [a,i], Martin Wikelski [c,d]

[a] Department of Biology, Box 351800, University of Washington, Seattle, WA 98195, USA
[b] Department of Neurobiology, Physiology and Behavior, University of California, One Shields Avenue, Davis, CA 95616, USA
[c] Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ, USA
[d] Max-Planck-Institut für Ornithologie, Seewiesen, and Univerzsity of Konstanz, Konstanz, Germany
[e] Department of Biology, Tufts University, Medford, MA, USA
[f] Center for Ecosystem Sentinels, University of Washington, Seattle, WA 95195, USA
[g] Cornell University, Ithaca, NY, USA
[h] Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada
[i] Department of Biology, Fairfield University, Fairfield, CT, USA

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ABSTRACT

El Niño Southern Oscillation events (ENSO) and the subsequent opposite weather patterns in the following months and years (La Niña) have major climatic impacts, especially on oceanic habitats, affecting breeding success of both land and sea birds. We assessed corticosterone concentrations from blood samples during standardized protocols of capture, handling and restraint to simulate acute stress from 12 species of Galapagos Island birds during the ENSO year of 1998 and a La Niña year of 1999. Plasma levels of corticosterone were measured in samples collected at capture (to represent non-stressed baseline) and subsequently up to 1 h post-capture to give maximum corticosterone following acute stress, and total amount of corticosterone that the individual was exposed to during the test period (integrated corticosterone). Seabird species that feed largely offshore conformed to the brood value hypothesis whereas inshore feeding species showed less significant changes. Land birds mostly revealed no differences in the adrenocortical responses to acute stress from year to year with the exception of two small species (<18 g) that had an increase in baseline and stress responses in the ENSO year – contrary to predictions. We suggest that a number of additional variables, including body size and breeding stage may have to be considered as explanations for why patterns in some species deviated from our predictions. Nevertheless, comparative studies like ours are important for improving our understanding of the hormonal and reproductive responses of vertebrates to large scale weather patterns and global climate change in general.

1. Introduction

At irregular intervals of several years, a warm water layer develops originating from the Pacific Ocean outward from the west coast of South America. As a result the layer of warm water overlays the cold water of the Humboldt current that normally passes through and around the Galapagos Islands. This warm water may be 10–18 °C above that of the Humboldt current and can cut a swath some 5000 km or more across the eastern Pacific Ocean. The event is officially termed the El Niño Southern Oscillation (ENSO, Trenberth, 1997; Enfield, 2001) and in some years it is so enormous that dramatic changes in weather can result worldwide (Ropelewski and Halpert, 1987). For example, in the Galapagos Islands, warm water accompanying an ENSO event results in extensive and heavy rains that can be prolonged for over a year compared with the "normal" wet season of about a month (in February). On land the prolonged rains during an ENSO event result in tremendous productivity of plant growth, arthropod emergence, fruits, seeds etc. Consequently, land birds such as Darwin's finches can breed for much longer periods – up to 9 months (Gibbs and Grant, 1987). ENSO events also can affect individual capacity to breed through an interaction with age, younger birds may not have an opportunity

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[*] Corresponding author at: Department of Neurobiology, Physiology and Behavior, University of California, Davis CA 95616 USA.
E-mail address: jcwingfield@davis.edu (J.C. Wingfield).

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to breed. Population density may also be a factor because at higher densities no individuals may breed (Grant et al., 2000; Wilson et al., 2007).

In contrast, for marine organisms ocean warming during ENSO events reduces the upwelling of nutrients and primary productivity in the ocean declines. As a result, algae production is reduced to virtually zero and the food chain collapses resulting in a virtually barren sub-tidal zone. Fishes migrate away or just wait out the ENSO event probably without feeding. Others such as Marine Iguanas, *Amblyrhynchus cristatus*, are dependent upon algal growth in the sub-tidal zone and may starve in large numbers during El Niño years (e.g. Laurie, 1989; Wikelski and Trillmich, 1997). Many seabirds that depend on fish, squid etc. to breed, may also leave or wait out the ENSO event while losing body condition sometimes accompanied by higher mortality. It is not unusual for breeding to cease resulting in total reproductive failure and the population to decline (Boersma, 1978, Boersma, 1998a). This may be especially true of seabirds that occupy inshore waters versus more pelagic species that may have access to other resources. When upwelling returns bringing nutrients to the surface, sea temperature drops and upwelling of sea water from the deep ocean brings nutrients (La Niña event). Then the algae bloom again, marine invertebrates and fish numbers increase and seabirds have sufficient food to breed successfully. On land, drought conditions prevail, much of the vegetation dies, trees lose their leaves and arthropod numbers decline. Rains are much more sparse and very restricted to January/February. As such, many of the land birds have restricted breeding seasons at these times – one month or so, or do not breed at all (Lack, 1950; Grant and Grant, 1980). Reproductive success may be low and survival is also reduced (Schluter, 1984).

In ENSO years land birds have favorable breeding conditions and seabirds do not, with the opposite conditions prevailing in La Niña years. However, all are subjected to essentially identical climatic conditions, but their responses in terms of coping with food shortage, or not, may be completely different. The Galapagos Penguin breeding biology is adapted to these highly variable and unpredictable food condition (Boersma, 1977). It molts before breeding, can breed at any time of the year and lays up to 3 clutches in a year (Boersma, 1977, Boersma et al. 2013). Other exceptions include the Great Frigatebirds, *Fregata minor*, that can breed at any time having a flexible diet over large distances at sea whereas on land, the Galapagos Hawk, *Buteo galapagoensis*, may breed in El Niño years feeding on the corpses of other animals such as marine iguanas that have starved to death. To what extent such changes in climatic conditions influence the hormonal responses to weather (i.e. at baseline levels in blood) and the acute stress response that allow vertebrates to cope physiologically and behavioral remain poorly known. The hypothalamo-pituitary-adrenal (HPA) axis plays a key role in coping mechanisms with glucocorticosteroids such as corticosterone playing a major role (e.g. Sapolsky et al., 2000; Wingfield, 2013; Romero and Wingfield, 2015). In this study we use the adrenocortical responses to acute stress of capture, handling and restraint as a tool to assess the responsiveness of the HPA axis in free-living bird populations on the Galapagos Islands.

An earlier study on a Blue-footed Booby, *Sula nebouxi*, colony on Isla Isabel, Pacific coast of Sinaloa, Mexico, showed that reproductive success was essentially zero during the ENSO event of 1993. Only about 20% of the colony actually attempted to breed and some pairs hatched chicks, but all young died by day 18 of age (Wingfield et al., 1999). In 1994, La Niña conditions prevailed and most of the colony bred successfully. There were differences in baseline levels of corticosterone in male and female Blue-footed Boobies that reflect variation in day-to-day energetic demand. But, the response to capture stress (capture, handling and restraint), representing responsiveness to acute stress was not modulated at all across ENSO and La Niña years (Wingfield et al., 1999). Testosterone levels were lower in males and females in the ENSO year as expected if most birds did not actually breed. No change in the adrenocortical response to acute stress in the Blue-footed Booby may be because brood reduction is the main response to adverse breeding conditions. Adults are affected minimally because they cease to feed chicks if food supply is reduced and invest all available food in self preservation.

In Marine Iguanas, mortality rates increase in ENSO (famine) years and they depend upon stored fat and protein to survive. A few may eat other terrestrial plants and survive this way, but most may not feed at all. In La Niña years algae are very abundant and marine iguanas thrive. During ENSO years, Marine Iguanas have higher baseline and stress levels of corticosterone in blood measured following standard procedures of capture handling and restraint (Romero and Wikelski, 2001). Furthermore, baseline circulating corticosterone predicted which individuals were likely to survive the El Niño period. Some Marine Iguanas even shrink their bodies during an ENSO event mobilizing protein and other nutrients for survival (Wikelski and Thom, 2000). Higher corticosterone levels at this time may be involved in body "remodeling" during famine allowing these individuals to survive prolonged periods with no food (Wikelski and Romero, 2003).

A recent comparative analysis of baseline and stress-induced concentrations of plasma corticosterone in over 100 avian species (Bókony et al. 2009) suggested that there is a classic trade off in which some species that are short lived, have a restricted breeding season or are threatened by severe environmental conditions, show lower stress-induced corticosterone concentrations during acute stress. Because high circulating levels of corticosterone can inhibit reproductive function, suppression of the adrenocortical response to acute stress is favored under such circumstances (after Bókony et al. 2009; see also Wingfield et al., 1995; Wingfield and Sapolsky, 2003; Hau et al., 2010). This is the high brood value hypothesis that favors successful breeding over self preservation in cases in which breeding opportunities are restricted. Other species that are long-lived and have many potential attempts at breeding, and/or have flexible breeding seasons, should remain responsive to acute stress and abandon breeding in favor of self preservation so that the individual can survive to breed again. More field data from diverse species in a variety of ecological contexts are needed to resolve the issue of population, seasonal and gender differences in the adrenocortical response to acute stress.

In 1998, one of the longest and most severe ENSO events (Overlauber et al., 1998; Enfield, 2001) came to an end and transitioned to La Niña conditions. We predict that in an ENSO year (1998) when the land birds have high food resources they will suppress the adrenocortical response to acute stress and favor maximum reproductive effort. For seabirds, some have greatly reduced food resources and are predicted to remain responsive to acute stressors thus decreasing reproductive effort. Yet other seabirds appear to have adequate access to food and are predicted to show no change in responsiveness to acute stress in El Niño and La Niña years.

2. Methods

2.1. Field sites

In May 1998 and 1999, we sampled 240 individuals from 12 species on the Galapagos archipelago in the eastern central Pacific Ocean approximately 0.5°N to 1.5°S and 89° to 92°W. All individuals sampled were adults, although in non-breeding populations it is possible that we sampled some individuals that had not yet bred. This is most pertinent to land birds and in the event we did sample some younger individuals, their morphology was essentially similar to adults. Furthermore, studies on other passerines have shown that hatch year and after hatch year birds show similar adrenocortical responses to stress in the non-breeding season (Wingfield et al., 1995). Seabird species were captured by hand, or in hand nets on land either in breeding colonies or roosting areas. Great Frigatebirds, *Fregata minor*, were sampled on breeding colonies on Isla Baltra, Isla Seymour and Isla Genovesa. Red-footed Boobies, *Sula sula*, were sampled on Isla Genovesa. All Galapagos Penguins, *Spheniscus mendiculus*, and Flightless Cormorants, *Phalacrocorax harrisi*, were sampled on Isla Fernandina and Isla Isabella. In 1999, Band-rumped (or Madeiran) Storm-Petrels, *Oceanodroma castro*, were sampled on Isla Genovesa. The penguins and cormorants represent inshore feeding seabirds whereas the boobies and petrels are more pelagic. Great Frigatebirds are local and pelagic, being able to shift diets depending upon prevailing conditions and food available. They also have a piratical foraging technique, chasing other seabirds forcing them to regurgitate food.

Land birds were captured in mist nets or by hand nets while they foraged. Sharp-beaked Ground-finches, *Geospiza difficilis*, Large Ground-finches, *G. magnirostris*, Large Cactus-finches, *G. conirostris*, Galapagos Doves, *Zenaida galapagoensis*, and Galapagos Mockingbirds, *Nesomimus parvulus*, were sampled on Isla Genovesa. Small Ground-finches, *G. fuliginosa*, and Medium Ground-finches, *G. fortis*, were sampled at the Charles Darwin Research Station, and at higher elevations on Isla Santa Cruz.

Field work was permitted by the Galapagos National Park Service (GNPS) as part of a long-term investigation into the ecophysiology of land and marine vertebrates. All procedures were approved by the University of Washington Institutional Animal Care and Use Committee. Sample sizes and sex (when known) for all species are presented in Table 1. Sexes were determined by presence of a brood patch when specific to females, presence of a cloacal protuberance, size of wings and/or tarsi, or were declared unknown.

2.2. Blood sampling

Blood samples were collected from the wing vein into heparinized micro-hematocrit tubes after puncture of the vessel with a 26 gauge needle (all land birds and Band-rumped Storm Petrels). For larger seabirds, blood was collected into heparinized syringes from the wing vein. In Galapagos Penguins blood was collected from subcutaneous veins in the foot in micro-hematocrit tubes after puncture with a 26 gauge needle. In all cases blood flow was stanched with cotton. In no case did total volume of blood collected from a single individual exceed 1% of body weight. All blood was stored on ice in small portable coolers until return to the ship or the laboratory. Blood was then centrifuged for up to 10 min, plasma harvested and frozen at −20 °C. At the end of the field season all samples were transported frozen to the University of Washington, Seattle and stored at −20 °C until assay.

To assess the adrenocortical response to acute stress, all birds were subjected to the "stress series" in which a blood sample is collected as soon as possible after capture (up to 3 min) and then further samples collected at 5, 10, 30 and 60 min of capture, handling and restraint. For cormorants and penguins we only collected 3, 5, 10 and 30 min samples because heat stress might be a deleterious factor if held captive for a longer period. For small land birds less than 15 g, Sharp-beaked (1999) and Small Ground-finches (1998 and 1999) we collected samples at 3, 5, 20 and 60 min. In 1998, we collected blood samples from Sharp-beaked Ground-finches at 3, 5, 10, 30, and 60 min but nonetheless remained within the limit of maximum amount of blood taken from an individual (less than 1% body weight). These standardized procedures have proven to be the most effective and repeatable method for assessing the adrenocortical response to acute stress (e.g. Wingfield, 1994). Corticosterone levels in the first sample (within 3 min of capture) represent the baseline concentration in blood just prior to capture. Subsequent samples allow the determination of the maximum level of corticosterone attained during capture handling and restraint. These series also allow estimates of the rate of increase and a measure of total amount of corticosterone secreted during the stress series (i.e. area under the curve above integrated baseline level).

Table 1
Sample sizes and numbers by sex (when known). U = unknown, M = male, F = female and n is total sample size.

Species	U	F	M	n
Small ground finch GEOFUL	14	2	2	18
Sharp-beaked ground finch GEODIF	24	1	1	26
Medium ground finch GEOFOR	8	–	–	8
Galapagos Dove ZENGAL	16	–	–	16
Galapagos mockingbird NESPAR	15	–	–	15
Large cactus finch GEOCON	8	–	2	10
Large cactus finch GEOMAG	9	–	–	9
Flightless cormorant PHAHAR	17	5	8	30
Galapagos penguin SPHMEN	7	13	13	33
Great Frigatebird FREMIN	–	8	23	31
Red-footed booby SULSUL	6	6	10	22
Band-rumped storm petrel OCECAS	8	–	–	8

2.3. Body measures

All birds were weighed using Pesola scales (Pesola AG; Baar, Switzerland). We estimated subcutaneous fat score in land birds in the furculum and abdomen on a scale 0 (no fat) to 5 (bulging fat bodies; see Wingfield and Farner, 1978). Wing length, culmen and tarsus (when appropriate depending upon species) were measured to the nearest millimeter. Status of molt was assessed in all birds if present. Replacement of remiges such as primaries, secondaries and body molt were noted (the latter especially in penguins that have no flight feathers).

2.4. Weather data

Sea surface temperature (daily mean) and daily total precipitation data from Puerto Ayora (0°34′0″S, 90°19′0″W) were obtained from the online Charles Darwin Foundation Meteorological Database (http://datazone.darwinfoundation.org/climate/). We assessed seasonal changes and annual differences using Generalized Additive Models (GAM; Hastie and Tibshirani, 1986) implemented in R 2.11.1 (R Development Core Team, 2009) with package "mgcv" (Wood, 2006). Generalized Additive Models are a family of models that used non-parametric smoothers to assess nonlinear patterns between variables without imposing a priori relationships on the data (as polynomial regression does) (Wood, 2006). To assess how climatic variables changed between and within years, we independently modeled daily sea surface temperature and daily precipitation data as functions of both year (parametric term) and a spline of date (nonparametric smoothed term with a cyclic cubic regression spline). We modeled data from

1997 to 2000 to accurately model temporal fluctuations in our focal years. Models for daily sea temperature and daily precipitation data were modeled using Gaussian and gamma error structures, respectively, to approximate the distributions of the raw climate data.

2.5. Corticosterone assay

A total of 409 samples were assayed for corticosterone concentrations using the radioimmunoassay procedures of Wingfield and Farner (1975) and Wingfield et al. (1992). Briefly, all plasma samples (up to 30 µl) were mixed with 200 µl distilled water and approximately 2000 cpm of tritiated corticosterone added to measure recovery following extraction in 4 ml of freshly re-distilled dichloromethane. The organic phase was aspirated and dried under a stream of nitrogen at 40 °C. Dry extracts were then reconstituted in 550 µl of phosphate buffered saline with gelatin (pH 7.0) with 220 µl aliquots taken to duplicate assay tubes and 100 µl to a scintillation vial for recovery determination. All samples were then assayed with corticosterone antiserum (Endocrine Sciences) and bound and free steroid separated with addition of dextran-coated charcoal.

Lower sensitivity of the standard curve was 10 pg per tube, and intra-assay variation is less than 15% and inter-assay variation was 2.3%. Recoveries ranged from 69% to 95% (mean = 88.5%). See Wingfield and Farner (1975); Wingfield et al. (1992) for further details.

2.6. Statistics

To assess the effect of year on corticosterone measures across all species in our dataset, we used generalized linear mixed models (GLMMs, package "nlme", Pinheiro et al. 2009) in R 2.11.1 (R Development Core Team, 2009). Analysis proceeded in two steps. First we conducted an analysis of all species to examine the relative influence of year (El Niño versus La Niña), mean mass of species, guild (land, inshore, or sea), as well as the interaction between year and guild and between year and species-specific mass. We used mean mass for each species because mass data for some individuals were lost during fieldwork. This allowed us to examine the influence of mass across the wide range of species-specific body masses in our dataset. We excluded two species (Band-rumped Storm Petrel and Medium Ground-finch) because sampling was only conducted in a single year. Mass data were log-transformed to improve normality of model residuals.

We sampled 12 species from eight families. We accounted for some, but not all, phylogenetic inertia by including a random effect consisting of species nested within family. The appropriateness of this hierarchical structure was assessed by comparing two null models (no fixed effects) – one with species nested within family and one with species as the sole random effect – for each stress measure (baseline corticosterone, integrated corticosterone, and maximum corticosterone). A variance components analysis indicated that 19% of total variation in baseline corticosterone occurred at the inter-familial level, whereas 91% and 90% of variation in integrated and maximum corticosterone respectively occurred at the inter-specific level. Relative Akaike's Information Criterion (Akaike, 1974) and likelihood ratio tests both indicated that the two-level hierarchical structure (species nested within family) improved the fit of the null baseline corticosterone model but failed to improve the fit of the null integrated and maximum corticosterone models. Given these equivocal results, we adopted a conservative approach and included species nested within family as the hierarchical random effect in each model.

For the second step of the analysis, we conducted a species-level analysis to assess the influence of year and body measurements on corticosterone. For each model, we standardized raw data to a mean of 0 and unity variance after applying an appropriate data transformation. Such standardization facilitated direct comparisons of the magnitude of fixed effects within and between species. We ran models that included all variables and second-order interaction terms, where appropriate (excluding the interaction between mass and the mass-derived index of body condition). Some interaction terms were not included because of lack of variation (e.g. all birds were molting in one year but not in the other). For visualization of overall effects, we also plotted the results of simple linear models of standardized corticosterone data for each species (as in Fig. 6).

For both between and within-species models, we used an information theoretical approach to assess model fit (Burnham and Anderson, 2002), using Akaike's Information Criterion corrected for small sample sizes (AICc, Akaike, 1974). Model comparison and averaging was performed using R package "MuMIn" (Barton, 2009). In the within-species models, model-averaged parameter estimates for year were plotted to compare the relative effect of year on corticosterone.

3. Results

3.1. Climatic comparison between El Niño and La Niña years

Sea surface temperature was significantly higher in 1998 during El Niño conditions (GAM: $t = 5.66$, $p < .0001$). Daily sea surface temperature ranged from 25 to 28.5 °C (median = 26.9 °C) during El Nino and ranged from 23.2 to 25.1 °C (median = 23.9 °C) during La Niña (Fig. 1). In both years, sea surface temperatures rose to a maximum in March and April and dropped to a minimum in August–September. The smoothed term of date in the Generalized Additive Model indicated that this seasonal fluctuation was statistically significant for each year (1998: estimated d.f. = 7.77, $F = 8.61$, $p < .0001$; 1999: estimated d.f. = 8.51, $F = 28.50$, $p < .0001$).

Total annual rainfall was significantly higher in 1998 (El Niño) compared to 1999 (La Niña) – 1687 mm versus 143 mm, respectively. In 1998, rainfall showed a strong seasonal fluctuation, as indicated by a highly significant smoothed term of date (GAM: estimated d.f. = 6.018, $F = 7.18$, $p < .0001$). Approximately 94% (or 1583 mm) of total annual precipitation occurred by the end of May at the end of the sampling period (Fig. 1). In contrast, rainfall in 1999 did not vary significantly as a function of date (GAM: estimated d.f. = 4.27, $F = 1.21$, $p = .304$), with only 53% (or 76 mm) of total annual rainfall occurring by the end of May.

Both daily sea surface temperature and daily rainfall during the study period (May of each year) were significantly different between years. In May 1998, sea surface temperature was 4.3 ± 0 .3 °C warmer than in May 1999 (GAM: $t = 17.18$, $p < .0001$). Total rainfall in the month of May was also significantly higher in 1998 than in 1999 (1998: 246 mm, 1999: 3 mm; GAM: $t = 8.45$, $p < .0001$).

3.2. Overall (among species) effects on adrenocortical responses to the stress of capture, handling and restraint

After controlling for taxonomic clustering in our data, there were strong effects of both year and guild on adrenocortical responses to standardized acute stress. Variation in baseline corticosterone was best explained by model that included year as the only fixed effect (Table 2). The Akaike weight indicated that this model had a 63% chance of being the most appropriate model. Other plausible models (<0.95 cumulative AIC weight) indicated only weak support for species-specific mass as a predictor of baseline corticosterone (AIC_c weight < 0.20, $\beta = -0.049 \pm 0.093$). For

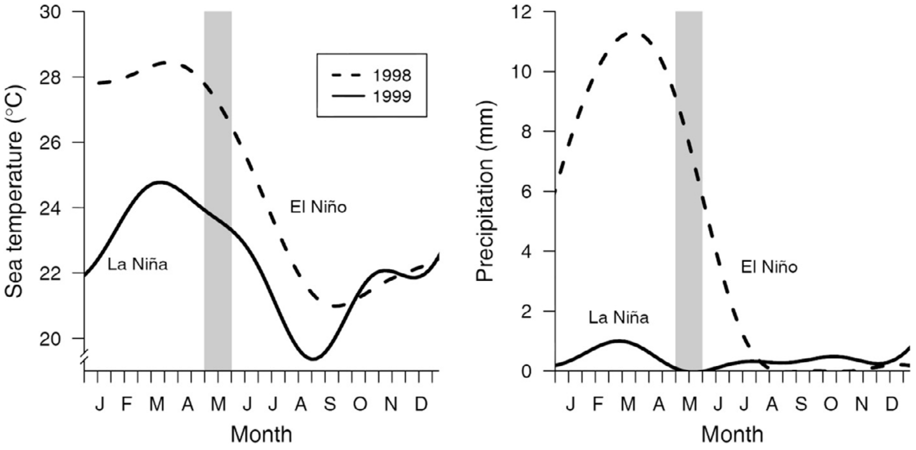

Fig. 1. Sea temperature and precipitation patterns in 1998 (El Niño) and 1999 (La Niña) at Puerto Ayora in the Galapagos Islands. Lines were fitted from average daily temperature and total daily precipitation using nonparametric smoothing. Each tick mark on the x-axis indicates approximately two weeks. The shaded region shows the range of dates when birds were studied in both years.

both integrated and maximum corticosterone levels, the model with the strongest support included guild, year, and their interaction (Table 1). Specifically, the transition from El Niño to La Niña had a strong negative effect on land birds' integrated and maximum corticosterone levels (model-averaged $\beta_{\text{integrated}} = -0.962 \pm 0.313$ standard error; model-averaged $\beta_{\text{maximum}} = -0.838 \pm 0.324$), no effect on the levels of inshore birds ($\beta_{\text{integrated}} = 0.213 \pm 0.337$; $\beta_{\text{maximum}} = 0.218 \pm 0.022$), and a positive effect in (offshore) sea birds ($\beta_{\text{integrated}} = 0.746 \pm 0.679$; $\beta_{\text{maximum}} = 0.694 \pm 0.236$). There was little evidence for species-specific mass as a predictor of either integrated or maximum corticosterone levels (Table 2).

3.3. Within species effects on adrenocortical responses to the stress of capture, handling and restraint

All species sampled showed an increase in plasma levels of corticosterone following capture, handling and restraint (Fig. 3). Corticosterone levels rose between 5 and 30 min in both males and females (when sex was known), and in both 1998 (El Niño) and 1999 (La Niña). Two species, Band-rumped Storm Petrel and Medium Ground-finch, were only sampled in 1999, and both showed typical adrenocortical responses to stress similar to other species sampled at this time (Fig. 3).

3.4. Offshore seabirds

In Great Frigatebirds, baseline levels of corticosterone were similar in 1998 and 1999 (median 1998 = 4.05 ng ml^{-1}, median 1 999 = 1.9 ng ml^{-1}, Fig. 3). Although males had higher overall baseline levels of corticosterone, sex differences among years were not supported in the top models. Integrated corticosterone (i.e. total corticosterone released over 60 min above baseline values; 1998: median = 1867 ng ml^{-1}, 1999: 1948 ng ml^{-1}, sexes combined) were also similar in both years (Fig. 4). Maximum corticosterone levels were not different across years (Fig. 5) but were positively associated with baseline corticosterone ($\beta = 0.778 \pm 0.153$, Table 3). Body mass was not significant in predicting baseline and integrated corticosterone, but there was moderate support of a negative association between body mass and maximum corticosterone ($\beta = -0.173 \pm 0.127$, Fig. 6)

Similar patterns were observed in the Red-footed Booby. There was evidence of weak sex differences among years in baseline corticosterone ($\beta = 1.030 \pm 0.553$, Fig. 3), with males exhibiting slightly higher levels than females (Table 2). There was also little support for year-to-year variation in baseline, stress-induced (integrated or maximum levels of corticosterone Figs. 4 and 5).

3.5. Inshore seabirds

In Flightless Cormorants (Figs. 2–5), there was little support for year-to-year differences in baseline corticosterone, although plasma corticosterone rose dramatically following capture and handling (Table 2). Integrated corticosterone of Flightless Cormorants was strongly influenced by baseline levels of corticosterone ($\beta = -3.254 \pm 0.0.693$) and year ($\beta = -0.577 \pm 0.163$), with high values being observed in 1998 (Fig. 4). Model selection also showed similar patterns for maximum levels of corticosterone. Additionally, there was evidence for increased body condition being associated with lower maximum levels of corticosterone ($\beta = -0.305 \pm 0.190$). In Galapagos Penguins, model selection and model-averaging indicated that body condition, year, and sex had negligible impacts on any corticosterone measure (Fig. 6). There was support for a positive association between baseline and maximum levels of corticosterone ($\beta = -0.423 \pm 0.203$). Penguins in 1999 had slightly raised levels of baseline but depressed levels of integrated, and maximum corticosterone (Figs. 3–5; sexes combined). There was no evidence for sex differences in any corticosterone measure among years, but in the El Niño year of 1998, corticosterone titers at 5 and 10 min post capture appeared elevated over the same time points in the La Niña year of 1999 but by the 30 min sample, circulating corticosterone levels were identical in both years (Fig. 2). Of the 21 adults sampled in 1998, 7 were in some stage of molt and in 1999, 11 of the 23 adults were in molt. We saw no juveniles in 1998, suggesting penguins did not successfully rear fledglings or the juveniles died. In 1999, we saw 3 juveniles and one had finished the molt suggesting the penguins had bred successfully in the last 6 months. Galapagos Penguins keep their juvenile plumage for about 6 months (Boersma, 1977). In neither1998 or 1999 did we find evidence that penguins were breeding and in both years they were in poor body condition. In 1998 their body condition was similar to their condition in the 1972 El Niño (Boersma, 1998b).

3.6. Land birds

In the Galapagos Dove (Figs. 2 and 3), baseline corticosterone levels were extremely low in 1999, compared to levels during the El Niño year (1998) (effect of year: $\beta = -1.538 \pm 0.542$). Of all

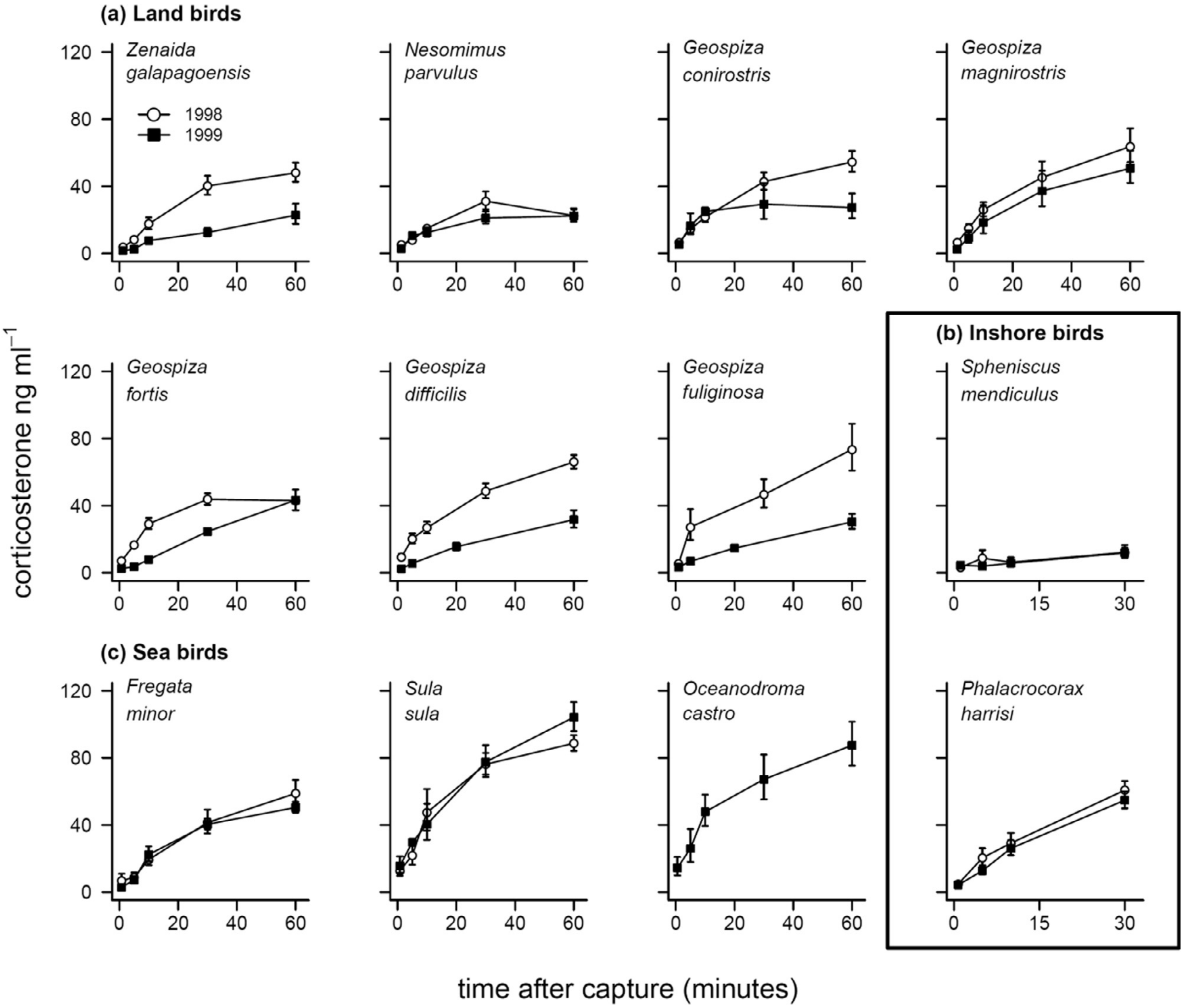

Fig. 2. Temporal changes in plasma levels of corticosterone following capture, handling and restraint in twelve bird species on the Galapagos Island during El Niño in 1998 (open white circles) and La Niña in 1999 (black squares). Species are arranged in by guild: (a) Land birds: Galapagos Dove (*Zenaida galapagoensis*), Galapagos Mockingbird (*Nesomimus parvulus*), Large Cactus-finch (*Geospiza conirostris*), Large Ground-finch (*Geospiza magnirostris*), Medium Ground-finch (*Geospiza fortis*), Sharp-beaked Ground-finch (*Geospiza difficilis*), and Small Ground-finch (*Geospiza fuliginosa*); (b) Inshore birds: Galapagos Penguin (*Spheniscus mendiculus*) and Flightless Cormorant (*Phalacrocorax harrisi*); (c) Sea birds: Great Frigatebird (*Fregata minor*), Red-footed Booby (*Sula sula*), and Band-rumped Storm Petrel (*Oceanodroma castro*). Note the different time scale (30 min) for the inshore species. Error bars equal standard errors of back-transformed normalized data and are thus asymmetrical. Sample sizes are unbalanced between and within years (mean = 9, range = 1–24 individuals).

species studied, the Galapagos Dove was the only species in which mass was measured where there was strong support for an overall effect of body mass (negative) on maximum and integrated levels of corticosterone (Fig. 6). In the larger passerines such as Galapagos Mockingbirds, Large Cactus-finches, and Large Ground-finches, the maximum and integrated corticosterone levels were similar across species (Figs. 2–5). In Large Cactus-finches, we found strong support for a positive association between baseline corticosterone and maximum corticosterone levels ($\beta = 0.701 \pm 0.277$) and a weaker association ($\beta = 0.553 \pm 0.296$) between baseline and integrated corticosterone levels (Table 3). Large Cactus-finches in 1999 (La Niña) had low levels of maximum and integrated corticosterone compared to the 1998 (integrated corticosterone by year – 1998: median = 1968 ng ml^{-1}, 1999: 800 ng ml^{-1}, sexes combined, Figs. 2–5). The pattern of corticosterone response to capture was similar in the Medium Ground-finch (Fig. 2) sampled in the La Niña year).

In Large Ground-finches, baseline corticosterone levels, but not maximum or integrated levels, were lower in 1999 relative to 1998 ($\beta = -1.702 \pm 0.397$). In contrast, the smaller passerines such as Sharp-beaked Ground-finches and Small Ground-finches, exhibited dramatically greater responses of circulating corticosterone to capture stress in the 1998 (El Niño) year (Fig. 2). For Sharp-beaked Ground-finches, year explained large amount of variation for baseline, maximum, and integrated corticosterone (Table 3). Maximum levels of corticosterone in response to capture were much greater in 1998 than in 1999 (Sharp-beaked Ground-finches: $\beta = -1.365 \pm 0.312$; Small Ground-finches: $\beta = -1.283 \pm 0.421$, Fig. 5); a similar pattern held for integrated levels of corticosterone. In Sharp-beaked Ground-finches but not Small Ground-finches, baseline corticosterone was also higher in 1998–contrary to predictions ($\beta = -1.141 \pm 0.345$, Fig. 3).

In Small Ground-finches, maximum levels of corticosterone were positively associated with baseline levels of corticosterone

Table 2
Inter-specific model selection for the influence of year, guild, and species mass (plus two-way interactions) on corticosterone levels during the El Niño to La Niña transition (1998–1999) in the Galapagos Islands. All candidate models are shown. Indicated are the number of model parameters (k), AIC_c, AIC_c difference (ΔAIC_c) relative to the best supported model (the model with the lowest AIC_c), and the AIC_c evidence weight for each model (w_i). Models with the greatest support ($\Delta\text{AIC}_c \leq 2$) appear in bold type.

Response	Model	k	AIC_c	ΔAIC_c	w_i
Baseline corticosterone	**Year**	**5**	**506.95**	**0.00**	**0.63**
	mass, year, mass × year	7	509.35	2.74	0.19
	guild, year, guild × year	9	511.50	4.89	0.07
	mass, year	6	512.47	5.86	0.04
	guild, mass, year, guild × year	10	514.22	7.61	0.02
	guild, mass, year, mass × year	9	514.37	7.76	0.02
	guild, year	7	514.70	8.09	0.01
	(.)	4	515.01	8.40	0.01
	guild, mass, year, guild × year, mass × year	11	515.73	9.12	0.01
	guild, mass, year	8	517.56	10.95	0.00
	Mass	5	520.44	13.83	0.00
	guild	6	522.60	15.99	0.00
	guild, mass	7	525.42	18.81	0.00
Integrated corticosterone	**guild, year, guild × year**	**9**	**513.46**	**0.00**	**0.45**
	Year	**5**	**514.23**	**1.79**	**0.30**
	guild, mass, year, guild × year	10	516.25	3.81	0.11
	mass, year, mass × year	7	517.36	4.92	0.06
	guild, mass, year, guild × year, mass × year	11	517.87	5.43	0.05
	mass, year	6	519.78	7.34	0.02
	guild, year	7	522.09	9.65	0.01
	guild, mass, year, mass × year	9	522.52	10.08	0.00
	guild, mass, year	8	524.99	12.55	0.00
	(.)	4	532.07	19.63	0.00
	Mass	5	537.52	25.08	0.00
	guild	6	539.74	27.30	0.00
	guild, mass	7	542.55	30.11	0.00
Maximum corticosterone	**guild, year, guild × year**	**9**	**517.93**	**0.00**	**0.41**
	Year	**5**	**518.28**	**1.37**	**0.34**
	guild, mass, year, guild × year	10	520.67	3.76	0.10
	mass, year, mass × year	7	521.58	4.67	0.07
	guild, mass, year, guild × year, mass × year	11	522.29	5.38	0.05
	mass, year	6	523.80	6.89	0.02
	guild, year	7	526.09	9.19	0.01
	guild, mass, year, mass × year	9	526.66	9.76	0.01
	guild, mass, year	8	528.95	12.05	0.00
	(.)	4	529.23	12.32	0.00
	Mass	5	534.67	17.76	0.00
	guild	6	536.89	19.99	0.00
	guild, mass	7	539.69	22.79	0.00

($\beta = 0.462 \pm 0.185$). We found strong support for the effect of molt and baseline corticosterone on both integrated and maximum levels of corticosterone (Table 3). Molt had a strong negative effect such that individuals observed with some molt exhibited lower integrated corticosterone values.

4. Discussion

There is growing evidence that climatic events and the weather that ensues can have dramatic effects on the adrenocortical responses to acute stresses (e.g. Wingfield and Ramenofsky, 2011). Elevated glucocorticoids following climatic events are thought to be adaptive by triggering the emergency life history stage that favors self preservation over reproduction and investment in chicks (e.g. Wingfield, 1994; Bókony et al., 2009; Romero and Wingfield, 2015), and have beneficial actions to redirect behavior associated with survival and suppress reproductive effort including territorial behavior (e.g. Wingfield et al., 1998; Sapolsky et al., 2000; Wingfield and Romero, 2001; Romero, 2002; Wingfield and Kitaysky, 2002; Wingfield, 2003). Questions remain, however, as to why not all species respond in the same way to perturbations of the environment. Such differences may have ecological bases and individuals could be responding directly to weather or to effects on food supply etc. Yet others may suppress the adrenocortical response to acute stress while breeding to maximize reproductive success (Wingfield, 1994; Bókony et al., 2009; Hau et al., 2010). To determine possible effects of climatic events on the adrenocortical responses to stress and to test hypotheses generated by theoretical approaches (Bókony et al., 2009; Hau et al., 2010) field investigations are key. In this study we investigated the effects of two climatic events, El Niño and La Niña, on the adrenocortical responses to acute stress.

4.1. Sea birds

The offshore feeding seabirds in the Galapagos Islands largely show changes in adrenocortical responses to acute stress that are consistent with the stress responses of many vertebrate species and similar to those found in other studies on these species (Lormée et al., 2003). Great Frigatebirds were breeding in both years of our study, and baseline corticosterone titers in males and females in 1998 were similar but tended to be higher in 1999. This contradicts what we would have predicted – no difference in baseline corticosterone – in offshore feeders that are probably not as severely affected by ENSO. Note that maximum and integrated corticosterone measures showed no year differences (Fig. 2). In Red-footed Boobies, as predicted, baseline corticosterone levels and the stress patterns did not differ between years (Fig. 2). In a study of a very different population of Red-footed Boobies, plasma corticosterone increased from the egg-laying period, through incubation to the highest level when brooding chicks. In

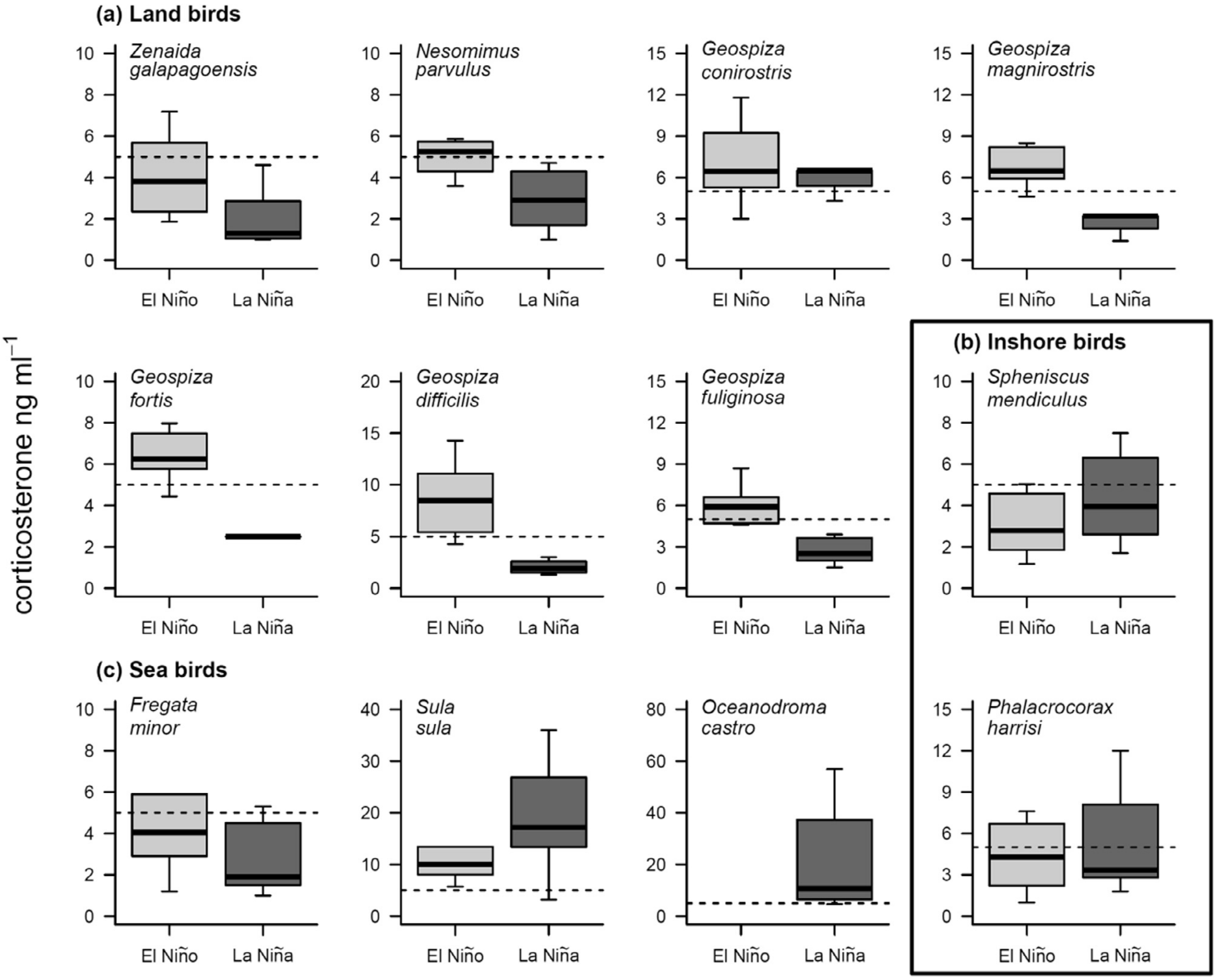

Fig. 3. Baseline levels of corticosterone for 12 species of birds in the Galapagos Islands in 1998 (El Niño) and 1999 (La Niña). The horizontal dashed line (5 ng ml^{-1}) facilitates comparisons between species, since scales vary between plots. Tukey boxplots show median (thick center line), first and third quartiles (lower and upper extent of grey boxes, respectively), and the lowest and highest datum within 1.5× the inter-quartile range from the first and third quartiles (whiskers). For clarity, outliers on boxplots are not shown. Species are arranged in by guild: (a) Land birds: Galapagos Dove (*Zenaida galapagoensis*), Galapagos Mockingbird (*Nesomimus parvulus*), Large Cactus-finch (*Geospiza conirostris*), Large Ground-finch (*Geospiza magnirostris*), Medium Ground-finch (*Geospiza fortis*), Sharp-beaked Ground-finch (*Geospiza difficilis*), and Small Ground-finch (*Geospiza fuliginosa*); (b) Inshore birds: Galapagos Penguin (*Spheniscus mendiculus*) and Flightless Cormorant (*Phalacrocorax harrisi*); (c) Sea birds: Great Frigatebird (*Fregata minor*), Red-footed Booby (*Sula sula*), and Band-rumped Storm Petrel (*Oceanodroma castro*).

other studies, this effect is higher in males than females (Lormée et al., 2003). These authors suggest that because male Red-footed Boobies are 15% smaller than females, they may lose condition because males incur greater flight costs and thus are less efficient foragers. We saw no difference in corticosterone concentrations of Red-footed Boobies on Isla Genovesa in May 1998 and 1999 – birds were not breeding at this time in either year. Thus there was no confound of breeding stage.

It is important to point out that many species of seabirds, especially at high latitudes that feed offshore may fast voluntarily for several days to weeks while they incubate eggs or brood young. Reduced food in turn may have an impact on body condition. For example, McQueen et al. (1999) found that baseline corticosterone was higher in Adélie Penguins, *Pygoscelis adeliae*, fasting during incubation than when they first arrived from a foraging trip. However, baseline corticosterone does not change during the actual fast of an incubation bout (Vleck and Vleck (2002). On the other hand in Magellanic Penguins, *Spheniscus magellanicus*, fasting during incubation showed no changes of baseline corticosterone, but maximum corticosterone (capture stress) increased with length of the fast, was higher in females and negatively correlated with body condition (Hood et al., 1998). Hence, periods of fasting and the accompanying decrease in body condition may obscure the relationship between corticosterone and reproductive effort.

Plasma profiles of corticosterone following capture stress in Band-rumped Petrels (Fig. 2) showed the typical increase over 30 min. There were no sex differences or changes with breeding sub-stage in baseline corticosterone levels in Grey-faced Petrels, *Pterodroma macroptera gouldi*), but there was a weak negative relationship of the adrenocortical response to capture stress (Adams et al., 2005). Stress responses were also higher during the incubation phase than other stages. Thus we have to be careful to control for stage in breeding to look for ENSO effects in future studies of these offshore feeding birds and further studies on Band-rumped Petrels on the Galapagos are needed.

In the inshore feeding seabirds, including Flightless Cormorants, differences in adrenocortical responses to standardized stressors were also similar in the ENSO and La Niña years (Figs. 2–5). Low levels of stress-induced corticosterone in Galapagos Penguins in both years could be related to the pre-breeding molt and their poor

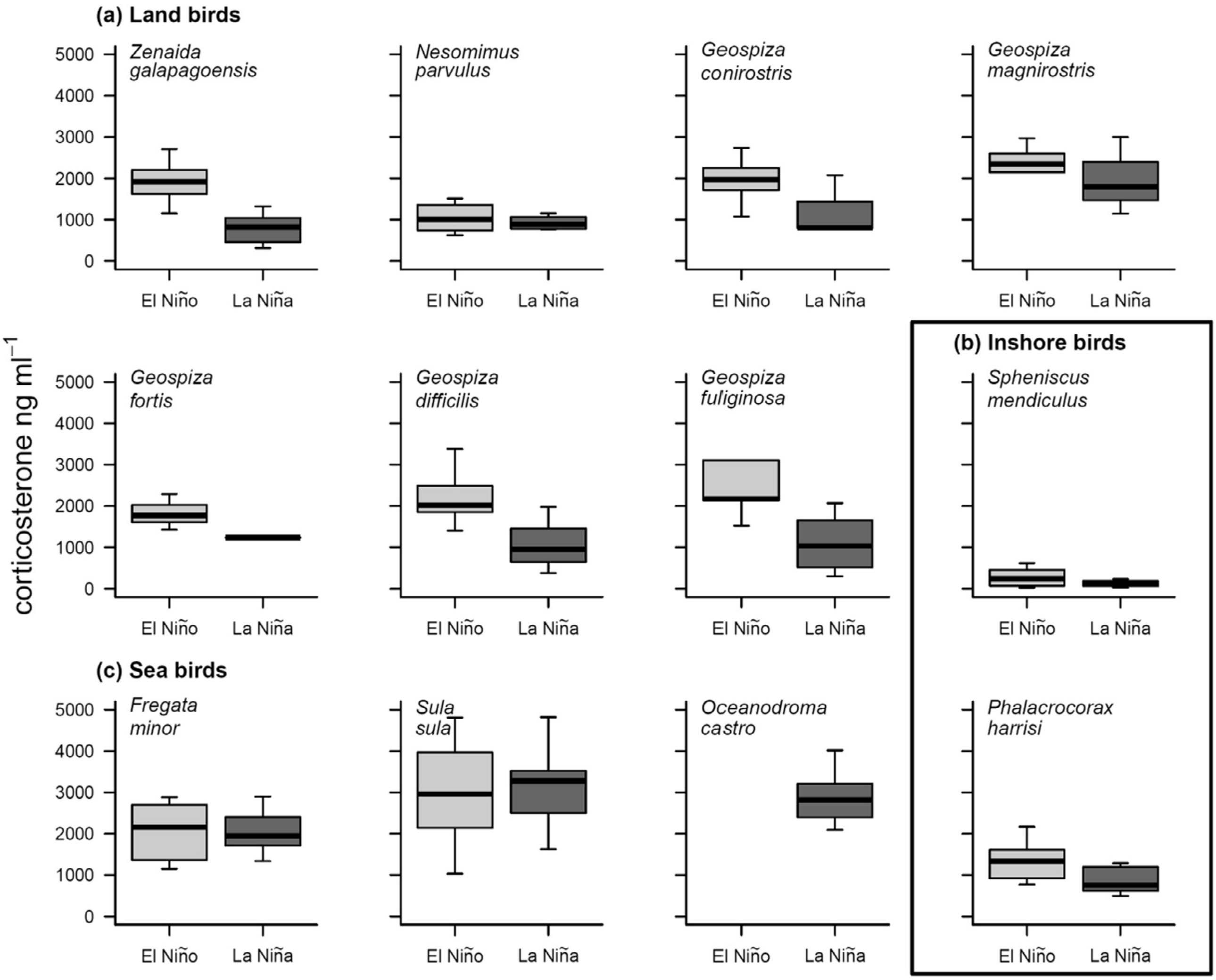

Fig. 4. Integrated levels of corticosterone for 12 species of birds in the Galapagos Islands in 1998 (El Niño) and 1999 (La Niña). Tukey boxplots show median (thick center line), first and third quartiles (lower and upper extent of grey boxes, respectively), and the lowest and highest datum within 1.5× the inter-quartile range from the first and third quartiles (whiskers). For clarity, outliers on boxplots are not shown. Species are arranged in by guild: (a) Land birds: Galapagos Dove (*Zenaida galapagoensis*), Galapagos Mockingbird (*Nesomimus parvulus*), Large Cactus-finch (*Geospiza conirostris*), Large Ground-finch (*Geospiza magnirostris*), Medium Ground-finch (*Geospiza fortis*), Sharp-beaked Ground-finch (*Geospiza difficilis*), and Small Ground-finch (*Geospiza fuliginosa*); (b) Inshore birds: Galapagos Penguin (*Spheniscus mendiculus*) and Flightless Cormorant (*Phalacrocorax harrisi*); (c) Sea birds: Great Frigatebird (*Fregata minor*), Red-footed Booby (*Sula sula*), and Band-rumped Storm Petrel (*Oceanodroma castro*).

body condition. Plasma corticosterone levels (baseline) were also maintained at low levels in molting King Penguins, *Aptenodytes patagonica*, and Emperor Penguins, *A. forsteri*, increasing only toward the end of molt when birds were beginning to move back to the ocean (Groscolas and Cherel, 1992; Groscolas and Robin, 2001). The low levels of corticosterone during the molt are thought to spare protein breakdown during a period of massive protein synthesis for feathers (DesRochers et al., 2009; Romero et al., 2005) and the increase in corticosterone at the end of molt may be a 'refeeding' signal in those species that also fast at this time.

In Blue-footed Boobies, *Sula nebouxi*, breeding in Mexico during an ENSO event in 1993, at least some of the birds in the colony actually initiated breeding but then failed (Wingfield et al., 1999). In our study in the Galapagos, with the exception of the offshore feeding Great Frigatebirds, the other seabird species did not even initiate breeding during the ENSO of 1998. Yet, there were no clear differences in the adrenocortical responses to acute stress in our study or that of Wingfield et al. (1999). Comparisons with other marine species show that in non-vitellogenic Green Sea Turtles, *Chelonia mydas*, corticosterone levels were highest in females in Australia Barrier Reef region in 1997 (first El Niño year) but lower in 1998 (also an El Niño year) suggesting at best a weak correlation with ENSO in this species (Hamann et al., 2005). Food availability, as a consequence of ENSO, may also be important. In the Common Murre, *Uria aalge* and the Black-legged Kittiwake, *Rissa tridactyla*, food abundance around breeding islands in the Gulf of Alaska predicted baseline corticosterone levels in breeding adults. Plasma corticosterone of adults was lower when food abundance was high (Kitaysky et al., 1999, 2007).

Likewise, for Nazca Boobies, *Sula granti*, breeding on the Galapagos Islands ENSO hardly had an effect on corticosterone concentrations. In contrast, moonlight may in turn affect corticosterone through its effects on the distribution of prey species – numbers and when they are available, and in turn affect corticosterone (Tarlow et al., 2003). Similarly, although a weak ENSO in 2003 affected lay date and the sex ratio of offspring in Rhinoceros Auklets, *Cerorhinca monocerata*, compared to 2004 (cooler waters and greater primary production in the north eastern Pacific Ocean), laying dates were earlier (Addison et al., 2008).

In seabirds in general baseline corticosterone appears to be an excellent indicator of foraging conditions with high corticosterone related to poor conditions and low corticosterone to good

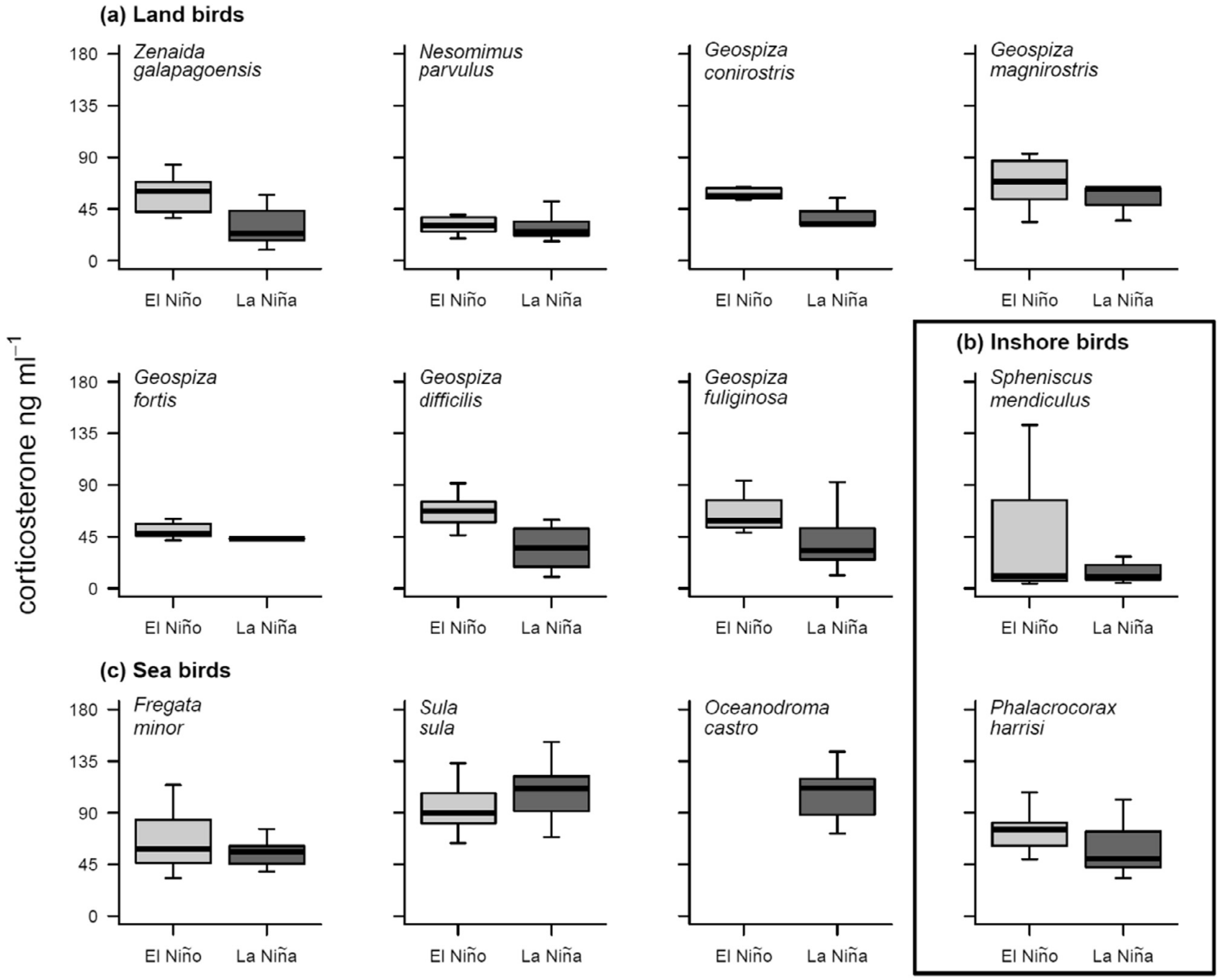

Fig. 5. Maximum levels of corticosterone for 12 species of birds in the Galapagos Islands in 1998 (El Nino) and 1999 (La Nina). Tukey boxplots show median (thick center line), first and third quartiles (lower and upper extent of grey boxes, respectively), and the lowest and highest datum within 1.5× the inter-quartile range from the first and third quartiles (whiskers). For clarity, outliers on boxplots are not shown. Species are arranged in by guild: (a) Land birds: Galapagos Dove (*Zenaida galapagoensis*), Galapagos Mockingbird (*Nesomimus parvulus*), Large Cactus-finch (*Geospiza conirostris*), Large Ground-finch (*Geospiza magnirostris*), Medium Ground-finch (*Geospiza fortis*), Sharp-beaked Ground-finch (*Geospiza difficilis*), and Small Ground-finch (*Geospiza fuliginosa*); (b) Inshore birds: Galapagos Penguin (*Spheniscus mendiculus*) and Flightless Cormorant (*Phalacrocorax harrisi*); (c) Sea birds: Great Frigatebird (*Fregata minor*), Red-footed Booby (*Sula sula*), and Band-rumped Storm Petrel (*Oceanodroma castro*).

conditions (e.g. in Least Auklets, *Aethia pusilla*, and Thick-billed Murres, *Uria lomvia*, Benowitz-Fredericks et al., 2008; Black-legged Kittiwakes, Kitaysky et al., 2007; Buck et al., 2007). Corticosterone was also high in years when Common Murres were feeding chicks and when fish populations were low, or were not coincidental with the seasonal increase in fish, and was lower in years when chick presence coincided with maximum fish populations (Doody et al., 2008). Alternatively, experimentally increased foraging effort (by clipping wing feathers) in Little Auks, *Alle alle*, resulted in a decline in body mass and increases in baseline corticosterone in both clipped adults and their chicks compared with controls (Harding et al., 2009). On the other hand, increasing wing loading by adding 45 g weights to Cory's Shearwaters, *Calonectris diomedea*, during incubation increased foraging time and resulted in a lower rate of mass gain while at sea but no change in baseline corticosterone compared with controls (Navarro et al., 2008). Much more investigation is needed to tease apart effects of poor versus good feeding conditions and reproductive function in seabirds in general, and in relation to the brood value hypothesis.

In Black-legged Kittiwakes, baseline corticosterone was high after arriving at the nest from a foraging trip and declined proportionally to the success of that foraging trip (Angelier et al., 2007a). In contrast in Adélie Penguins, post-foraging corticosterone was lower (McQueen et al., 1999: Angelier et al., 2008) suggesting that success of the foraging trip (environmental conditions) and length of the trip allowing replenishment of body condition were important factors. Beaulieu et al. (2009) also found no correlation of baseline corticosterone in Adélie Penguins with length of foraging trips associated with extent and rate of melt of sea ice (earlier melt means a shorter trip and later melt means greater distances to travel for food). Furthermore, several studies indicate that black-legged Kittiwakes breeding in poorer condition have higher baseline corticosterone. Curiously experimentally increased corticosterone resulted in greater body condition presumably through enhanced foraging activity (Kitaysky et al., 2003; Lanctot et al., 2003; Angelier et al., 2007b). In general it appears that in many seabirds there was a negative relationship of MaxCort with body mass. This fits with individuals in poor body condition being more 'on the edge' and/or more willing to suppress breeding.

4.2. Land birds

The adrenocortical responses to acute stress in land birds showed typical increases over 3–30 min (Fig. 2) as seen in virtually

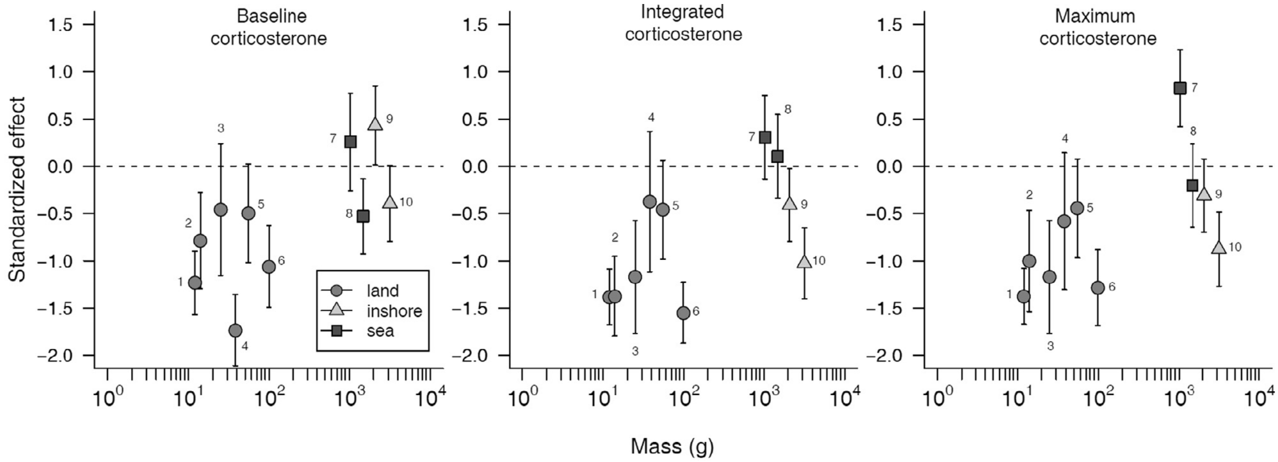

Fig. 6. Standardized effect of year (relative to El Niño) on baseline, stress-induced, and maximum levels of corticosterone for ten bird species in the Galapagos Islands, plotted on species-specific mass: *Geospiza difficilis* (1), *Geospiza fuliginosa* (2), *Geospiza conirostris* (3), *Geospiza magnirostris* (4), *Nesomimus parvulus* (5), *Zenaida galapagoensis* (6), *Sula sula* (7), *Fregata minor* (8), *Spheniscus mendiculus* (9), and *Phalacrocorax harrisi* (10). For each stress measurement, models were run on individual species with year as the only factor. Dashed line indicates no change in corticosterone from 1998 (El Niño) to 1999 (La Niña). Error bars are equal to one standard error of the parameter estimate. See legends to Figs. 2–5 for English names of species.

Table 3
Table of effects for ten species of birds on Galapagos Islands. Each model-averaged parameter estimates with an importance weight > 0.5 is shown with a double effect sign, whereas those with importance weights < 0.5 are indicated by a single effect sign. For interactions, only the direction of the effect is indicated. Abbreviations are as follows: Y = year, M = individual mass, B = baseline corticosterone, S = sex, F = fat score, C = body condition, Mo. = molt score.

Species	Stress measure	Y	M	B	S	F	C	Mo.	Interactions
Geospiza fuliginosa GEOFUL	Baseline	–						– –	
	Integrated	– –		+				–	
	Maximum	–		+ +				–	
Geospiza difficilis GEODIF	Baseline	– –				– –		–	
	Integrated	– –		+		–		–	
	Maximum	– –		–		–		–	
Zenaida galapagoensis ZENGAL	Baseline	– –	+			+		+	
	Integrated	– –	– –	+		+		– –	Y*B(–), B*Mo.(–)
	Maximum	–	– –	+		–		–	
Nesomimus parvulus NESPAR	Baseline	+	+					+	
	Integrated	–	+	–				+	
	Maximum	+	+	–				+	
Geospiza conirostris GEOCON	Baseline	–	+			+		–	
	Integrated	– –	– –	+		+		– –	Y*B(–), B*Mo.(–)
	Maximum	– –	+	+ +		+		–	
Geospiza magnirostris GEOMAG	Baseline	– –							
	Integrated	–		+					
	Maximum	–		+					
Phalacrocorax harrisi PHAHAR	Baseline	–					–	–	
	Integrated	– –		– –			–	–	Y*B(–), Y*Mo.(+)
	Maximum	– –		+ +			–		Y*B(+ +)
Spheniscus mendiculus SPHMEN	Baseline	+			+		–		
	Integrated	–		+ +	+		+		B*S(+)
	Maximum	–		+ +	+		+		
Fregata minor FREMIN	Baseline	– –			+		–		
	Integrated	+		+	+		–		
	Maximum	+		+ +	+		–		B*S(–)
Sula sula SULSUL	Baseline	–	+ +		+				
	Integrated	+	+	+	+				
	Maximum	+	+	+	+				

all vertebrates studied to date. However, the magnitude of those responses differed markedly from predictions. The larger species (i.e. over 20 g) showed little yearly difference in the adrenocortical response to capture stress with the exception of the Galapagos Dove that revealed a slightly elevated level of circulating corticosterone in 1998 compared with levels in 1999 (Figs. 2–5). These data are contrary to predictions from the brood value hypothesis (Bókony et al., 2009) that in the El Niño year when conditions for breeding are favorable, then the responses to acute stress should be suppressed to allow increased reproduction. One possible explanation is that in El Niño year of 1998, environmental conditions conducive to breeding were prolonged and birds were able to breed many times which in turn might favor abandonment of any single breeding attempt if exposed to perturbations so that another, potentially more successful, breeding attempt could be initiated. If this was the case, then a further prediction would be that in La Niña years with very limited rainfall, land birds should suppress their adrenocortical responses to acute stress in the short breeding season when one breeding attempt is only possible. A follow up study should sample birds when breeding in a La Niña year.

The enhanced adrenocortical response of the small land birds to acute stress during the El Niño year was unexpected. Hau et al. (2010) found a large effect of body mass, especially on maximum corticosterone, with smaller species having higher maximum corticosterone. This could be expected if one considers that smaller species have a higher mass-specific metabolic rate and so burn their resources faster than larger species, and also may accrue smaller energy reserves such as fat because body size is less. This might have resulted in faster adrenocortical responses to acute stress responses in ‘dire straights’ during an exceptionally strong ENSO and therefore should have a greater adrenocortical response to maximize survival in whichever way they can. It is also possible that these small passerines may be subjected to thermoregulatory stress during intense periods of rain. They do not have insulation because down feathers are largely absent and they carry little fat. Thus, heavy rain and wind may increase thermoregulatory costs in small land birds more than the larger species that showed no differences in corticosterone stress profiles. It would be interesting to document behavioral patterns and energy expenditure in small versus large Darwin’s Finches in relation to rain and wind in El Niño years. In contrast, in superb starlings, *Lamprotornis superbus*, of East Africa, spring rainfall correlated with baseline and maximum (stress) corticosterone with levels being highest in driest years. Dominant and subordinate animals responded differently with subordinates having higher levels of corticosterone (Rubenstein, 2007). Although tropical birds generally show similar corticosterone responses to stress as do mid- and high-latitude species, future sampling in relation to seasonal and weather events such as ENSO may show differences not apparent at present (Martin and Rubenstein, 2008) and influenced by a spectrum of ecological factors that remain to be identified.

4.3. Conclusions

It was predicted that in an ENSO year (1998) when the land birds have high food resources there would be widespread suppression of the adrenocortical response to acute stress to favor maximum reproductive effort. Longer lived seabirds, especially those feeding inshore, would experience greatly reduced food resources during ENSO and were predicted to remain responsive to acute stressors thus decreasing reproductive effort when conditions were poor. Breeding would then be deferred until a time when food resources favored successful reproduction. Yet other seabirds, i.e. those feeding offshore, have adequate access to food and were predicted to show no change in responsiveness to acute stress in El Niño and La Niña years. In sea birds, predictions were generally borne out although there were some confounds such as molting and in general poor body condition in Galapagos Penguins, which was not explicitly considered in this study. In land birds, predictions were generally not borne out with differences between larger land birds and smaller species. Possible ecological factors explaining these differences were discussed and clearly further studies will be needed to tease apart other factors involved.

The significance of these differences is not clear at present without data on corticosteroid binding globulin (CBG, see Schoech et al., 2013) that would allow us to determine whether free (unbound and thought to be available to enter target cells) corticosterone was different in 1998 and 1999. What role CBG may have in regulating how much free corticosterone (i.e. not bound to CBG) is available to enter target cells and to bind to receptors should be a focus of further studies (Breuner and Orchinik, 2002; Schoech et al., 2013). In Black-legged Kittiwakes variation in baseline and maximum corticosterone and CBG varied from year to year and colony to colony but appeared to be driven by such environmental cues as local food supplies as might be predicted for long-lived seabirds (Shultz and Kitaysky (2008). In the Laysan Albatross, *Phoebastria immutabilis*, corticosterone levels rise during incubation fasts while body condition declines, but this may be accompanied by an increase in CBG to protect the reproductive attempt (Sprague, 2009), whereas in Tufted Puffins, *Fratercula cirrhata*, total and free baseline and maximum corticosterone (after capture stress) were higher prior to egg laying than during incubation. CBG levels were positively correlated with body condition when parents had chicks whereas free corticosterone baselines were negatively correlated (Williams et al., 2008). Otherwise the seabirds investigated in our study showed no differences across years. Note that in the northern hemisphere breeding Laysan Albatrosses, opposite trends in baseline corticosterone and free corticosterone were not necessarily related to foraging bout length (Sprague, 2009). Northern albatrosses apparently show different foraging patterns from those of the southern hemisphere and this could partially explain these differences. Other factors such as changes in corticosteroid receptor numbers, and metabolizing enzymes could also be important (Wingfield, 2013; Romero and Wingfield, 2015) but were beyond the scope of this investigation.

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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.2017.10.015.

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