Corticosterone levels predict survival probabilities of Galápagos marine iguanas during El Niño events

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Plasma levels of corticosterone are often used as a measure of ''stress'' in wild animal populations. However, we lack conclusive evidence that different stress levels reflect different survival probabilities between populations. Galápagos marine iguanas offer an ideal test case because island populations are affected differently by recurring El Niño famine events, and population-level survival can be quantified by counting iguanas locally. We surveyed corticosterone levels in six populations during the 1998 El Niño famine and the 1999 La Niña feast period. Iguanas had higher baseline and handling stress-induced corticosterone concentrations during famine than feast conditions. Corticosterone levels differed between islands and predicted survival through an El Niño period. However, among individuals, baseline corticosterone was only elevated when body condition dropped below a critical threshold. Thus, the population-level corticosterone response was variable but nevertheless predicted overall population health. Our results lend support to the use of corticosterone as a rapid quantitative predictor of survival in wild animal populations.

Glucocorticoids, a class of steroid hormones, are released in response to a wide variety of stressful stimuli and conditions, including inclement weather (e.g., refs. 1 and 2), and are believed to help an animal survive these conditions (3). However, conclusive evidence for a relationship between plasma glucocorticoid concentrations and survival is still missing for wild animal populations. It is important to establish this link because glucocorticoids are increasingly used as indicators of population health (4). Glucocorticoids are important regulators of carbohydrate, lipid, and protein metabolism (5) and thus would be predicted to play a role during periods of food shortage. Recent techniques allow baseline glucocorticoid sampling from a variety of wild, free-living species (e.g., refs. 4, 6, and 7), and several earlier studies indicated that poor body condition can be correlated with elevated glucocorticoid concentrations (reviewed in ref. 4). However, most studies do not have the opportunity to study the glucocorticoid response to stress in a wild population facing extreme environmental conditions. We studied Galápagos marine iguanas (*Amblyrhynchus cristatus*), which are severely impacted by El Niño-induced famine conditions. El Niño is a recurring global climate event whose main impact in the Eastern Pacific is a change in trade winds and the subsequent failure of nutrient-rich upwelling (8). Iguana populations can decline by as much as 90% during severe El Niños (9, 10).

During 1997 and 1998, one of the longest and most severe El Niños on record struck the Galápagos Islands. Water temperatures in the Galápagos Archipelago, normally between 18°C and 23°C, remained elevated up to 32°C for nearly 18 months (11). This led to a severe reduction in the algal forage of the marine iguanas and resulted in widespread starvation, as observed during previous El Niños (9, 10). Severe environmental conditions can cause widespread mortality in many taxa (e.g., ref. 12) and would be predicted to elicit a robust physiological stress response in the iguanas as they attempted to survive. Corticosterone, the species-typical glucocorticoid in reptiles (13), is one of the quintessential hormones involved in the stress response (3).

We captured marine iguanas from six islands in the Galápagos, took blood samples, and assessed body condition within 1 week before widespread nutrient upwelling ended the 1998 El Niño conditions. Many animals were in extremely poor condition, and carcasses were abundant. Surprisingly, however, some animals appeared to be coping adequately. We also sampled animals exactly 1 year after the El Niño event, during a ''normal'' year. This report compares corticosterone responses to the acute stress of capture, handling, and restraint in iguanas that were in good and poor condition, as well as to iguanas captured a year later when the algal forage had returned and surviving animals had recovered.

Materials and Methods

We visited six islands in the Galápagos Archipelago from May 6 to May 19, 1998 and from May 19 to June 9, 1999. We captured iguanas from Punta Espinosa on Fernandina (91° 27′ W, 0° 16′ S), Darwin Bay on Genovesa (89° 59 W, 0° 19′ N), Elizabeth Bay on Isabela (91° 26′ W, 0° 36′ S), Academy Bay on Santa Cruz (90° 17′ W, 0° 46′ S), and Seymour (90° 18′ W, 0° 24′ S) and Santa Fe Island (90° 2′ W, 0° 50′ S). We selected animals at random throughout the day and captured them by hand within 30 s of approach.

Immediately upon capture, we placed each iguana head-first into an opaque cloth bag and took a blood sample from the caudal vein on the underside of the tail. Blood was collected into heparinized microhematocrit tubes from a hypodermic needle or into 2-ml heparinized vacutainer tubes (Becton Dickinson). The initial blood sample was usually collected within 1–2 min of capture, and never longer than 3 min after capture. Glucocorticoids such as corticosterone generally do not start to increase until ≈3 min after the initiation of an acute stressful stimulus in most species (3, 4), although increased corticosterone concentrations may not be detectable in some reptile species for 10 min (14, 15). Galápagos iguanas are very tame, and there is no evidence (from corticosterone concentrations; unpublished data) that capture is perceived as stressful to nearby (uncaptured) animals, so all animals could be considered unstressed before capture. All initial samples were grouped together for statistical purposes. After collecting the initial sample, iguanas were maintained in the bags for a 30-min period of restraint, with subsequent blood samples taken at 15 and 30 min. In addition, nine iguanas on Santa Fe were maintained in the bags for an additional 30 min, with a fourth blood sample collected at 60 min postcapture. At the end of the restraint period, iguanas were weighed, measured for their snout-to-vent length, sexed (for methods see ref. 10), and released.

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The use of handling and restraint as described above provides a standardized stressful stimulus that is the same for all animals. This standardized procedure allows the comparison of stress responses across populations (2). All procedures were conducted in accordance with guidelines from the American Society of Ichthyologists and Herpetologists and approved by the Tufts University Institutional Animal Care and Use Committee.

Blood samples were maintained on ice for up to 12 h and then centrifuged at $\approx 400 \times g$ for 6 min. Plasma was removed, frozen at -20°C, and shipped to Tufts University where corticosterone was analyzed by RIA using a previously described method (16). Briefly, plasma was equilibrated with a small amount of tritiated corticosterone to measure subsequent recovery, and then steroids were extracted with redistilled dichloromethane. Each sample was then assayed in duplicate, with intraassay and interassay variations of 6 and 12%, respectively.

To compare how well animals were coping with El Niño conditions, a body condition index was computed as (body mass/snout-vent length^3) $\times 10^6$. Although this condition index is crude, it adequately describes the physical condition of an iguana (9, 10) and has the additional advantage that animals of different island populations can be compared. Condition indices are indeed well comparable between islands as iguanas on all islands die at indices of less than $\approx$25 and are maximally fat at indices of 60. The entire range of indices is reached on each island.

Linear regression was then used to determine whether corticosterone levels were correlated with body condition. An inflection point in the relationship was determined through iterative testing of different inflection points, with the accepted point providing the highest R^2. Multiple factor analyses of variance (ANOVA) were used to test for sex, island, and year differences in corticosterone levels, body condition, and hematocrit. The inability to catch the same animals in consecutive years prevented analyzing these factors in a repeated measures ANOVA. Repeated measures ANOVAs were used to test increases in corticosterone levels over time in response to capture, handling, and restraint. A relationship between physiological state and demographic measures was tested with linear regression. To characterize physiological state, we used corticosterone levels after 15 min of handling stress because (*i*) corticosterone generally did not increase further after 15 min handling and (*ii*) the 15-min handling represents the least invasive (shortest) way of measuring physiological responses for future monitoring efforts. We included corticosterone data of all individuals for each island in the analysis.

To characterize marine iguana demography, we used estimates of population size from direct counts or mark recapture estimates. In short, to count iguanas we delineated a stretch of coastline at each site and counted all iguanas visible in this area. Counts were conducted shortly after low tide, when the maximum number of iguanas gather on exposed rocks to bask. Simple counts underestimated the total population size of iguanas by an average of 18 $\pm$ 3% compared with mark-recapture studies, but the repeatability of counts for postforaging, basking iguanas was very high ($\pm$6%; M.W., unpublished data averages from two islands and 10 field seasons). For the islands of Santa Fe and Genovesa, our long-term study sites, additional maximum likelihood estimates of survival were available based on modified Jolly–Seber models (10).

Results

There were no sex differences in either initial corticosterone levels ($F_{1,208} = 0.35, P = 0.56$), corticosterone levels after 15 min of restraint ($F_{1,191} = 0.06, P = 0.81$), corticosterone levels after 30 min of restraint ($F_{1,204} = 0.31, P = 0.58$), or body condition ($F_{1,210} = 0.02, P = 0.89$). Consequently, sex was ignored for the remaining analyses, and the multiple factor ANOVAs were recomputed after removing sex from the statistical models.

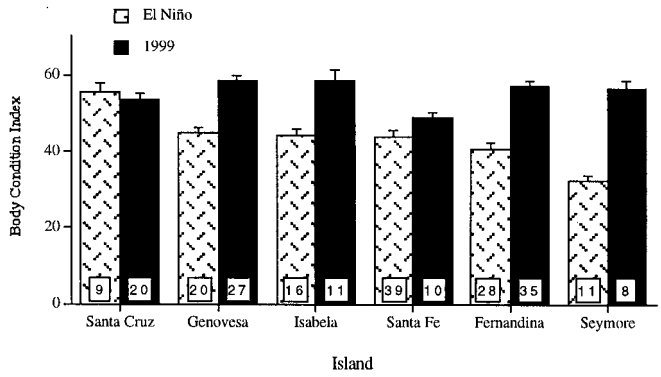

Fig. 1. Body conditions of iguanas captured on six different islands during 1999 and during the El Niño of 1998. Each bar represents the mean $\pm$ SE for each year on each island for the sample sizes indicated. The body condition index = (body mass/snout-vent length^3) $\times 10^6$.

Nine iguanas from Santa Fe were captured and bled over a 60-min period of restraint (data not shown). There was a tendency for corticosterone levels to increase after 15 min, but no further change in corticosterone was detected after 30 or 60 min ($F_{3,24} = 2.43, P = 0.090$).

El Niño conditions affected marine iguana body condition differently on each island (Fig. 1). During the El Niño, body condition indices ranged from extremely low at Seymour and Fernandina to relatively high on Santa Cruz (overall island effect: $F_{5,222} = 5.27, P < 0.0001$). During the El Niño, body conditions were lower on five of the six islands, with only iguanas on Santa Cruz having equivalent body conditions during both years (overall year effect: $F_{1,222} = 98.13, P < 0.0001$). This meant that not only were there normal island differences in body condition, but iguanas on different islands reacted differently to El Niño conditions (interaction between island and year effects: $F_{5,222} = 8.15, P < 0.0001$).

Corticosterone increased significantly over the period of restraint on all islands (Fig. 2) in both the El Niño year of 1998 (overall effect of sampling time: $F_{2,228} = 28.82, P < 0.0001$) and in 1999 ($F_{2,210} = 63.16, P < 0.0001$). However, the corticosterone response varied by island. In 1999, iguana populations on different islands varied in both overall corticosterone levels (overall island effect: $F_{5,87} = 8.86, P < 0.0001$) and in how corticosterone responded to acute stress with a change from baseline (interaction between island and sampling time: $F_{10,210} = 5.09, P < 0.001$). During the El Niño, overall corticosterone concentrations also varied by island (overall island effect: $F_{5,108} = 3.45, P < 0.007$). However, stress response did not differ by island (interaction between island and sampling time: $F_{10,228} = 1.56, P = 0.12$).

Baseline and stress-induced corticosterone levels were significantly higher during El Niño in iguanas on five of the six islands (Fig. 2). Only on Santa Cruz were corticosterone levels lower during the El Niño. Overall, corticosterone showed a significant difference between years (overall year effect) in the initial sample ($F_{1,221} = 7.71, P < 0.006$), the 15-min sample ($F_{1,203} = 6.68, P < 0.01$), and the 30-min sample ($F_{1,216} = 4.73, P < 0.03$). Furthermore, the El Niño conditions affected these corticosterone levels differently depending on the island (interaction between island and year effects: $F_{5,221} = 3.41, P < 0.006$; $F_{5,203} = 5.82, P < 0.0001$; and $F_{5,216} = 8.71, P < 0.0001$ for initial, 15-min, and 30-min samples, respectively).

Much of the differences in corticosterone responses between 1999 and the El Niño of 1998 (Fig. 2) might be explained by body condition. On the three islands where initial corticosterone levels were elevated during El Niño (Fernandina, Seymour, and

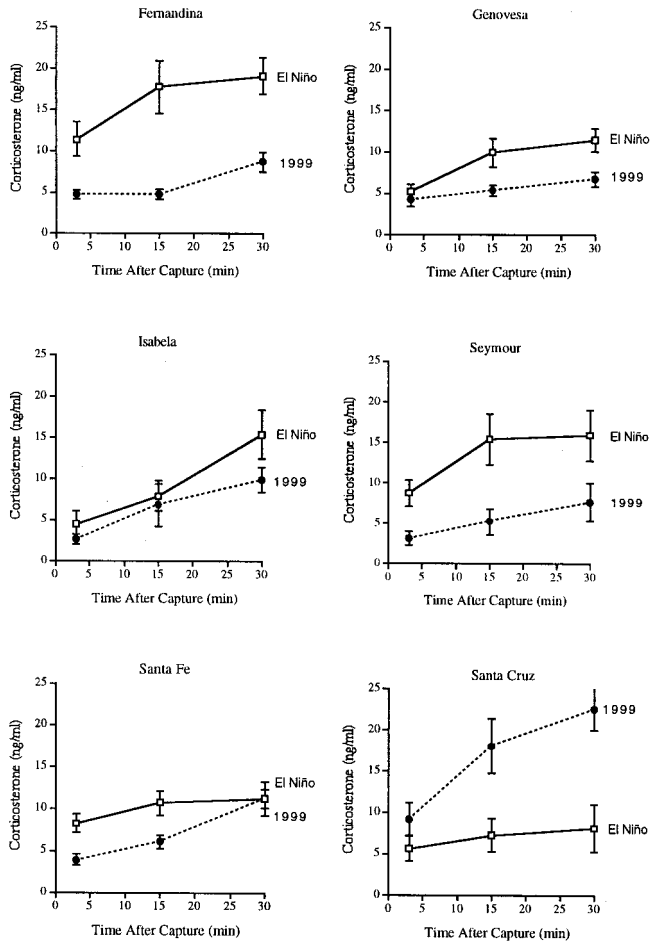

Fig. 2. Corticosterone responses to the stress of capture and handling in marine iguanas captured on six different islands during 1999 and during the El Niño of 1998. Each point represents the mean ± SE. Sample sizes for El Niño and 1999 are, respectively: n = 28 and 35 on Fernandina; n = 11 and 8 on Seymour; n = 18 and 26 on Genovesa; n = 36 and 10 on Santa Fe; n = 13 and 11 on Isabela; and n = 20 and 8 on Santa Cruz.

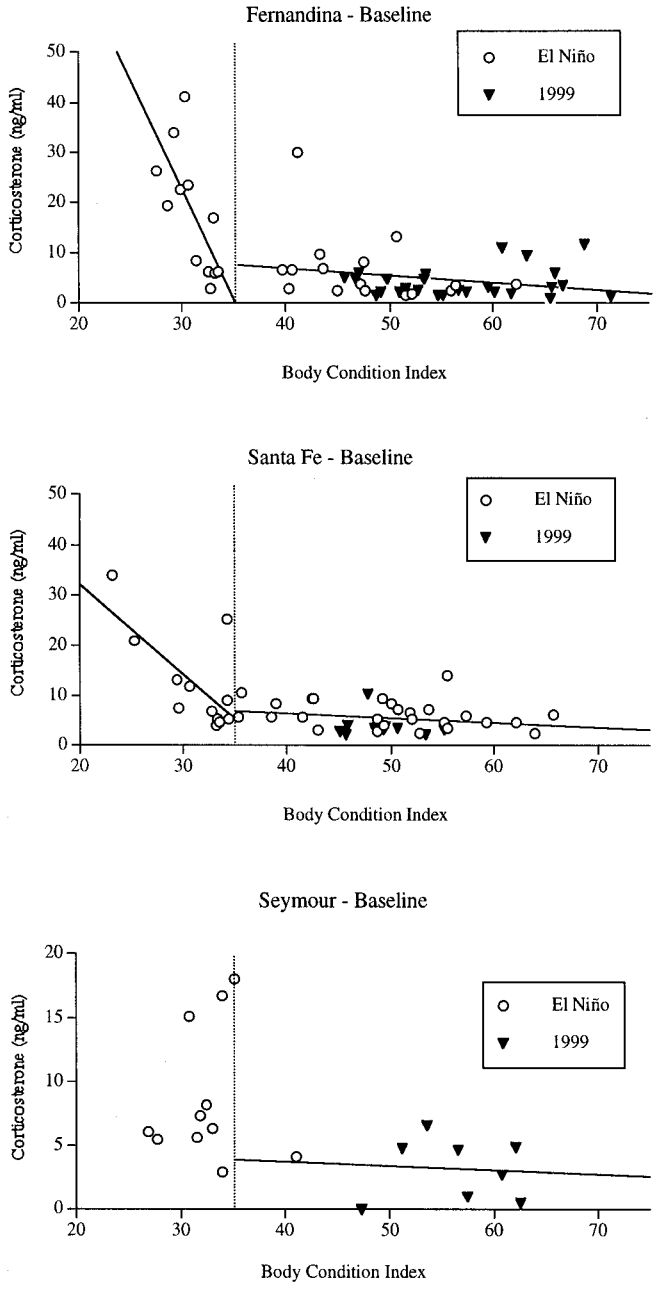

Fig. 3. Baseline corticosterone levels depend on the body condition of iguanas, as determined by (body mass/snout-vent length^3) × 10^6. At body conditions above ≈35, corticosterone is apparently low in all individuals. When body condition drops below 35, corticosterone levels increase dramatically. Vertical dashed line represents the proposed threshold in body condition. Lines represent the linear regression for body conditions above and below 35.

Santa Fe), iguanas also had the lowest body conditions. When comparing body condition to initial corticosterone levels (Fig. 3), there appeared to be a shift in circulating corticosterone levels when the body condition index fell below ≈35. When the data were divided between animals with body condition indices greater than 35 and those with indices less than 35, an index of 35 appeared to be a threshold for changing physiology. On all three islands, corticosterone had low variance and was not correlated with body condition when the index was greater than 35 (F = 1.02, P = 0.32, r^2 = 0.02, n = 51 for Fernandina; F = 1.66, P = 0.21, r^2 = 0.047, n = 36 for Santa Fe; F = 0.08, P = 0.79, r^2 = 0.01, n = 9 for Seymour), yet corticosterone was highly correlated with body condition when the index was less than 35 on two of the three islands (F = 9.56, $P < 0.02$, r^2 = 0.49, n = 12 for Fernandina; F = 9.04, $P < 0.02$, r^2 = 0.47, n = 12 for Santa Fe). Although there was no significant relationship between corticosterone and body conditions that were less than 35 on Seymour, the evidence of a threshold was still apparent (Fig. 3). We did not capture iguanas with body conditions less than 35 during the 1998 El Niño on the other three islands (Genovesa, Isabela, and Santa Cruz). Examples of iguanas at various body conditions are presented in Fig. 4.

Stress-induced (15-min) corticosterone levels were used to predict the survival of marine iguanas in island populations that were differently affected by El Niño conditions (Fig. 5). Whereas iguanas on Seymour and Fernandina were suffering heavy mortality and high corticosterone levels, animals on Santa Cruz and Isabela were only mildly affected by El Niño and barely reacted to handling stress. The iguanas on Genovesa and Santa Fe islands showed intermediate levels of both traits (linear regression: $F_{1,4}$ = 27.9, r^2 = 0.87, P = 0.006). Adding body condition to this statistical model improved the r^2 to 0.94 (P = 0.07 for body condition); corticosterone was better than body condition at predicting survival (linear regression for body

Fig. 4. Example of iguanas with a body condition near 60 in 1999 (*Upper*) and a body condition of ≈30 during El Niño (*Lower*).

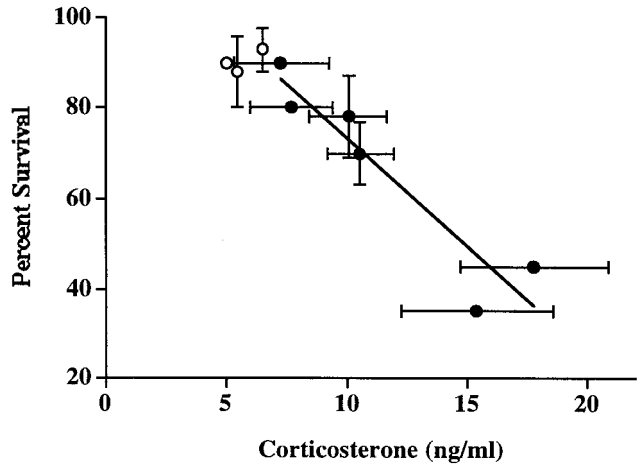

Fig. 5. Corticosterone levels measured after 15 min of handling stress predict the survival of Galápagos marine iguanas. Data show mean ± SE of local survival as determined by counts (circles without vertical error bar) or survival analysis (circles with vertical bars, for Santa Fe and Genovesa island only). Horizontal bars indicate SE of corticosterone measurements. ●, data for the El Niño period 1998 to 1999; ○, data for the subsequent La Niña period (1999 to 2000; islands Genovesa, Santa Fe, and Seymour only). The line represents the linear regression for the El Niño period only.

condition alone: $F_{1,4} = 15.75$, $r^2 = 0.80$, $P = 0.02$). Baseline corticosterone did not predict survival ($F_{1,4} = 7.65$, $P = 0.051$).

Discussion

The increase in corticosterone 15 min after capture and restraint is consistent with several other studies in reptiles (14, 17, 18). Corticosterone levels have been reported to be low in a variety of reptilian species when samples are collected within a few minutes of capture (reviewed in ref. 15), and restraint in a cloth bag has been shown to increase corticosterone concentrations, although stress-induced samples are often not taken for several hours (15, 19). The acute corticosterone response apparently plateaued between 15 and 30 min, as there was no further increase up to 60 min. This is a short time frame compared with data from many other reptile species. One species, however, showed a bimodal response with concentrations in some male tree lizards (*U. ornatus*) becoming elevated within 10–15 min and in others becoming elevated several hours to days after exposure to territorial aggression (18, 20).

It is likely that capture, handling, and restraint were potent stressors for these iguanas. Handling is never experienced by these animals, and in juvenile alligators handling and restraint produce extensive physiological changes (21). In addition, even though several studies have indicated that corticosterone responses in reptiles can change dramatically in different seasons (reviewed in ref. 19), all iguanas in this study were captured at the same time of year. Consequently, the island and year differences reported here likely reflect physiological changes rather than differences in the perceived stressfulness of the stimuli.

The El Niño conditions of 1998 clearly had a profound effect on corticosterone physiology on five of the six islands. Overall corticosterone levels were higher for all animals on these islands during El Niño, but as body condition worsened, corticosterone levels increased disproportionately (Fig. 3). Furthermore, poor-condition animals had visible muscle wastage (Fig. 4; see also ref. 22). Because high corticosterone levels are known to promote protein catabolism (5), these data suggest that corticosterone may have been helping mobilize protein reserves for survival. Even though most evidence suggests that corticosterone release is blunted, rather than elevated, during a fast (5), these iguanas may be described more accurately as enduring starvation conditions, which may require a robust corticosterone response. Corticosterone is elevated during starvation in many species (e.g., refs. 23–25), but it is currently unknown how long fasting conditions must continue in marine iguanas before it becomes starvation.

Importantly, *all* animals we captured in this study (even those in better condition) had little, if anything, to eat. Marine iguanas rarely, if ever, alter their feeding habits (26, 27), and their preferred algal food source was essentially absent in the intertidal during the El Niño year only (personal observations), similar to the 1982/1983 El Niño (9). And yet, only animals in poor condition with muscle wastage had elevated corticosterone levels. By itself, an enforced fast [even one lasting nearly 18 months (11)] appeared insufficient to maximally stimulate corticosterone release. One recent hypothesis on glucocorticoid function under severe environmental conditions is a reorientation of behavior to promote escape to more benign locales (4) through an increase in locomotor activity [in red-eared slider turtles (14) and in white-crowned sparrows (28)]. Marine iguanas cannot emigrate because their swimming stamina is insufficient to actively reach neighboring islands (29). Even if they could emigrate, El Niño conditions exist throughout the Archipelago, making this strategy ineffective. However, on an evolutionary time scale, there is a low level of gene flow, indicating that eventually animals drift with ocean currents from island to island

(30). Together, this suggests that in this species, corticosterone effects are primarily physiological rather than behavioral.

Surprisingly, for animals in better condition (body condition index > 35), body condition was indistinguishable from animals captured during 1999 or other non-El Niño years (9, 10), and their corticosterone levels are within the range of those reported for other free-living reptiles (15). A body condition index of 35 seems to be a consistent threshold for switching from normal to starvation corticosterone physiology, despite differences in the range of body sizes on the three worst-hit islands (10). Because iguanas in this poor condition are unlikely to survive, the surge in corticosterone may serve as a last-ditch effort to mobilize energy. It would seem, therefore, that animals would attempt to remain above an index of 35 at all costs, and this apparently occurs. Galápagos marine iguanas can shrink their body length during severe El Niños (31), presumably by absorbing bone from their axial skeleton. Because all glucocorticoids, including corticosterone, can have profound deleterious effects when present over long periods of time (3, 32), perhaps shrinking body length is a way to decrease the total amount of tissue that must be sustained and thereby delay an increase in corticosterone. As the body condition index used here is a rough approximation of actual condition, determining what parameters the iguanas may use to regulate corticosterone release (such as thresholds in protein levels or fat stores, an increase in plasma ketones, etc.) needs to be tested.

Perhaps not surprisingly, however, iguanas from different islands reacted differently to the El Niño. Part of these differences was environmental, as El Niño's effect on the algal forage was not as severe on Genovesa as it was on the other islands (R. Scheibling and M.W., unpublished observations). Consequently, the iguanas on Genovesa tended to be in better condition. The iguana populations on Isabela and Fernandina are very close, however, and yet they differed in body conditions. The marine iguana population on Isabela almost entirely consists of very large adults, as most small iguanas are killed by introduced predators (33). Thus, population density is predicted to be below carrying capacity; consequently, the large iguanas do not suffer from severe food shortage as most other (food-limited) populations do during El Niños (9, 10). The surprising finding that corticosterone levels were *lower* during the El Niño year on Santa Cruz can be explained by the microgeography of the sampling site, Academy Bay. This shallow bay receives substantial freshwater input via subterraneous lava fissures. During El Niño years, tropical rainfalls wash out large amounts of nutrients into the bay, presumably even from the highlands of Santa Cruz. Thus, algae growth and standing biomass can be enhanced inside the bay because of enhanced terrestrial nutrient input during El Niño events (R. Bustamante, personal communication).

Finally, individual differences in the regulation of corticosterone, especially in the ability to forestall deleterious situations when the body condition becomes too low, could be a substrate for natural selection. Not only could corticosterone play a role in the immediate survival of the animal by helping to mobilize fat and protein reserves, but, as in other taxa, elevated corticosterone levels in reptiles have been shown to inhibit gonadal function (17, 19, 34, 35) and reproductive behaviors (36, 37), thereby disrupting reproduction. Severe El Niño events have been shown to exert profound selective pressure on morphological traits (38), and these data suggest that El Niño events may exert selective pressure on physiological traits (i.e., corticosterone release and action) as well.

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