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# Corticosterone stress response shows long-term repeatability and links to personality in free-living Nazca boobies

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ABSTRACT

The concept of "coping styles", or consistently different responses to stressors, is of broad interest in behavioral ecology and biomedicine. Two critical predictions of this concept are individual consistency of neurophysiological and behavioral responses (relative to population variability) and a negative relationship between aggression/proactivity and hypothalamic–pituitary–adrenal axis reactivity. Recent studies failed to provide strong support for these predictions, especially outside of strictly controlled conditions, and long-term measures to test the first prediction are rare. Here, we demonstrate individual repeatability across 2–3 years of maximum circulating corticosterone concentration [CORT] and area under the [CORT] response curve ($AUC_I$) during a standard capture-restraint test in wild, free-living adult Nazca boobies (*Sula granti*). We also show that the stress response predicts the personality traits aggression and anxiety in these birds (measured in the wild); however, the strength of these results was weak. Maximum [CORT] and $AUC_I$ showed higher repeatability between years than baseline [CORT]. After controlling breeding status, sex, mass, date sampled, and their interactions, baseline [CORT] was most closely related to personality traits, followed by $AUC_I$, and then maximum [CORT]. The direction of these relationships depended on whether the testing context was social or non-social. [CORT] parameters had little to no relationship with cross-context plasticity in personality traits. Our results generally affirm two critical predictions of coping styles, but match the emerging trend that these relationships are weak in the wild, and may depend on testing context.



## 1. Introduction

The recognition of temporally consistent differences between individuals in behavior and physiology when confronted with a stressor (i.e., "coping style"; Koolhaas et al., 1999) is of broad scientific interest for biomedicine, livestock agriculture, behavioral ecology, and evolution (e.g., Carere et al., 2010; Gherardi et al., 2012; Wolf and Weissing, 2012). The traditional coping style model predicts correlated endocrine and behavioral responses that produce two distinct alternative patterns of response to stressors: proactive (high aggression, low hypothalamic–pituitary–adrenal (HPA) axis response) and reactive (low aggression, high HPA axis response; Koolhaas et al., 1999). Tests of this model are most often conducted by artificial selection in laboratory settings and typically support the traditional model (Carere et al., 2010; but see Thomson et al., 2011). Recent studies in natural settings have often not found the predicted bimodal distribution of coping styles, instead showing a continuum of proactive-reactive phenotypes (see Réale et al., 2007) or little relationship between neuroendocrine and behavioral responses to stressors (Ferrari et al., 2013; but see Baugh et al., 2013). In an update to the traditional model, Koolhaas et al. (2010) proposed a "two-tier" model of coping styles, in which the quality of response to a stressor ("proactive" or "reactive") and quantity of response to a stressor ("stress reactivity" including the HPA axis) operate on two independent axes. Both the traditional and two-tiered models of coping style predict consistent between-individual differences in behavioral and endocrine responses to stressors. However, the traditional model predicts a high correlation between these responses (proactive individuals exhibit low HPA axis response and reactive individuals exhibiting high HPA axis response). In contrast, the two-tiered predicts a more complicated relationship between behavior and hormones, in which behavioral and hormonal response to stressors may not be correlated (Koolhaas et al., 2010).

Tests of the endocrine aspect of the prediction common to both models – that individual HPA axis responses are consistent across some period of time – generally indicate high repeatability (effectively, the ratio of variance explained by between-individual effects and population variance) for stress-induced glucocorticoids,

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but lower repeatability of baseline glucocorticoids (Romero and Reed, 2008). Such studies are usually over a short time span on a semi-free-ranging (Kralj-Fiser et al., 2007) or laboratory population (Ouyang et al., 2011; Romero and Reed, 2008; Williams, 2008). Similar studies on organisms in the wild are rare (Garamszegi et al., 2012) and suggest that consistency of the HPA axis response depends on the timespan across which repeatability is calculated and on the phylogenetic group (e.g., amphibians exhibit high repeatability, Narayan et al., 2013a,b; Rensel and Schoech, 2011; birds show high variability in repeatability dependent on season, time of day, and species, Angelier et al., 2010; Ouyang et al., 2011; Rensel and Schoech, 2011; Romero and Reed, 2008). One might expect low repeatability of glucocorticoid measurements to be the norm in the wild because circulating baseline and stress-induced glucocorticoid concentrations vary in response to recent environmental conditions, which are highly variable in the wild compared to the lab (Kitaysky et al., 2007; Romero and Reed, 2008; but see Angelier et al., 2010 and Ferrari et al., 2013).

The second prediction of the traditional coping style model – that the endocrine stress-response is linked to personality traits – was generally supported by initial studies. Evidence from captive lines selected for behavior or physiological response to stressors provided the strongest support: proactive (bold) individuals displayed a lower glucocorticoid stress response than reactive (shy) individuals (Carere et al., 2010; Cockrem, 2007) and fewer baseline circulating glucocorticoids (Koolhaas et al., 1999). However, several recent studies of captives found no covariation between either stress-induced or baseline glucocorticoid concentration and personality (e.g., European starlings (*Sturnus vulgaris*); Apfelbeck and Raess, 2008), or gave mixed results within one species (e.g., rainbow trout (*Oncorhynchus mykiss*); Pottinger and Carrick, 2001; Schjolden et al., 2005; Thomson et al., 2011). Recent studies of wild animals have generally supported the modified "two-tier" model of independent behavioral and physiological response axes (Garamszegi et al., 2012; Ferrari et al., 2013; Koolhaas et al., 2010), while others have found support for the traditional coping style model (Baugh et al., 2013).

This study tests the two predictions of the traditional coping style model in a wild, free-ranging bird, the Nazca booby (*Sula granti*): (1) the corticosteroid stress response is temporally repeatable (predicted by both the traditional and modified models), and (2) the corticosteroid stress response can predict personality traits, specifically that lower corticosteroid activity and reactivity is related to proactive personality traits (aggression), and high activity and reactivity is related to shy/anxiety-related personality traits (displacement behaviors; predicted by the traditional model but not the two-tier model). Personality traits that we evaluate are repeatable in the short- and long-term (across years), show high between individual variance, and were performed in all testing situations, making them informative for between-individual comparisons (Grace and Anderson, 2014). We evaluate and compare the ability of three parameters of the corticosteroid stress response to predict personality in a wild population of Nazca boobies assayed in the field.

## 2. Methods

### 2.1. Ethical statement

All research reported here was permitted under the regulations of the Wake Forest University Institutional Animal Care and Use Committee (Protocol #A11-051) and the Galápagos National Park Service (PC-15-07), and adheres to NIH standards for animal use in research and the Ornithological Council's "Guidelines to the Use of Wild Birds in Research". All behavioral testing was done at the nest sites of incubating birds to minimize disturbance. At our long-term study site on Isla Española, Galápagos, Nazca boobies tolerate the proximity of humans well (Apanius et al., 2008). During incubation, birds typically do not respond to humans at a distance of two m or greater from the nest site. Females were not tested for personality during their first incubation bout (immediately following egg-laying) because the risk of abandonment due to disturbance is higher at this time. All blood sampling was conducted on non-breeders, or during late chick-rearing, when chicks are able to self-thermoregulate and are frequently left unattended by parents. Blood sampling was conducted between 0230 h and 0600 h when air temperature is lowest to prevent thermal stress in birds, and birds were held in commodious cages with grates that did not restrict airflow. Sampling was conducted at 15–20 m from the colony, researchers spoke in whispers and worked with minimal light (focused away from the colony) to minimize disturbance to other birds. Birds were returned to their roost site following blood sampling.

### 2.2. Study animals

The Nazca booby, a long-lived, pelagic seabird (Anderson and Apanius, 2003), has been the subject of recent work on personality (Grace and Anderson, 2014). Adults are identified by permanent numbered metal leg bands. Nazca boobies in our population can live at least 26 years (unpublished data) and, like most pelagic seabirds, spend approximately half of the year at sea, returning to land during the breeding season (October–June). Thus, all blood sampling and personality tests occurred during the breeding season; breeding state (non-breeder, breeder) was controlled statistically (see Section 2.6.2, and Section 2.6.5). Nazca boobies exhibit biparental incubation and care of their altricial offspring (Anderson and Ricklefs, 1992; Apanius et al., 2008), and are socially and genetically monogamous within a breeding season (Anderson and Boag, 2006; Maness and Anderson, 2007).

### 2.3. Personality tests

Data on personality of birds in this study came from earlier work during the breeding seasons between November 2008 and January 2012 (Grace and Anderson, 2014); those methods are described briefly here. Behaviors were counted for each of 479 incubating adults during four tests performed at the nest site: a nest intruder, two novel objects (a Red Bull™ can attached to a 3 m long pole placed on the ground at the nest site, and a plastic crate covered with a black cloth curtain), and a simulated social stimulus (mirror). The second novel object test immediately preceded the social stimulus, possibly violating the statistical assumption of independence. Thus, these tests were not combined in analyses. We focused on incubating individuals because they remain in place at their nest sites throughout tests and were relatively standardized in other respects (reproductive and behavioral history immediately preceding testing). Females were not tested for personality during their first incubation bout for ethical reasons (see Section 2.1) and because up-regulated hormones and neuropeptides associated with egg laying (Li et al., 1996) could influence behavior. Tests were performed between 0715–1200 h and 1330–1700 h to avoid the hot mid-day and the socially active dawn and dusk periods.

The covariation between personality traits within and across contexts was evaluated via model selection based on Akaike's Information Criterion values corrected for small sample size (AICc, see Section 2.6.2; Burnham et al., 2011), generated by structural equation models. This suggested a personality syndrome that was independent of context and that involved three repeatable traits: "Gardening," "Shaking", and "Aggression" (Table 1). These behavioral traits were expressed in all contexts, normally