**Supplementary Material for Early-life maltreatment predicts adult stress response in a long-lived wild bird**

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**SUPPLEMENTARY MATERIAL 1: METHODS**

*Study System*

Nazca boobies tolerate the proximity of humans at our long-term study site on Isla Española, Galápagos Islands (1°23.4' S, 89°37.2'W) [1], permitting easy observation and capture. Adults are identified by permanent numbered metal leg bands. Nazca boobies spend their juvenile years at sea, returning to the breeding colony on average 4-5 years after fledging [2], with a life expectancy of 14 yrs [3]. Like most pelagic seabirds, Nazca boobies spend approximately half of the year at sea and attached very loosely to the island breeding colony, but return to land during the breeding season (Oct.-June). Thus, all blood sampling occurred during the breeding season, but only current non-breeders were used. Nazca boobies exhibit bi-parental incubation and postnatal care of their altricial offspring [1,4] and are socially and genetically monogamous within a breeding attempt [5,6]. All nests within the colony were monitored and parents identified. Thus, breeders and non-breeders were easy to distinguish.

All birds used in this study were monitored as nestlings as part of our ongoing demographic study of this population. Age at reaching 1% down was measured in days since hatching and is the point at which only 1% of plumage remains as down. This is a proxy for duration of growth and was recorded for all birds. Birds attain full juvenile plumage within 3 d. of reaching the 1% down state [2]. A longer nestling growth period is associated with lower mass throughout the nestling period, indicating chronic food stress [1]. Characteristics of NAV interactions are known for all nestlings in this study from systematic monitoring during three breeding seasons (2000-2001, *n* = 183; 2001-2002, *n =* 88; 2002-2003, *n* = 200) [7]. Nests within a subsection of the colony were patrolled by one or two observers from 1300 h. to 1700 h., when the majority of NAV interactions occur [8], from Jan.-Mar., when nestlings are in the age range of NAV vulnerability (30-80 d. post-hatching; Anderson et al. 2004). NAVs were easily identified by uniquely numbered plastic leg bands. Their distinctive behavior when approaching nestlings coupled with the open terrain of the colony allowed easy observation and meant that nestling histories as victims of NAVs were essentially comprehensive [7].

Our final sample size was primarily limited by juvenile mortality (approximately 40% - 60% for the cohorts in this study; Maness and Anderson 2013), because natal philopatry is exceptionally high in this population (>98%; Huyvaert and Anderson 2004). Biological replicates were individual birds whose experience with NAVs as nestlings ranged along a continuum. For corticosterone assays, technical replicates were aliquots of serum samples.

### *Standardized capture-restraint tests*

In March of 2009 we conducted standardized capture-restraint tests [10] on birds with known NAV victimization and growth rate histories, 6-8 years after these nestling experiences (median = 6 years). We sampled blood between 0250 h and 0535 h, when circulating blood [CORT] is least affected by external stimuli [11], and when air temperature is minimized to prevent thermal stress in birds [10]. The first blood sample of 1 ml (baseline) from each bird was taken within 3 min of initial disturbance; then the bird was placed in a commodious cage (60 x 30 x 50 cm) and three successive samples of 400 μl were taken at 10, 25, and 40 min after caging (all within 3 min of approach to the cage). All blood samples were obtained by brachial and medial metatarsal venipuncture with unheparanized syringes and capillary tubes.

Because sampling was done at night, headlamps were used to identify birds and complete sampling. Two people were used for every blood sampling event (a blood sampler and a bird holder). These two people approached the colony with headlamps oriented on the ground, and set-up a sampling station off of the colony. During set-up, headlamps were oriented away from the colony and speech was conducted only in whispers. Birds typically awoke briefly on approach to the colony, and settled again within 5 minutes. Previous work in this colony determined that sleep is frequently punctuated by periods of arousal, preening, and other activities [12]; thus, this short arousal period was not unusual for this species. After setting up the sampling station, the sampler and handler turned off their headlamps and sat in the dark, without speaking for approximately five minutes. Initial disturbance was determined to be the moment that the handler turned their headlamp into the colony and moved toward it, to catch a bird. Birds had been identified at least 3 days earlier by an annual band re-sight, and had been marked with a spray paint mark on their chest to indicate they needed to be sampled. Our colony monitors - who were near and/or in the colony for most of every day during the breeding season - scanned the colony at dusk to locate where these birds were roosting and directed the handler during the night time blood sampling. Between blood samples, headlamps were turned off, and birds were placed in commodious cages approximately five meters from the sampling station.

*Corticosterone assay*

Serum was separated by centrifugation at 6000 rpm for 5 min within 4 h of collection and preserved in 95% ethanol [13] at ambient temperature. Total (bound and unbound) corticosterone concentration ([CORT]) was measured by quantitative competitive enzyme immunoassay (Enzo Life Sciences/Assay Designs, Cat. No. ADI-901-097), previously validated with Nazca booby serum for accuracy, precision, cross reactivity, and parallelism [14]. Each plate contained standard curves and samples were run in duplicate. CORT was extracted by dichloromethane double extraction. These samples were part of a larger sampling effort, all of which were analyzed simultaneously. Values reported below are calculated from this larger dataset. Efficiency of extraction averaged 82.5%, using the supplied CORT standard diluted to 1600 pg/ml in stripped chicken serum and ethanol (*X* ± SD = 82.5% ± 19.8, *n* = 7) as reported in Grace, et al. (2011). Immunoassay detection limit was provided by the manufacturer and was 26.99 pg/ml. The intra-assay coefficient of variation was 4.4% and was calculated by dividing the standard deviation by the mean of absorbance for each sample run in duplicate (862 samples), averaging these across each plate (25 plates), then averaging these across all plates. The inter-assay coefficient of variation was 6.2% and was calculated using the standard curves run on each plate and the method described above (range = 5.9% - 6.6% for 32 pg/ml, 160 pg/ml, 800 pg/ml, 4000 pg/ml, and 2000 pg/ml; 25 plates). Measurements reported in this study were calculated from means of absorbance and samples were re-assayed if duplicates differed by ≥ 0.04 optical density. Because the primary antibody did not cross-react significantly with other circulating steroids, we call measures “CORT” measurements.

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