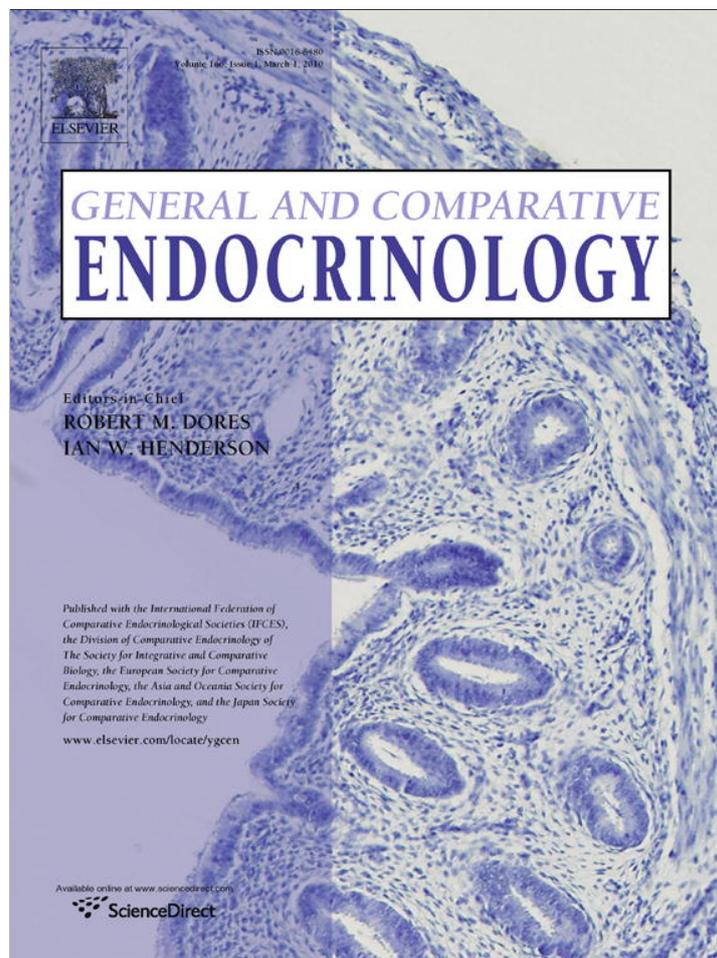


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Stress response of brown pelican nestlings to ectoparasite infestation

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ABSTRACT

Measurement of corticosterone has become a useful tool for assessing the response of individuals to ecological stressors of interest. Enhanced corticosterone levels can promote survival of stressful events; however, in situations where a stressor persists and corticosterone levels remain elevated, the adrenocortical response can be detrimental. A potential ecological stressor for wild birds is parasitism by ectoparasites. We studied the stress response of 11–23-day-old brown pelican (*Pelecanus occidentalis*) nestlings by measuring plasma corticosterone levels in relation to the presence of the soft tick *Carios capensis* at two colonies in South Carolina in 2005. We expected to see higher baseline and stress-induced levels of corticosterone for parasitized chicks compared to those nestlings with no ticks. Although nestlings mounted a response to capture stress, tick category was not associated with corticosterone levels at either colony. Our results appear to contrast those of previous studies and indicate that the adrenocortical response of the host is likely dependent on the type of ectoparasite and the degree of infestation.

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1. Introduction

Exposure to ecological stressors can trigger a stress response in affected individuals that stimulates energy mobilization and behavior modifications to facilitate survival. The stress response is activated via the hypothalamic–pituitary–adrenal (HPA) axis and ultimately results in the synthesis and secretion of glucocorticoids, which in birds is the steroid hormone corticosterone. Following an initial stress event, increased amounts of corticosterone are secreted and, as a result, energy is shifted away from homeostatic maintenance activities to those which promote immediate survival and an eventual return to the pre-stress state. Despite the benefits gained from a short-term increase in corticosterone, long-term elevations of corticosterone can be harmful in birds and may result in suppression of the immune system, catabolism of muscle (Wingfield et al., 1997; Sapolsky, 2002) and, for nestlings, impaired cognitive development (Kitaysky et al., 2003) and reduced growth (Hull et al., 2007; Wada and Breuner, 2008).

From an ecophysiological perspective, the stress response of pre-fledged birds presents an interesting system in which to study baseline and stress-induced levels of corticosterone and the trade-off between the benefits and potential costs of a functional stress response. Nestlings of species across the altricial-precocial spec-

trum are capable of mounting a functional stress response early in post-hatch development to cope with acute stressors (Nunez-de la Mora et al., 1996; Sockman and Schwabl, 2001; Pravosudov and Kitaysky, 2006). For example, begging rates of altricial nestlings may increase (Loiseau et al., 2008) in times of food shortage with the release of additional corticosterone. If stressors persist, nestlings may become vulnerable to the costs of chronically elevated corticosterone unless the HPA axis is down-regulated (Rich and Romero, 2005), resulting in lower baseline corticosterone levels and a suppressed response to acute stressors. Alternatively, nestlings of some altricial and semi-altricial species appear to delay maturity of the stress response until nestlings are capable of physically coping with stressors (Sims and Holberton, 2000; Walker et al., 2005) and thereby avoid the potential costs of high corticosterone levels. Thus, there appear to be at least two general developmental paths for the stress response of nestlings on the altricial end of the developmental spectrum. Measurement of corticosterone in free-ranging nestlings can enhance our understanding of these developmental pathways for the stress response and has become a useful tool for assessing the response of individuals to ecological stressors of interest (Wingfield et al., 1998; Romero, 2002).

A common ecological stressor for wild birds and particularly nest-bound chicks is parasitism by ectoparasites. Colonial birds are especially vulnerable to ectoparasite infestation due to the high density of breeding adults and nestlings and the long-term persistence of colony locations (Moller, 1990; Coulson, 2002). Numerous studies have demonstrated that ectoparasite infestation can alter the duration of incubation, nestling growth rates, or nestling sur-

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vival (Duffy, 1983; Chapman and George, 1991; Moller, 1993) and that ectoparasites can elevate the baseline level of corticosterone (Raouf et al., 2006), the stress-induced level of corticosterone (Quillfeldt et al., 2004), or both (Kitaysky et al., 2001) for infested nestlings. We sought to examine the stress response of brown pelican (*Pelecanus occidentalis*) nestlings in relation to the presence of the soft tick *Carios capensis*. *C. capensis* has widespread distribution and is a common nest ectoparasite of marine birds (Duffy, 1983; Hoogstraal, 1985), including the brown pelican. High levels of *C. capensis* infestation in pelican colonies have been associated with abandonment of nests (Duffy, 1983; Norcross and Bolen, 2002) and desertion of young (personal obs). In general, *C. capensis* feed for short durations; however, ticks can reach high densities within individual nests, require multiple blood meals to support their development, and remain in the nest material between feedings (Hoogstraal, 1985; Norcross and Bolen, 2002). The limited mobility of pelican nestlings during the first three weeks of development hinders their ability to escape parasitism in the nest. Therefore, ticks have the potential to decrease the energy reserves of nestlings in infested nests and may be acting as persistent stressors during development. Increases in corticosterone levels in response to tick presence may help alleviate the loss of resources in the short-term via intensified begging behavior, for example. However, if ticks act as chronic stressors, a functional stress response could further burden nestling energy reserves and produce long-term fitness consequences.

Our primary objective was to determine if baseline and stress-induced levels of corticosterone in altricial pelican nestlings increased with the severity of tick infestation. As the stress response of brown pelican nestlings has not previously been studied, we first sought to determine whether nestlings of brown pelicans exhibit a functional stress response. We predicted that pelican nestlings would display a functional stress response and that baseline and stress-induced levels of corticosterone would increase with increased tick infestation. We also explored whether factors included in our study design, including nestling age, insecticide treatment, and time of day, or ecological factors, such as body condition, hatch order, or brood size, affected corticosterone levels in nestlings.

2. Methods

2.1. Study site

We sampled brown pelican nestlings at the two largest pelican colonies in South Carolina in 2005. Marsh Island (32°59'N, 79°32'W) is a 19 ha island within the boundaries of Cape Romain National Wildlife Refuge. Deveaux Bank (32°32'N, 80°10'W) is an 87 ha island located in the mouth of the North Edisto River. Both islands are managed by South Carolina Department of Natural Resources and share similar habitat characteristics despite their difference in size. Pelican nests are located on the ground at both colonies and spaced approximately 1–3 m apart (Shields, 2002). In 2005, 611 and 1575 brown pelican nests were counted on Marsh Island and Deveaux Bank, respectively (Jodice et al., 2007). Pelicans nested near other species at both colonies including laughing gulls (*Larus atricilla*) and several species of wading birds. *C. capensis* is found at all breeding colonies in the state (Keirans et al., 1992; Wilkinson et al., 1994), and have been implicated in periodic abandonment of nests at both of our study sites (Wilkinson et al., 1994; LE personal observation). Hereafter, any mention of ticks refers to *C. capensis* unless stated otherwise.

2.2. Field procedures

Pelican nests at both study sites were treated with an insecticide as part of standard management practice to control tick

populations. A single application of approximately 175 ml of a 0.5% dilution of Rabon® 50 WP insecticide (active ingredient: Tetrachlorvinphos; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) was hand-sprayed directly onto the nest material during peak incubation of pelicans. Rabon is commonly used on poultry and is approved for direct application to birds and their physical environment. Insecticide treatment reduced, but did not eliminate, the presence of ticks on nestlings (Eggert and Jodice, 2008).

Study nests were randomly selected at each colony prior to treatment: half of the selected nests received insecticide treatment and the other half received no treatment. The remaining nests in the colony were treated. Although we included treated and untreated nests in our study, our primary objective was to determine the effect of tick presence on the adrenocortical response of pelican nestlings. The treatment effect was included in part to control tick abundance and hence allow for the study of nests under a range of conditions but was not included as a means of determining the effect of insecticide treatment on tick abundance. Our sample included 11 nestlings from 8 treated nests and 18 nestlings from 13 untreated nests at Marsh Island, and 11 nestlings from 6 treated nests and 9 nestlings from 6 untreated nests at Deveaux Bank.

At Marsh Island, nestlings in some study nests were part of a concurrent project that required regular handling and measurement (Eggert and Jodice, 2008). Nestlings in the remaining study nests were handled only for the purposes of this study. To examine and account for the potential effects of researcher handling on the stress response of these nestlings, we categorized nestlings as those handled at regular intervals ('high' contact) and those sampled only for this study ('low' contact).

Sampling for the stress response occurred between 0700–1300 on 22 and 24 June 2005 at Marsh Island and on 27 June 2005 at Deveaux Bank. Nestlings selected for analysis (Marsh Island $n = 29$; Deveaux Bank = 20) ranged in age from 11–23 days. Within this age range, nestlings are developing a cover of down feathers and ambulatory movement but generally stay in their nest (Shields, 2002). We selected first and second hatched nestlings only and sampled siblings on separate days whenever possible. A pre-established sampling sequence was followed that allowed researchers to minimize disturbance to study nests prior to sampling.

We used a standardized capture stress protocol (Wingfield et al., 1992) to sample for baseline and stress-induced levels of corticosterone. Approximately 100 μ l of blood was collected from each nestling within 4 min after removal from the nest (mean = 1.98 ± 0.15 min post-capture) by puncturing a wing vein with a sterilized 25-gauge needle. We found no significant effect of time until the initial sample was collected on the level of corticosterone (simple linear regression; $F_{1,47} = 0.09$; $P = 0.8$) and therefore assumed initial samples represented baseline corticosterone levels (Romero and Reed, 2005). Nestlings were held in individual containers for collection of subsequent blood samples at 30 and 50 min following the same procedure described above. All blood samples were immediately sealed with clay in micro-hematocrit tubes, stored on ice, and centrifuged later that day (5 min, 2000 rpm). Plasma was removed with a Hamilton glass syringe and placed in labeled micro-centrifuge tubes, then stored frozen until laboratory analysis. Two groups of researchers worked simultaneously to capture nestlings and collect blood samples. Body mass (electronic scale if <1000 g; 2500 \pm 20 g spring scale if >1000 g), culmen length (dial calipers \pm 1 mm), and tick categories were measured for each nestling by the same researcher (LE) after the final blood sample was collected. The number of immature ticks on the neck and pouch of each nestling was counted and grouped into three categories (0 ticks, 1–10 ticks, and >10 ticks) to examine the effect of different infestation levels. Adult ticks were not observed on nestlings. Nestling age was estimated

($n = 38$) using the model (age = 14.13^{*} In culmen –42.85; Eggert and Jodice, 2008) if it could not be determined from a known hatch date. Body condition index (BCI) was calculated as the residual of the linear relationship between body mass and culmen length for a larger sample of pelican nestlings of the same age range from colonies in South Carolina ($n = 568$, $r = 0.94$, $P < 0.0001$).

2.3. Laboratory procedures

Corticosterone concentration of plasma samples was measured by radioimmunoassay (based upon Wingfield et al., 1992). Dichloromethane was used to extract corticosterone from plasma samples (range: 9–27 μl). Antiserum and tritiated corticosterone were added to extractions prior to radioimmunoassay. To determine the amount of corticosterone in our samples, we generated a standard curve using a dilution series with known amounts of unlabeled corticosterone, tritiated corticosterone, and antiserum. Each sample was measured in duplicate, and recoveries were generated for all samples. Variation between assays ($n = 2$) was 10%; intra-assay variation was 3.8%.

2.4. Statistical analysis

Our study was designed to address three questions: (1) Was there an elevation in corticosterone levels of pelican nestlings during the 50-min handling session? (2) Did aspects of the study design (e.g. colony, age, insecticide treatment, time of day) have a significant effect on corticosterone levels? (3) Did the ecological factors of interest (e.g. tick category, BCI, hatch order, brood size) have a significant effect on corticosterone levels?

We compared corticosterone levels among baseline, 30-min, and 50-min samples to determine if nestlings displayed a stress response to the capture stress protocol. Data were analyzed using a two-way analysis of variance (ANOVA) that incorporated both a repeated measures structure and a mixed model structure. Fixed factors included sampling interval, colony, and their interaction. A random term for nest was included and we used an adjusted Tukey multiple-comparison procedure to assess differences among sampling intervals. A significant increase in corticosterone by either the 30-min or 50-min sample was interpreted as an active stress response.

We assessed the effects of the study design and ecological factors (Table 1) on the stress response by examining three measures of corticosterone: baseline, peak, and magnitude. The baseline measure was determined from the initial sample collected for each nestling. The peak measure is the highest corticosterone level reached for each individual at the 30- or 50-min sample. The magnitude measure is the difference between the maximum and min-

imum corticosterone level for each individual, and thus provides a conservative estimate of the stress response at the individual level. We used a backward selection approach with a mixed model design to assess the potential effects of study design and various ecological factors separately for each of the three corticosterone measures. We accounted for sampling of siblings by including a random term for nest in the mixed models. Other explanatory variables were included as fixed effects or covariates. Our sample size limited the inclusion of two-way interactions to those which were considered to be most biologically meaningful to our study design, to have the greatest possibility of yielding ecologically relevant relationships, and to be the least likely to produce confounding results (see Table 1). We did not include the interaction between tick category and BCI or the interaction between hatch order and brood size. Manual backward selection was used to eliminate the two-way interaction with the highest P -value ≥ 0.15 at each step. We continued in this manner until either all interaction terms were eliminated or until remaining interaction terms had a P -value < 0.15 .

Nestlings at Marsh Island categorized at 'high' and 'low' contact did not differ in any of the three corticosterone measures (two sample t -test for means; $t_{27} \leq 0.9$, $P \geq 0.3$ for each measure) and were pooled for subsequent analyses. Baseline corticosterone levels differed between colonies ($F_{1,15} = 6.0$, $P = 0.03$), and so the ecological model was run separately by colony. Differences among levels of significant variables in all mixed models were assessed using Tukey multiple-comparison procedures. One-way ANOVA was used for separate comparisons of age, body condition, body mass, and culmen length of nestlings between colonies and among tick categories within colonies. Concentrations of corticosterone (ng/ml) were log transformed for all analyses. Mean are presented as untransformed values ± 1 SE. Coefficient estimates are presented as the log value as calculated by the statistical software to avoid confusion associated with back-transformation of standard error values. All analyses were performed with SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Tick infestation

On Marsh Island, 13 nestlings had no ticks present at the time of sampling, 8 nestlings had 1–10 ticks, and 8 nestlings had >10 ticks (range: 13–153 ticks). Only two of 20 nestlings sampled at Deveaux Bank were infested (<10 ticks each). Body mass, culmen length, BCI, and age did not differ among tick categories when data were pooled between colonies ($n = 49$; $P \geq 0.22$ for each). However, there were differences in body mass, culmen length, and age of nestlings between colonies and among tick categories within colonies (Table 2). Results were similar when parasitism at Marsh Island was categorized as present or absent, however, there was only a trend in the difference between age categories ($P = 0.07$).

3.2. Stress response

There was a significant interaction effect of colony and sampling interval on corticosterone level ($F_{2,110} = 3.7$, $P = 0.03$). We subsequently analyzed corticosterone levels separately by colony to detail the differences. Corticosterone levels differed among the sampling intervals in pelican chicks at Marsh Island ($F_{2,64} = 51.3$, $P < 0.0001$) and at Deveaux Bank ($F_{2,46} = 13.0$, $P < 0.0001$; Fig. 1). At Marsh Island, corticosterone levels differed significantly and positively between the baseline and 30-min sample, between the baseline and 50-min sample, and between the 30- and 50-min sample ($P < 0.001$ for each; Fig. 1). At Deveaux Bank, the baseline

Table 1

Variables included in the mixed models to assess potential effects of study design and ecological factors on three measures of corticosterone (baseline, peak, and magnitude; see Methods for definitions of each) in brown pelican nestlings at Deveaux Bank and Marsh Island, South Carolina, June 2005. Nest was included as a random variable for all models. A backward selection process was used to remove interaction terms when $P > 0.15$. Model 1 included samples from both colonies while Model 2 was run separately by colony.

Model 1: study design ($n = 49$)	Model 2: ecological factors Deveaux Bank ($n = 20$), Marsh Island ($n = 29$)
Colony	Tick category
Treatment	Body condition index (BCI)
Time of day	Hatch order
Age	Brood size
Colony * Treatment	Brood size * Tick category
Age * Treatment	Brood size * BCI
Age * Colony	Hatch order * Tick category
	Hatch order * BCI

Table 2
Mean body mass (g), culmen length (mm), age (d), and body condition index (BCI) measurements (± 1 SE) of brown pelican nestlings at Marsh Island and Deveaux Bank, South Carolina, June 2005. Upper-case letters represent $P \leq 0.05$ difference in each measure of the total sample between colonies. Lower-case letters represent $P \leq 0.05$ difference in each measure by tick category within each colony.

	Tick category	N	Body mass	Culmen length	Age	BCI
Deveaux Bank	0	18	1344.7 \pm 87.7	84.1 \pm 3.2	19.7 \pm 0.6	-24.6 \pm 23.5
	1–10	2	1260.0 \pm 100.0	80.5 \pm 3.5	19.5 \pm 0.5	-23.1 \pm 16.4
	Total sample	20	1336.3 \pm 79.3 ^A	83.8 \pm 2.9 ^A	19.7 \pm 0.5 ^A	-24.4 \pm 21.2
Marsh Island	0	13	786.1 \pm 61.3 ^a	59.0 \pm 2.5 ^a	14.8 \pm 0.7 ^a	16.5 \pm 18.8
	1–10	8	912.6 \pm 171.1 ^{ab}	66.0 \pm 7.4 ^{ab}	15.3 \pm 1.4 ^a	-24.2 \pm 26.9
	>10	8	1300.0 \pm 114.4 ^b	81.3 \pm 4.7 ^b	18.9 \pm 0.7 ^b	-0.9 \pm 32.6
	Total sample	29	962.8 \pm 72.7 ^B	67.1 \pm 3.1 ^B	16.1 \pm 0.6 ^B	0.5 \pm 14.2

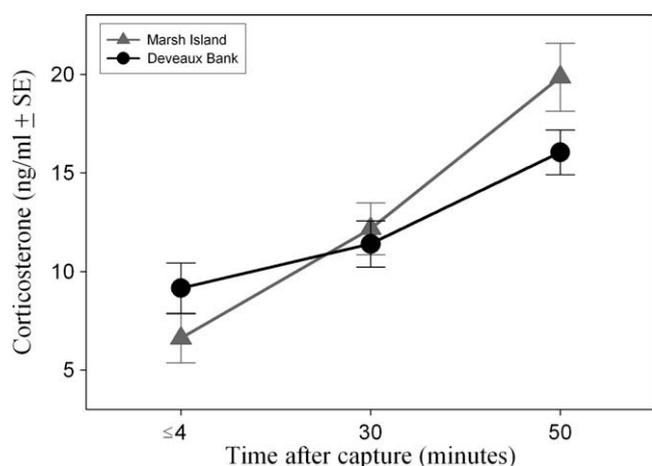


Fig. 1. Mean corticosterone levels (ng/ml \pm SE) of brown pelican nestlings at Marsh Island and Deveaux Bank, South Carolina, June 2005, at three sampling intervals: ≤ 4 min, 30-min, and 50-min post-capture. The rise in corticosterone over the 50-min handling period represents the stress response. At Marsh Island, corticosterone differed significantly between each sampling time ($P < 0.05$ for each). At Deveaux Bank, the baseline (≤ 4 -min) and 50-min samples differed significantly as did the 30- and 50-min samples ($P < 0.05$).

and 50-min samples differed significantly as did the 30- and 50-min samples ($P < 0.003$ for each; Fig. 1).

3.3. Study design effects

Baseline corticosterone differed significantly between colonies ($F_{1,15} = 6.0$, $P = 0.03$). Nestlings at Deveaux Bank had significantly higher baseline corticosterone (9.15 ± 1.28 ng/ml) compared to those at Marsh Island (6.61 ± 1.24 ng/ml). In contrast, the peak and magnitude of the stress response did not differ between colonies ($P \geq 0.11$ for each). Neither age, insecticide treatment, time of day, colony * insecticide treatment, age * insecticide treatment, or age * colony significantly affected baseline, peak, or magnitude measures of corticosterone ($P \geq 0.16$ for each).

3.4. Ecological effects

Tick category did not affect baseline, magnitude, or peak corticosterone values at Marsh Island or Deveaux Bank ($P > 0.15$ for each). There was a significant negative relationship (Fig. 2) between the BCI of pelican chicks and the baseline measure of corticosterone at Deveaux Bank (log 10 coefficient = -0.003 ± 0.0005 , $F_{1,5} = 25.1$, $P = 0.004$). There was no relationship between BCI and either the peak or magnitude levels of corticosterone at Deveaux Bank ($P \geq 0.22$ for each) or for all three measures of corticosterone at Marsh Island ($P \geq 0.22$ for each). There was a trend towards a greater magnitude in the stress response for first hatched nestlings

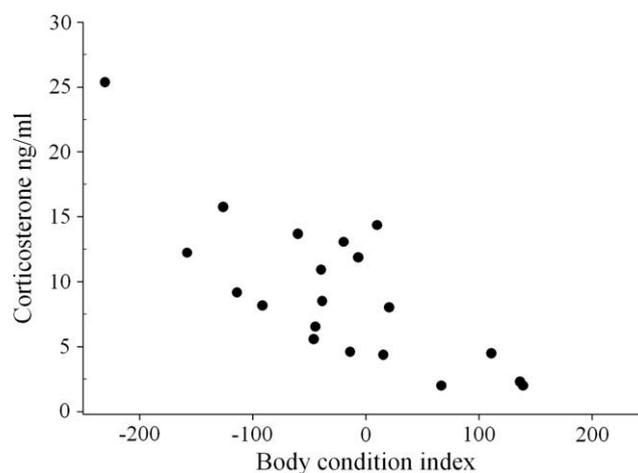


Fig. 2. Body condition index (BCI) and baseline corticosterone levels (ng/ml) of brown pelican nestlings at Deveaux Bank, South Carolina, June 2005. There was a significant negative relationship between the two variables ($F_{1,5} = 25.1$, $P = 0.004$).

at Deveaux Bank ($F_{1,5} = 5.79$, $P = 0.06$; 11.00 ± 1.42 ng/ml) compared to second hatched nestlings (7.20 ± 2.27 ng/ml). Corticosterone levels did not differ between hatch orders for the baseline or peak measures at Deveaux Bank ($P \geq 0.17$ for each) or for all three measures at Marsh Island ($P \geq 0.48$ for each). There was no relationship between brood size and the stress response of nestlings at either colony ($P \geq 0.27$ for each). None of the interaction terms tested (Table 1) significantly affected the baseline, magnitude, or peak measures of corticosterone in pelican chicks at either colony ($P \geq 0.24$ for each).

4. Discussion

4.1. Stress response

The stress response of brown pelican nestlings has not previously been studied. Corticosterone levels of pelican nestlings increased from baseline measurements during the 50 min study period, clearly demonstrating that pelicans of the ages studied here (11–23 days) are capable of a functional stress response. Despite being physically unable to escape their stressor at this age, our results suggest that a functional adrenocortical stress response provides benefits, such as the reallocation of energy stores or increased begging (Loiseau et al., 2008), that enhance nestling survival probability and outweigh the potential costs associated with elevated corticosterone (Sims and Holberton, 2000; Walker et al., 2005). A significant stress response has also been observed in nestlings of other altricial species, including the European white stork (*Ciconia ciconia*; Blas et al., 2005), barn swallow (*Hirundo rustica*; Saino et al., 2003) and western scrub-jay (*Aphelocoma californica*;

Pravosudov and Kitaysky, 2006). Baseline and stress-induced corticosterone levels of adults or juvenile brown pelicans are not available to assess the maturity of the adrenocortical response to stress in nestlings.

Though nestlings at both study sites displayed a stress response, baseline corticosterone levels and the stress response differed between colonies. Baseline corticosterone levels of nestlings from Deveaux Bank were higher than those from Marsh Island and did not increase significantly until the 50 min measure. By contrast, corticosterone levels increased significantly during each measurement interval (baseline to 30- to 50-min) for nestlings measured at Marsh Island. The observed differences between colonies in baseline corticosterone levels and the stress response itself could suggest a difference in previous exposure to stressors not measured in this study.

4.2. Tick infestation

C. capensis is a haematophagous ectoparasite that can reach high densities in the nest material of brown pelicans during the breeding season (Norcross and Bolen, 2002) and deplete energy reserves of their host. Pelican nestlings are vulnerable to parasitism by ticks during early development when they are unable to leave their nest. We hypothesized that ticks acted as stressors for pelican nestlings and that baseline and stress-induced levels of corticosterone would increase with increasing exposure to ticks. Our results, however, indicate that tick category is not a significant factor influencing baseline or stress-induced levels of corticosterone in pelican nestlings. Therefore our results appear to contrast those of previous studies, which found elevation in the level of baseline or stress-induced corticosterone in relation to ectoparasite infestation in red-legged kittiwakes (*Rissa brevirostris*, Kitaysky et al., 2001), Wilson's storm-petrels (*Oceanites oceanicus*, Quillfeldt et al., 2004), and cliff swallows (*Petrochelidon pyrrhonota*, Raouf et al., 2006).

Of the studies examining the adrenocortical stress response in relation to ectoparasite infestation in birds, no studies are specific to the soft tick species studied here. However, similar to our study, parasitized cliff swallows were exposed to haematophagous ectoparasites in the nest (swallow bugs, *Oeciacus vicarius*) (Raouf et al., 2006). This ectoparasite was associated with higher baseline corticosterone levels in nestling, juvenile, and adult cliff swallows compared to birds from parasite-free colonies, however, measures of the severity of infestation were not presented. Within our study nests, levels of tick infestation were sub-lethal and may not have been great enough to generate a strong, consistent adrenocortical response in the nestlings. Infestation may have been low due to the insecticide application (Norcross and Bolen, 2002; Eggert and Jodice, 2008) or we may have sampled nestlings in portions of the colonies or in a year with naturally low tick populations. A relative small number of ticks (1–16 ticks) were enough to reveal a positive linear relationship between baseline corticosterone and tick numbers in red-legged kittiwake nestlings (Kitaysky et al., 2001). However, the parasite was a species of hard tick, which generally feed for longer durations than soft ticks and thus may deplete more energy reserves from their host. This indicates that the adrenocortical response of the host is likely dependent on the degree of infestation and the type of ectoparasite.

It is also possible that ticks were acting as chronic stressors and the release of corticosterone was down-regulated as a result (Rich and Romero, 2005). However, we saw no difference in baseline corticosterone levels or measures of the stress response among parasitized and parasite-free nestlings. Rather, nestlings from infested nests may have habituated to tick presence. Nestlings were at least 11 days old during our study and may no longer perceive ticks as stressors after coping with the constant presence of ticks at the sub-lethal levels observed in this study. Habituation to ticks would

ultimately serve to reduce any negative consequences of elevated circulating levels of corticosterone including compromised growth (Hull et al., 2007). It is interesting to note that our companion study of parasitized pelican nestlings found that neither the growth rates of body mass or culmen length were negatively affected by tick infestation (Eggert and Jodice, 2008). The lack of a significant effect of ticks on corticosterone levels of parasitized nestlings appears to agree with those from our growth study. Further exploration of the adrenocortical response of pelican nestlings exposed to higher levels of tick infestation in combination with other measures of nestling condition, such as hematocrit, is needed to more fully understand this relationship.

There is evidence that corticosterone levels of parasitized nestlings may increase when exposed to multiple stressors. For example, Wilson's storm-petrel nestlings exhibited a positive correlation between ectoparasite (feather louse) loads and corticosterone levels, but only when a severe storm limited food delivery by parents. There was not a significant relationship between ectoparasites and corticosterone prior to the storm event (Quillfeldt et al., 2004). Additionally, Raouf et al. (2006) found that baseline levels of corticosterone were significantly lower in cliff swallow nestlings from large, treated (parasite-free) colonies compared to large, untreated (parasite infested) colonies and small colonies of both treatments. Although the brown pelican colony at Deveaux Bank was over twice the size of the colony at Marsh Island during the 2005 breeding season, the relationship between colony size and ectoparasite infestation could not be considered in this study.

4.3. Ecological effects

While our primary interest was to examine the effect of ticks on baseline and stress-induced corticosterone levels of nestlings, we also attempted to account for some of the additional ecological factors that might have influenced hormone levels. Although such an analysis makes for a complex interpretation of the results, it also allows for an exploration of background factors that may affect the stress response. There was a negative relationship between body condition and baseline levels of corticosterone levels at Deveaux Bank, such that nestlings in poor condition had elevated levels of corticosterone. No relationship was observed for nestlings at Marsh Island and body condition did not differ between the total samples, though nestlings at Deveaux Bank tended to have lower body condition scores than nestlings at Marsh Island. This suggests that baseline corticosterone is not a reliable indicator of body condition for pelican nestlings, as the relationship exists only for nestlings in poor condition. We are not aware of differences in pelican breeding phenology between the colonies or environmental conditions such as predation rates that may account for nestling condition at Deveaux Bank, but not Marsh Island. Body condition index can be indicative of food availability and provisioning rates (Benson et al., 2003). Several studies have indicated that nestlings provided a restricted diet have increased levels of circulating corticosterone, as well as a greater stress response (Nunez-de la Mora et al., 1996; Kitaysky et al., 2001; Pravosudov and Kitaysky, 2006). In the wild, food resources may be exhausted around seabird colonies during the breeding season, particularly near larger colonies and for near-shore foragers (Ashmole, 1963; Coulson, 2002). Food availability and provisioning rates were not measured in this study; however, food demands in the colony at the time of sampling would have been high, as most nestlings were hatched and growing at maximum rates (Eggert and Jodice, 2008). If food supplies were diminished, food shortages could have contributed to the higher levels of baseline corticosterone measured in nestlings in poor condition (as represented by low body condition index) at Deveaux Bank, which supported over twice as many pelican nests as Marsh Island.

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