GROWTH AND ADRENOCORTICAL RESPONSE OF BROWN PELICAN (*PELECANUS OCCIDENTALIS*) NESTLINGS IN RELATION TO ECTOPARASITE INFESTATION IN SOUTH CAROLINA

A Thesis

Presented to

the Graduate School of

Clemson University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

Wildlife and Fisheries Biology

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by

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May 2006

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ABSTRACT

Coloniality is relatively common among breeding birds, occurring in 13% of avian species and over 95% of seabirds. Ectoparasite infestation is a main disadvantage of coloniality that may affect growth, survival, and condition of nestling, particularly those of altricial species. I investigated the effect of the soft tick, *Ornithodoros capensis*, on the growth rates and adrenocortical stress response of altricial Brown Pelican (*Pelecanus occidentalis*) nestlings during early development. This research was conducted over two years at three colonies in South Carolina, and also considered the effect of insecticide treatment and select ecological variables. Growth rates of pelican nestlings were positively affected by tick load at the more heavily infested colony. There was no relationship between survival and level of tick infestation. The magnitude of the adrenocortical stress response, as measured by the release of corticosterone in reaction to an acute stress regime, was greater for tick-free nestlings than for those with a moderate tick load. The average number of ticks per nestling and the distribution of parasitized nestlings differed between insecticide treated and untreated nests, though results were not consistent between colonies or years. Other variables including nest effect, hatch order, and body condition index significantly affected growth rates and corticosterone levels, and colony differences were apparent in both studies. Together, these results suggest that soft ticks are not likely the major contributor to the observed declines in nesting effort of Brown Pelicans in South Carolina. However, a slight negative impact of ectoparasites over time, especially in conjunction with other ecological stressors, could affect long-term population dynamics of Brown Pelicans. A more detailed investigation of growth,

survival, and physiological condition of pelican nestlings during the extended developmental period may provide additional insight into the role of ectoparasites in this system and potential impacts to the long-term health and condition of Brown Pelicans in South Carolina.

ACKNOWLEDGEMENTS

I would first like to thank my advisor, Dr. Patrick Jodice, for the opportunity to conduct research on the coastal islands of South Carolina and for his guidance and support throughout this project. Dr. Kathleen O'Reilly generously contributed to all aspects of the corticosterone work, and provided patient instruction in the lab. I would also like to thank my committee members, Dr. Sidney A. Gauthreaux and Dr. J. Jeff Isely, for their support and contributions to this project, and Dr. Herman Senter and Dr. William Bridges, who assisted with statistical analysis. I am very grateful to all the individuals who helped with field work and offered feedback on this project. I am especially indebted to Lauren Bolte, Cristina Campbell, Felicia Sanders, and Mark Spinks. This project would not have been possible with out the dedication and hard work of these individuals.

I would like to acknowledge the USGS South Carolina Cooperative Fish and Wildlife Research Unit, in particular Carolyn Wakefield, who provided support in all forms. I would also like to acknowledge the support of South Carolina Department of Natural Resources and Cape Romain National Wildlife Refuge, in particular Sarah Dawsey and Matt Connolly. Coastal Expeditions kindly donated use of kayaks during both years of this project. The Association of Field Ornithologists supported the corticosterone work through the Bergstrom Award, and I was supported in part by the Clemson University Stackhouse Fellowship during the 2005 academic year.

Finally, I would like to thank my family and friends for their love, support, and encouragement.

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CHAPTER ONE

INTRODUCTION

Colonial breeding is relatively common among birds, occurring in 13% of avian species (Gill 1990). This breeding strategy is particularly widespread among seabirds, with over 95% of species classified as colonial (Coulson 2002). While the advantages of coloniality include maximization of limited nesting habitat and protection from predators, dense breeding aggregations do confer costs (Coulson 2002). One of the main disadvantages of coloniality is infestation of ectoparasites such as ticks, mites, fleas, and lice. These parasites capitalize on the seasonal reuse of breeding sites and the relatively long duration of incubation and chick rearing common to seabirds to exploit this highly reliable source of potential hosts (Rothchild and Clay 1952; Duffy 1983). Nestlings, particularly those of altricial species, are especially vulnerable to the detrimental impacts of nest parasites (Lehmann 1993).

Infestations of ectoparasites in breeding colonies can result in obvious and severe losses such as nest and colony abandonment (King et al. 1977a, b; Duffy 1983) as well as less apparent but equally detrimental effects such as decreased health and survival of adults and nestlings (McKilligan 1996; Gauthier-Clerc et al. 1998; Ramos 2001). For example, ectoparasite infestation has been shown to negatively impact breeding success (McKilligan 1996; Mangin et al. 2003), incubation period (Fitze et al. 2004), growth rates (Morbey 1996; Ramos 2001), and colony recruitment (Boulinier and Danchin 1996) in a variety of colonial taxa. The physiological condition of avian hosts may also be negatively affected by ectoparasites (Saino et al. 2003; Simon et al. 2004). For instance,

ectoparasite infestation may contribute to elevated levels of the stress hormone corticosterone (Kitaysky 2001; Quillfeldt et al. 2004), which over time may suppress the growth, immunity and neurological development of nestlings (Wingfield et al. 1997). Hence, ectoparasite infestation may affect the immediate survival and condition of individuals as well as the long-term fitness of individuals and population trends (Boulinier and Danchin 1996; Gebhardt-Henrich and Richner 1998).

Here, I examine the relationship between ectoparasite infestation and the growth and physiological condition of Brown Pelican (*Pelecanus occidentalis*) nestlings at three colonies off the coast of South Carolina (Figure 1.1). The impact of ectoparasites on the population of Brown Pelicans in South Carolina is important because (1) there is a declining population of breeding Brown Pelicans in the state, (2) colonies are known to be infested with ectoparasites, and (3) colonies are currently treated with insecticide in an effort to control ectoparasite infestation.

The Brown Pelican is the only true seabird among the eight pelican species with a breeding range that extends from the southern coasts of North America to the northern coasts of South America, and the Caribbean (Shields 2002). In the United States, the southeastern population of Brown Pelicans (including the Atlantic coast and Gulf coasts of Florida and Alabama) was removed from the Endangered Species List in 1985 after the population rebounded from drastic declines as a result of eggshell thinning from exposure to DDT and other organochlorines (Wilkinson et al. 1994). In South Carolina, pelicans have nested at nine different sites since 1969, although only four have been used regularly. Nest counts of Brown Pelicans increased steadily throughout the 1970s and 1980s, but since that time have progressively declined (Figure 1.2; Jodice et al. in

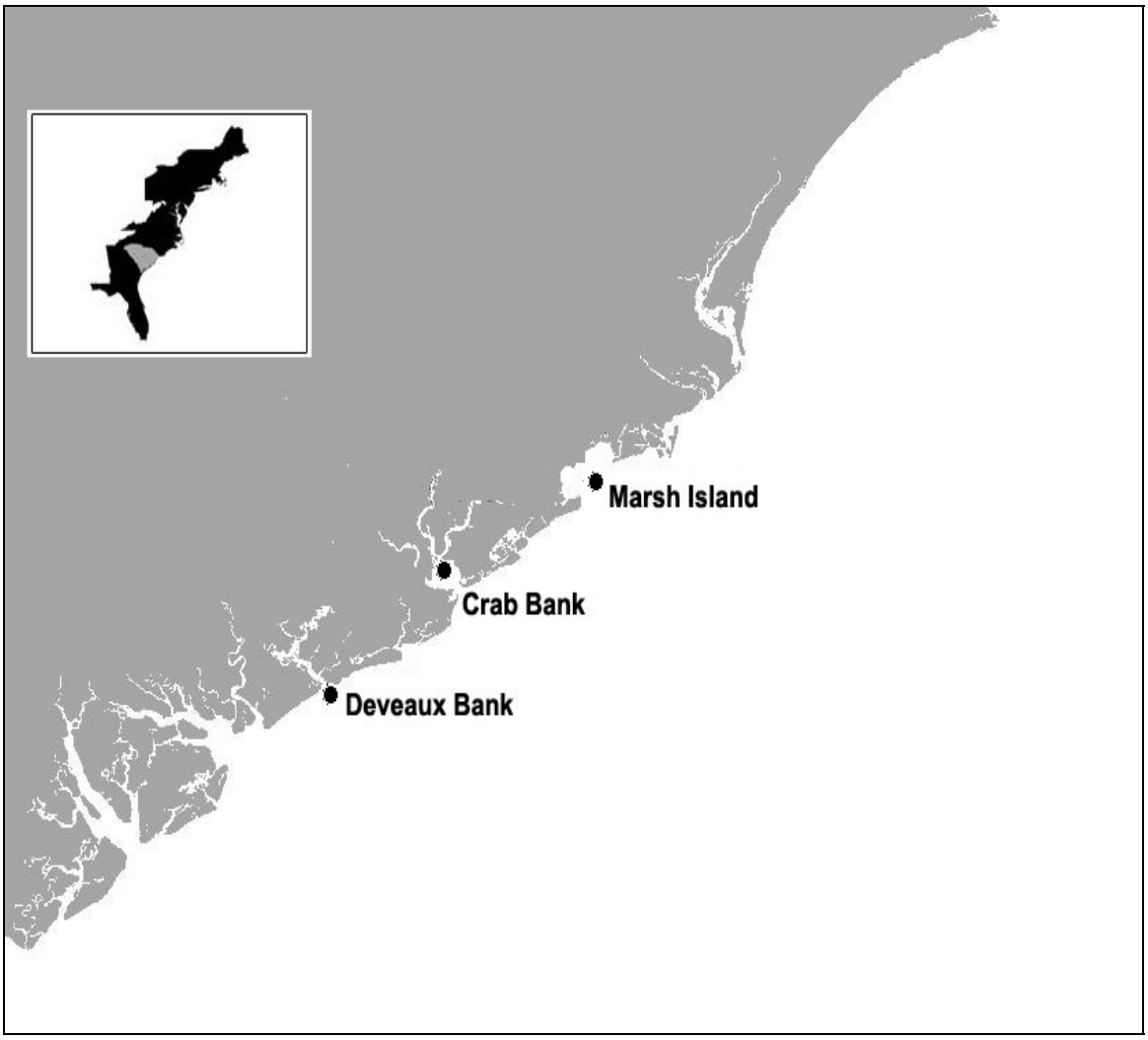

Figure 1.1 Three South Carolina Brown Pelican breeding colonies used as sites for the 2004 and 2005 study of the effect of ectoparasites on growth (Marsh Island and Crab Bank) and physiological condition (Marsh Island and Deveaux Bank) of nestlings.

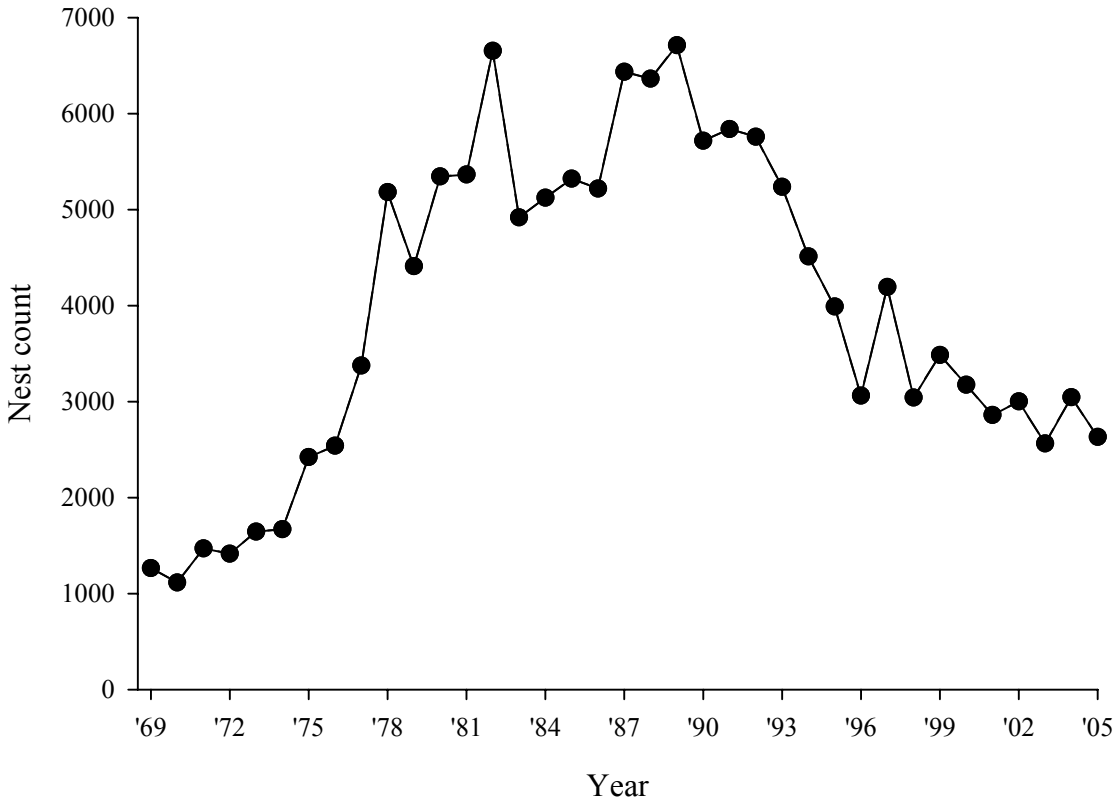

Figure 1.2 Annual statewide nest counts of Brown Pelicans in South Carolina, 1969 – 2005 (from Jodice et al. in review).

review). The mechanisms underlying this period of decreased population size are poorly understood and have not been well studied. Brown Pelicans, like many seabirds, exhibit high fidelity to traditional breeding sites and typically only abandon established colonies when conditions change (Coulson 2002; Shields 2002). Heavy ectoparasite infestation, poor reproductive success, beach erosion, and reduced food availability are factors that may trigger pelicans to desert traditional breeding colonies for new sites (Shields 2002; Jodice et al. in review).

A common ectoparasite occurring at Brown Pelican colonies in the southeastern U.S. is the soft tick *Ornithodoros capensis*. *O. capensis* are infected by numerous arboviruses and may transmit infectious agents and toxins (Hoogstraal 1985). *O. capensis* was first positively identified in South Carolina in 1987 from a Brown Pelican colony and has since been observed at all colonies within the state (Keirans et al. 1992; Wilkinson et al. 1994). Nest abandonment associated with soft tick infestation has been documented at Brown Pelican colonies within the state (Keirans et al. 1992; Wilkinson et al. 1994) and elsewhere in the species' range (King et al. 1977a, b; Duffy 1983; Norcross and Bolen 2002). In an effort to curb ectoparasite populations at seabird colonies, the South Carolina Department of Natural Resources has annually applied the organophosphate insecticide Rabon® to pelican nests during incubation for the past decade. The impact of insecticide treatment and resulting tick infestation levels at these colonies, however, have not been studied. Therefore, I sought to investigate the effect of ectoparasites on the growth and physiological condition of Brown Pelican nestlings in relation to insecticide treatment.

Chapter two of this thesis, "Effect of soft ticks on growth rates of Brown Pelican nestlings," examines the relationship between *O. capensis* infestation and rates of growth of Brown Pelican nestlings at two South Carolina colonies: Marsh Island and Crab Bank (Figure 1.1). I measured a suite of growth metrics for approximately three weeks post-hatch and survival to 21 days during the 2004 and 2005 breeding seasons. I compared measures of growth and survival among individuals carrying varying load of ticks. I also considered possible effects of insecticide treatment, hatch order, location, and year. Although increased chick mortality at a colony may provide an obvious visual cue to the consequences of tick infestation, effects of ectoparasites on growth rates may be equally important. Therefore, an investigation of growth rates provides a valuable assessment of potentially non-lethal yet biologically important effects of ectoparasite infestation.

Chapter three, "Adrenocortical response of Brown Pelican nestlings to ectoparasite infestation," investigates levels of the stress hormone corticosterone in Brown Pelican nestlings in relation to *O. capensis* infestation at two colonies in South Carolina: Marsh Island and Deveaux Bank (Figure 1.1). Ecological stressors such as ectoparasites may trigger an adrenal stress response in the affected individual resulting in increased levels of circulating corticosterone. If altricial pelican nestlings are capable of a physiological response to stress, young from infested nests may suffer adverse physiological effects associated with chronically elevated corticosterone, such as suppressed immunity, reduced rates of growth (Wingfield et al. 1997), and impaired cognitive development (Kitaysky et al. 2003). Ectoparasites could therefore have great consequences on the fitness of individuals in an infested colony. Similar to the approach

in Chapter 2, an analysis of corticosterone provides a means to assess potential non-lethal, yet adverse, effects of ectoparasites on nestling condition.

Results of this research will improve efforts to monitor and manage Brown Pelican nesting colonies, enhance our understanding of local and regional population trends in this species, increase the foundation of biological knowledge of the host-parasite relationship between *O. capensis* and seabirds, and contribute to a growing body of research on ecological tools for studying parasitized individuals.

CHAPTER TWO

EFFECT OF SOFT TICKS ON GROWTH RATES OF BROWN PELICAN NESTLINGS

Introduction

Ectoparasites are a noxious biotic component of many avian breeding colonies that may have a negative effect on the reproductive success of colonial species (Duffy 1983; Moller 1990; Chapman and George 1991; Saino et al. 2002; Fitze et al. 2004). Because colonies concentrate high densities of adults and nestlings during a relatively brief time period, ectoparasites gain ready access to numerous potential hosts at colony sites (Rothchild and Clay 1952). The consequences of infestation may be most severe for young birds (Lehmann 1993), especially those restricted to the nest during development. Such nest-bound chicks may experience parental desertion, increased exposure to arboviruses, delayed fledging, and increased mortality due to infestation of ectoparasites (King et al. 1977a, b; Duffy 1983; Hoogstraal 1985; Morbey 1996; Ramos et al. 2001). Additionally, blood-feeding ectoparasites may deplete nutrients and energy resources that are crucial for post-natal development, and several studies have documented reduced growth rates for parasitized nestlings of colonial species (Chapman and George 1991; McKilligan 1996; Morbey 1996; Ramos et al. 2001).

Most seabirds are long-lived, breed in dense colonies, have relatively lengthy chick-rearing periods, and exhibit high fidelity to breeding sites (Coulson 2002; Schreiber and Burger 2002). Hence, seabird colonies are prime habitat for ectoparasites (Duffy 1983) and, in fact, they appear to be prevalent throughout this group. Hard or soft ticks

have been recorded in at least 12 of the commonly recognized 15 seabird families and have been reported from all major ocean systems. In South Carolina, all colonies of Brown Pelicans (*Pelecanus occidentalis*) are infested with the soft tick *Ornithodoros capensis*. This nest parasite was first identified in the state at a Brown Pelican colony in 1987 (Keirans et al. 1992). Coincidentally, the breeding population of Brown Pelicans in South Carolina has experienced a decline in nest numbers that began in the late 1980s (Jodice et al. in review).

The reproductive costs of ectoparasites identified in other seabirds (Duffy 1983; Morbey 1996; Ramos et al. 2001; Mangin et al. 2003) suggest that tick infestation may be contributing to the current decline in pelican nest numbers in South Carolina, though this relationship has not been studied. Soft tick infestation has been implicated in periodic, large scale desertions of the Brown Pelican across the breeding range (King et al. 1977a, b; Duffy 1983), including South Carolina (Wilkinson et al. 1994). Norcross and Bolen (2002) did not find a negative relationship between *O. capensis* and condition (as measured by hematocrit levels) or survival to two weeks of Brown Pelican nestlings, though growth rates were not studied. The effects of ectoparasites are often subtle however. Measurement of post-natal growth of pelican nestlings can provide a means of assessing the potential effect of ectoparasites on development (Gebhardt-Henrich and Richner 1998) and ultimately on population dynamics.

I examined mass and structural growth rates of altricial Brown Pelican nestlings in relation to *O. capensis* infestation. Although infestation levels were not manipulated, nests were subjected to an experimental treatment whereby samples were either treated with a single insecticide application during incubation or left untreated. This experiment

was conducted as part of the management of South Carolina pelican colonies that consists of annual application of insecticide to all nests in an effort to control tick populations. A recent study suggests this course of treatment should reduce the levels of *O. capensis* infestation in Brown Pelican nests (Norcross and Bolen 2002).

I hypothesized that there would be a negative relationship between growth rates and tick loads on nestlings, and that nestlings from insecticide treated nests would have higher rates of growth compared to those from untreated nests. I also considered the effects of colony, hatch order, brood size, and year on growth rates.

Methods

Study Organisms

The breeding range of the Brown Pelican extends from the southern coasts of North America to the northern coasts of South America, and the Caribbean (Shields 2002). In South Carolina, Brown Pelicans generally construct nests synchronously on the ground in distinct clusters. One clutch, typically with three eggs, is laid per breeding season, although replacement clutches can be laid. Asynchronous hatching affords the first hatched nestling a size advantage and dominant position over its siblings (Pinson and Drummond 1993; Shields 2002). Nestlings are altricial and spend at least the first three weeks of development confined to the nest. Thereafter, chicks are mobile and may move short distances from the nest or may form crèches with siblings and neighboring chicks. This is particularly common in colonies with ground nests. Nestlings rely on parental provisioning at or near the nest during the entire developmental period of approximately 11 weeks.

The soft tick *O. capensis* infests a large variety of marine birds, including Brown Pelicans (Duffy 1983; Hoogstraal 1985). Nesting material provides *O. capensis* with shelter and access to adults and nestlings as hosts. *O. capensis* larvae are slow feeders and may attach to their host for several days at a time, whereas nymphal instars and adults tend to feed rapidly for no more than one hour (Hoogstraal 1985). High densities of soft ticks (e.g. > 1,000 per nest) have been recorded in pelican nests during the breeding season (King et al. 1977a; Norcross and Bolen 2002). Exposure of nest-bound pelican chicks to *O. capensis* of all stages can therefore be great. Hereafter, any mention of soft ticks refers to *O. capensis* unless stated otherwise.

Study Sites

Brown Pelican colonies at Marsh Island and Crab Bank (Figure 1.1), both located within Charleston County, South Carolina, served as study sites for this research. Marsh Island (32°59'N, 79°32'W) is a 19 ha island that lies approximately 4.6 km off the coast of Awendaw, SC. Marsh Island is located in a shallow bay within Cape Romain National Wildlife Refuge, is closed to the public, and has hosted a pelican colony for over 50 years. The island is comprised primarily of sandy beaches, vegetated upland, and salt water marshes. In 2004 and 2005, 789 and 611 pelican nests were counted on Marsh Island during peak incubation, respectively (Jodice et al. in review). These counts indicate a continued decline in colony size from nearly 3,500 nests in 1989, a trend that is apparent in state-wide censuses as well (Jodice et al. in review).

Crab Bank (32°46'N, 79°53'W) is an 6.5 ha sand-spit island located within Charleston Harbor, near the mouth of Shem Creek (Wando River), approximately 1.5 km from Mount Pleasant. Crab Bank is a Heritage Site managed by South Carolina

Department of Natural Resources (SCDNR). There is a high volume of boat traffic in Charleston Harbor and the island itself receives frequent disturbance from visitors. Crab Bank was first colonized by Brown Pelicans in 1994 and the colony has since fluctuated in size. In 2004 and 2005, 290 and 444 pelican nests were counted on Crab Bank during peak incubation, respectively (Jodice et al. in review).

Both colonies are infested with *O. capensis* and are treated annually with insecticide to reduce tick populations. Prior to spraying and after nests were established each year, I delineated six study plots within each colony. Plots represented the range of ground nesting habitat used each year and tended to reflect natural clusters of nests. Because sections of Marsh Island and Crab Bank used for nesting in 2004 experienced some degree of habitat modification from storms during autumn 2004, the location of plots differed somewhat between years. Within each plot I randomly selected 8 study nests: 4 remained unsprayed and 4 received the insecticide treatment. This design resulted in 4 treated and 4 untreated study nests in each of six plots per colony per year. All other nests in the colony were sprayed, including those nests not selected as study nests within plots. Nests were hand-sprayed with approximately 175 ml of a 0.5% dilution of Rabon® 50 WP insecticide (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) during the third week in May in both 2004 and 2005, corresponding with peak incubation. Study nests were marked with numbered flags secured in the underside of nest bowls and out of view of incubating adults. I recorded clutch size for all study nests and noted presence or absence of ticks in the upper layers of nest bowl material prior to spraying.

Field Procedures

I visited study nests at each colony approximately once every four days to monitor hatching, chick growth, and survival, and to determine presence and abundance of soft ticks on nestlings. Visits began on 15 May 2004 and 3 May 2005 and ended in early August in both years. Disturbance to breeding pelicans was minimized by going to the colonies on a rotating basis and maximizing time between visits to different sections within each colony whenever possible. I took care to enter nesting areas slowly, avoid colony work during the warmest hours of the day, and keep colony visits as short as possible. Adults often stayed at their nest upon approach so disruption was limited to adults at the targeted nest. Nest visits ceased when most chicks in an area became mobile (approximately 3 weeks old).

During each nest check, I measured body mass (electronic scale if ≤ 1000g; 2500 ± 20g or 5000 ± 50g spring scale if > 1000g), and culmen, tarsus, and wing length (dial calipers ± 1mm, wing bar ± 1mm). I then examined all nestlings and recorded number and location of ticks before returning chicks to their nests. Adult ticks were rarely seen on nestlings. Larval and nymph stages were difficult to distinguish in the field and, therefore, were grouped together for the total count. Similar to Duffy and de Duffy (1986), I assumed that the number of ticks counted on nestlings was representative of the overall tick population within the nest. All structural measurements and tick counts were performed by the same researcher (LF) in both years.

There was a strong correlation between the total number of ticks counted on parasitized nestlings and the number counted just on the neck and pouch combined at Marsh Island in 2004 ($r = 0.91$, $P < 0.0001$). Therefore, in 2005, I only counted the

number of ticks on the neck and pouch, and noted the presence of ticks elsewhere on the body. This method reduced the length of handling time for chicks and allowed for a more accurate comparison of ticks on nestlings after feathers developed at approximately 10 d. All references to tick counts from both years are restricted to those counted on the neck and pouch. Tick counts were categorized into tick loads as follows: None (0 ticks), Low (1-10 ticks), Moderate (11-50 ticks), and High (> 50 ticks). The low category contained approximately 50% of all counts of parasitized nestlings and the moderate and high categories each contained approximately 25% of all counts of parasitized nestlings. Based upon McKilligan (1996) and Norcross and Bolen (2002), I defined tick infestation as the average number of ticks counted on each nestling during all checks.

To differentiate hatch order among siblings, I applied a small amount of nail polish to the culmen and non-toxic permanent marker to the feathers at each nest check. Hatchlings were designated as alpha (first hatched), beta (second hatched), or gamma (third hatched) based on nest contents at the previous check, relative size among siblings, and hatchling appearance (Schreiber 1976; Shields 2002). As the size advantage of the alpha chicks is typically maintained for at least the first two weeks of development (Shields 2000), the largest nestling was assumed to be the first hatched nestling when marking techniques did not last between nest checks.

Age of nestlings within study plots was determined in one of two ways. When possible, hatch dates were based on hatching stages observed during nest checks. Nestlings recently hatched (naked, pink skin) or in the process of hatching were considered to have hatched that day. The hatch date for pipped or starred eggs was designated as the day following the nest check, based on Shields (2000). The majority of

colony visits occurred in the morning hours; therefore, I rounded hatch date to the nearest 24 h period. I refer to nestlings with a hatch date based on an observed stage of hatching as the known age sample (n = 114; combined years and colonies). When neither hatching nor stages of hatching were observed (e.g., a nest with 3 chicks had 3 eggs at the previous check), I designated hatch date using a multi-tiered approach based upon that described in Nisbet et al. (1995). First, hatch date was set as the mid-point between nest checks. This estimation was then refined using the known hatch date of siblings, with alpha nestlings hatching approximately one and three days prior to beta and gamma siblings, respectively (Ploger 1992; Shields 1998). Finally, in cases where the brood consisted of only a single nestling or contained no nestlings of known age, the initial culmen length was used to estimate hatch date based on the mean culmen length of all known age chicks that survived beyond 21 d (n = 54). I refer to nestlings with a hatch date based on the multi-tiered approach as the estimated age sample (n = 249; combined years and colonies). First estimated age was never greater than seven days. To check the accuracy of the multi-tiered approach, I used the method described above to estimate the hatch date of the known age sample and found no difference in hatch dates based on observed stages of hatching and the multi-tiered approach (two tail paired t-test; $t_{113} = 0.95$, $P = 0.35$). Known and estimated age samples were therefore pooled for all subsequent analyses.

Statistical Analysis

The majority of nestlings were measured at regular intervals during the first three weeks of development while still confined to the nest, but longer whenever possible. With the exception of the first 4-5 d of growth, the period of development I tracked can best be characterized as the linear phase of growth. I determined the linear growth rate

(LGR) using a simple linear regression to fit the slope of age to body mass and culmen length, which was the most accurately measured structural variable, for each individual (Nisbet et al. 1995). In this study, body mass and culmen length of chicks that survived to 21 d began to increase at a linear rate at approximately four days (Figure 2.1), which is consistent with results from previous growth studies of Brown Pelicans (Schreiber 1976; Shields 2002). The culmen length continues to grow linearly throughout development, while asymptotic body mass is achieved at approximately 50 d (Schreiber 1976). I included all nestlings with three or more measurements after three days post-hatch for LGR analyses (age range: Marsh Island: 4 – 45 d; Crab Bank: 4 - 46 d). One individual from Marsh Island in 2004 that had three growth measurements after two days post-hatch and five individuals from Crab Bank in 2005 that had two measurements after four days post-hatch also were included in the sample to provide a representation of treated and untreated individuals within each plot as required by the mixed model design used for these analyses. These exceptions represented 0.9 and 4.4% of the total sample at Marsh Island (n = 112) and Crab Bank (n = 113), respectively.

I used a mixed model (SAS Version 9.1; SAS Institute Inc., Cary, NC, USA) to separately analyze the LGR of body mass and culmen length. Fixed factors included year (Y), treatment (T), tick load, and hatch order. Random terms included plot nested within year (PY); nest nested within treatment, plot, and year (NTPY); and a treatment and plot within year interaction (T*PY). *F* and *P*-values for the variables Y, T, PY, and T*PY were adjusted for the appropriate error term: PY, T*PY, T*PY, and NTPY, respectively. The mean sum of squares was used to calculate test statistics as the error term for the remaining variables. Tick infestation at Crab Bank was low in both years; therefore, I

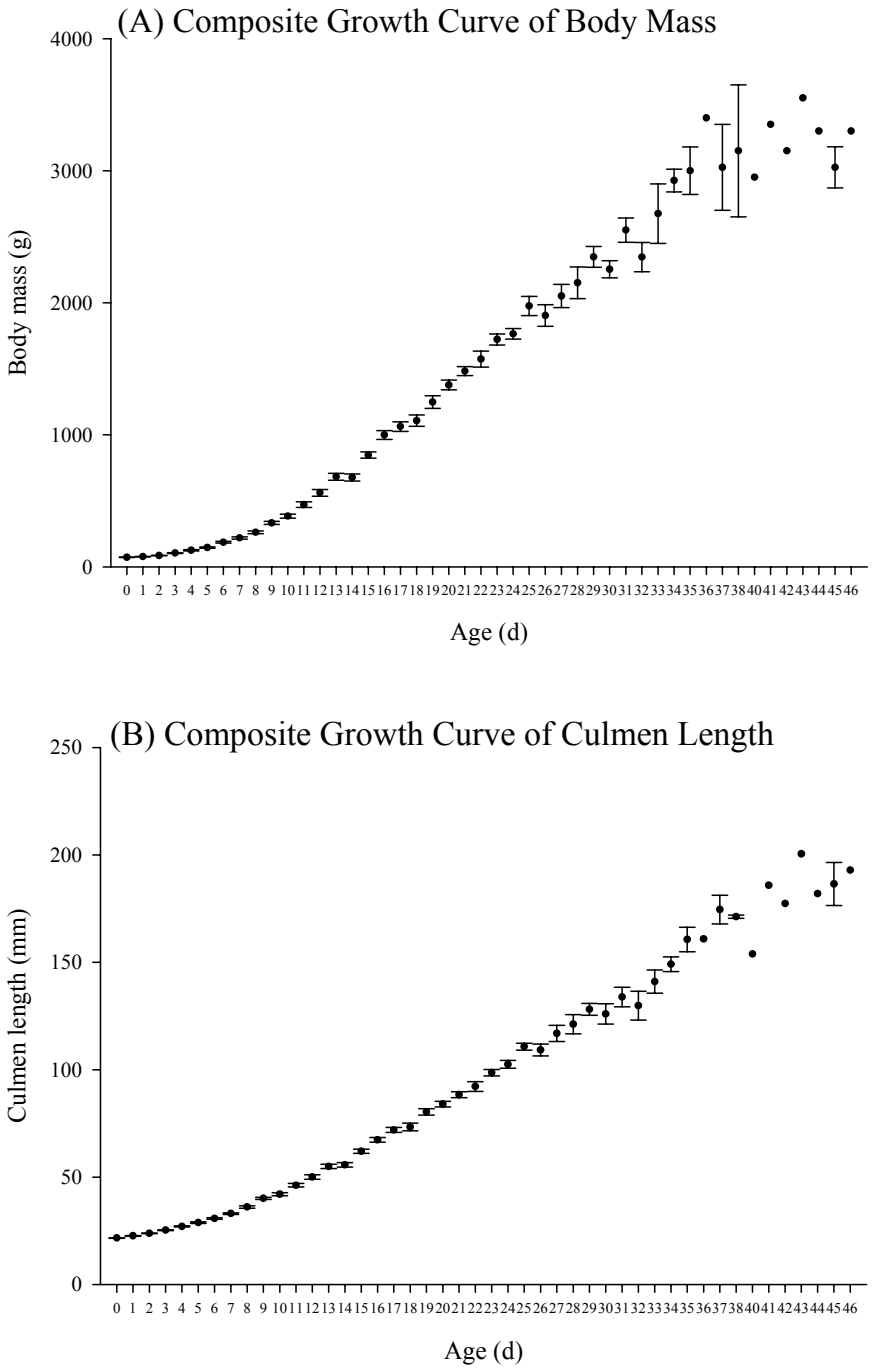

Figure 2.1 Composite growth curve of mean (± SE) (A) body mass and (B) culmen length of Brown Pelican nestlings that survived ≥ 21 days at Marsh Island and Crab Bank, South Carolina in 2004 and 2005. Error bars occur for each day but may be obscured by symbol.

analyzed each colony separately. Data were tested for equal variance and no transformations were necessary. All growth analyses were performed on a reduced sample of nestlings used for LGR comparisons as described above (3 or more checks past 3 days; n = 225); otherwise, I used the entire sample of nestlings (n = 363) monitored and measured throughout the study for analyses. Survival was estimated to 21 d, which was found to be tightly correlated with fledging success of Brown Pelicans in North Carolina (Shields 1998). Means are presented ± standard error. I refer to results as significant when $P \leq 0.05$, and moderately significant when $0.05 < P \leq 0.10$, although actual *P*-values are presented throughout.

Results

I monitored a total of 48 and 55 nests at Marsh Island during the 2004 and 2005 breeding seasons, respectively. In late May 2005, seven study nests (with a total of 11 chicks) were lost to flooding and were replaced with an equal sample to balance treatments. At Crab Bank I monitored a total of 48 nests in both breeding seasons. Soft ticks were common throughout the Marsh Island colony and were present but less common in the Crab Bank colony (Table 2.1). Average tick counts on all nestlings at Marsh Island were significantly greater than those on nestlings at Crab Bank in both years (2004: $t_{191} = -3.56$, $P < 0.0005$; 2005: $t_{168} = -3.50$; $P \leq 0.0006$). Average tick counts on all nestlings did not differ between years at either colony (Marsh Island: $t_{167} = -1.5$, $P = 0.13$; Crab Bank: $t_{192} = 1.2$, $P = 0.23$).

Insecticide treatment during late incubation did not eliminate ticks from pelican nests at either colony. Although differences in tick counts between treatments did occur,

Table 2.1 Average tick counts for all Brown Pelican nestlings measured at Marsh Island and Crab Bank, South Carolina in 2004 and 2005. Mean of average ticks (± SE) counted per individual during all checks are presented for the full sample of nestlings, as well as for only those carrying ticks. The range of average tick counts for infested nestlings is also presented.

Colony	Year	No. of chicks (No. of these chicks with ticks)	Mean ± SE average tick count for all chicks	Mean ± SE average tick count for chicks with ticks	Range of mean average tick counts for chicks with ticks
Marsh Island	2004	95 (43)	6.0 ± 1.67	13.3 ± 3.5	0.2 – 96.8
	2005	74 (52)	11.9 ± 3.86	16.9 ± 5.4	0.1 – 233.7
Crab Bank	2004	98 (10)	0.07 ± 0.03	0.7 ± 0.3	0.2 – 3.0
	2005	96 (7)	0.03 ± 0.01	0.4 ± 0.1	0.1 – 1.0

these varied by year. The mean tick count on chicks from untreated nests at Marsh Island in 2004 (10.4 ± 3.0; n = 50) was greater compared to chicks from insecticide treated nests (1.1 ± 0.8; n = 45; t_{93} = -2.9, P = 0.01). The mean tick count on chicks from untreated nests at Crab Bank in 2004 (0.1 ± 0.1; n = 50) was moderately greater compared to the mean tick count on chicks from treated nests (0.01 ± 0.01; n = 48; t_{96} = -1.8, P = 0.1). Tick average did not differ between treatments at Marsh Island or Crab Bank in 2005 (Marsh Island: t_{72} = 1.4, P = 0.2; Crab Bank: t_{94} = 10.5, P = 0.6). The effect of insecticide treatment in relation to the presence or absence of ticks on nestlings also varied between colonies. There was a significantly greater proportion of parasitized chicks present in untreated nests than in treated nests at Marsh Island (χ^2 = 10.97, P = 0.001). No significant difference in parasite proportion was observed at Crab Bank (P = 0.11).

Growth Rates

Effects of year, plot, nest, treatment, hatch order, and tick load on the LGR of mass and culmen length of Brown Pelican nestlings from Marsh Island and Crab Bank are presented in Table 2.2. The sample of nestlings that fit the criteria for growth rate analyses of mass and culmen length included nestlings with no (Marsh Island: n = 34; Crab Bank: n = 100), low (Marsh Island: n = 40; Crab Bank: n = 13), moderate (Marsh Island: n = 23; Crab Bank: n = 0), and high (Marsh Island: n = 15; Crab Bank: n = 0) tick loads. At Marsh Island, growth rate of mass and culmen length was significantly related to tick load (P < 0.01 for both). At Marsh Island, LGR of mass for nestlings with no ticks (77.0 ± 3.47 g/day; Figure 2.2A) was significantly lower than nestlings with low (87.23 ± 2.62 g/day) and high tick loads (88.72 ± 4.85 g/day; P < 0.04 for both), and moderately

Table 2.2 Effects of select ecological factors on the linear growth rate (LGR) of body mass and culmen length of Brown Pelican nestlings at Marsh Island (n = 112) and Crab Bank (n = 113), South Carolina, in 2004 and 2005. Random variables for all mixed models included plot nested within year; nests nested within treatment, plot, and year; and a treatment and plot within year interaction. *F* and *P*-values for year, plot nested within year, treatment, and treatment and plot within year interaction were determined using appropriate error terms (see Methods). *F* and *P*-values for the remaining variables are listed as they appeared in the final model. *P*-values ≤ 0.05 are in bold and italic print; *P*-values ≤ 0.10 are in bold print.

	Marsh Island		**Crab Bank**	
	Mass F_{df} ; *P*	Culmen F_{df} ; *P*	Mass F_{df} ; *P*	Culmen F_{df} ; *P*
Year	**$4.00_{1,10}$; 0.07**	$2.74_{1,10}$; 0.13	$0.76_{1,10}$; 0.40	$0.31_{1,10}$; 0.59
Plot (Year)	$0.68_{10,11}$; 0.73	$0.62_{10,11}$; 0.77	$1.32_{10,11}$; 0.32	**$2.52_{10,11}$; 0.07**
Treatment	$0.74_{1,11}$; 0.41	$0.82_{1,11}$; 0.39	$1.43_{1,11}$; 0.26	***$6.53_{1,11}$; 0.03***
Treatment * Plot (Year)	$0.92_{11,38}$; 0.53	$0.80_{11,38}$; 0.64	$0.89_{11,33}$; 0.56	$0.42_{11,33}$; 0.94
Nest (Treatment * Year * Plot)	***$3.14_{38,45}$; 0.0001***	***$3.72_{38,45}$; <0.0001***	***$1.88_{33,53}$; 0.02***	***$1.70_{33,53}$; 0.04***
Tick load	***$4.04_{3,45}$; 0.01***	***$5.47_{3,45}$; 0.003***	$0.05_{1,53}$; 0.82	$2.01_{1,53}$; 0.16
Hatch order	***$21.38_{2,45}$; 0.01***	***$39.53_{2,45}$; <0.0001***	***$30.16_{2,53}$; <0.0001***	***$31.06_{2,53}$; <0.0001***
	$R^2 = 0.84$	$R^2 = 0.88$	$R^2 = 0.79$	$R^2 = 0.78$

lower than nestlings with a moderate tick load (83.43 ± 4.81 g/day; $P = 0.09$). LGR of culmen length for nestlings with no ticks (3.49 ± 0.13 mm/day; Figure 2.2B) was significantly lower than nestlings with low (3.94 ± 0.09 mm/day) and moderate tick loads (3.71 ± 0.15 mm/day; $P < 0.04$ for both), and moderately lower than nestlings with a high tick load (3.86 ± 0.14 mm/day; $P = 0.07$). At Crab Bank, LGR of mass and culmen length was not significantly affect by tick load ($P > 0.16$ for both). To test for merely an effect of the presence or absence of ticks at Crab Bank (i.e., given the low tick numbers there) I substituted tick presence (Yes or No) for tick load in the model and found no significant relationship with the LGR of mass or culmen length ($P > 0.15$ for both).

The LGR of mass and culmen length differed significantly among all hatch orders at both colonies ($P < 0.0001$ for all). At Marsh Island, LGR of mass for alpha (n = 59), beta (n = 40), and gamma (n = 13) nestlings was 90.4 ± 2.2 g/day, 79.0 ± 2.9 g/day, and 66.1 ± 6.3 g/day, respectively (Figure 2.3A); LGR of culmen length was 4.04 ± 0.06 mm/day, 3.52 ± 0.10 mm/day, 3.07 ± 0.20 mm/day, respectively ($P \leq 0.002$ for all; Figure 2.3B). At Crab Bank, LGR of mass for alpha (n = 55), beta (n = 42), and gamma (n = 16) nestlings was 90.6 ± 2.0 g/day, 79.5 ± 3.1 g/day, and 55.2 ± 5.0 g/day, respectively; LGR of culmen length was 4.04 ± 0.05 mm/day, 3.65 ± 0.09 mm/day, 2.81 ± 0.17 mm/day, respectively ($P \leq 0.003$ for all). Post hoc t-tests of growth rates by hatch order indicated no significant differences between colonies (alpha: $t_{112} = 0.05$, $P = 0.96$; beta: $t_{80} = 0.12$, $P = 0.90$; gamma: $t_{27} = -1.37$, $P = 0.18$). There was no effect of hatch order on tick average at either colony (Marsh Island: $F_{2,109} = 1.59$, $P = 0.21$; Crab Bank: $F_{2,110} = 0.77$, $P = 0.47$).

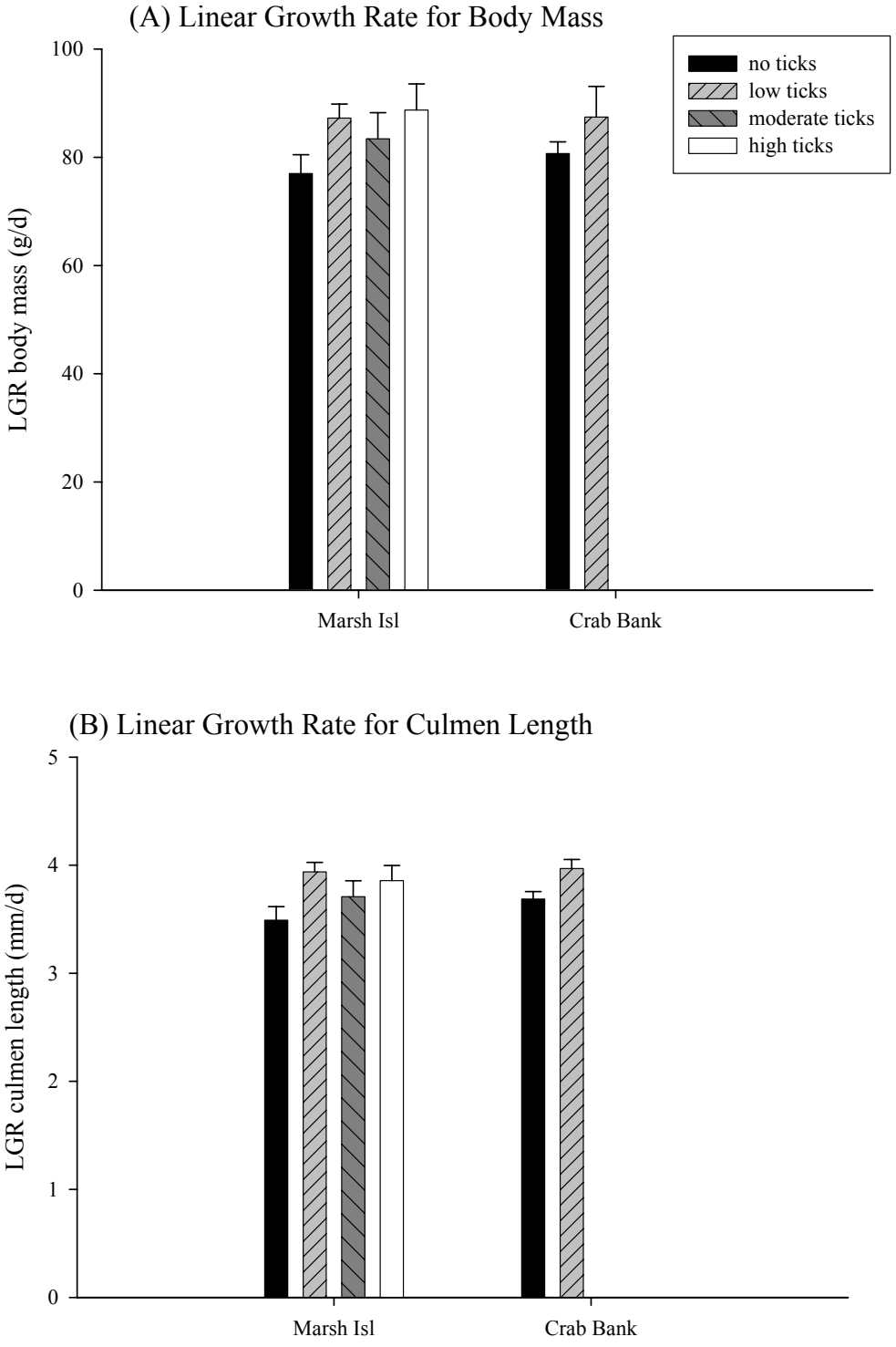

Figure 2.2. Linear growth rate of (A) body mass and (B) culmen length of Brown Pelican nestlings at Marsh Island and Crab Bank, South Carolina, in 2004 and 2005 in relation to tick load. LGR are presented as mean ± SE.

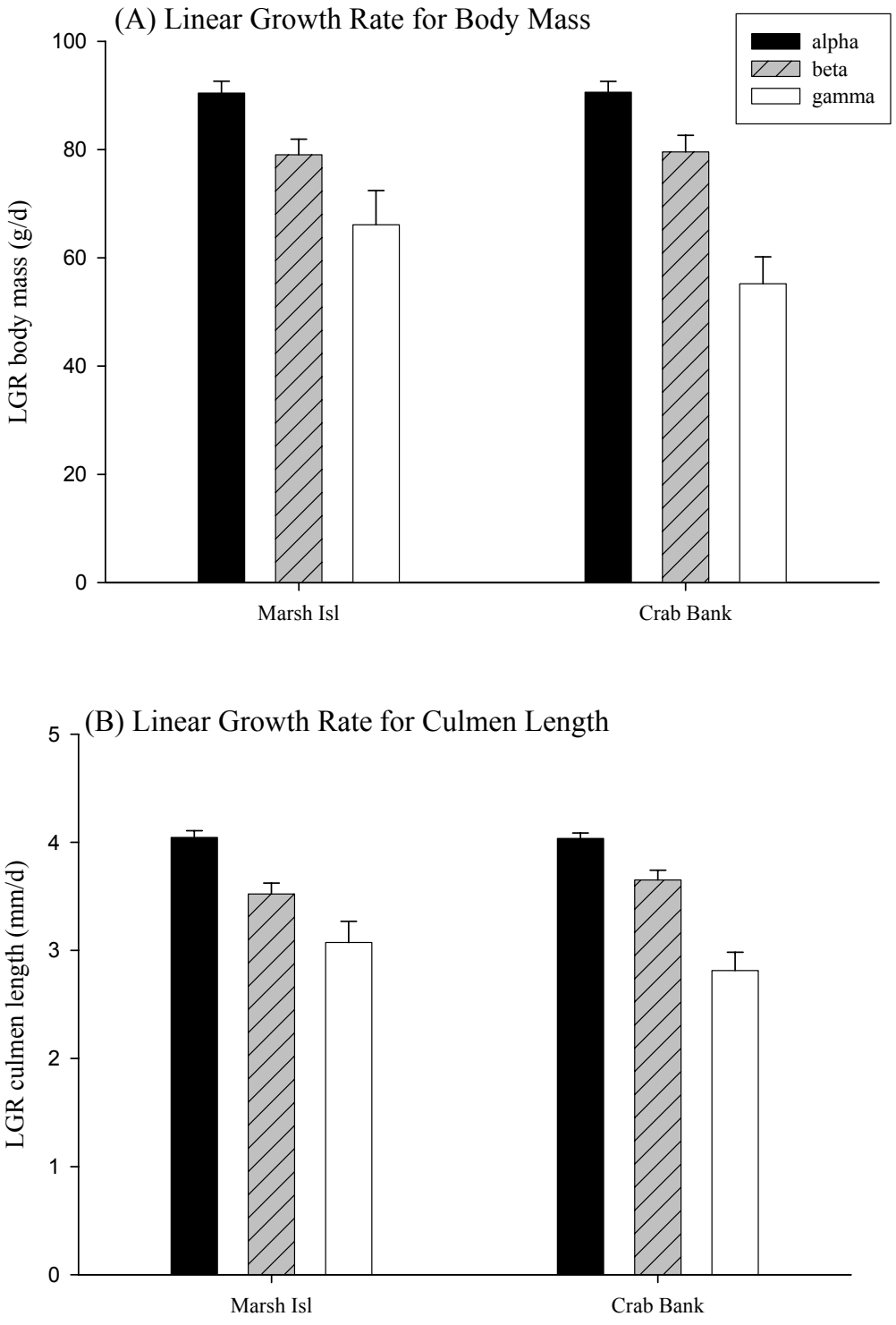

Figure 2.3. Linear growth rate of (A) body mass and (B) culmen length of Brown Pelican nestlings at Marsh Island and Crab Bank, South Carolina, in 2004 and 2005 in relation to hatch order. LGR are presented as mean ± SE.

There was a moderate relationship between LGR and year. The LGR of mass at Marsh Island in 2004 was moderately lower compared to 2005 ($P = 0.07$). Growth of mass and culmen length during the linear phase did not differ between treatments or among plots at Marsh Island. At Crab Bank, LGR of culmen length varied moderately by plot ($P = 0.07$) and significantly by treatment ($P = 0.03$). LGR of mass or culmen length did not differ between colonies within or between years ($P > 0.21$ for all). There was a significant effect of nest, which was included as a random variable in the model, on the LGR of mass and culmen at both colonies.

Because the structure of the model did not allow for inclusion of brood size, I performed a separate test of brood size on LGR using ANOVA. Brood size had no effect on LGR of mass at either colony (Marsh Island: $F_{2,109} = 1.25$, $P = 0.29$; Crab Bank: $F_{2,110} = 1.84$, $P = 0.16$). Brood size had a significant effect on LGR of culmen length at Crab Bank (Crab Bank: $F_{2,110} = 4.33$, $P = 0.02$), and a moderately significant effect at Marsh Island (Marsh Island $F_{2,109} = 2.69$, $P = 0.07$). Growth rates of culmen length for a brood size of three were lower than that for brood size of one for both colonies ($P < 0.1$). There was a moderate significant statistical difference in brood size between colonies ($t_{223} = 1.83$, $P = 0.07$), but this difference was not biologically significant (Marsh Island: 2.41 ± 0.07 eggs/brood; Crab Bank: 2.57 ± 0.05 eggs/brood).

Hatching Success and Survival

Mean clutch size for both colonies and years combined was 2.7 ± 0.4, and did not differ between colonies ($P = 0.85$). The number of eggs that hatched per nest was significantly greater at Crab Bank (2.1 ± 0.1) than at Marsh Island (1.8 ± 0.1; $t_{197} = 2.4$, $P = 0.02$). Within colonies, hatching success was greater in 2004 at Marsh Island (2.40 ±

0.13) than in 2005 (1.5 ± 0.2; $t_{101} = 2.82$, $P = 0.006$), but greater in 2005 at Crab Bank (2.3 ± 0.1) than in 2004 (2.0 ± 0.2; $t_{94} = -2.01$, $P = 0.5$).

Of the total number of nestlings followed in both years, the fate to 21 d was known for 136 nestlings at Marsh Island and 140 nestlings at Crab Bank. Survival was not related to average tick count within or between years at Marsh Island or Crab Bank (t-test: $P > 0.18$ for all). I found 22 nestlings dead at Marsh Island in 2004 and 2005. The number of dead chicks per tick load category was: none = 7, low = 11, moderate = 2, and high = 2. The four chicks found dead at Crab Bank in 2004 and 2005 did not have ticks during any previous nest check.

Discussion

The models I tested explained, on average, 85% of the variability in growth rates of Brown Pelican nestlings from the two study colonies. Nest effect, which has not been included as an explanatory term in many growth studies, explained a significant degree of variability within each colony during the early period of chick growth examined here. Variability in growth rates among nestlings may be due in large part to attributes that are unique to each nest. Nonetheless, growth rates for mass and culmen length did not differ between the study sites despite dissimilarity between colonies in the adjacent marine habitat, nesting history, level of human disturbance, and level of tick infestation.

Composite growth curves derived from measurements of mass and culmen length of Brown Pelican nestlings from both Marsh Island and Crab Bank were similar to those from nestlings measured in Florida over four years (Schreiber 1976). Though growth rates differed among hatch orders during early development in this study, Schreiber (1976) found that the shape of complete growth curves did not vary among hatch orders

for those nestlings that fledged. Comparison of growth rates among hatch orders measured over a longer period may mask the differences observed during early development in the present study. In younger siblings especially, survival of chicks that fledge in poor condition (i.e. smaller body size, fewer fat reserves) or relatively late in the breeding season may be compromised (Perrins et al. 1973; Coulson and Porter 1985) if growth rates do not increase later in development or if an extended developmental period is required for younger chicks to fledge.

Previous studies that have examined the growth of Brown Pelican nestlings make no mention of ectoparasite presence at the colonies or on nestlings (Schreiber 1976; Ploger 1992; Shields 2000), though colonies in these systems may have been infested with soft ticks (Wilkinson et al. 1994). Norcross and Bolen (2002) investigated the effect of soft tick infestation on nestling survival at ground nesting colonies of Brown Pelicans in North Carolina in relation to insecticide treatment, though they did not measure growth rates. In that system, soft tick infestation did not affect survival measured to two weeks, which is similar to results observed over approximately three weeks of development in this study. Because survival to 21 days is positively correlated with fledging success (Shields 1998), fledging likely was not compromised by the levels of tick infestation observed here. However, cumulative effect of ticks on development and survival may not be manifest until later developmental stages (Moller 1993; Simon et al. 2004), and, therefore, may not have been detected in either study. Future research that measures growth metrics, tick infestation levels, and survival of pelican nestlings throughout the entire duration of development would further elucidate the effect of ticks on the development and breeding success of Brown Pelicans.

Ectoparasite infestation of nest-bound chicks may result in depletion of energy or nutrients that are crucial during development. Several studies have reported higher incidence of mortality and/or reduced rates of growth for parasite infested nestlings compared to those without parasites. For example, Cattle Egret (*Ardeola ibis*) nestlings infested with avian ticks had a lower probability of survival and reduced body mass during the later stages of development compared to tick-free chicks (McKilligan 1996). In the current study of Brown Pelican nestlings, tick load was significantly related to linear rates of growth for both mass and culmen length at Marsh Island where ticks were common in both years. I found a positive relationship between growth rates of pelican nestlings and tick loads that was consistent between years. At least 8 parasitized nestlings died early in development and could not be included in the Marsh Island growth rate sample. These nestlings were included in survival analyses, however, and there was no difference in survival for infested and parasite-free nestlings. Although tick load affected growth rates at Marsh Island but not Crab Bank, growth rates did not differ between the colonies. This suggests that at the observed levels of infestation ticks did not impact growth of pelican nestlings at the population level.

The positive relationship I observed between tick loads and growth rates of pelican nestlings at Marsh Island is somewhat unprecedented and the causal mechanism remains unclear. Few examples exist of a positive or null effect of ectoparasite load on nestling growth (but see Loye and Carroll 1991; Rogers et al. 1991). Examinations of the effect of ectoparasites on growth rates, however, have typically been conducted on colonial swallows and passerines that were infested with different ectoparasites than studied here. Here I suggest three mechanisms that may explain the positive relationship

observed in this study: (1) ticks benefit nestlings during development and hence positively affect growth rates, (2) ticks select the best quality hosts and hence growth rates are positively correlated with tick load, or (3) nestlings are compensated for resources lost to ticks and hence growth rates are positively correlated with tick load. Each suggestion is explored in turn.

Ectoparasites are, by definition, harmful to their hosts and so it would appear counterintuitive to suggest that any benefits of parasitism exist for the host. Recurring exposure to a sublethal infestation feeding larva ticks such as those in this study can result in acquired immunity for the host which may provide protection against acute viral infection during development and perhaps as adults (Hoogstraal 1985). Brown Pelican chicks from dense ground-nesting colonies likely have a relatively high probability of contact with an infectious agent not only from the numerous internal and external parasites known to be associated with the species (Dyer et al. 2002; Shields 2002), but also from the proximity to conspecifics within colonies. Soft tick infested nestlings in our study may have acquired some level of immunity as suggested by Hoogstraal (1985) and hence may not have suffered reduced growth rates stemming from other infectious agents.

A second hypothesis to consider is that tick infestation is in fact a consequence of improved body condition. Under such a scenario ticks would select chicks in better condition (i.e. higher growth rates) because those individuals present a more stable and faithful host. This would result in a positive relationship between tick loads counted on nestlings and growth rates, as was observed. I also observed a similar pattern in an investigation of levels of the stress hormone corticosterone in relation to tick infestation

(see Chapter 3). Brown Pelican nestlings in better physiological condition had moderate tick loads while nestlings in poor physiological condition had no ticks. Each of these results contradict the expected results of the Tasty Chick Hypothesis, which suggests that ectoparasites target the last hatched nestling in asynchronous broods and thereby increase the potential for growth and survival of older siblings (Christe et al. 1998).

Parasitized pelican nestlings at Marsh Island were clearly not allocating resources away from growth. Nutrients and energy resources necessary for growth and survival lost to soft ticks may be compensated for via increased food intake, which would require greater effort on the part of pelican parents. Enhanced begging by parasitized nestlings might stimulate parents to increase provisioning rates (Christe et al. 1996). Abundant food availability can mask the effects of ectoparasites on nestling growth, as demonstrated by the effect of ectoparasites on metabolic measures and growth of nestling Blue Tit (*Parus caeruleus*; Simon et al. 2004). However, because there may be a negative relationship between energy expenditure and adult survival (Golet et al. 2000), parents should only increase provisioning effort when the probability of accruing a benefit increases. This suggests that parents may compensate for costs of infestation when food availability is high but may not when food availability is low. Measures of food quality, provisioning rates, begging behavior, and ectoparasite loads on Brown Pelican nestling growth and condition would provide a test of this hypothesis.

The level of infestation that occurred during these study years may not have been great enough to exert a large impact on the reproductive success of the colony. Though several nests at Marsh Island were deserted both seasons presumably from heavy ectoparasite infestation, large-scale abandonment that has been observed within South

Carolina (Wilkinson et al. 2004; Ferguson pers. obs.) and elsewhere (King et al. 1977a, b; Duffy 1983; Norcross and Bolen 2002) did not occur at either colony in either year. While treatment was not consistently related to growth rates, annual insecticide treatment of colonies may keep tick populations below a threshold level that triggers nest abandonment. Norcross and Bolen (2002) found that a similar course of treatment decreased levels of soft tick infestation in Brown Pelican nests in North Carolina. Reduced tick averages on nestlings from treated nests at both colonies in 2004 indicate that treatment may be effective in limiting tick populations, but the lack of a significant finding in 2005 suggests that other factors may play a role.

For example, Danchin (1992) showed that tick populations at seabird colonies can increase with colony age. *O. capensis* was first identified in high numbers at pelican colonies in South Carolina in 1987 (Keirans et al. 1992), though it is unknown when colonies were first infested. Nestlings at Marsh Island, which has supported a pelican colony since the 1940s, had higher tick averages than nestlings at Crab Bank, which was first colonized by pelicans in 1994 (Jodice et al. in review). Colony age may account for the higher tick averages on nestlings at Marsh Island compared to Crab Bank. Nevertheless, growth rates were not negatively affected by the levels of tick infestation observed at these colonies during this study. The long-term effects of soft ticks on breeding Brown Pelicans in South Carolina, however, remain unclear.

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CHAPTER 3

ADRENOCORTICAL RESPONSE OF BROWN PELICAN NESTLINGS TO ECTOPARASITE INFESTATION

Introduction

Ectoparasite infestation is one of the main costs of coloniality (Rothchild and Clay 1952; Moller 1990; Coulson 2002). The relationship between avian breeding biology and ectoparasite infestation has been well studied. Negative effects of ectoparasites on incubation duration, nestling growth rates, nestling survival, nest abandonment, and breeding site fidelity have been documented across a range of taxa (King et al. 1977a, b; Duffy 1983; Chapman and George 1991; Moller 1993; McKilligan 1996; Morbey 1996; Ramos et al. 2001). Each of the aforementioned biological or ecological reactions, however, likely represents the expression of an underlying physiological response by adults or nestlings to the presence and activity of the ectoparasites. For example, recent studies indicate that nestlings respond to ectoparasites as stressors that can trigger a physiological stress response that in turn may contribute to reduced individual condition or behavioral modifications as noted above (Kitaysky et al. 2001a; Quillfeldt et al. 2004; Raouf et al. 2006). Whether in adults or nestlings, this stress response is activated via the hypothalamic-pituitary-adrenal (HPA) axis, which ultimately results in the synthesis and secretion of the steroid hormone corticosterone. The secretion of this hormone may play a critical role in avian breeding biology and ultimately in avian population dynamics.

The functional role of corticosterone is to maintain homeostasis (Sapolsky 2002). Following a stress event, increased amounts of the hormone are secreted from the adrenal cortex in response to adrenocorticotropic hormone (ACTH). Release of corticosterone stimulates a mobilization of energy and modification of behaviors which facilitate survival. For example, Black-legged Kittiwake (*Rissa tridactyla*) nestlings exposed to a period of food deprivation exhibited elevated levels of corticosterone and increased rates of begging (a behavior which stimulates parental feeding) compared to nestlings that were not food limited (Kitaysky et al. 2001b). Physiological and behavioral adjustments resulting from enhanced corticosterone levels shift energy away from maintenance activities to those which promote immediate survival and an eventual return to a balanced state. However, in situations where a stressor persists and corticosterone levels remain high, the adrenocortical response may work to the detriment of the individual. Suppression of the immune system, catabolism of muscle (Wingfield et al. 1997; Sapolsky 2002), and impaired cognitive development of nestlings (Kitaysky et al. 2003) are all possible consequences of chronically elevated corticosterone.

Because the ultimate outcome of the adrenocortical stress response (i.e., the release of corticosterone) is generally the same regardless of the stressor (Siegel 1980), measurement of corticosterone has become a useful tool for assessing the reaction of individuals to ecological stressors of interest. Corticosterone measurements are also valuable for establishing baseline values of the physiological condition of populations of concern (Wikelski and Cooke 2006). Field techniques for measuring corticosterone and the adrenocortical stress response in birds are well developed (Wingfield et al. 1992). Initial blood samples collected upon capture (typically within 3 minutes) represent the

circulating amount of corticosterone in the plasma, and are regarded as a baseline sample. Additional blood samples are then collected as the adrenocortical response to capture and handling is realized. Together, these additional samples provide a measure of the sensitivity of the stress response to acute stressors. Individuals with a low adrenocortical response to a period of capture and handling are assumed to be in good condition and have little prior exposure to acute stressors (Wingfield et al. 1997).

Of the research conducted to date on the adrenocortical stress response in birds, few examples exist for altricial young that are restricted to the nest bowl and dependent upon parental care for extended periods. Among altricial young, the manifestation of an adrenocortical response to stressors appears to be inconsistent. For example, Blas et al. (2005) found that corticosterone levels in nestlings of the European White Stork (*Ciconia ciconia*) increased in response to capture stress. In contrast, Sims and Holberton (2001) found no evidence of an adrenocortical response to a capture stress protocol in very young Northern Mockingbird (*Mimus polyglottos*) nestlings. Details of the maturation process of the HPA axis and the benefits of a stress response in altricial young, therefore, remain unresolved (Schwabl 1999; Sims and Holberton 2001; Walker et al. 2005). Because negative impacts associated with chronic elevation of corticosterone in nestlings may lead to reduced survival, measurement of the physiological condition of nestlings may offer a useful means of assessing the potential impacts of ecological stressors on the breeding biology of colonial birds. The goal of this study was to examine the adrenocortical response of altricial nestlings exposed to ectoparasite infestation, which I assumed acted as a chronic ecological stressor.

I studied the effects of the soft tick *Ornithodoros capensis* on the adrenocortical stress response of altricial Brown Pelican (*Pelecanus occidentalis*) nestlings in South Carolina. The breeding population of Brown Pelicans in South Carolina is experiencing a 15 year decline (Figure 1.2; Jodice et al. in review). All breeding colonies in the state are infested with soft ticks, a nest parasite first identified in the state in 1987 (Keirans et al. 1992; Wilkinson et al. 1994). Most pelicans in South Carolina are ground-nesters and young remain in the nest for at least the first three weeks of post-natal development; hence exposure to soft ticks is likely great. My first objective was to determine whether Brown Pelican nestlings exhibited a stress response while nest-bound. If Brown Pelican nestlings respond to nest parasites as stressors, chronic exposure to parasitism may have long-term health consequences as noted above. Therefore, it would not be advantageous for altricial young to respond to stressors they are unable to avoid or escape with an increase of corticosterone (Sims and Holberton 2001). In contrast, though altricial nestlings may not be physically able to evade the stressor (i.e., tick infestation in this study), a functional adrenocortical response may allow nestlings to offset resources siphoned by ticks through an increase in available energy or changes in behavior. The second objective was to examine how various aspects of the adrenocortical response of pelican nestlings were affected by tick infestation and other ecological factors influencing their development while in the nest, such as brood size, hatch order, and body condition.

Methods

Study Organisms

The Brown Pelican is a large marine bird that breeds colonially on off-shore islands. In South Carolina, Brown Pelicans are typically ground-nesters with 3 egg

clutches. Young hatch asynchronously, with the first nestling hatching on average one day before the second chick and three days before the third chick. First hatched nestlings usually maintain a dominant rank in the brood through aggressive behavior, and siblicide is not uncommon (Pinson and Drummond 1993; Shields 2000). Nestlings are altricial and dependent on parental care throughout the 11 weeks of their development. Approximately 10 days after hatching nestlings begin to develop feathers, and by 3 weeks of age may venture from the nest and form crèches with neighboring chicks.

The soft tick *O. capensis* is a common ectoparasite of a variety of marine birds, including adult and nestling Brown Pelicans (Hoogstraal 1985; Duffy 1983). *O. capensis* larvae are slow feeders and may attach to their host for several days at a time, whereas nymphal instars and adults only feed for approximately one hour (Hoogstraal 1985). All stages of *O. capensis* live in nesting material of Brown Pelicans and counts may number in the thousands (King et al. 1977a; Norcross and Bolen 2002). Nest-bound pelicans can therefore be repeatedly exposed to multiple stages and high densities of soft ticks. Hereafter, any mention of soft ticks refers to *O. capensis* unless stated otherwise.

Study Sites

I sampled Brown Pelican nestlings at the Marsh Island colony and the Deveaux Bank colony in South Carolina in 2005 (Figure 1.1). Marsh Island (32°59'N, 79°32'W) is a 19 ha island approximately 4.6 km off the coast of Awendaw, Charleston County, South Carolina, within the boundaries of Cape Romain National Wildlife Refuge (CRNWR). Deveaux Bank (32°32'N, 80°10'W) is a 87 ha island located in the mouth of the North Edisto River and is managed by South Carolina Department of Natural Resources (SCDNR) as a Heritage Site. In 2005, 611 and 1,575 Brown Pelican nests

were counted on Marsh Island and Deveaux Bank, respectively (Jodice et al. in review). Both colonies are infested with *O. capensis*.

Pelican nests at Marsh Island and Deveaux Bank are treated annually with approximately 175 ml of a 0.5% dilution of Rabon® 50 WP insecticide (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) in an attempt to control the population of *O. capensis*. In 2005, as in previous years, nests at Marsh Island and Deveaux Bank were hand-sprayed during the third week in May corresponding with peak incubation. Spraying occurred when winds were light and the immediate weather forecast predicted little to no chance of precipitation. Prior to spraying, I randomly selected 39 nests at Marsh Island and 50 nests at Deveaux Bank to remain unsprayed for the purposes of this study; a balanced sample of sprayed nests was also randomly selected at this time to serve as study nests. These study nests occurred throughout the colony in plots that were representative of used nesting habitat and also were used as part of an experiment investigating chick growth rates and tick infestation levels (see Chapter 2). Nests were therefore categorized as Treated (single insecticide application) or Untreated (no insecticide application).

Marsh Island also served as a study site for the aforementioned growth study and many nestlings there were regularly exposed to researcher handling. To account for the potential effect of regular handling on the stress response, a sample of the selected study nests at Marsh Island were not exposed to regular handling by researchers. Therefore, nests are also defined as High Contact (nestlings handled at regular intervals) and Low Contact (nestlings handled infrequently by researchers). Deveaux Bank was not used for the growth study; therefore all nestlings at this colony were considered Low Contact.

Field Procedures

Sampling for the stress response occurred on 22 and 24 June 2005 at Marsh Island and on 27 June 2005 at Deveaux Bank. Sampling was timed so the majority of nestlings were in the second or third week of development. Two groups of researchers handled nestlings within a colony simultaneously to limit sampling hours to approximately 0700-1300 and thus control for possible diel fluctuations in corticosterone. Prior to entering the colony, I established a sequence for sampling nestlings based on nest location within each colony. This sequence allowed researchers to minimize disturbance to nestlings prior to sampling and avoid sections of the colony that would be sampled later that day. I only selected 1st and 2nd hatched nestlings, and siblings were sampled on separate days whenever possible to reduce the chance of parental abandonment caused by the removal of all nestlings. Nestlings in this study (n = 49) ranged in age from 11-23 days. Within this age range, nestlings are still nest-bound, are developing a cover of down feathers, and have coordinated muscle movement (Shields 2002).

I used a standardized capture stress protocol (Wingfield et al. 1992) to measure both baseline levels of corticosterone and the stress response of nestling Brown Pelicans. I collected approximately 100µl of blood from each nestling immediately after removal from the nest (≤ 4 min post-capture; mean = 1.98 ± 0.15 min) by puncturing a wing vein with a sterilized 25-gauge needle. These samples represent baseline corticosterone levels of nestlings. I found no significant effect of time until the initial sample was collected on the log transformed level of corticosterone (simple linear regression; $F_{1,47} = 0.09$; $P = 0.8$) and, therefore, categorized all initial samples as 'baseline'. Nestlings were then held in individual containers for collection of subsequent blood samples at 30 and 50 minutes

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 same 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.

After the final blood sample was collected, I measured body mass (electronic scale if < 1000g; 2500 ± 20g spring scale if > 1000g), culmen length (dial calipers ± 1mm), wing length (wing bar ± 1mm), and tick load of each chick before returning them to their nests. Tick load was determined by counting the total number of ticks on the neck and pouch of all nestlings (see Chapter 2). Larval and nymph stages were difficult to distinguish in the field and were therefore grouped together for the total count; adult ticks were not observed on nestlings. Tick loads were then categorized as follows: None (0 ticks), Low (1-10 ticks), Moderate (11-50 ticks), and High (> 50 ticks). These categories were based on infestation loads observed in a larger sample of nestlings included in the aforementioned growth study (see Chapter 2). Similar to Duffy and de Duffy (1986), I assumed that the number of ticks counted on nestlings was representative of the overall tick population within the nest. All structural measurements and tick counts were performed by the same researcher (LF).

Several nestlings sampled from growth study nests were of known age (n = 11). For the remaining nestlings (n = 38), age was estimated using the model (age = 14.13*ln culmen - 42.85). This model is based on a composite growth curve of 54 known age nestlings measured at two South Carolina colonies during 2004 and 2005 (see Chapter 2). The accuracy of this model to ± 1 day and ± 2 days was 76% and 96%, respectively.

Laboratory Procedures

Corticosterone concentration of plasma samples was measured by radioimmunoassay (based upon Wingfield et al. 1992). Dichloromethane was first used to extract corticosterone from plasma samples (range: 9-27µl). Antiserum and tritiated corticosterone were then added to extractions prior to radioimmunoassay. To determine amount of corticosterone in our samples, I 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%; interassay variation was 3.8%.

Statistical Analysis

Statistical analyses were designed to address three questions: (1) Did Brown Pelican nestlings demonstrate an elevation in corticosterone during the 50-min handling session (i.e., was there a stress response)? (2) Did any aspect of the study design (e.g. colony, age, insecticide treatment, time of day) have a significant effect on corticosterone levels? (3) Did any of the ecological factors we considered (e.g. hatch order, brood size, tick load, body condition index) have a significant effect on corticosterone levels? The analysis procedure for each is briefly described below.

To determine if nestlings demonstrated a stress response to the handling protocol I compared the mean corticosterone levels among baseline, 30-min, and 50-min samples. Data were analyzed separately by colony using a one-way analysis of variance (ANOVA) followed by Tukey multiple comparison procedure. A significant increase in corticosterone by the 50-min sample was interpreted as an active stress response.

I assessed the effects of various study design and ecological factors (i.e., questions two and three above) on the stress response by examining three measures of corticosterone: baseline, peak, and magnitude. The baseline measurement is the corticosterone concentration from the first sample collected for all nestlings within 4 minutes of capture. The peak measurement is the highest corticosterone level reached for each individual at the 30 or 50 minute sample. The magnitude measurement is the difference between the maximum and minimum corticosterone concentration for each individual regardless of the time it occurred. I found no difference in any of these measures between high and low contact nestlings at Marsh Island (two sample t-test for means; $t_{27} \leq 0.9$, $P \geq 0.3$ for each measure) and, therefore, pooled these groups for all subsequent analyses.

To assess the influences of design and ecological factors on corticosterone, I conducted a series of mixed models (SAS Version 9.1, SAS Institute Inc., Cary, NC, USA) whereby each of the three measures of the adrenocortical stress response were analyzed separately (Wingfield et al. 1997). I used the same procedure for both the study design and the ecological model. Nest was included in all models as a random variable. Other explanatory variables were included as fixed effects or as covariates. I also included a suite of biologically meaningful two-way interactions. I did not include all two-way interactions because of sample size restrictions. I used a manual backward selection process and, at each step, eliminated the two-way interaction with the highest *P*-value ≥ 0.15. I continued in this manner until either all interaction terms were eliminated or until remaining interaction terms had a *P*-value < 0.15.

The initial model testing for study design effects included the fixed effects treatment and colony, age, and the time of day the nestling was captured. Because of a significant difference in baseline corticosterone between colonies ($F_{1,15} = 6.0$, $P = 0.03$), the final or ecological model was run separately by colony. The final model included the fixed effects tick load, hatch order, and brood size. Body condition index, calculated as the residual of the linear relationship between mass and culmen length, was also included as a covariate. This relationship met the assumptions of a strong linear relationship ($r = 0.98$, $P < 0.0001$) and no correlation with any other size metric (wing: $r = 0.09$, $P = 0.5$), as explained in Green (2001). Tick load was not included in the final model for Deveaux Bank because only two of 20 nestlings harbored ticks (1 and 8 ticks each). I assessed differences among levels of significant variables in all mixed models using Tukey multiple comparison procedures. Response variables (concentrations of corticosterone in μg/ml) were log transformed for all analyses. Mean and regression coefficients are presented as log transformed values ± 1 standard error unless otherwise noted. I refer to results as significant when $P \leq 0.05$, and moderately significant when $0.05 < P \leq 0.10$, although actual *P*-values are presented throughout.

I also examined the relationship between corticosterone levels and growth rates (see Chapter 2) for those chicks where measures for each were available. I conducted an ANCOVA using each of the 3 measures of corticosterone as dependent variables and included as independent variables a measure of growth rate, hatch order, and the interaction of growth rate and hatch order. I ran separate ANCOVAs for the linear rate of growth measured in body mass and in culmen length (see Chapter 2).

Results

Tick Infestation

Soft ticks occurred throughout the Marsh Island colony. Ticks were observed on 63 of the 84 Brown Pelican nestlings (75%) checked during growth measurements in 2005 (see Chapter 2). At the time of sampling on Mash Island, 13 nestlings had no ticks present, and 16 nestlings were parasitized with ticks (range: 1-153). Of these, eight nestlings had a low tick load (1-10 ticks), two had a moderate tick load (11-50 ticks), and six had a high tick load (> 51 ticks). Tick load varied significantly with body mass, culmen length, and age ($F_{3,25} > 3.5$, $P < 0.03$ for each). Compared to nestlings with no ticks, nestlings carrying a high tick load had greater mass (1353.3 ± 143.7 g vs. 786.0 ± 61.3 g), longer culmen (82.5 ± 5.6 mm vs. 59.0 ± 2.5 mm), and were older (19.0 ± 0.9 d vs. 14.8 ± 0.7 d). Although large sections of the Deveaux Bank colony had extremely high levels of tick infestation and abandoned nests during the 2004 breeding season, this was not the case during 2005. Only two nestlings sampled at Deveaux Bank were infested by soft ticks (1 tick and 8 ticks per individual; n = 18 non-parasitized nestlings).

Stress Response

There was a significant increase in corticosterone during the 50 minute handling protocol at both Marsh Island ($F_{2,84} = 27.5$, $P < 0.0001$) and Deveaux Bank ($F_{2,57} = 8.3$, $P < 0.0007$; Figure 3.1). At Marsh Island, corticosterone differed significantly and positively between each sampling time ($P < 0.05$ for each; Figure 3.1A). At Deveaux Bank, the baseline and 50 minute samples differed significantly ($P < 0.05$; Figure 3.1B).

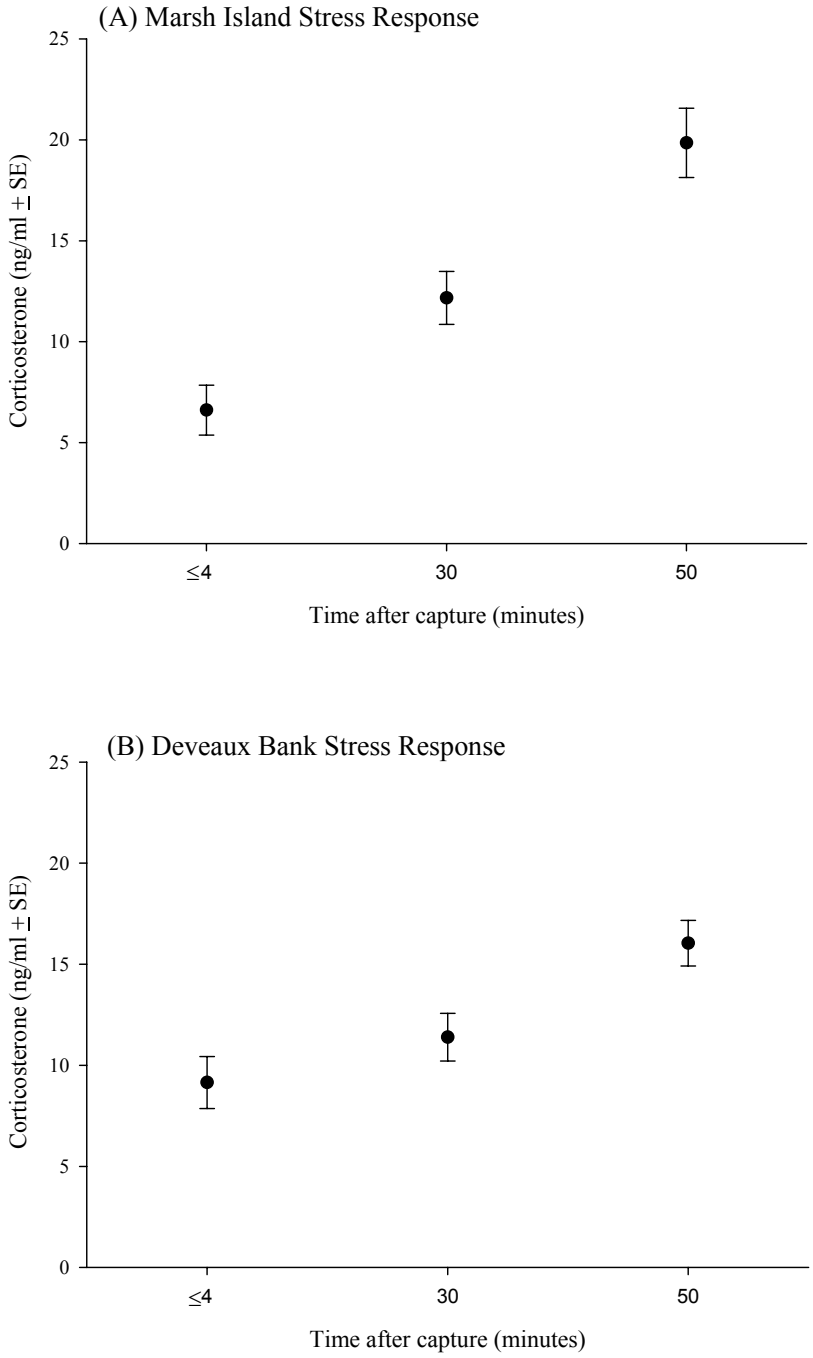

Figure 3.1. Mean corticosterone levels (ng/ml ± SE) of Brown Pelican nestlings at (A) Marsh Island and (B) Deveaux Bank, South Carolina, June 2005, at three sampling intervals: ≤ 4 minutes, 30 minutes, and 50 minutes post capture. The rise in corticosterone over the 50 minute handling period represents the stress response.

Study Design Effects

Baseline corticosterone differed significantly by colony (Table 3.1). Nestlings at Deveaux Bank had significantly higher baseline corticosterone (log corticosterone = 0.91 ± 0.08 ng/ml) compared to those at Marsh Island (log corticosterone = 0.63 ± 0.07 ng/ml). The peak and magnitude of the stress response did not differ, however, by colony ($P > 0.1$; Table 3.1). Age (which differed between colonies: Marsh Island = 16.07 ± 0.60 days; Deveaux Bank = 19.70 ± 0.53 days; $t_{47} = 4.26$, $P < 0.0001$), treatment, and time of day did not significantly affect baseline, peak, or magnitude measurements ($P > 0.1$ for each; Table 3.1). All subsequent analyses were conducted separately by colony.

Primary Effects

Effects of tick load, body condition index, hatch order, and brood size on the three measures of corticosterone in pelican nestlings at Marsh Island and Deveaux Bank are presented in Tables 3.2 and 3.3, respectively. Tick load (only measured at Marsh Island) had a significant effect on the magnitude of the stress response ($F_{3,5} = 6.32$, $P = 0.04$) and was greater for nestlings with no ticks (log corticosterone = 1.02 ± 0.10 ng/ml) compared to those with a moderate tick load (log corticosterone = 0.52 ± 0.61 ng/ml; $t_5 = -3.91$, $P = 0.01$). Corticosterone was negatively related to body condition index (BCI), although BCI affected different measures of the stress response at the two colonies (Figure 3.2). At Marsh Island, the magnitude of the stress response was significantly and negatively related to BCI (coefficient = -0.003 ± 0.001; $F_{1,5} = 9.0$, $P = 0.03$; Table 3.2) while peak corticosterone showed a moderate negative relationship with BCI (coefficient = -0.001 ± 0.001; $F_{1,5} = 4.9$, $P = 0.08$). At Deveaux Bank, baseline corticosterone was significantly and negatively affected by BCI (coefficient = -0.003 ± 0.001; $F_{1,6} = 26.0$, $P = 0.002$).

Table 3.1 Effects of study design on baseline, peak, and magnitude of LOG corticosterone concentration in altricial Brown Pelican nestlings (n = 49) at Marsh Island and Deveaux Bank, South Carolina, June 2005. Data were analyzed using mixed models. *F* and *P*-values for all main variables are listed as they appeared in the final model. Also included are *F* and *P*-values for two-way interaction terms prior to removal using manual backward selection. Nest was included as a random variable for all models. *P*-values ≤ 0.05 are in bold and italic print; *P*-values ≤ 0.10 are in bold print.

	Corticosterone (F_{df}; *P*)		
Effect	Baseline	Peak	Magnitude
Colony	***$6.00_{1,15}$; 0.03***	$0.44_{1,15}$; 0.52	$2.86_{1,15}$; 0.11
Treatment	$2.65_{1,15}$; 0.36	$0.70_{1,15}$; 0.42	$2.18_{1,15}$; 0.16
Time of day	$0.23_{1,15}$; 0.95	$1.06_{1,15}$; 0.32	$0.54_{1,15}$; 0.47
Age	$4.13_{1,15}$; 0.20	$0.09_{1,15}$; 0.76	$0.74_{1,15}$; 0.40
Colony * Treatment	$1.11_{1,14}$; 0.31	$0.07_{1,15}$; 0.80	$0.24_{1,14}$; 0.64
Age * Treatment	$1.30_{1,14}$; 0.27	$0.01_{1,13}$; 0.93	$0.23_{1,13}$; 0.64
Age * Colony	$0.36_{1,13}$; 0.56	$0.07_{1,14}$; 0.80	$0.66_{1,14}$; 0.43

Table 3.2 Effects of select ecological factors on baseline, peak, and magnitude of LOG corticosterone concentration in altricial Brown Pelican nestlings (n = 29) at Marsh Island, South Carolina, June 2005. Data were analyzed using mixed models. *F* and *P*-values for all main variables are listed as they appeared in the final model. Also included are *F* and *P*-values for two-way interaction terms prior to removal using manual backward selection. Nest was included as a random variable for all models. *P*-values ≤ 0.05 are in bold and italic print; *P*-values ≤ 0.10 are in bold print.

	Corticosterone (F_{df} ; *P*)		
Effect	Baseline	Peak	Magnitude
Tick load	$0.37_{3,5}$; 0.78	$1.84_{3,5}$; 0.26	***$6.32_{3,5}$; 0.04***
Body condition index (BCI)	$0.08_{1,5}$; 0.79	**$4.90_{1,5}$; 0.08**	***$8.96_{1,5}$; 0.03***
Hatch order	$0.04_{1,5}$; 0.84	$0.21_{1,5}$; 0.67	**$5.01_{1,5}$; 0.08**
Brood size	$0.87_{2,5}$; 0.47	$0.57_{2,5}$; 0.60	$0.41_{2,5}$; 0.68
Brood size * Tick load	$0.21_{4,1}$; 0.91	$1.10_{4,4}$; 0.46	$0.20_{4,1}$; 0.91
Brood size * BCI	$0.66_{2,1}$; 0.66	$0.92_{2,4}$; 0.47	$0.48_{2,1}$; 0.71
Hatch order * Tick load	$1.03_{2,2}$; 0.49	$0.20_{2,2}$; 0.84	$2.33_{2,3}$; 0.24
Hatch order * BCI	$0.94_{1,4}$; 0.39	$0.02_{1,1}$; 0.91	$1.03_{1,2}$; 0.42

Table 3.3 Effects of select ecological factors on baseline, peak, and magnitude of LOG corticosterone concentration in altricial Brown Pelican nestlings (n = 20) at Deveaux Bank, South Carolina, June 2005. Data were analyzed using mixed models. *F* and *P*-values for all main variables are listed as they appeared in the final model. Also included are *F* and *P*-values for two-way interaction terms prior to removal using manual backward selection. Nest was included as a random variable for all models. *P*-values ≤ 0.05 are in bold and italic print; *P*-values ≤ 0.10 are in bold print.

	Corticosterone (F_{df} ; *P*)		
Effect	Baseline	Peak	Magnitude
Body condition index (BCI)	***$26.01_{1,6}$; 0.002***	$1.91_{1,6}$; 0.22	$0.19_{1,6}$; 0.68
Hatch order	$0.59_{1,6}$; 0.47	$1.62_{1,6}$; 0.25	**$4.70_{1,6}$; 0.07**
Brood size	$0.87_{2,6}$; 0.46	$1.04_{2,6}$; 0.41	$0.32_{2,6}$; 0.74
Brood size * BCI	$0.60_{2,5}$; 0.59	$1.65_{2,5}$; 0.28	$1.47_{2,5}$; 0.31
Hatch order * BCI	$0.18_{1,4}$; 0.69	$0.84_{1,4}$; 0.41	$0.10_{1,4}$; 0.77

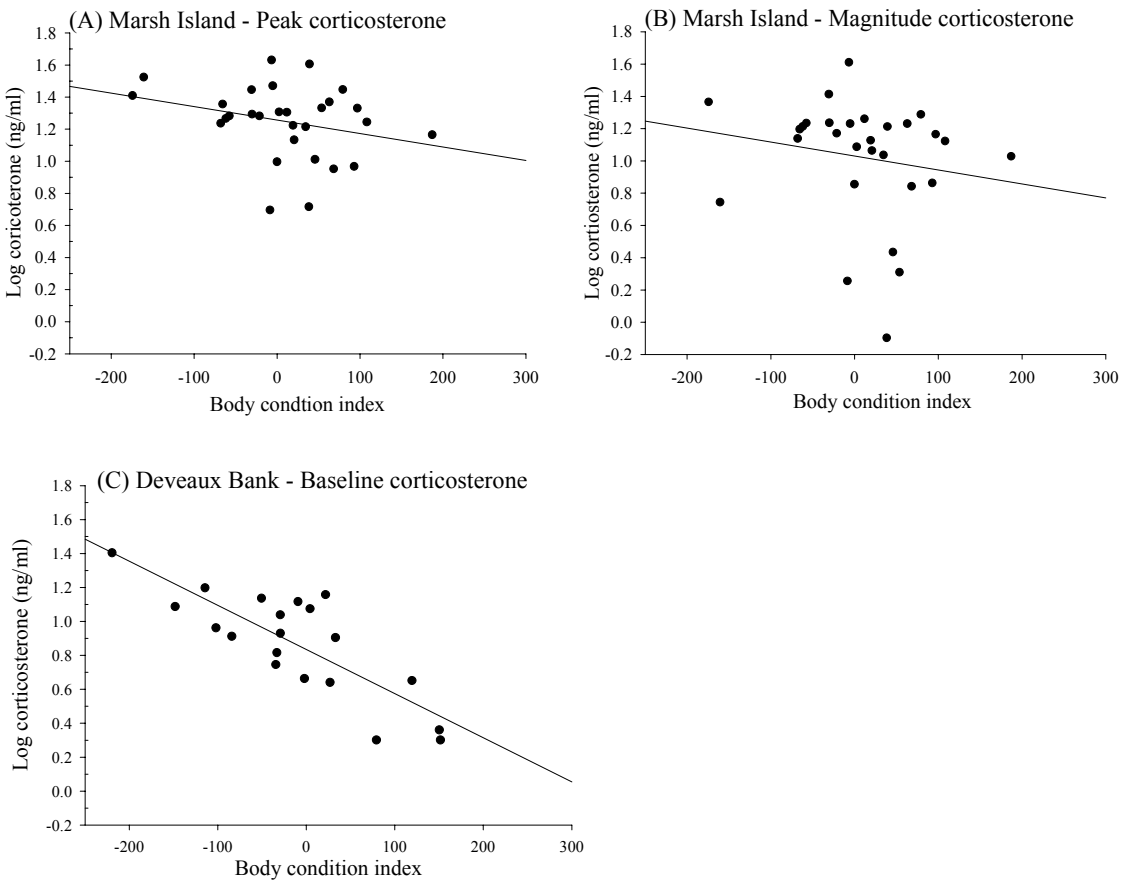

Figure 3.2 Body condition index (BCI) and corticosterone levels (log ng/ml) of Brown Pelican nestlings at Marsh Island and Deveaux Bank, South Carolina, June 2005. (A) The peak and (B) magnitude of the corticosterone response in pelican nestlings measured over the 50 min handling period at Marsh Island and, (C) the baseline level of corticosterone in pelican nestlings at Deveaux Bank. Plots represent significant relationships based upon mixed model results (see Tables 3.2 and 3.3), although simple linear regression plots are presented here.

At both colonies the hatch order of nestlings had a moderate effect on the magnitude of the stress response (Marsh Island: $F_{1,5} = 5.0$, $P = 0.07$; Deveaux Bank: $F_{1,6} = 4.7$, $P = 0.07$; Figure 3.3). First hatched nestlings (Marsh Island: log corticosterone = 1.05 ± 0.09 ng/ml; Deveaux Bank: log corticosterone = 1.02 ± 0.10 ng/ml) showed a greater magnitude in their stress response compared to second hatched nestlings (Marsh Island: log corticosterone = 1.05 ± 0.09 ng/ml vs. 0.95 ± 0.12 ng/ml; Deveaux Bank: log corticosterone = 1.96 ± 0.11 vs. 0.59 ± 0.61 ng/ml). Brood size did not affect the stress response of nestlings at either colony.

I found no significant relationship between corticosterone levels and growth rates for those chicks where measures for each were available (n = 20). None of the models that included the growth-hatch interaction were significant ($F_{3,16} < 0.6$, $P > 0.6$ for each) nor were any models that included just the main terms significant ($F_{2,17} < 0.4$, $P > 0.7$ for each). I also did not find any significant correlations between either of the growth measures and any of the corticosterone measures when hatch order was ignored ($|r| < 0.17$ for each).

Discussion

Brown Pelican nestlings at both study sites exhibited an increase in circulating levels of corticosterone in response to a period of capture and handling. A significant stress response has also been observed in altricial nestlings of European White Stork (Blas et al. 2005), Barn Swallow (*Hirundo rustica*; Saino et al. 2003) and Western Scrub-jay (*Aphelocoma californica*; Pravosudov and Kitaysky 2006). These results suggest that rather than being suppressed to avoid the potential costs associates with elevated

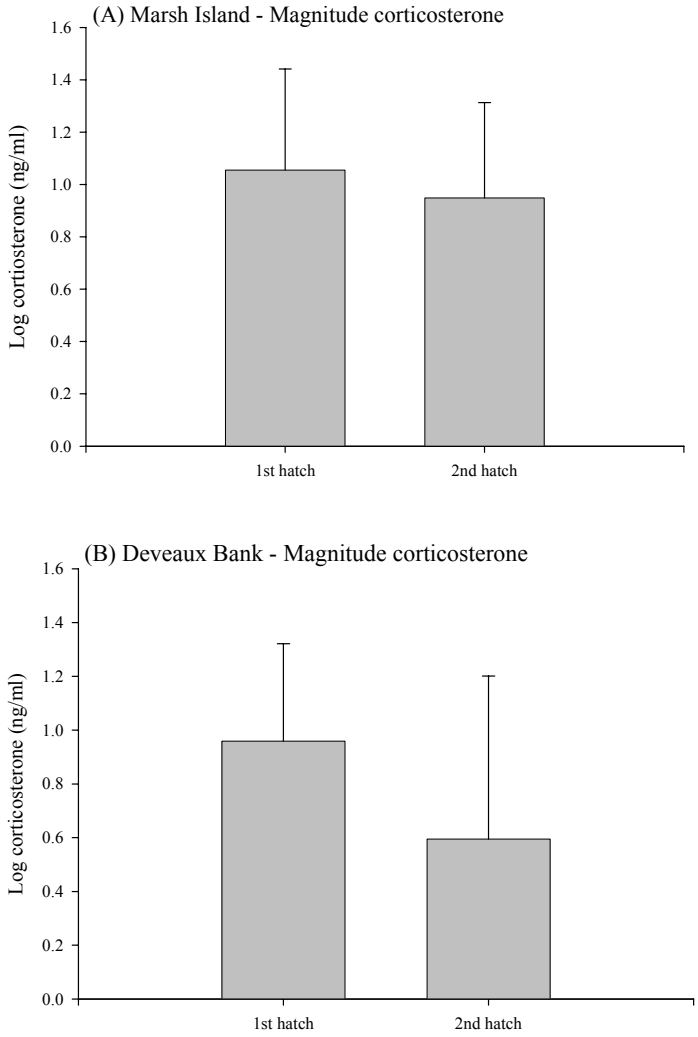

Figure 3.3 Hatch order and corticosterone levels (log ng/ml) of Brown Pelican nestlings at Marsh Island and Deveaux Bank, South Carolina, June 2005. (A) The magnitude of corticosterone response in first and second hatched pelican nestlings measured over the 50 minute handling period at Marsh Island; (B) the magnitude of the corticosterone response in first and second hatched pelican nestlings measured over the 50 minute handling period at Deveaux Bank. Plots represent moderately significant differences based upon mixed model results (see Tables 3.2 and 3.3).

corticosterone (Sims and Holberton 2000), a functional adrenocortical stress response likely provides benefits such as enhanced of survival probability for altricial nestlings. However, other studies have found a suppressed or reduced stress response in altricial young of Northern Mockingbirds (Sims and Holberton 2000) and Redpolls (*Carduelis flammea*; Romero et al. 1998), and semi-altricial young of Magellanic Penguin (*Spheniscus magellanicus*; Walker et al. 2005). Clearly there is variation in the responsiveness of the HPA axis of nestlings to stressors among the species studied to date. Comparative studies of closely related species or those with varying lengths of development would provide valuable information regarding the development of the adrenocortical stress response in altricial species.

Baseline corticosterone levels and the sensitivity of the response to capture stress differed between nestlings at Marsh Island and Deveaux Bank. The varying patterns of the response to stress highlight the benefit of measuring both baseline values and a series of samples taken in conjunction with a standardized capture stress regime. Multiple measures of the stress response may indicate different types of stress operating in the system (e.g. chronic versus acute). At Marsh Island, the concentration of corticosterone increased significantly from the baseline to 30 to 50 minute samples. The magnitude of change in corticosterone over the 50 minute period was significantly related to body condition index and tick load. Because the stress response of nestlings at Marsh Island that were regularly measured prior to sampling did not differ from those nestlings that had low exposure to researchers, it is reasonable to assume that the capture stress protocol served as a novel acute stressor to all nestlings. Nestlings measured at Marsh Island thus have a highly responsive reaction to acute stressors. In comparison, nestlings

at Deveaux Bank had a less sensitive adrenocortical response to stressors (i.e. baseline and 30 minute samples were not significantly different) though the corticosterone levels during the handling period did eventually increase significantly. However, baseline corticosterone levels at Deveaux Bank were significantly higher than those at Marsh Island. This may indicate that nestlings at Deveaux Bank were chronically stressed and unable to effectively metabolize corticosterone to reduce levels to a lower baseline. Over time, persistently elevated levels of corticosterone, such as those observed at Deveaux Bank, could adversely affect the health and development of nestlings (Wingfield et al. 1997).

The strong negative relationship of body condition index and baseline corticosterone levels observed in Deveaux Bank nestlings could suggest that a limited food supply is acting as a chronic stressor in that system. While food availability and provisioning rates were not explicitly measured in this study, body condition index is indicative of these two metrics (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 2001a; 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). In this study food demands in the colony at the time of sampling would have been high, as most nestlings were hatched and in the linear phase of growth (see Chapter 2). It is possible that diminished food supplies 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.

Pelican nestlings with a moderate load of *O. capensis* had a lower magnitude of stress response than nestlings carrying no ticks at the time of sampling. There have been few studies examining the adrenocortical response in relation to ectoparasite infestation, and no studies specific to the soft tick studied here. Of the studies that have been conducted on birds, all have found a positive relationship between corticosterone levels and ectoparasite infestation (Kitaysky et al. 2001a; Quillfeldt et al. 2004; Raouf et al. 2006). While all ectoparasites have a negative effect on the condition of their host, the extent of the repercussions likely varies depending on the type of ectoparasite (i.e. feather lice, haematophagus ticks or fleas) and the degree of infestation.

It is also possible that nestlings as young as those included in this study are not able to perceive factors that are a part of their environment, such as ticks, as stressors (Walker et al. 2005). If nestlings do not distinguish ticks as stressors, no differences in corticosterone between tick loads would be expected. This explanation, however, does not account for the significant difference in the magnitude of the stress response between parasitized and non-parasitized nestlings at Marsh Island. A more likely explanation is that chicks from infested nests become habituated to persistent stressors (i.e. ticks) and therefore have a decreased stress response which ultimately would serve to reduce any negative consequences of stressors (Sapolsky 2002). Habituation to ectoparasites would account for the suppressed adrenocortical response of moderately parasitized individuals. It is not clear, however, why this pattern of adrenocortical response to stress was not detected in nestlings carrying high tick loads.

There is evidence that corticosterone levels of parasitized nestlings may increase when exposed to multiple environmental stressors. For example, Wilson's Storm Petrel (*Oceanites oceanicus*) 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). During the breeding season studied here, I was not aware of any severe weather events or sudden environmental changes that may have acted additively with tick loads to produce a 'natural experiment' similar to that observed by Quillfeldt et al. (2004). Additionally, Raouf et al. (2006) found that baseline levels of corticosterone were significantly lower in Cliff Swallow (*Petrochelidon pyrrhonota*) nestlings from large, treated (parasite-free) colonies compared to large, untreated (parasite infested) colonies and small colonies of both treatments. Though the Brown Pelican colony at Deveaux Bank was over twice the size of that at Marsh Island during the 2005 breeding season, the relationship between colony size and ectoparasite infestation could not be considered in this study due to the unavailability of parasitized nestlings at Deveaux Bank.

Though the findings of this study suggest that ectoparasites are a biologically relevant variable to consider, the adrenocortical response of Brown Pelican nestlings needs to be further explored with a larger sample size and wider range of tick infestation. Additional information regarding the role of ectoparasites on reproductive success could be gained by exploring the stress response in relation to adult breeding behaviors such as chick provisioning rates, feeding frequency, and time spent away from infested nests. The role of the adult stress response in relation to ectoparasites as a causal mechanism of nest

abandonment could be explored at different breeding stages. Nest desertion has been associated with both heavy ectoparasite infestation (Duffy 1983; King 1977 a, b) and high corticosterone (Love et al. 2004).

The implications of a multiple sibling brood, such as fewer feedings per nestling, reduced growth rates, and increased ectoparasite loads, are often assumed to increase stress among nestlings. In this study, however, there was no relationship between any of the measures of corticosterone and brood size at either colony, or its interaction with body condition index and tick load. Blas et al. (2005) also found no relationship between corticosterone levels of two and three chick broods in the White Stork. Together, these results suggest that studies should not operate under the assumption that brood size is a stressor, but rather should examine the role of brood size in relation to other variables of interest.

The relationship of siblings, especially those in asynchronous hatching species with an extended developmental period in the nest, such as the Pelecanidae, may play a role in the physiological condition of nestlings. In Brown Pelicans, asynchronous hatching affords the first hatched nestling a size advantage and dominant rank that is constantly contested by younger siblings (Pinson and Drummond 1993; Shields 2002). In this study, first hatched nestlings at both study colonies exhibited a trend toward a greater magnitude in the stress response than second hatched siblings. Similar results were detected in altricial canaries (*Serinus canaria*) and were posited to be a consequence of asynchronous hatching or exposure to different levels of maternal stress hormone in the yolk (Schwabl 1999). Other studies, however, have found higher baseline stress hormone levels in subordinate nestlings, including measurements in Nazca Booby (*Sula granti*;

Tarlow et al. 2001), Blue-footed Booby (*Sula nebouxii*; Nunez-de la Mora et al. 1996), and Magellanic Penguin (Walker et al. 2005) chicks. Stability of the sibling hierarchy may account for differences in the direction and strength of the stress response among these species (Sapolsky 2002). In obligate siblicidal species such as the Nazca Booby, subordinates may contend with a greater amount of stress than dominant siblings. For facultative siblicidal species like the Brown Pelican, maintenance of rank and associated behaviors (e.g. begging and aggression against siblings; Kitaysky et al. 2001b, 2003) may confer a greater amount of stress to the dominant individual. Stress levels and aggression within a brood may be further amplified by limited food supplies (Nunez-de la Mora et al. 1996; Kitaysky 2001a), which may contribute to variation in results among studies.

In summary, altricial Brown Pelican nestlings showed a significant adrenocortical stress response to a capture stress regime at both study colonies. This suggests that nest-bound pelicans are capable of responding to ecological stressors encountered during early development with an increase in levels of corticosterone. However, because pelican nestlings have a responsive HPA axis, they are also vulnerable to the detrimental effects of prolonged elevation of corticosterone. Although first hatched nestlings showed a trend toward a greater magnitude of stress response at both colonies in comparison with their younger sibling, the other ecological and biological variables considered in this study appeared to affect the populations at the two study colonies differently. The stress response of nestlings at Marsh Island was highly sensitive to the stress regime, and was significantly affected by tick load and marginally so by body condition index. In comparison, baseline levels of corticosterone were significantly higher in the Deveaux Bank sample and were more strongly related to body condition index compared to Marsh

Island. These results suggest that nestlings at Marsh Island were adapted to ecological conditions, while nestlings at Deveaux Bank appeared to suffer from chronic stress. Differences in colony size may have contributed to these results, although the exact mechanisms remain unclear. A more detailed investigation of the change in the stress response of pelican nestlings during the extended developmental period may provide additional insight into the role of ectoparasites in this system and the potential impacts they may have on the long-term health and condition of Brown Pelicans.

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CHAPTER FOUR

CONCLUSION

Brown Pelicans nest at several colonies off the coast of South Carolina that are known to be infested with the soft tick *Ornithodoros capensis.* Nests within these colonies are sprayed annually with an insecticide to reduce parasite populations. While *O. capensis* has been implicated in massive nest abandonment, the direct effect of these ticks on nestling condition or the current decline in nesting effort within South Carolina (Figure 1.2) has not been explored. In this study, I examined the growth, survival, and physiological condition of nestlings in relation to levels of infestation in insecticide treated and untreated nests.

The second chapter of this thesis, "Effect of soft ticks on growth rates of Brown Pelican nestlings," examined the relationship between levels of *O. capensis* infestation and growth rates of Brown Pelican nestlings at two South Carolina colonies: Marsh Island and Crab Bank (Figure 1.1). I measured a suite of nestling growth rate metrics for approximately three weeks post-hatch and survival to 21 days during the 2004 and 2005 breeding seasons for pelican nestlings from insecticide treated and untreated nests. Tick infestation varied between study colonies, and growth rates of nestlings at the more heavily infested colony were positively affected by tick load. Survival was not related to average tick count within or between years at Marsh Island or Crab Bank. Mean tick count on chicks from untreated nests was greater compared to chicks from insecticide treated nests. Growth rates differed between hatch orders, though tick averages did not.

Chapter three, "Adrenocortical response of Brown Pelican nestlings to ectoparasite infestation," investigated levels of the stress hormone corticosterone of Brown Pelican nestlings in relation to *O. capensis* infestation at two colonies in South Carolina: Marsh Island and Deveaux Bank (Figure 1.1). I measured corticosterone levels in altricial nestlings in response to a 50 minute capture stress regime and found that nest-bound young at both colonies were capable of a significant increase in corticosterone. I found variation between colonies in the adrenocortical stress response in nestlings in relation to ecological variables. Nestlings at Marsh Island were highly sensitive to the acute stress regime, though the magnitude of the response was lower for parasitized nestlings at Marsh Island, the more heavily infested colony. Nestlings at Deveaux Bank had higher baseline levels of corticosterone that were significantly and negatively related to body condition index. These results suggest that nestlings at Marsh Island were adapted to ecological conditions, while nestlings at Deveaux Bank appeared to suffer from chronic stress.

The levels of soft tick infestation observed during this study, which may be mediated by annual insecticide treatment, did not negatively impact the growth or physiological condition of Brown Pelican nestlings during early development. Thus, ectoparasite infestation of nestlings does not appear to be the primary mechanism driving the population decline in South Carolina. However, this study provides further evidence that measures of growth, survival, and physiological condition are useful for assessing the condition of altricial pelican nestlings in relation to ectoparasites and a suite of other ecological variables, and therefore can help managers monitor and manage breeding colonies within the state.