

Ecological Drivers of Brown Pelican Movement Patterns, Health, and Reproductive Success in the Gulf of Mexico [Sample Report]

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Ecological Drivers of Brown Pelican Movement Patterns, Health, and Reproductive Success in the Gulf of Mexico [Sample Report]

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Authors:

First S. Last1
First G. Last2
First A. Last3
First G.R. Last4

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by
U.S. Geological Survey
South Carolina Cooperative Fish and Wildlife Unit
260 Lehotsky Hall
Clemson University
Clemson, SC 29634

DISCLAIMER

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ABOUT THE COVER

An eastern brown pelican (*Pelecanus occidentalis carolinensis*) returns to its nest on Raccoon Island, Louisiana. Photo credit: S. Last5 2014.

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List of Abbreviations and Acronyms

A:G	albumin-to-globulin ratio
A1G	Alpha 1 globulin
A2G	Alpha 2 globulin
AIC	Akaike's information criterion
ALT	alanine aminotransferase
ANOVA	analysis of variance
ASE	accelerated solvent extraction
AST	aspartate aminotransferase
BCI	body condition index
BOEM	Bureau of Ocean Energy Management
BPR	biomass provisioning rate
BUN	blood urea nitrogen
CBC	complete blood count
CI	confidence interval
CO ₂	carbon dioxide
CORT	corticosterone
CPK	creatine phosphokinase
CV	coefficient of variation
d	day(s)
DDT	dichlorodiphenyltrichloroethane
DSR	daily survival rate
DWH	<i>Deepwater Horizon</i>
EPR	energy provisioning rate
ft	foot/feet
g	gram(s)
GGT	Gamma glutamyl transferase
GLM	generalized linear model
GMT	Greenwich mean time
GOM	Gulf of Mexico
GoMAMN	Gulf of Mexico Avian Monitoring Network
GoMMAPPS	Gulf of Mexico Marine Assessment Program for Protected Species
hr	hour(s)
HDLc	high density lipoprotein cholesterol
HMM	hidden Markov model
HSD	honestly significant difference
in	inch(es)
J	joule(s)
kg	kilogram(s)
km	kilometer(s)
L	Liter(s)
lb	pound(s)
m	meter(s)
mEq	milliEquivalent

mi	mile(s)
min	minute(s)
MRPP	multi-response permutation procedure
NOAA	National Oceanic and Atmospheric Administration
OCS	Outer Continental Shelf
OMI	outlying mean index
oz	ounce(s)
PAH	polycyclic aromatic hydrocarbon
PC1	first principal component axis
PCA	principal component analysis
PCR	polymerase chain reaction
PTT	platform transmitter terminal
SD	standard deviation
sumALK	sum of alkylated PAHs
sumPAH	sum of all PAHs
sumPAR	sum of parent PAHs
UD	utilization distribution
UPLC	ultra-performance liquid chromatograph
VHF	very high frequency
VLDLc	very low density lipoprotein cholesterol
w	week(s)
WBC	white blood cells

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1 Introduction

The number of marine wind and tidal energy (Pelc and Fujita 2002) developments and proposals (Pelc and Fujita 2002), as well as petroleum extractions (Freudenburg and Gramling 1994), is increasing to meet the growing global demand for energy. However, the rapid progress of energy extraction and development often outpaces the understanding of these actions' effects on marine systems and their organisms (Ward et al. 1979, Burke et al. 2012). Assessments of post-installation offshore energy projects document that effects on marine species, whether positive or negative, can be more significant than anticipated (Boesch and Rabalais 1987, Daan and Mulder 1996, Sammarco et al. 2004). Energy extraction can impact marine species directly (e.g., adult mortality) and indirectly through various pathways, including: compromised condition from contaminants exposure, altered availability or distribution of prey, altered behavior, or reduced reproductive output (Alonso-Alvarez et al. 2007, Dean et al. 2017, Haney et al. 2014).

Marine birds are valuable and commonly used models for studying the impacts of threats on their environment, such as offshore development influences on the broader marine ecosystem (Furness and Greenwood 1993). Seabirds are relatively accessible compared to other marine vertebrates, are wide-ranging migrators, and their foraging behaviors increase the likelihood for interactions with energy installation (Wiese and Jones 2001). Seabirds also rely on a variety of above- and below-water habitats, including both terrestrial breeding colonies and pelagic foraging grounds (Hunt 1990, Pinaud and Weimerskirch 2005). As top-level marine predators they are particularly vulnerable to bioaccumulation of contaminants (Walker 1990, Pérez et al. 2008) and may provide indications of perturbations at lower trophic levels (Thompson et al. 1998, Wiese and Jones 2001). Understanding the effects of existing development and predicting the impacts of future development on seabirds requires a thorough understanding of seabird population dynamics, behavior, physiology, and habitat use under baseline conditions (Ballance 2007, Soanes et al. 2013, Jodice et al. 2019). However, such information is often not collected until after development or contamination has altered baseline processes. Also, the direct influence of anthropogenic stressors on demographic parameters in the marine environment varies widely and can be difficult to estimate (Burger 1993, Uhlmann et al. 2005).

The Gulf of Mexico (GOM) Outer Continental Shelf (OCS) Region contains a high density of oil and gas infrastructure and coastal development. It also has a rich assemblage of nearshore seabirds, pelagic seabirds, wading birds, migratory waterfowl, and shorebirds (Duncan and Havard 1980). The region is of year-round importance to seabirds, including local breeding populations and breeders from distant locations that winter along the Gulf Coast (Mikuska et al. 1998, Montevecchi et al. 2012, Haney et al. 2014, Jodice et al. 2019). Many terrestrial areas of known importance to breeding, migrating, and wintering waterbirds have been designated for protection at state and federal levels. However, few marine protected areas have been designated in the GOM, and much of the marine environment there, including offshore foraging and migratory habitat of seabirds, is open to oil development, ship traffic, fishing, and contaminants release (Coleman et al. 2004, Davis et al. 2000).

Because of its distribution patterns, behavior, and known sensitivity to chemical and oil contaminants exposure (Blus 1982, King et al. 1985, Shields 2014), the brown pelican (*Pelecanus occidentalis*) is potentially a good indicator of species-level impacts from interactions with coastal and marine development (Wilkinson et al. 1994, Jodice et al. 2019). The species is widespread throughout the Northern GOM and common during all phases of the annual cycle. Brown pelicans nest along the Gulf Coast from Corpus Christi Bay, Texas through southwestern Florida. During the non-breeding season, the species can be found throughout the Northern GOM as well as along the Yucatan Peninsula, Cuba, and as far south as Guatemala. Because of the spatial extent of this annual range, the species is exposed to a substantial array of environmental and anthropogenic stressors that may influence its health, habitat use,

and survival. The species is generally regarded by managers and stakeholders in the region as a good indicator of ecosystem health for estuarine and nearshore habitats. For example, brown pelicans were recently included as a priority species for monitoring in the Seabird Monitoring Plan for the Gulf of Mexico Avian Monitoring Network (GoMAMN) (Jodice et al. 2019). Despite the species' long history as a focus for conservation and restoration efforts, much of the information required to understand pelican population dynamics and habitat requirements (i.e., adult and fledgling mortality, dispersal, site fidelity, diet composition, foraging behavior, migration patterns, and non-breeding habitat use) remains unknown or is poorly understood (Briggs et al. 1981 for habitat use, Schreiber and Mock 1988 for survival rates, Wood et al. 1995 for Florida colony site fidelity, and Shields 2014, Jodice et al. 2019 for addressing multiple data gaps).

In this study, we used several unique research avenues to address questions regarding movement, habitat use, physiology, and reproductive ecology of brown pelicans. Our research encompassed six principal objectives: (1) assessing reproductive ecology; (2) assessing baseline habitat use by the species in this region, particularly individual and regional variability; (3) assessing baseline health and exposure to contaminants in this region, particularly individual and regional variability; (4) predicting overlap of pelicans with anthropogenic risk factors; (5) understanding pathways by which changes to adult movement patterns might influence reproductive ecology and how to best measure such effects in wild populations; and (6) assessing movement ecology in the context of interactions with key prey resources. The remainder of the report is organized with a common introduction (Chapter 1), common methods (Chapter 2), and overall summary (Chapter 8). Chapter 3 through Chapter 7 are focused on specific data streams and objectives. Within Chapters 3 through 7, the structure includes a brief introduction to the topic, followed directly by a combination of individual results and interpretation.

1.1 Baseline Habitat Use

Animals use various habitats for different needs, including foraging, sheltering from predators, thermoregulating, raising young, moving among patches, and migrating stopovers (Börger et al. 2008, Morrison et al. 2012). Each need requires specific habitat characteristics and features; thus, an animal's interaction with its environment varies depending on its location on the landscape and its fine-scale movement and behavioral patterns (Garthe and Hüppop 2004). Seabirds are unique among many avian species in that they regularly require terrestrial and aquatic habitats, although the extent to which each is used varies between nearshore and pelagic seabirds. For example, in wide-ranging pelagic and semi-pelagic seabirds, habitat use typically changes between the breeding season, when birds are central-place foragers based in terrestrial colonies, and the non-breeding season, when birds rely primarily on marine habitats (Weimerskirch and Wilson 2000). Within each stage of the breeding cycle, habitat use also depends on individual characteristics (Bearhop et al. 2006), phenology (Catry et al. 2009), colony size and location (Lewis et al. 2001), and environmental features (Tew Kai et al. 2009). These factors all contribute to variation in individual energy requirements, resulting in differences in foraging strategies and habitat preferences (Daunt et al. 2006, Phillips et al. 2009).

Compared to pelagic species, nearshore seabirds, such as brown pelicans, generally occupy smaller foraging ranges that extensively overlap human-dominated marine and coastal areas year-round (Thaxter et al. 2012). These smaller areas contain a higher diversity of habitat features and prey species assemblages (Becker and Bessinger 2003) and respond to different oceanographic processes than do large marine ecosystems (Gray 1997). Despite these habitat differences, many of the same individual, colonial, and environmental factors that influence habitat choice in pelagic species also operate within nearshore seabird populations (e.g., Erwin 1977, Suryan et al. 2000). However, the role of density-dependent prey depletion in determining movement patterns has been well-established in pelagic seabirds (e.g., Ainley et al. 2004, Ford et al. 2007, Ballance et al. 2009), but has received little study in nearshore seabirds. Additionally, partial migration (Lack 1944) influences individual differences in year-round seabird movements but has received little study in nearshore systems.

A principal goal of our work was to establish a framework for understanding pelican movement patterns under baseline conditions (i.e., not immediately associated with a recent disturbance event), including preferred habitat characteristics, sources of individual variation in movement, and dispersal and habitat selection throughout the year (breeding and the lesser studied non-breeding periods). This work provides an important comparison point for studying the effects of any future changes to the GOM marine environment on brown pelican movements and energetic expenditure, as well as addressing key ecological questions in relation to the spatial ecology of nearshore seabirds.

1.2 Risk Exposure

Evaluating the effects of environmentally heterogeneous stressors on mobile wildlife requires understanding of the spatial and temporal overlap between individuals and threats as well as the extent of risk individuals encounter in relation to adverse effects based upon their habitat use and behavior (Desholm and Kahlert 2005, Jaeger et al. 2005, Beaudry et al. 2010). Increases in the spatial and temporal resolution of individual tracking technologies have resulted in a shift toward individual-based analysis of habitat requirements (Hebblewhite and Haydon 2010); however, habitat assessments derived from individual tracking data often incorporate only presence or absence across landscapes and do not account for behavior (Tremblay et al. 2009). Nearshore seabirds experience higher levels of human disturbance and habitat modification of breeding, resting, and foraging grounds than pelagic species (Croxall et al. 2012). Habitat features that concentrate nearshore seabirds and their prey may also concentrate risk factors such as pollutants, bycatch, and anthropogenic disturbance. Temporal variation in habitat needs and movement patterns can significantly contribute to the likelihood of risk exposure and the degree to which risk factors impact individuals and populations (Beaudry et al. 2010). The effects of environmental perturbations on seabirds depend on temporal factors (e.g., breeding stage) that influence their behavior and use of affected areas (Eppley and Rubega 1990, Montevecchi et al. 2012).

Due to its large size and persistence along human-dominated coastlines, the brown pelican represents one of the most high-profile nearshore seabirds for much of the GOM and southeastern U.S. The species was reduced to near-extinction by exposure to dichlorodiphenyltrichloroethane (DDT) during the mid-twentieth century (McNease et al. 1992) and continues to experience high mortality rates during oil spills (USFWS 2011, DWH NRDAT 2016). Despite these factors, baseline assessments of health and exposure to petroleum-based contaminants are minimal for the species in the region (Jodice et al. 2019). Furthermore, within the GOM, the source of data on brown pelican movements are from observations of a small number of marked and banded birds across limited geographic areas (Schreiber and Mock 1988, Stefan 2008, King et al. 2013, Walter et al. 2014), despite their prevalence throughout the region.

The discrete nature of existing data makes it difficult to reliably predict how, or at what spatial and temporal scales, individuals may interact with current or future acute and chronic contamination from oil spills or other pollution sources. For example, until recently, home range size for the species was based on limited data from very high frequency (VHF) telemetry or inferred from observations of individuals foraging in relation to the nearest colony, neither of which provide the spatial or temporal resolution needed to assess risk exposure. Therefore, if significant winter and migratory ranges of pelicans from different breeding colonies overlap with each other, and if these areas also overlap with contaminants (i.e., spilled oil) then relatively localized oiling events in certain GOM areas during the non-breeding season could affect birds from multiple colonies and result in population-level impacts. Moreover, though efforts to restore injured populations following stressor events, such as oil spills, generally target colony sites, most threats associated with marine energy development (e.g., acute or chronic spills) also affect offshore foraging grounds and therefore the risk to individuals and populations extends across multiple ecosystems (Campagna et al. 2011). Developing reference ranges for various health metrics (e.g., hematology and serum chemistry) will improve management, conservation, and response activities (e.g., responses to oil spills, hurricanes) for the species. Understanding the year-round overlap of brown pelicans with risk factors and contaminants (e.g., polycyclic aromatic hydrocarbons, PAHs) throughout the region could improve targeted mitigation efforts. Affected at-sea habitats could be linked to individual breeding colonies and improvements can be made in predicting which portions of the GOM-wide metapopulation are likely to be affected by contamination events.

1.3 Ecology and History of Reproduction

Brown pelicans have been a species of high conservation concern in the GOM for decades (Schreiber 1980, Nelson 2005). Following the 2010 *Deepwater Horizon* (DWH) oil spill, which caused widespread mortality of pelicans and other coastal birds (Haney et al. 2014), restoration activities for seabirds increased across the Northern GOM. However, to be successful, continued restoration efforts will require data beyond levels currently available. For example, detailed data are required on the relationship between environmental conditions at the nest site and reproductive success, which can be affected by numerous characteristics (e.g., density of breeders, exposure to inclement weather, vegetation characteristics, landscape features, and weather) (Robinson and Dindo 2011, Walter et al. 2013, Lamb 2016). Reproductive output may also be limited by environmental variables beyond the nest site or even the nesting island. For example, weather and stochastic events, such as storms and flooding, can decrease egg and chick survival either directly (e.g., through overwash) or indirectly (e.g., through exposure) (Ramos et al. 2002, Frederiksen et al. 2006, Sherley et al. 2012, Bonter et al. 2014). Understanding which site-specific factors contribute to the success of nests will inform restoration efforts and better allow projects to maximize population-level impacts for the focal species.

Impacts of acute or chronic environmental stressors on wildlife are typically quantified directly using mortality rates derived from carcass counts (Piatt et al. 1990, Burger 1993) or multi-year census data (Wiens et al. 1996, Yaukey 2012). Data are subsequently incorporated into demographic models to estimate the population-level effects of stressors (Haney et al. 2014). In addition to causing immediate mortality, stressors can impact seabirds sublethally through secondary pathways, including: reduced habitat quality (Cheng et al. 2009, Williams et al. 2010), compromised physical condition (Romero and Wikelski 2001), physiological and genetic modifications (Møller and Mousseau 2011), and/or increased susceptibility to existing threats (e.g., disease or environmental fluctuation) (Balseiro et al. 2005, Whitehead 2013). Many indirect and sublethal stressors subsequently impact demographic processes by reducing reproductive fitness in surviving individuals (Krebs and Burns 1977, Peterson 2001), but are often not explicitly or adequately addressed in demographic calculations and projections. Moreover, the breeding process itself is likely to compound impacts of environmental stress as reductions in the adult condition and habitat suitability make it less likely for breeders to meet the energetic demands of territory defense, gestation, and provisioning young (Butler et al. 1988, Gannon and Willig 1994). Demographic

models that do not accurately incorporate secondary effects of environmental stressors on breeding success and recruitment cannot accurately predict or quantify the complex population-level impacts of environmental perturbations (Peterson et al. 2003, Haney et al. 2014).

Despite widespread understanding of the capacity of sublethal environmental stress to negatively affect reproduction and recruitment, it can be difficult to determine the most appropriate endpoints for measuring these effects (Smits and Fernie 2013). There must be a pre-existing understanding of the level of variation in reproductive parameters expected under baseline conditions for post-disturbance measurements to be informative (Teal and Howarth 1984, Velando et al. 2005). Such data are not always available for species of interest before catastrophic events (Eppley 1992). Moreover, the collection of reproductive data can be time- and labor-intensive and can involve researcher disturbance, which may make it difficult to implement rapidly in the wake of an unexpected external change (Wiens et al. 1984). Snapshot measures of reproductive health (Jakob et al. 1996, Benson et al. 2003), which can be collected during a single visit with minimal disturbance, allow for rapid data collection across large areas after disturbance events; however, the relationship of such snapshot measurements to demographic parameters of interest (e.g., reproductive success) must be evaluated to select appropriate metrics.

Stress hormone production offers a broadly applicable metric for assessing the impacts of environmental stressors on free-living wildlife populations (Romero and Wikelski 2001). CORT is the principal glucocorticosteroid stress hormone in birds, rodents, reptiles, and amphibians, and is frequently used as a measure of individual stress responses to environmental conditions and disturbance (Marra and Holberton 1998, Kitaysky et al. 2001, Blas et al. 2005, Bonier et al. 2006, Almasi et al. 2009). Stress hormones are upregulated in response to perceived stressors, prompting short-term behavioral and physiological modifications (McEwen et al. 1997). Over time, however, chronic elevation in CORT levels in response to chronic stress may negatively affect organism health by compromising immunosuppression, growth rates, body condition, and behavior (Sapolsky et al. 2000). CORT levels can be complicated by individual physiology (Angelier et al. 2007) and may change over life stages (Williams et al. 2008, Bonier et al. 2009). Within avian taxa, measuring CORT in altricial young controls for some of these influences because their exposure to stress is localized and their range of behavioral responses is restricted (Kitaysky et al. 2003, Eggert et al. 2010). Elevated stress in early life can result in severe developmental consequences (Kitaysky et al. 2003, Müller et al. 2009, Spencer et al. 2008, Butler et al. 2009). Therefore, the CORT stress response can be used to test whether chick development, condition, growth, and/or survival are affected by acute and/or chronic environmental stress during nestling development. CORT stress response can also explore mechanisms underlying survival, reproductive performance, and population dynamics (Kitaysky et al. 2010).

Though CORT levels in blood plasma can be elevated by short-term factors (e.g., stress resulting from capture; Love et al. 2003, Romero and Reed 2005), CORT in avian feathers provides a more sustained record of stress levels over days or weeks (Bortolotti et al. 2008, Harms et al. 2010). Feather CORT measurements allow for a direct comparison of nestling condition between different breeding habitats, where variations in nutrition, contamination, predation, and parental attendance may affect chronic chick stress even if no physiological differences are apparent (Bortolotti et al. 2008, Harms et al. 2010). Recent laboratory and field studies have demonstrated that chronic nutritional stress elevates feather CORT levels in both captive and free-living seabirds (Will et al. 2015). We undertook a direct comparison of body condition index (BCI) with feather CORT as a predictor of fledging success and post-fledging survival. This information will help to create rapid evaluation metrics for brown pelicans and other seabirds following environmental perturbations and is already being considered as a monitoring tool by the GoMAMN (Jodice et al. 2019).

1.4 Foraging Ecology

The ability of apex marine predators to survive and reproduce depends primarily on the production and availability of sufficient food resources at lower trophic levels to meet the energetic requirements of both adults and young (Frederiksen et al. 2006). Both the quantity and quality of available prey can influence survival, reproduction, and population dynamics in apex predators, and reductions in either prey availability or quality can affect demographic parameters (Trites and Donnelly 2003, Jodice et al. 2006, Hjernquist and Hjernquist 2010). A switch to nutrient-poor prey may cause reduced fitness even if abundant prey is available (Rosen and Trites 2000, Österblom et al. 2008). Both experimental (Rosen and Trites 2004, Romano et al. 2006) and field (Golet et al. 2000, Kadin et al. 2012, Cohen et al. 2014) studies have found that switching high-lipid prey for lower-energy alternatives can result in measurable reductions in breeding parameters, even when the amount and rate of delivery are unchanged. Most of these data come from cold-water systems, where prey species are likely to have higher lipid reserves on average than warm-water species (Stickney and Torres 1989). Few data are available from tropical systems (waters $\geq 23^{\circ}\text{C}$ average temperature: Ballance and Pitman 1999), in which the relatively low variation in lipid levels among fish species may reduce the range of energetic values in prey species available to top predators.

Even in a prey community with limited interspecific variation in energy density, differences in prey quality may still exist. For example, the junk-food hypothesis posits that energy density, particularly as represented by lipid density, is positively related to productivity. Optimal foraging theory (MacArthur and Pianka 1966) takes into account the energy a predator obtains from prey and the energy it expends in finding, capturing, handling, and digesting prey. An optimal forager is expected to maximize the net energy gain, calculated as the difference between energy obtained from prey and energy expended in foraging. Thus, differences in both predator foraging strategies and prey behavior could result in variation in the amount of energy predators obtain from different prey types, even among prey species with similar energy content. Marine predators employ a wide variety of foraging strategies, which allow them to exploit different prey types and forage in different sections of the water column (Ashmole 1971, Spear and Ainley 1998). Tropical seabirds, most of which forage near the water's surface, compete for limited prey resources using a variety of capture techniques including skimming, surface-plunging, surface-seizing, plunge-diving, and, occasionally, pursuit-diving (Ballance and Pitman 1999). Though the various modifications of surface-feeding techniques allow some partitioning of prey, species at tropical latitudes do not partition prey species as extensively as high-latitude species that forage at a wider variety of depths and often specialize on different prey items. Thus, the definition of junk food should include not only the energy density of prey but also how readily prey can be captured given the foraging techniques used by the species of interest. Differences in availability between prey species reflect both abundance, which is an absolute measure, and accessibility, which can differ from predator to predator both within and among species.

Studies of brown pelicans in the tropical waters of the GOM have suggested reliance on a single prey species, Gulf menhaden (*Brevoortia patronus*), which can constitute over 95% of biomass in diet samples in the central Northern GOM (Arthur 1919). The Gulf menhaden is one of the most abundant forage fish species in the region and supports the second-largest fishery in the United States (Vaughan et al. 2007). Samples collected from eastern portions of the species' GOM range, where menhaden are naturally less abundant than in the central and western portions of the GOM, show a decreasing trend in the predominance of menhaden in pelican diets (Fogarty 1981). Although this suggests that relative availability plays a role in the frequency of menhaden in pelican diets, it is unclear how or whether this underlying variation in diet composition affects demographic parameters, or how menhaden compares energetically to other available alternatives. Furthermore, pelicans may benefit from land- and ship-based supplemental feeding at fishing piers or trawlers (Wickliffe and Jodice 2010), although the extent of this behavior and its effect on nutritional status remains unclear. Because of the role of brown pelicans as an indicator species for assessing the effects of contamination and oil pollution in the region (Shields 2014), understanding underlying dietary and demographic variation provides a crucial reference point for quantifying the effects of environmental stressors.

2 Methods

2.1 Study Area

2.1.1 Colony Selection

Research was conducted at pelican colonies within each of the Bureau of Ocean Energy Management (BOEM) planning areas (east, central, and west) within the GOM (**Figure 2.1**). Study sites extended from the Florida panhandle in the east to the central coast of Texas in the west. We selected colonies within each of the three BOEM planning areas to sample among different levels of oil and gas development (central = most developed, east = least developed, and western = intermediate development between east and central). We did not extend our research onto colonies in peninsular Florida due primarily to logistical constraints and to avoid adding additional ecological variability into the data (e.g., the addition of mangrove ecosystems).

Individual tracking was conducted from seven colonies in the GOM. We selected 2–3 colonies each in the western (Texas), central (Louisiana, Mississippi, and Alabama), and eastern (Florida) planning areas of the GOM and selected 2–3 colonies each in the western (Texas), central (Louisiana, Mississippi, and Alabama), and eastern (Florida) planning areas of the GOM between 83° and 98° W and 27° and 31° N (**Figure 2.1**; hereafter, with respect to colonies and the coastal zone in general, the term “Northern GOM” refers to this area). Within planning areas, colonies were 50–150 km (31–93 mi) apart, and colony groups in separate planning areas were 500–600 km (311–373 mi) apart. The number of breeding pairs at each study site was obtained from the most recent (i.e., 2013) colonial waterbird censuses for each planning area (Colibri Ecological Consulting and R. G. Ford Consulting, 2015, Texas Colonial Waterbird Survey¹). We also sampled and observed nestlings between 2013 and 2015. Colony locations varied between years but included nine colonies throughout the study area.

We extracted environmental variables, including two fixed parameters (bathymetry and bottom substrate) and three seasonally averaged parameters (salinity, sea surface temperature, and chlorophyll a) to compare underlying environmental conditions between colonies. Salinity, sea surface temperature, and chlorophyll a were measured at distances of 10, 20, 50, and 150 km from the colony, bounded by the coastline and up to 50 km offshore, sea surface temperature, and chlorophyll a were measured at distances of 10, 20, 50, and 150 km from the colony, bounded by the coastline and up to 50 km offshore. These distances were chosen post-hoc to GPS tracking to represent the range of movements we observed among individuals. We used a multivariate hierarchical clustering approach (K-means clustering; MacQueen 1967) to compare environmental characteristics between sites, and tested the resultant clusters using multi-response permutation procedure (MRPP) on a Euclidean distance matrix (McCune and Grace 2002). All statistical analyses were conducted in R (R Core Team 2014).

¹ See the Texas Colonial Waterbird Survey here:
https://tpwd.texas.gov/huntwild/wild/wildlife_diversity/tcws/data.phtml

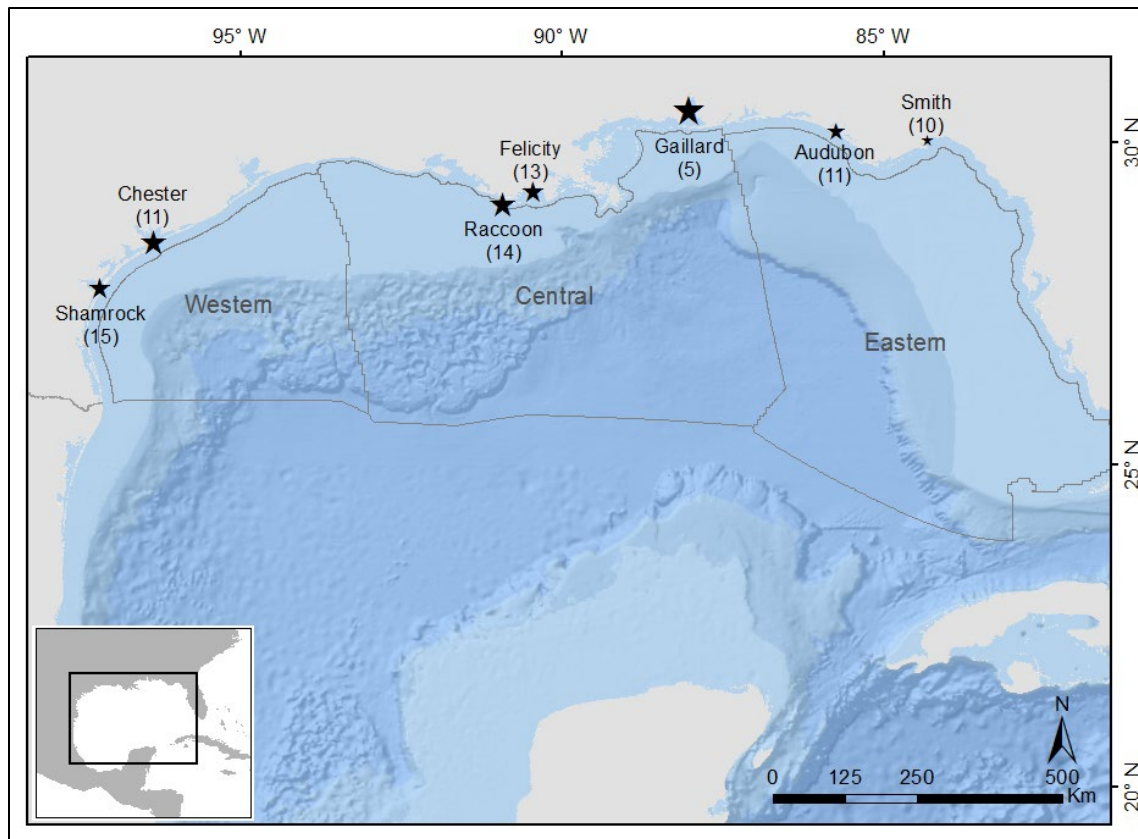


Figure 2.1. Map of colony locations of brown pelicans fitted with GPS transmitters, GOM, 2013–2015

Note: Number of adult pelicans tracked through the end of the breeding season from each colony is indicated in parentheses. Marker sizes represent relative colony size (75–5,000 nesting pairs). Planning areas are delineated by dashed lines (Base layer: Esri™, DeLorme, GEBCO, NOAA, NGDC, and other contributors).

2.1.2 Data Collection Schedule

Between 26 April and 3 July 2013, we captured 60 adult pelicans that were breeding on colonies throughout the study area and equipped them with GPS transmitters. During the same period, we collected physical measurements and feather samples from 3–4-week-old chicks at the six colonies used for adult tracking.

Between 26 April and 29 May 2014, we captured 25 additional adult pelicans that were breeding on colonies throughout the study area and equipped them with GPS transmitters. We also conducted chick sampling and monitored nest productivity at four colonies along the central and northern Texas coast from 8 May to 31 July 2014.

Between 5 May and 31 July 2015, we conducted chick sampling and monitored nest productivity at three colonies in the Florida panhandle and one in Alabama. We did not capture nor GPS tag any additional adults in 2015.

On 26 June 2016, we captured and attached GPS transmitters to five additional adult pelicans on Gaillard Island, Alabama.

Between 20 April and 15 August 2017, we monitored nests and chicks on Gaillard Island and Cat Island, Alabama.

Between 20 April and 15 August 2018, we monitored nests and chicks on Gaillard Island, Alabama. Cat Island did not support nesting pelicans in 2018.

2.2 Individual Tracking

2.2.1 Capture Technique

We captured and attached GPS transmitters to 90 breeding adult eastern brown pelicans, with a maximum of one adult captured per nest. Capture and handling techniques were approved by the Clemson University Institutional Animal Care and Use Committee, in consultation with a veterinarian. All adults were captured on nests using leg nooses during the late incubation and early chick-rearing stages. If eggs were present in the nest, they were replaced with porcelain eggs during capture to prevent damage. If chicks were present, they were moved to the nest edge to avoid injury. Following successful captures, a plastic laundry basket was placed over the nest to protect nest contents from weather and predation during the adult's absence. The basket was used to eliminate the possibility of predation and we did not observe chicks behaving abnormally during or following this procedure. Median handling time was 17.5 min (range = 11–35 mins) from capture to release. After release, we observed individuals for several minutes to ensure that they displayed normal flight, swimming, and balance capabilities. Observation methods and results are described below.

2.2.2 Measurements and Sampling

We collected physiological measurements from all individuals while captured. Immediately following capture, we measured body mass using a 5,000 g Pesola spring scale (Pesola, Switzerland) to ensure that the transmitter weight represented less than 3% of total body mass. The minimum body mass necessary to attach a 65 g GPS unit was 2,167 g, therefore pelicans falling below this threshold were released without a transmitter. Immediately after weighing, we collected 5 mL of metatarsal blood in a heparinized VacuTainer for later analysis of contaminants and blood chemistry. We also collected 0.1 μ L of metatarsal blood on filter paper, which was later used to determine the sex of all captured adults through polymerase chain reaction (PCR) (Itoh et al. 2001). Then, we obtained three contour feathers for contaminant analysis. Finally, we measured three indices of skeletal size: wing chord length (last wing joint to tip of longest primary feather) and culmen length (forehead to bill tip) using a 600 mm wing rule, and tarsus length (intertarsal to metatarsal joint) using a 150 mm caliper.

2.2.3 Tracking Devices

2.2.3.1 Transmitter Specifications and Duty Cycle

We tracked adults using GPS- platform transmitter terminals (PTTs) (65 g, GeoTrak, Inc., Apex, North Carolina: 65 units) and GPS-GSM (65 g, NorthStar Science and Technology, Oakton, Virginia: 20 units), which records GPS locations and uploads data to Argos satellites and cellular towers, respectively, for remote download. Transmitters were programmed to collect 12 locations day⁻¹ during breeding (April–August; every 90 min from 1030 to 0130 GMT), 10 locations day⁻¹ during pre- and post-breeding (September–October and February–March; every 90 min from 0700–0100 GMT), and 8 locations day⁻¹ during winter (November–January; every 120 min from 0700–0100 GMT). We obtained an average error estimate for GPS points from transmitters at known locations (N = 220) of 4.03 ± 2.79 m (13.22 ± 9.15 ft).

2.2.3.2 Transmitter Attachment

Transmitters were attached dorsally between the wings using a backpack-style Teflon ribbon harness (Dunstan 1972; **Figure 2.2**). Transmitters were constructed with sloped fronts, to minimize resistance while diving. Transmitters ranged from 1.5–2.9% of individual body mass ($\mu = 1.9\%$), below the generally accepted 3% threshold for seabirds (Phillips et al. 2003). To elevate the transmitters and prevent feathers from covering the solar panels and antenna, we mounted each device on a 6 mm thick neoprene pad that also extended 6 mm beyond the perimeter of the transmitter in all directions.

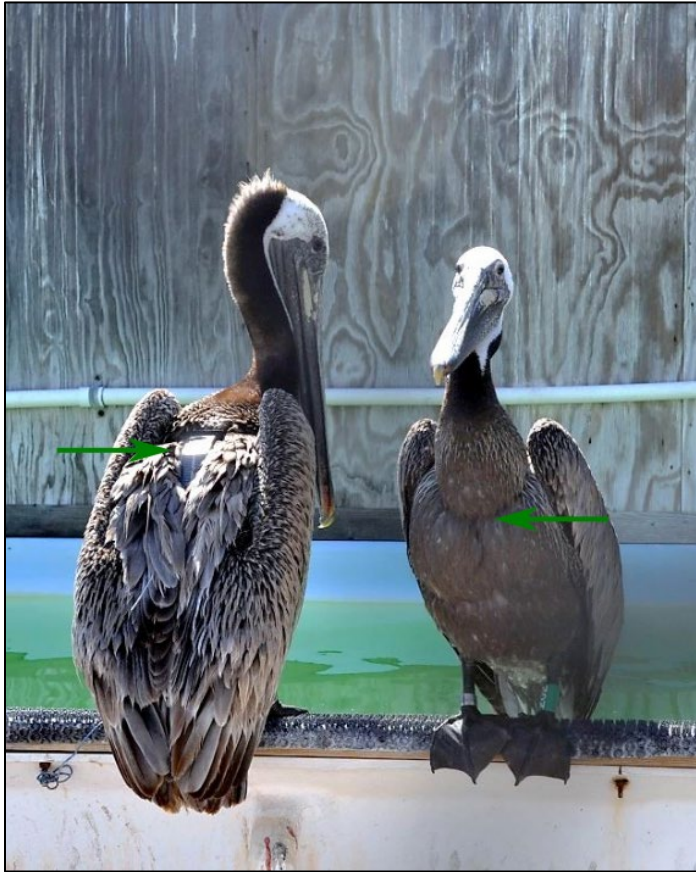


Figure 2.2. Positioning of GPS transmitter and harness

(Left) Dorsal location of GPS transmitter and (right) ventral location of harness. Los Angeles Oiled Bird Care and Education Center, San Pedro, California, 11 June 2015 (F. Last1). Green arrows point to the locations of the dorsal and ventral attachments.

2.2.3.3 Transmitter Effects

2.2.3.2.1 Captive Trial

Because captured birds often leave the colony area after release to forage or loaf offsite (i.e., short-term absence), we chose to assess the immediate behavioral responses of pelicans to transmitter attachment in a captive setting. Five adult California brown pelicans were fitted with transmitters at the Los Angeles Oiled Bird Care and Education Center rehabilitation facility in San Pedro, California on 11 June 2015. All GPS-tagged pelicans were released into a $6 \times 13 \times 5$ m outdoor net enclosure containing a large pool and several perches 4 m in elevation, and filmed for 142 min pre- and 167 min post-transmitter attachment, for a total of approximately 5 hr (309 min) per individual and 25 total observation hours. Four additional adult pelicans that did not receive transmitters were housed in the same enclosure and filmed during the

same time period to serve as behavioral controls. We used EthoLog 2.2 software (Ottoni 2000) to record behaviors of all pelicans during the pre- and post-attachment phases. Behaviors included six mutually exclusive state events: resting (standing or crouching with neck folded and head down), loafing (standing or crouching with head up), perching (standing or crouching in a location accessible only by flight), preening (using beak or feet to rearrange feathers), swimming (floating or paddling on water), and flying and nine instant events: walking, flapping (extension and rapid movement of wings while standing), stretching (brief extension of neck, leg or wing), scratching, eating, shaking (brief, rapid movement while stationary), bathing (splashing in water), diving (completely underwater) and interacting (behaviors directed at or responding to other individuals). To minimize observer bias, all coding was done by the same observer (JSL).

2.2.3.2.2 Field Trial

1–3 days after capture, we conducted 3 hr behavioral observations on all adults present at their respective nests during return visits to the colony ($N = 35$ individuals; 105 observation hours). The remaining individuals were not present during return visits, either due to nest abandonment or because their mates were attending the nest at the time. Before beginning the observation, we selected a nearby (≤ 2 m; ≤ 6.6 ft distance) nest at the same phenological stage as each focal nest (i.e., incubation, small chick-rearing, or large chick-rearing) to act as a control for comparison of behaviors.

During the observation, we recorded the behavior of the tagged and control adults at 5 min intervals, classifying behaviors as resting, preening, alert (moving nest material, interacting with chicks or neighboring birds; comparable to loafing behavior in the captive trials), or agitated (alert and exhibiting signs of stress). For each individual observed, we calculated the percent of time spent in each behavior. We then separated the data by behavior and used paired t-tests to compare frequency of each individual behavior between GPS-tagged and untagged individuals.

Using transmitter data, we recorded the duration in days of subsequent nest attendance by all GPS-tagged individuals. Nests were considered active for as long as adults continued to visit the nesting colony at least once a day. We inferred approximate hatching dates from nest stage at date of capture and considered breeding successful if adult attendance continued for at least 60 days after hatch. This represents the minimum age at which nestlings are likely to fledge (Shields 2014). For pelicans that re-nested following capture, we interpreted the start of attendance at the new site as the beginning of incubation and used a 90-day cutoff for successful breeding, incorporating 30 days of incubation time (Shields 2014) in addition to the 60-day fledging period.

2.2.3.2.3 Statistical Analyses

To assess post-capture nest survival and breeding success, we used a generalized linear modeling (GLM) framework to model the probability that parents would attend the nest for at least 60 days after hatch, which we interpreted as likely brood success (binomial function, Bernoulli with logit link). To test which factors most influenced post-capture nest persistence and reproductive success, we included handling time, nest stage, sex, BCI (residual of the linear relationship between mass and culmen length), capture date, and capture location (i.e., breeding colony) as predictor variables. We used a Hosmer-Lemeshow Goodness of Fit test to assess the fit of the global model and compared models using Akaike's information criterion (AIC) values. Models were preferred if they resulted in a decrease in AIC of ≤ 2 relative to the best-fitting model; models with Δ AIC of 4–7 were considered weakly supported (Burnham and Anderson 2004). We estimated means-parameterized model-averaged coefficients over the suite of preferred models, weighted by AIC weights.

2.3 Annual Habitat Use by Adults

Unless otherwise specified, all statistical manipulation of spatial data was conducted using the `adehabitat` family of packages (Calenge 2006) in R 3.2.3 (R Core Team 2014).

2.3.1 Data Screening and Interpolation

Of the 90 transmitters deployed in 2013 and 2014, 74 recorded at least one full breeding season of GPS data (87% of tagged individuals). Only these were included in subsequent analyses of reproductive success. We manually identified and removed outlying data points using a speed cutoff of 65 km hr^{-1} between successive points, which is the maximum travel speed recorded for brown pelicans (Schnell and Hellack 1978). Cleaned locations for each individual were then interpolated to regular 90-min intervals. Because location data were not collected overnight, we chose not to interpolate tracks between successive days, and we differentiated each day as a separate trajectory by cutting tracks between each set of two successive points separated by a gap of greater than 6 h. Subsequent analyses focused on off-colony locations (i.e., habitat use at the nest site was not analyzed here).

2.3.2 Habitat Variables

Because the scale of movement that we observed was relatively small (on the order of tens of kilometers per day, rather than hundreds of kilometers as is commonly observed in pelagic seabirds), we selected environmental variables likely to relate to the distribution of prey rather than those that might facilitate long-distance movement (e.g., prevailing winds) or visual identification of foraging areas (e.g., ocean color). We measured environmental characteristics of brown pelican habitat using seven habitat variables. Four habitat variables were constant year-round for any given point (distance to coastline, distance to river outflow, bathymetry, and bottom substrate) and three habitat variables varied by month (net primary production, sea surface salinity, and sea surface temperature) (**Table 2.1**). We selected these variables to represent a suite of likely drivers of nearshore habitat variation, particularly the distribution of pelican prey populations (e.g., Deegan 1990). Because limited data are available on fine-scale variations in oceanographic features (e.g., currents and eddies), and because these features have a high degree of short-term variability in coastal areas (Kaltenberg et al. 2010), we used the distance to physical features that influence the movement of water (coastline, river outflow) as proxies for these processes. Depth and bottom substrate can influence both prey distributions and oceanographic characteristics. Net primary production, which integrates chlorophyll concentrations over a range of depths (Behrenfeld and Falkowski 1997), provides an index of oceanographic productivity that influences the distribution of consumers at higher trophic levels. Salinity and temperature also influence the distribution of aquatic prey species, depending on their osmotic and thermal tolerances. Because some data were reported at finer spatial resolutions than others (**Table 2.1**), we standardized all variables to a resolution of 0.1 degree (approximately 10 km) grid squares. Distance values were calculated as the distance from the grid square centroid to the feature of interest. For all other variables, we resampled the data using the mean value for each 0.1 degree grid square.

Table 2.1. Environmental data layers and data sources used for analysis of annual habitat use by adult brown pelicans

Variable name	Layer name	Data source	Resolution
Distance to coast	World Vector Shoreline, Intermediate Resolution	Global Self-consistent Hierarchical High-Resolution Geography Database, National Oceanic and Atmospheric Administration (NOAA) (Wessel et al. 1996)	1:25,000
Distance to river outflow	North American Rivers and Lakes	North American Data Atlas, U.S. Geological Survey	1:100,000
Bathymetry	2-min Gridded Global Relief Data, (ETOPO2) v2	NOAA (National Geophysical Data Center 2006)	0.033
Bottom substrate	Dominant Bottom Types and Habitats	GOM Data Atlas, NOAA	-
Net primary production	Vertically Generated Production Model	Ocean Productivity, Oregon State University (O'Malley 2012)	0.083
Sea surface temperature	Sea Surface Temperature, Climatological Mean, 10 m depth	NOAA National Centers for Environmental Information (Boyer et al. 2011)	0.1
Sea surface salinity	Sea Surface Salinity, Climatological Mean, 10 m depth	NOAA National Centers for Environmental Information (Boyer et al. 2011)	0.1

2.3.3 Habitat Selection

We mapped preferred habitat characteristics in ecological space using a multivariate ordination of all habitat variables using a Hill-Smith principal components analysis (PCA; Hill and Smith 1976), which allows the inclusion of both categorical and continuous variables. For each grid square, we calculated habitat suitability as the squared Mahalanobis distance of that point from optimal location of the species in the multivariate ordination (i.e., higher distances indicate less suitable habitat) (Clark et al. 1993, Calenge et al. 2008). We projected habitat suitability as the probability of obtaining a higher squared Mahalanobis distance for that cell than the calculated value. Thus, in the final suitability scores, values closer to one indicate lower distance from the multivariate optimum location and higher habitat suitability.

To characterize individual responses to the measured habitat variables, we used an Outlying Mean Index (OMI) analysis (Dolédec et al. 2000). OMI is an ordination technique that characterizes available sites based on a set of environmental variables. It sets the mean of all conditions at zero in n-dimensional space and determines the axis that describes the maximum amount of marginality (difference from the mean) of individual animals or species in ecological space. Thus, the first axis of the OMI is the combination of environmental characteristics that best explains the position of animals across available resources. Similarly, the position of each habitat characteristic on the first axis of the OMI represents that variable's contribution to animal distributions; that is, the strength of selection on that characteristic. OMI does not assume specific resource selection functions and allows differences in individual niche selection to be taken into account when describing the distribution of a group of animals. We conducted OMIs for each month on all individuals and habitat variables for each behavioral state. We then averaged the scores of individuals on the first OMI axis to calculate niche location and breadth for groups within the population. We also examined the spatial distribution of breeders from different planning areas. We determined 50% and 95% kernel density estimates (utilization distributions [UD]) for all individuals from each planning area using the “ks” package (Duong 2015) with a plugin bandwidth estimator (Wand and Jones 1994,

Gitzen et al. 2006). We then used an Albers Conic Equal-area Projection to calculate the areas included within each planning area's 95% kernel contour, and to estimate the intersection areas between kernels from different planning areas.

2.3.4 Movement States

We fit a two-state Hidden Markov Model (HMM; Patterson et al. 2009) to the regularized movement trajectories using the moveHMM R package (Michelot et al. 2015) to distinguish resident behavior from commuting behavior. HMMs are a particularly flexible and efficient way of characterizing behavioral states from precise and regularized tracking data (Langrock et al. 2012), and thus are a good fit for GPS tracked locations. Briefly, the model assumes a priori that observed movement data are driven by underlying movement "states," characterized by a distribution of step lengths (distance between successive points) and turning angles. A Markov chain is used to describe the state parameters and classify data according to its most probable state membership.

Because we intended to characterize patterns of movement between days rather than within, we fit the model to a reduced data set of one location per day (i.e., the centroid of all locations for that day). We assumed that local (i.e., resident) movement would be characterized by short step lengths and sharp turning angles, and commuting movement by long step lengths and wide turning angles. Therefore, initial step lengths were set at 5 ± 5 km (3.1 ± 3.1 mi) for State 1 and 10 ± 10 km (6.2 ± 6.2 mi) for State 2. Initial turn angles were set at π radians for State 1 and 0 radians for State 2. Angle concentration for each state was initially set at one. In subsequent analyses, all points along the trajectory for a given day were assigned to the movement state associated with that day.

2.3.5 Statistical Analyses

We modeled individual adult home ranges (50% UD, 95% UD) and migratory parameters (migration strategy, migration distance) using full-factorial generalized linear models as a function of colony size, environmental characteristics (principal component 1 and/or 2), and individual characteristics (body size (culmen length), sex, and BCI). In all cases, the global model including all five predictor variables fit the data well (Hosmer-Lemeshow Goodness of Fit tests, $p > 0.1$ for all). We selected the best candidate models using Akaike's information criterion (AICc) values. Models that increased AICc by ≤ 2 relative to the top model were substantially supported, while models with Δ AICc of 4–7 received weak support (Burnham and Anderson, 2004). We calculated means-parameterized model-averaged coefficients and importance values for each predictor based on the full 95% confidence set of tested models. We conducted model selection using the "AICcmodavg" package in R (Mazerolle 2016). To assess relationships between individual predictor and response variables, we used univariate linear models.

2.4 Risk Exposure

2.4.1 Surface Pollutant Data Layer

We created a combined index of potential pollutant sources to calculate surface pollutant concentrations for each grid square (**Table 2.2**). These potential sources included: an ocean pollution data layer generated from shipping traffic and port locations (Halpern et al. 2008), locations of oil drilling rigs and platforms, and locations of oil and gas pipelines (BOEM; State of Alabama Oil and Gas Board; Louisiana: Strategic Online Natural Resources Information System; Texas General Land Office). Together, these potential pollutant sources (i.e., non-plastic pollutants) account for the majority of acute and chronic pollution in this region (NOAA Incident news c2016). After restricting the dataset to active platforms and pipelines, we calculated oil infrastructure concentrations using values of platform counts and total lengths of pipeline per grid square. We assumed each layer contributed equally to pollution risk;

therefore, we summed evenly across the three pollutant layers and normalized the resulting values to create a combined surface pollutant and oil infrastructure data layer.

Table 2.2. Surface pollutant data layers and data sources used for risk analysis

Variable name	Layer name	Data source	Resolution
Surface pollution	Ocean Pollution (Ship Traffic and Ports)	Global Map of Human Impact Project, National Center for Ecological Analysis and Synthesis (Halpern et al. 2008)	0.01
Platforms (fed)	Drilling Platforms—federal waters	Bureau of Safety and Environmental Enforcement GOM OCS Region	NA
Platforms (state)	Drilling Platforms—state waters	Texas General Land Office; Louisiana Strategic Online Natural Resources Information System; Alabama Oil and Gas Board	NA
Pipelines	Oil and Gas Pipelines—Gulf of Mexico	Bureau of Safety and Environmental Enforcement GOM OCS Region	NA

2.5 Ecology and Physiology of Reproduction

2.5.1 Nest Monitoring

During 2013–2015, we visited nesting colonies close to the end of the incubation period and selected 3–4 groups of focal nests per colony, each group containing 20–30 nests. In colonies containing both elevated (shrub) and ground nests, we selected closely spaced groups (i.e., with nests < 2 m from each other within the group) such that nests of both types were represented to allow for comparison. During our initial visit, we photographed the nest group from marked observation points that could be accessed without disturbance to focal nests, assigned an identifying number to each nest, and recorded nest contents. During return visits, we identified nests using the numbered photograph and checked the contents of each nest from the observation point. Once nestlings reached 3–4 weeks of age, based on either hatch dates (when known) or plumage development (fully developed scapular contour feathers, remiges and rectrices in pin), we captured all monitored nestlings for sampling. Nestlings were readily captured by hand at or near nest sites. We collected physical measurements (culmen length, tarsus length, wing chord, and mass), checked for the presence of ectoparasites, and counted all ticks found on the underside of the left wing. We banded nestlings on the left tarsus with a permanent plastic band (Haggie Engraving, Crumpton, Maryland: 2014–Green; 2015–Blue) engraved with a unique three-digit white alphanumeric code. We also banded nestlings on the right tarsus with a metal engraved U.S. Geological Survey Bird Banding Lab band, with a unique nine-digit identifying code for later recovery outside the study area. Once nestlings began to disperse away from nest locations, we searched the surrounding areas of the colony with binoculars for banded chicks and recorded all bands observed. We continued observations until chicks reached at least 60 d of age.

During 2017 and 2018, we established productivity plots within brown pelican colonies on Cat Island (2017: n = 2 plots) and Gaillard Island (2017: n = 4 plots; 2018: n = 7 plots), Alabama, during early incubation. Each plot contained 10–30 nests, depending on nest configuration and proximity. All plots were spaced based on natural contours and aspects of the islands, resulting in distance between plots ranging from 60–260 m (197–840 ft). Plots were visited as often as possible given weather conditions and logistics (range: 2–11 d) although cameras were also used to record activity and status daily. During each visit, we enumerated and recorded nest contents. We subsequently banded all nestlings of approximately 21 d of age with a permanent plastic band on the left tarsus (Haggie Engraving, Crumpton, Maryland; 2017: n = 145; 2018: n = 156) engraved with a unique three-digit white alphanumeric code. We also banded nestlings on the right tarsus with a metal engraved U.S. Geological Survey Bird Banding Lab band, with a unique nine-digit identifying code for later recovery outside the study area.

2.5.2 Nestling Health

During 2013–2015, we compared two different assessments of nestling health: BCI (a measurement of the ratio of mass to skeletal size) and feather CORT (a measurement of stress hormone levels in nestling feathers).

2.5.2.1 Body Condition

We ran a PCA on the three measures of skeletal size (tarsus length, culmen length, and wing chord) to calculate BCI (Benson et al. 2003). Using each individual's score on the first principal components axis (PC1) as an index of overall skeletal size, we calculated the best-fitting regression equation for the relationship between mass and PC1 score. We chose a second-order polynomial to accurately represent the asymptotic nestling growth process, which is initially linear but reaches a peak and descends slightly before fledging. Finally, we calculated BCI as the standardized residual of actual body mass from the value predicted by the regression equation.

2.5.2.2 Stress Hormone Levels

At capture, we collected 3–4 scapular contour feathers from each nestling. Feathers were bagged and stored at room temperature until processing. We used a random number generator to select 150 samples per year for CORT analysis, divided equally among study colonies. Following the recommendations of Lattin et al. (2011), we restricted the range of sample sizes analyzed by excluding samples that were extremely small (< 20 mg), and dividing samples larger than 160 mg into separate units for analysis.

We closely followed the methods for feather CORT extraction and analysis originally described by Bortolotti et al. (2008). Briefly, we removed the calamus from each feather, weighed and measured feathers individually, and prepared the sample for analysis by snipping feathers into small (< 0.5 mm) pieces with scissors and transferring the entire sample into a 16 mL test tube. Each sample received 7 mL of methanol and was placed in a sonicating water bath overnight at 30 °C. Then we pipetted the methanol into a separate 13 mL tube and conducted two additional washes, each with 2.5 mL methanol. The cumulative methanol sample, totaling 12 mL, was dried down under N₂, reconstituted in 200 µL buffer, and centrifuged to ensure that all accumulated CORT was dissolved in buffer. We conducted a radioimmunoassay (MP Biomedicals, LLC, Solon, Ohio: ImmuniChem™ Double Antibody CORT 125I RIA Kit) on diluted samples. Simultaneous parallelism tests indicated that the assay accurately detected CORT, and we used a standard sample with known CORT to measure intra-assay variation (1.7–1.9%) and subsampled a single feather sample to measure inter-assay variation (11%). We assessed feather CORT in a total of 365 chicks (2013: N = 126; 2014: N = 144; 2015: N = 95).

2.5.3 Nest and Fledging Success

During 2013–2015, beginning approximately 8 w after hatching, we conducted regular searches of the colony for dead banded chicks and recovered all bands found. Nestlings that were observed alive at least 60 d after hatching and disappeared from the colony, but were not found dead, were presumed to have successfully fledged (Shields 2014). We used this information to determine apparent fledging success (fledglings nest⁻¹). We calculated plot- and colony-wide fledge success as the number of chicks fledged from observation nests, divided by the total number of nests observed. Because detectability of fledglings is high in this species and habitat, we considered this method to accurately represent overall fledging success.

During 2017–2018 (i.e., Gaillard and Cat Islands, Alabama), we enumerated and recorded nest contents during each visit. During subsequent visits, we searched for banded chicks at the colony site and by observations from a small power boat within 70 m of the shore until all banded chicks were located and identified. We continued re-sighting efforts until $\geq 80\%$ of the banded chicks were > 70 (2017) or 65 (2018) days post hatch, which we defined as “fledged” (Schreiber 1980). All monitored nests were assigned a final fate of either successful (≥ 1 egg hatched) or failed (0 eggs hatched) and all broods were assigned a final fate of either successful (≥ 1 chick fledged) or failed (0 chicks fledged). We refer to these fates as nest success and brood success, respectively.

At Gaillard and Cat Islands, we measured 10 environmental variables to assess in relation to daily survival rate (DSR) of nests and broods. Nest-based variables that remained fixed throughout the breeding season (n = 3) included substrate beneath nest (rock from rip rap or bare ground), nest elevation above sea level (low = 0 - 0.59 m / 1.94 ft, medium = 0.60 - 0.75 m / 1.97–2.46 ft, high = 0.76–1.0 m / 2.49–3.28 ft, and berm > 1.0 m / 3.28 ft; except brood stage of 2017, when low = 0–0.75 m / 0–2.46 ft, high > 0.75 m / 2.45 ft due to restricted sample sizes within categories), and distance from nest to Mobile Bay waters (range = 1.5–127.7 m). These are hereafter referred to as fixed variables and we recorded these once at the start of the nesting season. Nest-based variables that could change during the breeding season (n = 2) included nest height above ground and vegetation cover directly above the nest. These are hereafter referred to as dynamic variables and we measured these approximately every 3 weeks (range 2–4 weeks)

beginning with the establishment of the plots. We used the average value of the dynamic variables in subsequent analyses. Nest height above ground level (range = 0–156 cm) was measured by placing a level across the nest, then measuring the distance from the ground to the edge of the level (i.e., the rim of the nest). Vegetation cover (range = 0–100%) was measured using photographs taken from the center of the nest, with the lens facing the sky. These photographs were analyzed in Adobe® Photoshop® by overlaying a grid of 100 squares on each photo and enumerating the grids that contained vegetation to establish percent cover.

2.5.4 Post-fledging Survival

We relied on opportunistic re-sighting of banded chicks by colony monitors and birders along the coast of the GOM to determine survival post-fledging of birds banded in 2013–2015. We received band re-sightings and recoveries reported to the U.S. Geological Survey Bird Banding Lab, as well as directly through a dedicated web portal. Sightings and recoveries were obtained throughout the U.S. Gulf Coast and from Mexico through January 2016. To calculate colony-wide survival rates, we used a joint live recapture–dead recovery model (Burnham 1993). We assessed survival rates at two time steps: survival to fledge (3 months after hatch) and post-dispersal survival (6 months after hatch). Dead individuals were recovered in the intervals between time steps, and individuals were considered to have survived to a new time step if they were re-sighted alive after that period ended. Because re-sightings and recoveries occurred across the entire range of the population, we fixed dispersal parameters (F) at a value of one (i.e., 100% probability that banded individuals remained in the sampling area). We derived parameter estimates for survival (S), recovery (r), and re-sighting (p) during each time interval using Markov chain Monte Carlo estimators with a burn-in of 1,000 samples, followed by 4,000 tuning samples and 10,000 runs.

2.5.5 Statistical Analysis

For data collected in 2013–2015, we conducted a logistic regression with a binary outcome (fledged/died) on each metric and assessed the fit of the resulting models to evaluate health metrics as predictors of individual survival to fledge. We ran independent GLMs, each with a binary outcome (fledged/died; re-sighted alive/ recovered dead) and logit link, with health parameters (CORT, BCI) and individual covariates (nest elevation, nesting colony, date, hatch order, and number of siblings) as fixed factors to assess the utility of measured covariates as predictors of individual nestling survival. We used a GLM framework (Gamma, log link) with fledge success as the response variable and health metrics as predictor variables to compare the relative value of different metrics for predicting aggregate nest productivity and survival rates at the colony level. We computed AICc values to account for the small sample sizes that resulted from using colony as the sampling unit and used these values for model comparison. Models were considered to receive strong support if they resulted in a $\Delta AICc \leq 2$ (Burnham and Anderson 2004).

For data collected from 2017–2018 in Alabama, we assessed reproductive success by calculating the DSR of nests (incubation stage, laying to hatch) and broods (chick-rearing stage, hatch to fledge) using package RMark (Laake and Rexstad 2014). The nest survival module models the survival probability (i.e., DSR) over the course of each breeding stage as a function of user-specified covariates using generalized linear models with a logit link function and binomial errors. Before analyses, we compared the DSR of nests and broods between Gaillard and Cat Islands and, finding no difference ($P > 0.10$ for each), pooled data from both islands in subsequent analyses. We report DSR and apparent survival to allow for comparisons to previous studies.

We modeled the relationships of the independent variables with DSR separately for incubation and brood rearing. We also included the following independent variables: Julian date, nest age (nest success models), and age of first chick hatched (brood success models) (calculated in RMark using AgeFound and AgeDay1). We tested both linear and quadratic terms for the age and time covariates and used the best-

performing term for each variable (quadratic for age covariates in all breeding stages except for 2017 brood rearing; linear for all time covariates in all models) in subsequent models (Streker 2019). We developed a suite of hypotheses to assess the relationship between the independent variables and daily survival rates (**Table 2.3**). Variables that were highly correlated ($|r| \geq 0.5$) were not included in the same model and therefore multiple global models were developed to separate correlated values. For each year for incubation data we re-ran the top-performing models (see below on identification of top-performing models) on the subset of nests within which temperature was recorded to assess whether the addition of nest-specific temperature variables substantially improved model fit. Temperature variables were not tested during chick-rearing due to the small sample size of broods that failed that also had temperature loggers (2017: $n = 1$ nest with temperature logger + brood failure; 2018: $n = 7$ nests with temperature logger + brood failures).

Table 2.3. Models assessed in relation to daily survival rates of eggs and broods of brown pelicans breeding on Gaillard and Cat Islands, Alabama, 2017 and 2018

Model name	Hypothesis	Variables included
Time	Survival has a linear relationship with time	Julian date
Age	Survival has a nonlinear relationship with age	Nest age*
Weather [^]	Survival has a positive relationship with mild weather	Average humidity + Average barometric pressure
Storm	Survival has a negative relationship with increasing storm activity	Average humidity + Average barometric pressure + Distance to water
Location	Survival has a nonlinear relationship with location	Distance to water + Elevation + Location+ Julian date + Nest age*
Habitat [^]	Survival as a positive relationship with habitat variables	Nest height + Vegetation cover +Substrate + Julian date + Nest age*
Null	Survival is constant	~1
Global	Survival has a linear relationship with all variables	All variables
Temperature [^]	Survival has a linear relationship with temperature within the nest	Average temperature + Maximum temperature + Julian date + Nest age*

*Quadratic age term for all years and breeding stages except for 2017 chick-rearing, when a linear term was used

[^] Ran both the models listed and additional models that included average daily temperature and maximum daily temperature as recorded by loggers placed in a subset of nests.

2.6 Nestling Diet

2.6.1 Diet Sampling

In Year 1 (2013), we collected meals opportunistically from chicks captured for banding and sampling. In years 2–3 (2014–2015), we visited each study colony regularly (every 5–7 days). We selected recently fed nestlings, based on either having seen a feeding occur or observing that the nestling had a visible bolus or engorged throat, to obtain meals from nestlings. We approached the nest from the colony edge and waited for the nestling to voluntarily regurgitate the meal. If the target nestling did not regurgitate, we selected a different nestling and repeated the process until we had obtained approximately 10 complete meals. We targeted different areas of the colony on subsequent visits to limit disturbance to individual nests; we also varied the time of day at which samples were collected. We collected meals throughout the chick-rearing period, from hatch (late April) through fledging (early August). We targeted nestlings at the same stage of feather development to limit chick age variation within each sample, indicating similar hatch dates, and recorded overall nestling age for the sample as estimated from feather growth (*sensu* Walter et al. 2013).

We did not collect samples from recently hatched nestlings (1 week old or less), both to limit disturbance and because pelican nestlings do not consume whole fish until several days after hatch (Sachs and Jodice 2009). Additionally, because nestlings regurgitated food less readily as they reached adult size, we were not able to sample chicks older than approximately 10 weeks of age. Samples were stored on ice in the field and then moved to a freezer within ~6 hr of collection.

2.6.2 Diet Composition

During processing, we thawed each sample in a warm-water bath, removed it from plastic, dried off surface water using paper towels, then weighed, measured, and identified the species of each individual fish. We based species identifications on descriptions in McEachran and Fechhelm (2010), relying on soft tissue and external characteristics. We also classified each fish as whole (no visible damage), partial-whole (total or standard length obtained, but some soft tissues missing), and partial (length could not be obtained). For samples containing large numbers (50–1,000) of small fish of the same species (26% of samples), we counted the total number of individuals of the species, weighed and measured a subsample of 10 individual fish, and obtained a total weight and overall classification (whole, partial-whole, partial) for each species group. For samples containing extremely large numbers (> 1,000) of small fish (<1% of samples), we weighed and measured a subsample of 10 fish per species, weighed the overall sample, and used the average weight per fish to approximate the total number of fish in the sample. For samples in which individual fish were no longer intact, we counted the number of heads and tails present in the sample and used the larger of the two numbers as an approximate count. We did not analyze samples for which the digestive process was too advanced to identify fish to species (< 1% of all samples collected).

Where needed, we corrected standard lengths of fish to total lengths using the best-fitting regression equation between standard and total length for that species calculated from whole samples. We calculated the length-weight relationship as the best-fitting regression equation between log total length and log mass of whole fish for each species by year to estimate the mass of partial-whole and partial fish. For partial-whole fish (i.e., degraded fish for which we were able to measure total length), we used the regression line to estimate the corrected mass of the whole fish from its length. For partial fish (i.e., degraded fish for which total length was not measurable), we used the mean total length of whole and partial-whole individuals collected from the same breeding colony on the same day to estimate a corrected mass from the regression equation. Finally, we totaled the corrected masses of individual fish within each meal to obtain a total corrected meal mass.

2.6.3 Provisioning Rates

To assess meal delivery rates, we conducted 3 hr nest observations during each colony visit throughout the chick-rearing period (i.e., every 5–7 d from hatch through fledging, late April to early August). We selected groups of 15–20 nests, varying both the location within the colony and the time of day of observations. During each 3 hr period, we recorded all direct feedings in which a nestling's head entered an adult's throat and the nestling's throat was subsequently engorged. Indirect feedings in which parents regurgitate prey onto the floor of the nest as opposed to the chick directly (Sachs and Jodice 2009) appeared to take place only within the first few days after hatch. Because chicks are brooded by adults during this time and are hidden from view, the frequency of such feedings was difficult to quantify; thus, we excluded recently hatched nests from observation.

We calculated meal delivery rates on a per-nest basis. This measure reflects the rate of provisioning by adults, but not necessarily the rate at which each individual nestling consumes food. Pelicans can raise up to three young, hence meals delivered to a nest may be shared among as many as three nestlings. However, each nestling may not receive an equal share, because nestlings that hatch earlier can often consume a larger share of feedings based on superior competitive ability (larger body size, more advanced muscle development and mobility) or preferential feeding by adults. Because we were not able to

consistently distinguish first, second, and third-hatched chicks in the field throughout the extended chick-rearing period and subsequently allocate feedings to individual chicks, we chose to assess delivery rates by nest with number of chicks as a covariate. We standardized delivery rates to a 15-hr day, representing the average day length (civil twilight) during the study period. Pelicans are visual foragers and are considered not to forage at night (Shields 2014), and our observations suggest that adult activity diminishes shortly after sunset.

2.6.4 Proximate Composition and Energy Density

We measured proximate composition and energy density of common prey species using methods described by Anthony et al. (2000). Briefly, we dried fish to a stable mass in a 60 °C oven and homogenized samples using a mortar and pestle. We then extracted lipids from the sample using a Soxhlet apparatus with a 7:2 (v:v) hexane: isopropyl alcohol solvent. Following the 10-hr extraction, the sample was left to dry for 24 hr and re-weighed to determine lean mass. We then extracted proteins from the sample by ashing at 600 °C for 12 hr. The mass of the remaining skeletal ash was subtracted from the pre-ashing mass to determine the ash-free lean dry mass, which is composed primarily of proteins (94%: Anthony et al. 2000). We then multiplied the lipid and protein contents by standard energetic values based on their relative assimilation efficiencies (lipids: 39.5 kJ g⁻¹; proteins: 17.8 kJ g⁻¹; Schmidt-Nielsen 1997) to obtain the overall energy density of the sample.

We measured energy densities in both regurgitated fish that we identified as whole during processing and bait fish purchased live or freshly caught from fishing suppliers close to study colonies. For the three most common prey species (Gulf menhaden; Atlantic croaker, *Micropogonius undulatus*; and pinfish, *Lagodon rhomboides*), we ran ANOVAs to determine whether energy content differed among planning areas or sample types (bait fish compared to regurgitated fish). Because energy values for one of the three species, Atlantic croaker, differed significantly between the eastern and western planning areas, we calculated energy densities separately for the two planning areas where possible. However, we did not find differences in energy content between bait and regurgitated samples, and therefore combined all samples within each planning area during further analysis. Gulf menhaden had an apparent difference in energy content between bait samples and regurgitated fish ($p = 0.056$). In this case, regurgitated fish were higher in energy than bait samples, so we chose to use only regurgitated samples to determine energy content for this species. We also tested for differences in energy density between locations within planning areas and found over time that the energetic content in Atlantic croaker and Gulf menhaden did not differ within planning areas and did not change as the season progressed. Therefore, we considered energy density of prey to be consistent throughout the breeding season and within each planning area. Because Gulf menhaden were the only prey species to show a bimodal size distribution, we measured energy content of juveniles (< 110 mm total length: Ahrenholz 1991) and adults (> 110 mm) separately.

We multiplied the total mass of each prey species in the meal by the mean energetic value of that species to determine meal-specific energy density. For species without directly measured energy density, we obtained energetic values for the same or closely related species from published literature. Species with directly measured energy content accounted for 93% of the total biomass, while species with inferred values from closely related species measured directly (4%) and those with values obtained from scientific literature (3%) constituted the remaining 7%. We then summed the energy derived from each individual species and divided the sum by the total meal mass to obtain an energetic value (kJ g⁻¹) for the full meal. We calculated meal-specific lipid content using the same process.

2.6.5 Energy Provisioning Rates

We compared values of meal mass (g meal⁻¹), nest-specific provisioning rate (meals nest⁻¹ hr⁻¹), and energy density of meals (kJ g⁻¹) for each colony using ANOVAs with post-hoc Tukey's Honestly Significant Difference (HSD) tests to assess nutritional stress by colony. The product of these three

components is the energy provisioning rate (EPR: $\text{g nest}^{-1} \text{hr}^{-1}$, Jodice et al. 2006). We modeled energy-days for each colony, similarly to Jodice et al. (2006), by randomly selecting (with replacement) 100 values for provisioning rate (meals day^{-1}) from the set of measured values to obtain a combined measure of EPR by colony. The model then randomly selected (with replacement) a mass and an energetic value for each meal, multiplied the meal mass by energy density to obtain the total energy content per meal, and summed the total energy across all meals for each modeled day to obtain a set of EPRs (kJ day^{-1}). We calculated the mean and standard deviation of EPR for each colony by averaging values obtained from 1,000 runs of the model. We calculated EPR on a per-nest basis rather than a per-chick basis, to avoid the confounding relationship between higher provisioning rates and increased longevity of second- and third-hatched chicks (Jodice et al. 2006). Then we evaluated the relationships of individual provisioning metrics to EPR using ANOVAs on nested sequential linear models. Finally, we conducted non-metric multidimensional scaling on proportional composition of meals by species to assess the relationship between species composition and rate of energy delivery to nestlings, and overlaid provisioning metrics on the resulting ordination.

2.7 Health and Physiological Parameters

2.7.1 Blood Analytes

2.7.1.1 Sample Collection

Adult brown pelicans were sampled from active nests during the breeding seasons of 2013–2015 from six different colonies in the Northern GOM. We collected samples from Audubon and Smith Islands, Florida; Gaillard Island, Alabama; Felicity and Raccoon Islands, Louisiana; and Chester and Shamrock Islands, Texas. We collected blood smears from 90 adults and blood samples from 81 of the 90 adults for complete blood counts (CBCs). Not all samples, however, were suitable for complete analyses and so sample size varies among analytes and blood smears. We measured body mass ($\pm 50 \text{ g}/1.76 \text{ oz}$), culmen length ($\pm 1 \text{ mm}/0.04 \text{ in}$), tarsus length ($\pm 1 \text{ mm}/0.04 \text{ in}$), and wing length ($\pm 5 \text{ mm}/0.20 \text{ in}$). These variables were not assessed individually; instead they were used to create a new variable, BCI ($n = 64$), which provides an index for the mass of the bird in relation to its size (see 2.5.2.1). The more positive the BCI, the better the condition of the individual. In brown pelicans, sex cannot be easily determined in situ. Therefore, the distribution of samples between sexes is opportunistic. Sex was later determined from collected blood samples through PCR (Itoh et al. 2001).

Brown pelican chicks were sampled from active nests during the breeding seasons of 2014–2015 from seven colonies in the Northern GOM. We sampled from Audubon and Ten Palms Islands, Florida; Gaillard Island, Alabama; Marker 52 and North Deer (regrouped as Galveston Bay colonies), Chester, and Shamrock Islands, Texas. We collected blood smears and blood samples for CBCs from 35 individuals. As in adults, we measured body mass, culmen length, tarsus length, and wing length, and used these variables to assess BCI ($n = 35$). Sex was not determined for chicks.

For both adults and chicks, blood samples were collected within 2 min of capture from the tarsometatarsal vein. After sterilizing the collection site, we collected a 5 mL blood sample using a 23-gauge needle and VacuTainer tube (Becton Dickinson, Franklin Lakes, New Jersey) with lithium heparin anticoagulant. Samples were stored over cold packs until returning from the field (~5–10 h).

2.7.1.2 Sample Processing

In the lab, we created blood smears from stored samples, filled three capillary tubes for hematocrit analysis, and spun down both samples and capillary tubes using a centrifuge (Becton Dickinson, Franklin Lakes, New Jersey). We recorded hematocrit percent volume from each of the three capillary tubes. We

separated plasma from red blood cells in centrifuged samples by pipetting. All plasma and red blood cell samples were then stored frozen until analysis.

Biochemical, protein electrophoresis, and serological tests were conducted at the University of Miami (Department of Pathology, Miami, Florida). A full biochemical analysis was conducted on plasma samples on a dry-slide chemistry analyzer (Ortho Vitros 250 XR, Ortho Clinical Diagnostics, Rochester, New York) controlled daily for quality and ran per manufacturer's instructions. Evaluated analytes included alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine phosphokinase (CPK), gamma glutamyl transferase (GGT), lactate dehydrogenase, amylase, bile acids, blood urea nitrogen (BUN), calcium, cholesterol, carbon dioxide (CO₂), creatinine, glucose, lipase, phosphorus, potassium, sodium, total protein, triglycerides, and uric acid. Lipoprotein analysis included high-density (HDLc) and very low density (VLDLc) lipoprotein cholesterol. Plasma samples were analyzed following procedures provided in the Helena SPIFE 3000 system with the use of Split Beta gels (Helena Laboratories, Inc. Beaumont, Texas). Protein electrophoresis were scanned and analyzed by Helena software for pre-albumin, albumin, and Alpha 1 (A1G), Alpha 2 (A2G), Beta and Gamma globulins. Percentages for each fraction were determined by this software, and absolute concentrations (g dL⁻¹) for each fraction were obtained by multiplying the percentage by the total protein concentration. The albumin to globulins ratio (A:G) was calculated by dividing albumin by the sum of the globulin fractions. Concentrations of CORT were measured by radioimmunoassay (MP Biomedicals Double Antibody Corticosterone radioimmunoassay, Santa Ana, California). We classified each analyte as a blood gas, electrolyte/mineral, enzyme, lipid, metabolite, plasma protein, or stress hormone. We also noted typical indications from each analyte (e.g., nutrition, hepatic damage).

Blood smears were stained with Diff-Quik (Siemens Healthcare Ltd., Ontario, Canada) and reviewed at 1,000x to determine differential counts of white blood cells (WBC). We also measured the concentration (*10³ μ L⁻¹) of all WBC and the concentration of heterophils, lymphocytes, monocytes, eosinophils, and basophils.

2.7.1.3 Statistical Analysis

Among the 30 blood analytes, we identified any with a binomial distribution and separated the data into "high" and "low" categories, then treated those as two separate analytes (i.e., analyzed each category separately). We tested for differences in the independent variables between the low and high categories using t-tests, χ^2 tests, and Wilcoxon rank tests. Sodium as measured in samples from adults was the only analyte for which this bimodal treatment was necessary. Measures of sodium classified as "low" ranged from 111–156 mEq L⁻¹ and measures of sodium classified as "high" included only the maximum measured value for sodium of 250 mEq L⁻¹.

We assessed outliers for all data that did not have a binomial distribution (i.e., all analytes other than adult sodium) using the Dixon outlier range statistic. Following guidance in Geffré et al. (2011), we retained rather than deleted an outlier unless it seemed likely to be an aberrant observation. We examined the distance between points identified as potential outliers and non-outlying points to make this decision. We also compared points identified as potential outliers to published values for species within the same order. Once aberrant outliers were removed, we checked normality of the data using the Anderson-Darling test. We then calculated reference values using Reference Value Advisor (Geffré et al. 2011). When sample sizes were sufficient, we computed a nonparametric reference interval, calculated the lower and upper confidence intervals on the reference intervals using a bootstrap approach, and provided descriptions of alternate approaches when appropriate (Geffré et al. 2011). We reported reference values from raw data for all analytes. For analytes that did not meet assumptions of normality, we followed guidance from Geffré et al. (2011) and conducted a Box-Cox transformation for subsequent statistical analyses. The Box-Cox transformation is commonly used for data such as concentrations of blood analytes that are often heteroscedastic (i.e., non-constant variance).

We assessed the relationship between each blood analyte and a suite of independent variables using GLMs. Independent variables for analyses of blood analytes and smears included: sex (adults; categorical), BCI (adults and chicks; continuous), planning area (adults and chicks; categorical), and home range size (adults; continuous). Planning area was classified using the planning areas for BOEM in the GOM. Oil and gas development are highest in the central planning area (coasts of Louisiana, Mississippi, and Alabama), least in the eastern planning area (Florida panhandle), and moderate in the western planning area (Texas coast). Home range was reported as the 50% core area for any individual that was equipped with a satellite transmitter ($n = 64$). We selected the 50% core area as opposed to the 95% use area for analysis with blood analytes to assess the overall individual condition. We suggest the 50% core area better represents the conditions encountered regularly by an individual compare to the 95% use area and therefore the core area is most likely to affect an individual's overall condition. Deployment methods for satellite tags and calculation of home range size are detailed in Chapter 2 and Chapter 3. Note, the sample of animals with home range data is a subsample of those reported in Chapter 3 and therefore reported measures of home range size are not identical between Chapter 3 and Chapter 8. Continuous variables were scaled before running logistic models. Therefore, coefficient estimates were on a standardized scale with respect to independent variables and on either a raw or Box-Cox scale with respect to dependent variables. However, all figures used a raw scale for both dependent and independent variables. Correction procedures for repeated tests were not conducted (Moran 2003, García 2004, Nakagawa 2004).

We built eight models to assess the relationships among blood analytes and independent variables and compared them using AIC. We reported coefficient estimates from the top-ranked model when coefficient estimates \pm SE did not overlap 0. Transformed data were used for analyses when appropriate, but raw data were presented for ease of interpretation.

2.7.2 Polycyclic Aromatic Hydrocarbons

2.7.2.1 Sample Collection

Adult brown pelicans were sampled from active nests during the breeding seasons of 2013–2015 from seven different colonies in the Northern GOM. We sampled from Audubon and Smith Islands, Florida; Gaillard Island, Alabama; Felicity and Raccoon Islands, Louisiana; and Chester and Shamrock Islands, Texas. We collected feathers from 92 individuals and randomly selected blood from a subset of 33 individuals (from the pool of sample available described in 2.8.1.1) for PAH analysis. We measured body mass (± 50 g/ 1.76 oz), culmen length (± 1 mm/ 0.04 in), tarsus length (± 1 mm/ 0.04 in), and wing length (± 5 mm/ 0.2 in) of every individual. These variables were not assessed individually; instead they were used to create a new variable, BCI ($n = 79$), which provides an index for the mass of the bird in relation to its size. The more positive the BCI, the better the condition of the individual. In brown pelicans, sex cannot be easily determined in situ. Therefore, the distribution of samples between sexes is opportunistic. Sex was determined from collected blood PCR (Itoh et al. 2001).

We collected blood samples within 2 min of capture from the tarsometatarsal vein. After sterilizing the collection site, we collected a 5 mL blood sample using a 23-gauge needle and VacuTainer tube (Becton Dickinson, Franklin Lakes, New Jersey) with lithium heparin anticoagulant. We then stored samples over cold packs until returning from the field (~5–10 h). We collected 3–4 scapular feathers from each adult and chick. Feathers were stored at room temperature until processing.

2.7.2.2 Sample Processing

PAH analyses were conducted at the University of Connecticut Center for Environmental Sciences and Engineering (Storrs, Connecticut). In the lab, we weighed 0.2 g (0.007 oz) of blood sample into a 1.5 mL plastic centrifuge tube. Samples were spiked with quality control standard solutions and vortexed for 1

min at 2,500 rounds per min. Methanol or acetonitrile (500 μ L) were added to each tube along with $MgSO_4$. Samples were then vortexed for 5 min at 2,500 rounds per min, then centrifuged for 10 min at 14,000 rounds per min. Next, 190 μ L of the supernatant were transferred to a 300 μ L liquid-chromatography vial. These samples were then spiked with an internal standard and vortexed.

Following extraction, the samples were analyzed for alkylated PAHs using an Agilent 6890 gas chromatograph (Agilent Technologies, Inc., Santa Clara, California) equipped with a Restek Rxi-5Sil MS column (Restek, Bellefonte, Pennsylvania; 30 m) using splitless injection coupled to a Waters QuattroMicro triple quadrupole mass spectrometer (Waters Corporation, Milford, Massachusetts). Parent PAHs were quantified using a Waters Acquity ultra-performance liquid chromatograph (UPLC; Waters Corporation, Milford, Massachusetts) with fluorescence and photo diode array detection, which was equipped with an Acquity UPLC BEH C18 column (Waters Corporation, Milford, Massachusetts; 1.7 μ m, 2.1 \times 100 mm). All peaks were quantified against the internal standard, and the extraction efficiency was evaluated using a surrogate standard of naphthalene-d8. Standard quality assurance procedures were employed, including analysis of duplicate samples, method blanks, post-digestion spiked samples, and laboratory control samples.

Feathers were washed three times in acetone, three times in high performance liquid chromatography water, and one additional time in acetone before allowing them to dry overnight (ca. 10 h). Feathers were weighed (\pm 0.2 g/ 0.007 oz) on folded weighing paper and transferred directly into the accelerated solvent extraction (ASE) cell using forceps when needed. Hydromatrix powder was added to pack the 11 mL ASE cells. Using gelatin as the matrix for the blank and laboratory control samples, a 0.2 g (0.007 oz) sample was weighed out and transferred to ASE cells. Samples were then spiked with quality control standards. ASE extracts were subsequently run and collected utilizing acetonitrile solvent, and the solution was transferred into the pre-marked conical evaporation vials and evaporated to just below 0.5 mL under a gentle nitrogen stream (set flowrate on N-Evap unit to 180 mL/min). Samples were spiked again with internal standard. The volume was then brought up to 500 μ L with acetonitrile and vortexed for a few seconds to mix. Filtered samples were injected into liquid-chromatography vials using 1 mL plastic syringes and 4 mm, 0.2 μ m syringe filter.

The detection limit was 5 ng g^{-1} (i.e., part per billion) and values for PAHs were reported as wet weight for blood and dry weight for feathers.

2.7.2.3 Statistical Analysis

For subsequent PAHs analyses, we considered three dependent variables: the sum of all PAHs detected (sumPAH), the sum of parent PAHs detected (sumPAR), and the sum of alkylated PAHs detected (sumALK). We assessed the relationship between each and a suite of independent variables, including: BCI (adults and chicks; continuous), planning area (adults and chicks; categorical), sex (adults; categorical), migration class (adults; categorical) and home range size (adults; continuous). Planning area was classified using the planning areas for the BOEM as defined above. Home range was reported as the 95% use area for any individual that was equipped with a satellite transmitter. We selected the 95% use area for PAHs analysis rather than the core (50%) use area because we were interested in assessing the overall exposure of the individuals and suggest that this is best represented by the full extent of the area used. Methods for deployment of satellite tags and calculation of home range size are detailed in Chapters 2 and 3. Note, the sample of animals with home range data is a subsample of those reported in Chapter 3; therefore, reported measures of home range size are not identical between Chapters 3 and 8. Migration distance was calculated as the distance between the center of breeding home range and the center of winter home range, and classified as short (i.e., resident: < 200 km/ 124 mi), medium (200–800 km/ 124–497 mi), and long (> 800 km/ 497 mi).

We used a hurdle modeling approach to assess relationships between PAHs and independent variables. Step one of the hurdle model used a binomial logistic regression with a log link function, using the presence or absence of each of the three PAH variables as the response variable. Step two of the hurdle model used a GLM with a gamma distribution and a log link function, using the sum of the concentration of each of the three PAH variables as the response variable. The gamma model included individuals where sumPAH, sumPAR, or sumALK were superior to detectable limit of PAHs (i.e., sum \neq 0).

We built 12 models for adult blood samples, 16 models for adult feather samples, and 3 models for chick feather samples and compared them using AIC to assess the relationships among PAHs and independent variables. We reported coefficient estimates from all models within $\Delta AIC \leq 2.0$ and average coefficient estimates if they appeared in > 1 of the top models. We reported coefficient estimates when estimate \pm SE did not overlap 0. We provided odds ratios for coefficient estimates from binomial logistic models (the odds of a PAH being detected for a change in categorical levels, or for a one unit increase in a continuous variable) and from gamma models (the odds of the concentration of a PAH increasing by 1 ng g⁻¹ for a change in categorical levels, or for a one unit increase in a continuous variable).

3 Individual Tracking

Between 2013 and 2015 we deployed 86 transmitters on breeding pelicans throughout the Northern GOM (**Figure 2.1; Table 3.1**). Transmitters were evenly distributed between the eastern (Smith, Audubon, and Gaillard Islands, Florida-Alabama), central (Felicity and Raccoon Islands; Louisiana), and western (Shamrock and Chester Islands, Texas) planning areas, with colony sizes ranging from 40 to 4,500 breeding pairs. Transmitters typically collected data for between six months and three years, with most transmitting for 1–2 years before tag failure or mortality occurred. Transmitters collected data for breeding and non-breeding movements, including staging and migration. Brown pelicans used local habitat during the breeding season but showed substantial movement during the non-breeding season (**Figure 3.1**). Individuals breeding in the eastern GOM wintered as far west as southern Louisiana and as far south as central Cuba. Individuals breeding in the central GOM wintered along the entire GOM coastline, traveling as far south as Chiapas, Mexico and Belize. Individuals breeding in the western GOM staged as far east as southeastern Louisiana and wintered as far south as Chiapas, Mexico.

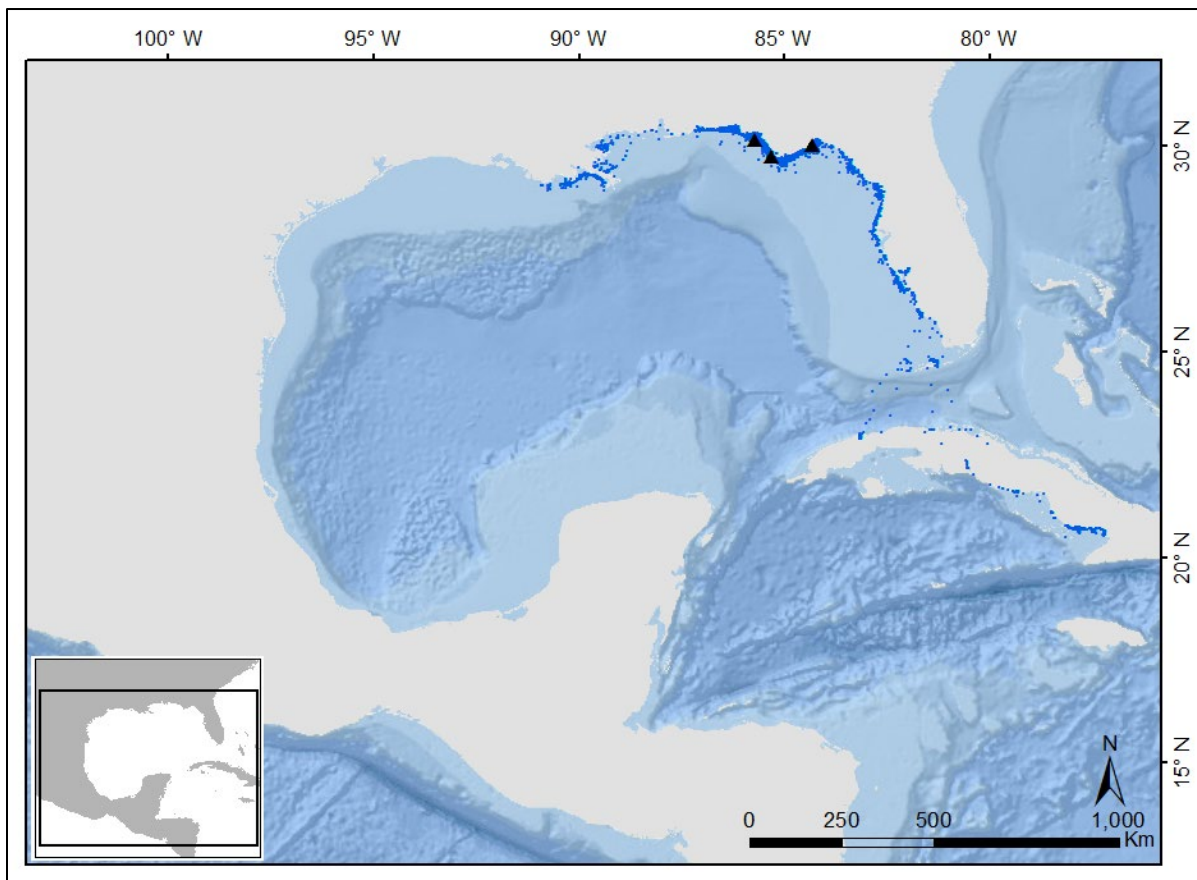


Figure 3.1. GPS locations of brown pelican originally captured at breeding colonies in the Eastern, Central, and Western Planning Areas of the Northern GOM, 2013–2016, Eastern Planning Area
Note: Black triangles indicate capture locations. Base layer: Esri, DeLorme, GEBCO, NOAA, NGDC, and other contributors.

Table 3.1. Colony characteristics and measurements of tracked adults captured at six brown pelican breeding colonies, GOM, 2013–2014

	Smith		Audubon		Gaillard		Felicity		Raccoon		Shamrock		Chester	
Colony size (breeding pairs)	40 ^a	-	100 ^a	-	4,500 ^b	-	1,800 ^c	-	4,300 ^c	-	1,400 ^d	-	3,200 ^d	-
# of adults tracked	9	-	11	-	5	-	12	-	14	-	11	-	10	-
% male	78	-	64	-	40	-	50	-	57	-	55	-	30	-
Mass (g)	3,414	± 432	3,414	± 558	3,190	± 329	3,448	± 36	3,546	± 353	3,459	± 562	3,070	± 508
Culmen length (mm)	322	± 22	315	± 21	312	± 20	313	± 23	316	± 23	321	± 25	309	± 19
BCI ^e	-141	± 273	-241	± 205	-131	± 343	77	± 195	121	± 263	-19	± 306	-147	± 281

Data sources:

^a Florida Fish and Wildlife Conservation Commission (<https://public.myfwc.com/crossdoi/shorebirds/>)

^b Dauphin Island Sea Labs

^c Walter et al. (2014)

^d Texas Colonial Waterbird Census (https://tpwd.texas.gov/huntwild/wild/wildlife_diversity/tews/data.phtml)

^e Body Condition Index (BCI) is a derived parameter representing the relationship between mass and skeletal size. Positive values indicate higher mass than predicted by the regression between mass and skeletal size, while negative values indicate lower mass than predicted.

3.1 Tag Effects

3.1.1 Behavioral Effects

Before treatment, captive pelicans spent the majority of time loafing (18–47%), preening (11–32%), or resting (20–49%). Swimming, perching, and flying each occupied less than 10% of individual time budgets. In the first 1–2 hr after receiving transmitters, GPS-tagged individuals spent an increased percentage of time preening (mean = + 16.4%, $F_{(1,7)} = 6.41$, $p = 0.038$) and decreased time resting (mean = -29.1%, $p = 0.047$, $F_{(1,7)} = 5.62$) relative to individuals that had not been tagged or handled. Changes in time spent swimming, flying, loafing, and perching did not differ from zero. We did not find significant differences in frequency (events hr^{-1}) after tagging for any of the instant events we quantified; also see Lamb et al. 2017). In free-ranging pelicans 1–3 days post-capture, we did not observe differences between tagged individuals and untagged neighbors in the proportion of observation time spent in preening ($t_{31} = -0.59$, $p = 0.56$), resting ($t_{31} = -0.88$, $p = 0.38$), alert/loafing ($t_{31} = 1.60$, $p = 0.12$), or agitated ($t_{31} = -1.42$, $p = 0.17$) behavioral states.

3.1.2 Effects on Nesting Success

Overall, GPS-tagged pelicans ($N = 74$) continued attending nests for an average of 50 d (standard deviation [SD] ± 34 ; Range 0–113) after capture, with a 51% apparent success rate for breeding ($N = 38$ successful nests). Apparent success rates of tagged breeders were slightly lower than but not significantly different from success rates of untagged adults measured in the same colonies in 2014–2015 (62%; $N = 482$; $X^2_1 = 3.46$; $p = 0.06$). The majority (88%; $N = 65$) continued breeding at their original nest sites following capture. The remaining adults either abandoned the breeding colony within one day of capture and did not re-nest that season ($N = 3$), re-nested at a different nest site in the same breeding colony ($N = 3$), or re-nested at different breeding colonies between 30 and 65 km (18.64–40.40 mi) from the original nesting colony ($N = 3$). Successful breeders attended colony sites for an average of 83 d after hatch (SD ± 13 d), while unsuccessful breeders attended on average 18 d (SD ± 14.7 d). We observed successful breeding in pelicans that re-nested elsewhere as well as pelicans that remained at their original nest sites. Breeding success was similar in the eastern (76%) and central (67%) planning areas and lower in the western (15%) planning area. In the eastern planning area, breeding success of tagged pelicans in 2013–2014 was similar to that of untagged pelicans at the same study colonies in 2015 (72%; $X^2_1 = 0.23$; $p = 0.63$). In the western planning area, breeding success was lower in tagged pelicans in 2013–2014 than in untagged pelicans in 2014 (45%; $X^2_1 = 9.91$; $p = 0.002$). We did not measure breeding success of untagged pelicans at the central colonies during any of the three study years.

The global model predicting breeding success of tagged birds was a good fit for the observed data, indicating that the full suite of parameters effectively explained variation in breeding success ($X^2_8 = 1.85$, $p = 0.99$). The four best-performing models for breeding success included capture location (**Table 3.2**), an index of underlying variability among planning areas. The model-averaged coefficient estimates (\pm SE) for location, with the eastern planning area set as the reference location, were -0.43 ± 0.66 for the central planning area and -2.83 ± 0.75 for the western planning area. Two of the top models also included handling time (-0.64 ± 0.54), and two included sex (0.67 ± 0.56). Phenological variables (capture date and nest stage), year of capture, physical condition (BCI), and percent body mass of transmitters were not included in the best-performing models for breeding success.

Table 3.2. Candidate models for breeding success of brown pelicans, GOM, 2013–2014

Model ID	Terms	AIC	Δ_i (AIC)	w_i (AIC)	Σw	L_i (AIC)
10	Location	85.75	0	0.30	0.30	1
13	Sex + Location (2 + 10)	86.3	0.55	0.23	0.53	0.76
16	Handling + Location (9 + 10)	86.97	1.22	0.16	0.69	0.54
19	Sex + Handling + Location (2 + 9 + 10)	87.56	1.81	0.12	0.81	0.40
15	Phenology + Location (8 + 10)	88.81	3.06	0.07	0.88	0.22
18	Sex + Phenology + Location (2 + 8 + 10)	89.46	3.71	0.05	0.93	0.16
20	Phenology + Handling + Location (8 + 9 + 10)	90.15	4.4	0.03	0.96	0.11
21	Global (2 + 8 + 9 + 10)	90.91	5.16	0.02	0.98	0.08
11	Sex + Phenology (2 + 8)	95.29	9.54	< 0.01	-	< 0.01
8	Phenology (5 + 6)	95.45	9.7	< 0.01	-	< 0.01
15	Sex + Phenology + Handling (2 + 8 + 9)	96.72	10.97	< 0.01	-	< 0.01
14	Phenology + Handling (8 + 9)	96.75	11.0	< 0.01	-	< 0.01
5	Nest stage	97.8	12.05	< 0.01	-	< 0.01
2	Sex	103.9	18.15	< 0.01	-	< 0.01
12	Sex + Handling (2 + 9)	104.3	18.55	< 0.01	-	< 0.01
6	Capture date (Julian)	104.5	18.75	< 0.01	-	< 0.01
22	Null model	104.5	18.75	< 0.01	-	< 0.01
9	Handling time	104.6	18.85	< 0.01	-	< 0.01
1	BCI	105.1	19.35	< 0.01	-	< 0.01
3	Payload (% body mass of transmitter)	106.5	20.75	< 0.01	-	< 0.01
7	Capture year	106.5	20.75	< 0.01	-	< 0.01
4	Individual (BCI + sex + payload)	107.5	21.75	< 0.01	-	< 0.01

Note: Models are ranked in order of increasing AIC values with model weights (w_i), cumulative weights (Σw) and relative likelihoods (L_i). Models above the dashed line were considered strongly preferred (Δ AIC < 2). Terms used in models are defined in Chapter 2 (Methods). Numbers in parentheses represent model IDs.

Handling time at capture was significantly longer in unsuccessful than successful breeders ($t_{55} = 1.7$, one-tailed $p = 0.047$), with a significant decrease in breeding success among birds that were handled for more than 20 min (Fisher's Exact Test, one-tailed $p = 0.045$). Sex did not differ significantly between successful and unsuccessful breeders (Fisher's Exact Test, one-tailed $p = 0.33$); however, females were more likely than males to abandon or re-nest within one day of capture (Fisher's Exact Test, one-tailed $p = 0.045$).

We observed short-term behavioral effects of handling and transmitter attachment in a captive setting 1–2 hr post-release, but not in a field setting 1–3 d post-release. Captive and free-ranging groups were observed under different conditions and had different histories. Because of these differences, the behavioral patterns we observed in captive birds may differ from those of free-ranging individuals. However, both captive and free-ranging pelicans were observed relative to control individuals under the same conditions that were not captured or GPS-tagged. Because we observed behavioral changes immediately after transmitter attachment but not within several days of capture, we suggest that behaviors indicative of stress or discomfort in our study (due to either the attached device, the harness, the capture process, or any combination of the above) diminished rapidly. Although we did not separate handling from device effects (i.e., include procedural controls), the process of fitting an individual with a transmitter inevitably involved both handling and device effects. A meta-analysis by Barron et al. (2010) found that behavioral effects of transmitter attachment are generally indistinguishable between studies with and without procedural controls, indicating that most effects can be attributed to the device alone.

Immediately after transmitter attachment, we observed differences in tagged captive birds' time spent preening and resting relative to the controls. Because both handling and harness attachment may disrupt plumage and reduce waterproofing, increased preening behavior suggests an attempt to restore feather

structure and represents a potential short-term increase in energy expenditure following handling and transmitter attachment. Other behaviors (e.g., swimming, perching, flying, loafing, and instantaneous events) did not increase or decrease following transmitter attachment. As swimming and flight are particularly critical to foraging, migrating, provisioning chicks, and escaping predators, these behaviors are often tested for adverse effects of transmitter attachment (Pennycuick et al. 2012; Matyjasiak et al. 2016). Our results suggest that individuals fitted with external transmitters continued to engage in swimming and flight at similar rates to control individuals immediately post-capture. However, our observations are limited to captive birds in a small enclosure, and we did not measure foraging movements or flight and swimming behavior in the field. Further, we did not assess the speed or efficiency of either swimming or flight, which can be altered by the presence of an external transmitter (Barron et al. 2010; Vandenabeele et al. 2011).

3.2 Foraging Movements and Home Range During the Breeding Period

During the breeding period, brown pelicans used coastal areas in the vicinity of their nesting site. We found some degree of overlap between neighboring colonies in both 50% UD and 95% UD home ranges. This suggests that individuals from neighboring colony sites were not partitioning foraging habitat. During the breeding season, colony size alone was the top predictor of individual 50% UD and 95% UD areas. Overall, the linear relationship between colony size and breeding season home range size was significantly positive for both 50% UD ($t_{65} = 3.65, p = 0.005$) and 95% UD home ranges ($t_{65} = 3.56, p = 0.007$). For each increase of 100 breeding pairs at a colony, mean core home range size of individual breeders increased by approximately 3 km² (1.86 mi²) and mean full home range size increased by approximately 19 km² (11.81 mi²). A model including both colony size and body condition also received substantial support as a predictor of 95% UD areas. The relationship between body condition and 95% UD area was positive, indicating an increase in 95% UD area with increasing body condition. However, condition was not a significant predictor of 95% UD area ($t_{65} = 1.20, p > 0.2$).

4 Habitat Use and Risk Exposure

4.1 Annual Habitat Use of Breeding Adults

Overall, 61.5% of bird-days were classified as resident and 38.5% as transient. The proportion of time individuals spent in each state did not differ significantly by sex (ANOVA, $F_{1,76} = 2.12$, $p = 0.15$). Between planning areas, individuals tagged in the eastern planning area spent relatively more time in the resident state ($\mu = 0.73 \pm 0.04$) than did individuals tagged in the central ($\mu = 0.53 \pm 0.03$) or western ($\mu = 0.65 \pm 0.05$) planning areas (ANOVA, $F_{2,74} = 6.61$, $p = 0.002$). Both states were observed year-round; however, resident behavior was relatively more common between December and March and between May and August. Transient behavior was more frequently observed during the remaining months. Niche position and breadth on measured habitat variables did not change depending on behavioral state (Figure 4.1).

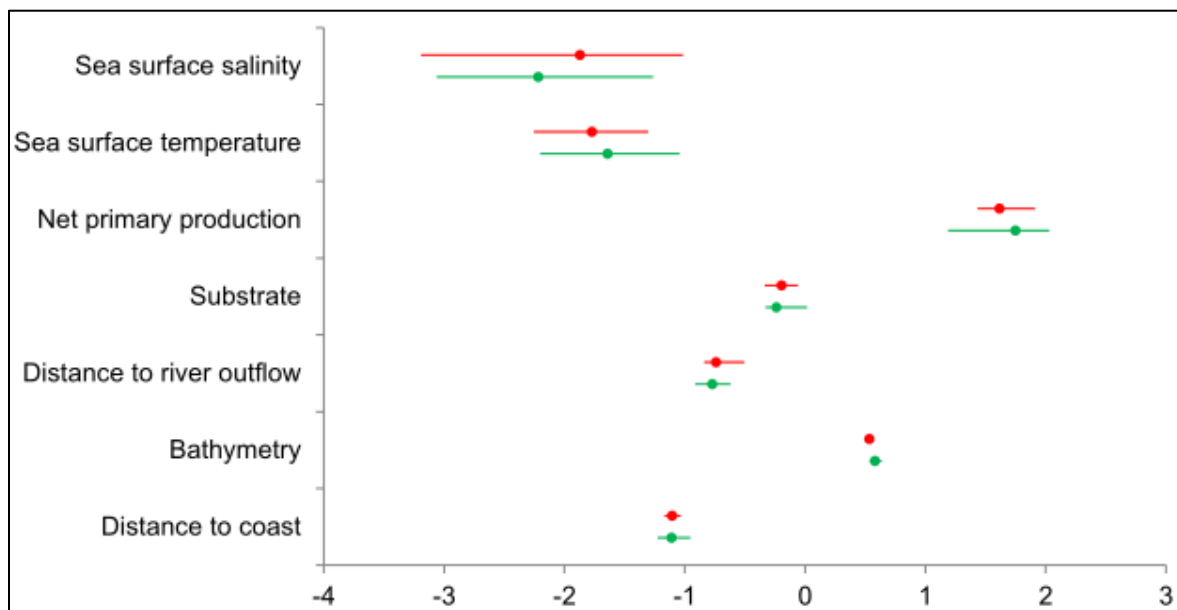


Figure 4.1. Niche center and breadth of resident and transient behavioral states of brown pelicans on measured habitat variables in the GOM, 2013–2016

Note: Resident behavioral state is shown in red, and transient behavioral state is shown in green.

The habitat variables most strongly associated with brown pelican residency year-round were net primary production (positive) and sea surface salinity (negative) (Figure 4.2). Sea surface temperature was negatively associated with residency during non-breeding, but the association diminished to near zero during the breeding season. Compared to seasonally dependent variables, fixed factors were less associated and less variable in their relationship to pelican habitat use and did not vary during the year. Bathymetry had a positive relationship with residency (i.e., pelicans were more likely to occupy shallower waters), while distance to coastline and distance to river outflow were both negatively associated with use by brown pelicans.

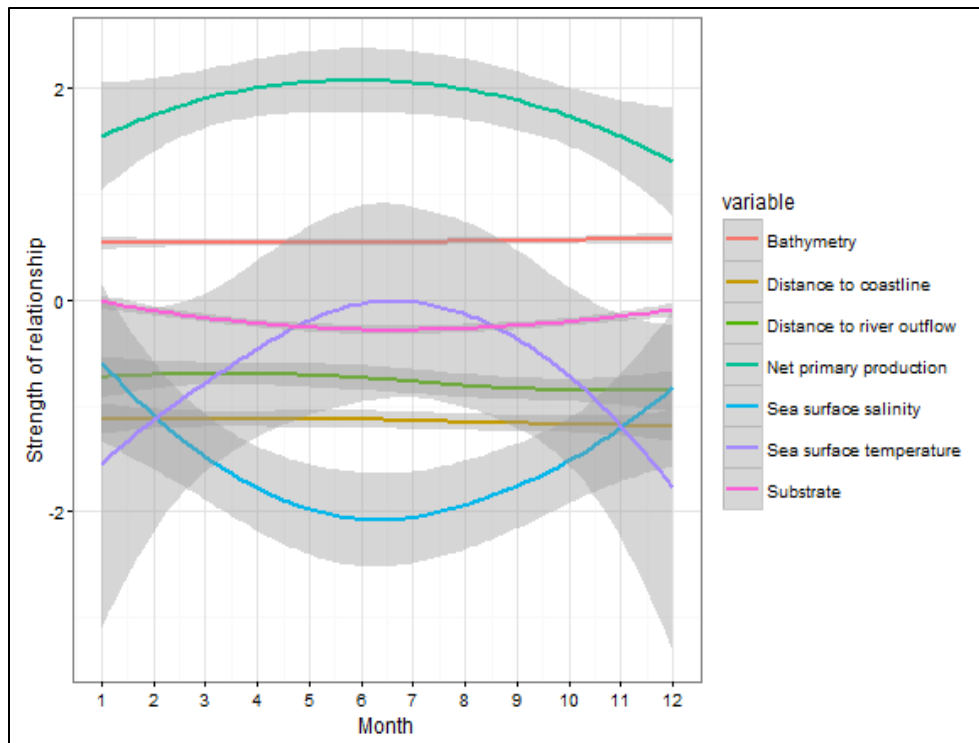


Figure 4.2. Annual patterns of strength and direction of selection by brown pelicans on measured habitat variables, GOM, 2013–2016

Note: Strength of selection (positive or negative) is generated from OMI and increases with distance from zero. Lines represent generalized additive model regressions (smoothing parameter = 1.3) of monthly averages for each variable, and gray bars are 95% confidence intervals of regression lines.

Patterns of association with seasonally dependent habitat variables varied between planning areas. Pelicans breeding in the central planning area of the GOM exhibited the highest degree of variation in environmental characteristics of selected habitat. Pelicans were more associated with waters characterized by high productivity and low salinity during summer (breeding) than during winter (non-breeding). Pelicans from the central and eastern planning areas selected habitat with a lower degree of seasonal variation in environmental characteristics, although pelicans from all planning areas associated more with sea surface temperature during breeding than during non-breeding.

Overall, areas of highest habitat suitability year-round were located in the Northern GOM, particularly the central and western planning areas. The total area of preferred habitat was narrowly restricted to coastal areas of the Northern Gulf during the summer; however, suitable habitat characteristics also occurred from the nearshore region out to approximately 600 km (372.8 mi) offshore during the fall and winter. Breeders from central GOM colonies shared 41% of their total habitat with breeders from other locations, western GOM breeders shared 36%, and eastern GOM breeders shared 15%. Habitats shared by central and western breeders accounted for 94% of total shared habitat.

4.2 Risk Exposure

4.2.1 Spatial and Temporal Aspects of Risk Exposure

Hot spots of overlap between preferred brown pelican habitat and surface pollutants (i.e., areas of high overlap) were consistent throughout the year and included most of the central and western planning areas of the Northern Gulf, particularly the Mississippi Delta and Galveston Bay (Texas) areas. Other hot spots

varied seasonally in intensity and included Corpus Christi Bay (Texas), Tampa Bay (Florida), the Florida Keys, the mouth of the Apalachicola River (Florida), and locations along the Yucatan Peninsula (Mexico) and in the Caribbean.

Pollutant exposure through the annual cycle varied by breeding location and sex among individuals (Table 4.1). Average overlap between individuals and pollution sources was lowest during non-breeding, increased at the start of the breeding season, and reached a maximum during post-breeding. Overlap rates differed significantly by planning areas (ANOVA: $F_{2,74} = 11.97$, $p < 0.001$). Breeders from the eastern planning area experienced lower year-round exposure to potential surface pollutants, while central and western breeders had similar year-round exposure rates (Table 4.1). Exposure varied seasonally in both central and western breeders, but individuals breeding in the eastern planning area experienced lower overall exposure and seasonal variation. Between sexes, males averaged higher exposure than females (ANOVA, $F_{1,75} = 4.48$, $p = 0.037$), which was driven by higher levels of overlap with surface pollutants during the non-breeding season.

Table 4.1. Mean pollutants overlap for observed brown pelican locations in the Northern GOM, 2013–2016

Planning Area	Mean	Standard Deviation	Number of Individuals
Eastern	0.082	0.023	23
Central	0.133	0.034	26
Western	0.122	0.049	28
Sex	Mean	Standard Deviation	Number of Individuals
Female	0.102	0.043	33
Male	0.123	0.041	44
Month	Mean	Standard Deviation	Number of Individuals
January	0.050	0.059	44
February	0.041	0.056	31
March	0.057	0.054	28
April	0.119	0.068	27
May	0.136	0.051	56
June	0.127	0.048	63
July	0.125	0.058	69
August	0.115	0.053	64
September	0.109	0.060	63
October	0.119	0.063	60
November	0.103	0.075	63
December	0.074	0.076	51

4.2.2 Individual Behavior and Model Structure

Spatial distribution and habitat use of seabirds are often used in combination with threat distributions to assess exposure to risk (e.g., Le Corre et al. 2012, Tranquilla et al. 2013, Renner and Kuletz 2015); however, overlap models have generally accounted for exposure only in terms of co-occurrence of birds and threats. The likelihood of threat exposure also varies depending on how birds interact with their environments, which can differ from species to species (Garthe and Hüppop 2004) or between phenological states within a species (Eppley and Rubega 1990). We used an HMM to distinguish resident behavior (individuals were restricted to limited habitat areas) from transient behavior (more frequent and longer-distance movements). This technique can improve predictive risk models by incorporating a priori

biological understanding of expected behavioral states (Patterson et al. 2009) to better predict the likelihood that co-occurrence of individual locations with threats will result in exposure.

5 Ecology and Physiology of Reproduction

5.1 Individual Nestling Survival

CORT concentrations from feathers of nestlings were significantly negatively correlated to BCI (linear model: coefficient = -194 ± 31.6 , $F_{1,364} = 37.7$, $p < 0.001$, $R^2 = 0.09$). Chicks that died before fledging had lower BCI ($F_{1,239} = 6.1$, $p = 0.01$) and higher CORT deposited in feathers ($F_{1,239} = 24.7$, $p < 0.001$) at 3–4 w of age than chicks that were presumed fledged (i.e., survived until at least 60 d after hatching). Of the other covariates we tested, only nest height (linear model, ground relative to elevated: coefficient = -2.79 ± 0.80 , $z_{109} = -3.76$, $p < 0.001$) and body size (linear model: coefficient = 1.25 ± 0.43 , $z_{109} = 2.88$, $p = 0.004$) were significantly correlated with individual fledging success. Nestlings from ground nests had significantly lower BCI (ground: $\mu_{74} = -97.2 \pm 479$; elevated: $\mu_{117} = 72.0 \pm 363$; $F_{1,191} = 7.74$, $p = 0.006$) and higher feather CORT (ground: $\mu_{74} = 2.08 \pm 0.71$; elevated: $\mu_{117} = 1.72 \pm 0.64$, $F_{1,191} = 17.8$, $p < 0.001$) than nestlings from elevated nests. We did not find a significant effect of colony, planning area, year, sampling date, hatch order, number of siblings, or their interactions on fledging probability (linear models: $p > 0.10$ for each).

Survival probabilities of individual nestlings > 60 d post hatch were positively related to BCI and negatively related to CORT. Chicks found dead at the colony post-fledging had significantly lower BCI (ANOVA: $F_{1,40} = 11.4$, $p = 0.002$) and significantly higher CORT (ANOVA: $F_{1,40} = 18.4$, $p < 0.001$) at 3–4 w after hatching than did chicks that were re-sighted alive after fledged.

5.1.1 Colony-specific Nest Productivity and Chick Survival

CORT levels were correlated with nest productivity at individual observation plots within breeding colonies. Nest productivity and nestling feather CORT, but not nestling BCI, differed significantly between ground and elevated subplots at two of the four colonies with both ground and elevated nests. Two of the three remaining colonies contained only shrub nests; the third contained too few ground nests to assess differences in productivity relative to shrub nests.

Overall, colony-wide productivity rates were positively correlated with average BCI and negatively correlated with average CORT of sampled chicks.

The strongest model predicting colony-specific nest productivity as a function of chick health parameters contained CORT alone. This was also the only model supported by comparison to AIC_c values. The top model explained 84% of the observed deviance (null = 1.91; residual = 0.31).

Modeled chick survival to fledge (3 m after hatch) at individual colony sites was negatively correlated with average CORT. The strongest model predicting chick survival to fledge as a function of chick health parameters, which was also the only model supported by comparison of AIC_c values, contained CORT alone. The top model explained 91% of the observed deviance (null = 0.144; residual = 0.013). The relationship between BCI and survival to fledge showed a non-significant positive trend, and BCI was not supported as a predictor of average colony-wide survival rates.

We found a weak negative correlation between colony size and nestling CORT levels ($t_{253} = -2.00$, $p = 0.05$). Colony size and nestling BCI were not significantly correlated ($t_{253} = -1.04$, $p > 0.20$). We did not find a significant relationship between environmental conditions or environment-colony size interactions and either of the chick health parameters ($p > 0.20$ for all variables).

Our first objective was to assess the relationship between feather CORT and a more traditional measure of nestling health, BCI (Benson et al. 2003), as predictors of nestling survival. In accordance with recent work on other avian taxa, we found that nestling feather CORT was negatively correlated to both body

condition (Fairhurst et al. 2013; López-Jiménez et al. 2016) and fledging probability (Fairhurst et al. 2013; Lodjack et al. 2015) at the individual level. Although both feather CORT and BCI were significantly correlated with chick survival to fledge, feather CORT predicted the fate of individual nestlings slightly better than BCI. At the colony level, models containing only feather CORT were favored over models containing BCI with and without feather CORT as predictors of nest productivity, survival to fledge, and post-dispersal survival. Additionally, feather CORT predicted within-colony differences in fledging success by habitat type that were not apparent in comparisons of BCI. The enhanced explanatory power of CORT compared to BCI may be due to both the longer time frame over which CORT integrates physiological condition and the sensitivity that BCI has to short-term variation in nutritional stress. For example, at the Shamrock Island colony the average mass of chicks was 2,660 g (93.8 oz) and average meal mass was 181 g (6.4 oz), or about 7% of body weight. This relatively high ratio of meal mass to body mass, combined with the daily variation we observed in mass of meals (range = 5.6–1039.8 g [0.2–36.7 oz], confidence interval [CV] = 0.76), makes BCI highly sensitive to feeding frequency and time since feeding. Meal delivery rates and the size of meals in relation to chick mass can vary by more than one order of magnitude both among and within avian species (Ricklefs et al. 1985; Anderson and Ricklefs, 1992). Therefore, the use of BCI as a measure of nestling condition requires consideration of how these short-term factors may influence its usefulness in describing long-term patterns of chick condition. Feather CORT integrates a longer time series of conditions (Bortolotti et al. 2008) and thus may be less susceptible than BCI to short-term variation. The fact that we measured feather CORT early in development (about 20–30 d into a 60–90 d nesting period) and found a strong relationship to fledging probability further indicates that feather CORT levels during early development can accurately predict survival through the breeding season.

We also assessed the relationship between feather CORT and variation in local (site- and nest-specific) conditions. Although nestling feather CORT is strongly correlated to environmental conditions during development (e.g., Harms et al. 2010; Will et al. 2015; Lodjack et al. 2015), site- and nest-specific factors can still confound the environment-stress relationship (Fairhurst et al. 2012; Lodjack et al. 2015). We did not find a significant influence of either hatch order or number of siblings on feather CORT. A previous study of plasma CORT in brown pelican nestlings (Eggert et al. 2010) also found no effect of brood size or hatch order on stress levels; however, sibling dynamics have been found to affect feather CORT levels in nestling raptors (Yosef et al. 2013; López-Jiménez et al. 2016). We did find an influence of microhabitat characteristics (elevated compared to ground nest location) on feather CORT. Nestlings at elevated nests may benefit from improved passive thermoregulation, reduced energy expended in movement, and reduced aggressive interactions with neighboring adults and nestlings that subsequently act to maintain lower levels of feather CORT. Our study concurs with data on brown pelican nest productivity in Louisiana (Walter et al. 2013), suggesting that nestlings from elevated nests tend to survive longer than nestlings from ground nests, contributing to increased nest productivity at elevated sites. If elevated nest sites offer improved fledging success, positive reinforcement may occur at these sites if experienced or dominant breeders preferentially select and defend elevated nesting sites.

5.1.2 Nutritional Stress

EPR showed a non-significant positive trend in relation to BCI (linear model, coefficient = 1.04 ± 0.52 , $t_5 = 2.02$, $p = 0.10$) and a significant negative relationship to feather CORT (linear model, coefficient = -613 ± 155 , $t_5 = 3.97$, $p = 0.01$). The two biomass components of EPR, feeding frequency (meals chick⁻¹ day⁻¹, $\mu = 2.51$, $N = 142$) and meal mass (g meal⁻¹, $\mu = 157.6$, $N = 583$), had similarly high levels of overall variation (CV frequency = 0.64; CV mass = 0.76), while energy density of meals (kJ g⁻¹, $\mu = 4.34$, $N = 583$) was less variable (CV = 0.10). EPR explained 76% of observed variance in colony-wide average feather CORT and 45% of observed variance in colony-wide average BCI. Of the separate components of EPR, meal delivery rate explained the largest portion of variance in each of the two chick health metrics (CORT: 30.5%; BCI: 33.0%), followed by meal mass (CORT: 22.1%; BCI: 3.7%) and energy density

(CORT: 3.2%; BCI: 0.1%). EPR was positively correlated to nest productivity (coefficient = 739 ± 258 , $t_5 = 2.85$, $p < 0.04$, $R^2 = 0.62$) and nestling survival to fledge (coefficient = $3,365 \pm 580$, $t_4 = 5.80$, $p = 0.002$, $R^2 = 0.87$), and the relationship between EPR and post-fledging survival rates showed a positive but non-significant trend (coefficient = $6,482 \pm 3,042$, $t_4 = 2.13$, $p = 0.09$).

6 Nestling Diet

6.1 Taxonomic Composition of Diet

Over three years, we collected 641 chick meals (Year 1: N = 27; Year 2: N = 423; Year 3: N = 191), totaling 98 kg (212 lb) of prey. We identified 46 prey species representing 25 families (**Table 6.1**). Thirty-six of the prey species represented less than 1% each of biomass collected; of these, 16 species represented less than 0.05% each of biomass collected (**Table 6.1**). Gulf menhaden was the most common prey species by weight overall, as well as at each study site. The proportion of menhaden in total biomass varied by colony, with higher proportions of menhaden within the central GOM. Other common prey species did not show a pattern of abundance in meals across sites, except for anchovy (*Anchoa spp.*), which increased from the western to the eastern GOM, and spot (*Leiostomus xanthurus*), which declined from the western to eastern GOM. The majority of meals (76%) contained a single fish species, and the maximum number of species in a meal was seven.

The overall proportion of menhaden in chick diets declined through the breeding season, (coefficient = -0.34 ± 0.10 , $F_{1,596} = 12.3$, $p < 0.001$), driven by a decrease in juvenile menhaden < 110 mm (4.33 in) total length (coefficient = -0.75 ± 0.09 , $F_{1,596} = 66.0$, $p < 0.001$). The proportions of adult menhaden, anchovies, and pinfish increased over the same period ($p < 0.01$ for all). The remaining prey species showed no seasonal trends in proportional occurrence.

Energetic content ranged from 3.3 to 5.5 kJ g⁻¹ among all species with a mean (\pm SD) of 4.38 ± 0.98 kJ g⁻¹ wet mass. Protein content had low variation across measured samples (CV = 8%) and correlated weakly with energy density per wet gram of fish ($F_{1,217} = 22.3$, $p < 0.001$, $r^2 = 0.09$); lipid content was variable both between and within species (CV = 75%) and was highly correlated with energy density ($F_{1,217} = 1,929$, $p < 0.001$, $r^2 = 0.90$). First-year menhaden had significantly lower-energy densities and lower lipid content than adult menhaden in the northeastern and northwestern GOM ($p < 0.1$ for all).

Table 6.1. Fish species occurring in the diets of brown pelican chicks in the Northern GOM, 2013–2015

Order	Family	Species	Common	Year	% biomass
Atheriniformes	Atherinidae	<i>Menidia beryllina</i>	Inland silverside	1,2,3	0.1
Aulopiformes	Synodontinae	<i>Sybodius foetens</i>	Inshore lizardfish	1,2	*
Beloniformes	Belonidae	<i>Tylosurus crocodilus</i>	Houndfish	3	*
"	Hemiramphidae	<i>Hemiramphus brasiliensis</i>	Ballyhoo halfbeak	1,2	0.1
Clupeiformes	Clupeidae	<i>Brevoortia patronus</i>	Gulf menhaden	1,2,3	61.0
"	"	<i>Harengula jaguana</i>	Scaled sardine	1	0.3
"	"	<i>Opisthonema oglinum</i>	Atlantic threadfin Herring	2,3	1.7
"	Engraulidae	<i>Anchoa hepsetus</i>	Striped anchovy	2,3	1.5
"	"	<i>Anchoa lyolepis</i>	Dusky anchovy	3	2.2
"	"	<i>Anchoa mitchilli</i>	Bay anchovy	1,2,3	7.5
Cyprinodontiformes	Cyprinodontidae	<i>Cyprinodon variegatus</i>	Sheepshead minnow	2	0.2
"	"	<i>Fundulus majalis</i>	Striped killifish	2	*
Decapoda	Penaeidae	<i>Farfantepenaeus duorarum</i>	Pink shrimp	2,3	*
Mugiliformes	Mugilidae	<i>Mugil cephalus</i>	Striped mullet	2,3	4.8
Perciformes	Carangidae	<i>Caranx crysos</i>	Blue runner	1	0.1
"	"	<i>Chloroscombrus chrysurus</i>	Atlantic bumper	1,2,3	0.6
"	"	<i>Decapterus punctatus</i>	Round scad	3	0.1
"	"	<i>Hemicaranx amblyrhynchus</i>	Bluntnose jack	2	*
"	"	<i>Selene setapinnis</i>	Atlantic moonfish	2	*
"	Gobiidae	<i>Gobioides broussonetii</i>	Violet goby	2	*
"	Haemulidae	<i>Orthopristis chrysoptera</i>	Pigfish	1,2	*
"	Lutjanidae	<i>Lutjanus campechanus</i>	Red snapper	15	0.3
"	Sciaenidae	<i>Bairdiella chrysoura</i>	Silver perch	1,2	0.4
"	"	<i>Cynoscion arenarius</i>	Sand seatrout	2,3	1.2
"	"	<i>Cynoscion nebulosus</i>	Spotted seatrout	2,3	1.1
"	"	<i>Larimus fasciatus</i>	Banded drum	2	*
"	"	<i>Leiostomus xanthurus</i>	Spot	1,2,3	2.9
"	"	<i>Menticirrhus americanus</i>	Southern kingfish	2	0.7
"	"	<i>Micropogonias undulatus</i>	Atlantic croaker	1,2,3	3.8
"	"	<i>Sciaenops ocellata</i>	Red drum	2,3	0.5
"	Scombridae	<i>Auxis thazard</i>	Frigate mackerel	3	0.2
"	"	<i>Scomberomorus cavalla</i>	King mackerel	2	0.1
"	"	<i>Scomberomorus maculatus</i>	Spanish mackerel	2	0.3
"	Serranicae	<i>Diplectrum formosum</i>	Sand perch	3	0.2
"	Sparidae	<i>Calamus proridens</i>	Littlehead porgy	1	*
"	"	<i>Lagodon rhomboides</i>	Pinfish	1,2,3	2.4
"	"	<i>Stenotomus caprinus</i>	Longspine porgy	1	*

Order	Family	Species	Common	Year	% biomass
"	Stromateidae	<i>Peprilus burti</i>	Gulf butterfish	2,3	0.1
"	"	<i>Peprilus paru</i>	American harvestfish	2	0.1
"	Trichiuridae	<i>Trichiurus lepturus</i>	Atlantic cutlassfish	1,2,3	3.6
Pleuronectiformes	Cynoglossidae	<i>Symphurus urospilus</i>	Spottail tonguefish	3	0.1
"	Paralichthyidae	<i>Citharichthys spilopterus</i>	Bay whiff	2,3	0.1
Scorpaeniformes	Triglidae	<i>Prionotus tribulus</i>	Bighead searobin	2,3	*
Siluriformes	Ariidea	<i>Bagre marinus</i>	Gafftopsail catfish	1,2,3	0.3
Tetraodontiformes	Diodontidae	<i>Diodon holocanthus</i>	Longspine porcupinefish	2	*
Teuthida	Loliginidae	<i>Lolligunculla brevis</i>	Atlantic brief squid	1,3	*
Other	"	"	Isopod	3	*
"	"	"	Bait (chicken)	3	*
"	"	"	Unknown	-	1.2

Note: An asterisk (*) in the biomass column denotes less than 0.05 % of total biomass. Year 1: 2013; Year 2: 2014; Year 3: 2015.

6.2 Meal Attributes

Average meal mass, meal delivery rate, and energy density of meals each differed significantly among colony sites. The two biomass components of EPR—feeding frequency and meal mass—had similarly high levels of overall variation (CV frequency = 0.67; CV mass = 0.76), while energy density of meals was less variable (CV = 0.10). Relative to averages within planning areas, individual colony sites showed a generally opposing pattern between meal mass and meal delivery rates. Colonies with below-average meal delivery rates tended to have above-average meal masses, and conversely. Energy densities followed a similar pattern to meal masses but did not deviate more than 10% from the overall mean. Site-specific variation in all three provisioning metrics tended to covary, with below-average variability toward the central and eastern GOM and higher variability in the west.

Provisioning metrics also varied seasonally within the chick-rearing period. Both meal mass and energy density increased over the course of the breeding season (meal mass: coefficient = 2.48 ± 0.24 , $F_{1,596} = 104$, $p < 0.001$; energy density: coefficient = 0.007 ± 0.001 , $F_{1,596} = 29.5$, $p < 0.001$), while meal delivery rate decreased during the same period (coefficient = -0.036 ± 0.005 , $F_{1,135} = 46.1$, $p < 0.001$). However, rates of energy delivery, calculated as the product of daily average meal mass, energy density, and meal delivery rates, neither increased nor decreased during the breeding season ($F_{1,40} = 0.60$, $p > 0.20$).

Mean biomass provisioning rate (BPR) varied by colony from 454 ± 294 to $1,106 \pm 587$ g d⁻¹. Mean EPR varied by colony from 1,977 to 4,876 kJ d⁻¹. BPR and EPR were highly correlated (coefficient = 4.48 ± 0.34 , $F_{1,5} = 168.0$, $p < 0.001$). Of the individual provisioning covariates measured at each colony, meal delivery rate explained 38% of variance in EPR, followed by meal mass (24%) and energy density of meals (1%). Both feeding frequency and meal mass improved model fit when added sequentially to the intercept-only model, but adding energy density did not significantly improve the fit of the model (Table 6.2).

Table 6.2. ANOVA comparisons of nested models for colony-specific mean brown pelican nestling energy provisioning rates and nest productivity based on feeding rate, meal mass, and energy density of meals, Northern GOM, 2014–2015

Energy provisioning rate

Terms	Residual df	Residual Deviance	df	Deviance	F	p
Intercept only	6	7,236,805	-	-	-	-
+ feeding rate	5	4,498,564	1,5	2,738,240	24.79	0.016
+ meal mass	4	379,699	1,5	4,118,866	37.3	0.009
+ energy density	3	331,316	1,5	48,383	0.44	0.56

Nest productivity

Terms	Residual df	Residual Deviance	df	Deviance	F	p
Intercept only	6	1.397	1,5	-	-	-
+ feeding rate	5	0.714	1,5	0.683	47.83	0.006
+ meal mass	4	0.056	1,5	0.658	46.12	0.007
+ energy density	3	0.043	1,5	0.896	0.90	0.41

Note: Terms are added sequentially, and a p-value of < 0.05 indicates a significant improvement in fit compared to the previous model.

Meal delivery rates increased with increasing proportions of menhaden and anchovy, which were also associated with decreasing energy density of meals. By comparison, meals containing higher proportions of spot, croaker, and pinfish were associated with lower delivery rates and higher energy densities. Meal masses were highest for meals containing striped mullet (*Mugil cephalus*) or Atlantic cutlassfish (*Trichiurus lepturus*) and lowest for meals containing anchovies. The proportion of biomass represented by small size-class fish (< 110 mm (4.33 in) total length) at individual colonies correlated to feeding frequency ($F_{1,5} = 7.18$, $p = 0.04$, $r^2 = 0.59$, coefficient = 0.108 ± 0.04), but not to meal mass or energy density ($F_{1,5} = 1.82$, $p > 0.20$ for both).

Average fledging success (chicks nest⁻¹) was strongly correlated to both mean EPR and BPR at the colony level. Of the individual components of EPR, feeding frequency explained the largest portion of variance in nest productivity (49%), followed by meal mass (15%) and energy density of meals (0.1%). Both feeding frequency and meal mass significantly improved the fit of a null model for average fledging success by colony, while energy density did not improve model fit (**Table 6.2**). Diet composition (% menhaden) did not correlate with fledging success ($F_{1,5} = 0.89$; $p > 0.20$).

6.3 Nutritional Stress

We found that, in comparison to seabirds at high latitudes, brown pelicans in the GOM experience a narrow range of variation in energy content between prey species. Furthermore, our results suggest that nest productivity of brown pelicans is more closely associated with feeding frequency, followed by meal mass, and that species composition and energy content of meals have little effect on productivity. Combined, these results suggest that brown pelicans provisioning nestlings in this system use a feeding strategy that prioritizes frequent deliveries of highly available prey regardless of energy density. Though our results indicate that the junk-food hypothesis may not be useful for explaining the relationship between nestling provisioning and nest productivity in this system, our study also highlights the key importance of small, highly abundant schooling fish for breeding brown pelicans in the GOM.

Although brown pelicans delivered a wide variety of prey species to nestlings, both lipid content and overall energetic value of prey items in nestling diets varied within a narrow range. Compared to results from previous work in temperate and subpolar systems, average energetic content of prey species in our study was 15–30% lower, with 55–78% less variation between species. Our observations accord with previous work on mesopelagic fish species in the GOM (Stickney and Torres 1989) and the South Atlantic Bight (Jodice et al. 2011), which suggest that fish species in the tropical northwest Atlantic have

relatively higher protein levels, lower lipid reserves, and lower overall energetic values than species at northern and southern latitudes characterized by cooler oceanic temperatures and higher inter-seasonal variability. Despite the wide longitudinal variation of our sampling area and the variation in prey species composition relative to prey distribution, energetic content of meals fed to pelican chicks varied little between colonies. As a result, colony-specific EPRs closely reflected a combination of meal mass and frequency of meal deliveries (i.e., BPR), but did not relate to energy content of meals. Our results suggest that prey energy content is not a significant driver of energy delivery rates to nestlings for brown pelicans in this system, given the lack of variation in energy density between prey species.

Our results support previous observations of the predominance of Gulf menhaden in brown pelican diets (e.g., Arthur 1919, Fogarty 1981); however, the proportions of menhaden consumed by pelicans in our study varied both spatially and temporally depending on underlying distribution and inferred availability. The proportion of juvenile menhaden in nestling diets declined over the course of the chick-rearing period, during which young-of-the-year menhaden move gradually from shallow estuarine waters to offshore habitats, decreasing their availability to foraging pelicans (Ahrenholz 1991). Other prey, including pinfish, anchovy, and adult menhaden, increased proportionally during the same period, and overall rates of energy delivery to nestlings remained consistent throughout the breeding season. Gulf menhaden constituted 60–84% of pelican nestling diets in colonies at the core of its range (i.e., the central Northern GOM), but less than 40% of diets in colonies at the eastern and western margins of its range. Notably, first-year menhaden (individuals hatched during the previous winter) represented 56% of nestling pelican diets at the colony closest to the core of their range and 3% or less outside the range margins. As the proportion of menhaden in nestling diets declined, other prey species, principally anchovy in the eastern GOM and spot, croaker, and pinfish in the western GOM, contributed more significantly to nestling diets. The comparatively larger size of pelican breeding colonies at the core of the Gulf menhaden range than at its margins may indicate that areas with high menhaden availability can support larger aggregations of breeding pelicans. However, further study is required to distinguish the effects of variation in prey availability among planning areas on population size from those of conservation history (e.g., King et al. 1985, Wilkinson et al. 1994) and breeding habitat availability (e.g., Walter et al. 2013).

Overall, we found that higher meal delivery rates were driven by the proportion of diet biomass composed of fish less than 110 mm (4.33 in) total length, regardless of species. Despite being among the least energy-rich prey items observed, juvenile menhaden constituted over 50% of pelican diets at the core of their range, suggesting that pelicans target accessible and highly aggregated prey without regard for energetic content. The importance of small, abundant schooling fish to brown pelican reproductive output is of potential conservation interest. Recruitment rates in Gulf menhaden are highly sensitive to temperature and precipitation, with warmer and wetter winters producing comparatively fewer recruits in the next year class (Deegan 1990). Given that winter temperatures and precipitation are expected to rise under current climate change projections (Biasutti et al. 2012), the biomass of larval fish available to upper-level predators (e.g., Muhling et al. 2011) could become more limited or more variable in future climatic conditions. Additionally, pollution events can significantly depress survival of larval fish (Incardona et al. 2014) and could have indirect effects on prey dynamics that compound the direct effects of pollutants exposure to predators.

7 Hematology, Plasma Chemistry, and PAHs

7.1 Hematology and Plasma Chemistry

CBCs provide a wealth of data within an individual and across populations. The half-life for red blood cells in birds is ~13 d (Maceda-Veiga et al. 2015), therefore blood samples provide insight into the recent condition of individuals (e.g., on the order of 2–4 w). For data in this study, analytes are indicative of condition during the incubation and early chick-rearing stage for adults, and for the early development stage for chicks. Furthermore, analytes of chicks may reflect nutritional conditions, and can be responsive to overall food quality as well as short-term changes in provisioning. It is not uncommon, therefore, to observe a substantial level of variability within an analyte among individuals (e.g., Ferguson et al. 2014, Fiorello 2019). Caution should be applied when interpreting such data, since attempting to provide detailed ecological explanations for the values of each analyte can be misleading due to the high levels of variability. We concentrated our interpretation on groups of analytes (e.g., plasma metabolites, enzymes) rather than individual analytes for each of the independent variables we assessed: BCI, planning area, sex, and home range size. This approach allows for a more ecologically focused assessment of the data, in contrast to an analyte-specific clinical review. We also compare the reference levels from this study to other data available from brown pelicans.

7.1.1 Individual Attributes, Hematology, and Plasma Chemistry of Adults

7.1.1.1 Individual Attributes of Adults

BCI of adults ranged from -515.0–491.2 (mean = -6.3 ± 253.7). BCI of adults differed by sex ($F_{1,63} = 4.1$, $P = 0.04$) but not by planning area or sex * planning area ($P > 0.10$). Males had higher BCI compared to females (**Figure 7.1**). The 50% core use area (i.e., home range) ranged from ~1km²–909 km² (mean = 102.9 ± 157.9 km²) (0.62–564.8 mi², mean = 63.9 ± 98.11 mi²). Home range size differed by planning area ($P < 0.05$). Home range did not differ with BCI, sex, or sex * planning area ($P > 0.10$). Correlated independent variables were not used within the same models, although all variables of interest were included in the overall suite of models.

7.1.1.2 Blood Analytes of Adults

Table 7.1 includes reference values for adults for all blood analytes. Sodium, which was the only analyte with a bimodal distribution, was treated as two separate analytes. Of the 30 analytes examined, four had outliers removed (high values were removed for corticosterone (CORT), potassium, and alanine aminotransferase; a low value was removed for low density lipoprotein cholesterol), 12 required transformation, and all had sufficient sample sizes to use a nonparametric reference interval (**Table 7.1**).

Reference intervals were calculated for 30 blood analytes. Among the 30 analytes there were nine cases of moderate to strong correlation. Beta globulin was most commonly correlated with other analytes ($n = 3$ pair), followed by A:G, BUN, uric acid, and creatinine ($n = 2$ pair each). Pooled among all 30 blood analytes, the models that were most often highly supported or for which $AICc \leq 2.0$ of the top-ranked model were (1) home range + sex ($n = 13$ analytes), (2) home range ($n = 10$ analytes), and (3) BCI + sex ($n = 10$ analytes).

Table 7.1. Statistical values for mass and serum chemistry for adult brown pelicans sampled from breeding colonies in the Northern GOM, 2013–2014

Analyte (units)	n	Mean	Median	SD	Min	Max	Reference Interval	Lower 90% CI	Upper 90% CI
Sodium (mEq L ⁻¹)	72	205.9	250.0	54.5	111	250	116.8–250.0	111.0–130.0	250.0–250.0
Potassium (mEq L ⁻¹) **^	72	3.95	3.65	1.79	1.2	10.9	1.37–9.66	1.20–1.65	6.42–10.90
CO ₂ (mEq L ⁻¹)	73	16.2	16.0	3.5	9	24	9.0–24.0	9.0–10.7	22.2–24.0
Calcium (mg dL ⁻¹) ^	72	8.30	8.55	1.54	4.5	13.1	4.83–11.20	4.50–5.64	10.09–13.10
Phosphorus (mg dL ⁻¹) ^	71	4.98	4.70	1.63	2.5	12.6	2.74–9.88	2.50–3.18	7.00–12.60
Glucose (mg dL ⁻¹)	73	204.8	210.0	43.4	25	307	98.1–293.4	25.0–145.8	264.9–307.0
BUN (mg dL ⁻¹)	73	4.3	3.0	3.9	1	18	1.0–16.3	1.0–1.0	13.2–18.0
Creatinine (mg dL ⁻¹) ^	71	0.7	0.7	0.3	0.2	1.9	0.28–1.42	0.20–0.38	1.20–1.90
Blood urea nitrogen:Creatinine ratio^	68	6.0	5.0	4.32	1.7	23.3	1.70–19.46	1.70–1.92	13.38–23.30
Amylase (U L ⁻¹)	72	1,166.2	1,171.0	145.2	760	1,637	801.3–1,553.7	760.0–942.0	1,377.4–1,637.0
Lipase (U L ⁻¹) ^	73	23.8	19.0	16.1	1	74	3.6–62.9	1.0–5.0	55.8–74.0
Cholesterol (mg dL ⁻¹)	73	151.9	153.0	31.7	80	252	86.8–223.1	80.0–103.4	199.4–252.0
Triglycerides (mg dL ⁻¹) ^	72	57.4	49.0	29.2	27	142	28.7–140.4	27.0–30.0	134.4–142.0
High density lipoprotein cholesterol (mg dL ⁻¹)	72	83.5	84.5	15.1	50	111	52.5–110.2	50.0–58.0	104.0–111.0
Low density lipoprotein cholesterol (mg dL ⁻¹) **^	72	11.4	10.0	5.8	5	28	5.8–28.0	5.0–6.0	27.0–28.0
Uric acid (mg dL ⁻¹) ^	72	12.15	9.55	7.81	0.7	32.6	2.19–32.19	0.70–3.27	27.73–32.60
Total protein (g dL ⁻¹)	74	4.34	4.20	0.90	2.4	6.7	2.93–6.26	2.40–3.19	5.90–6.70
Aspartate aminotransferase (U L ⁻¹) ^	72	175.9	163.5	57.1	71	380	93.3–371.8	71.0–109.0	265.9–380.0
Alanine aminotransferase (U L ⁻¹) *	70	28.5	28.5	7.3	12	44	12.0–43.2	12.0–15.9	39.1–44.0
Lactate dehydrogenase (U L ⁻¹)	72	4,674.3	4,592.5	1,403.3	1,764	7,960	1,953.8–7,521.1	1,764.0–2,632.8	7,218.1–7,960.0

Analyte (units)	n	Mean	Median	SD	Min	Max	Reference Interval	Lower 90% CI	Upper 90% CI
Creatine phosphokinase (U L ⁻¹)	72	964.4	942.0	442.5	48	1,854	143.7–1,737.7	48.0–256.3	1,617.3–1,854.0
GGT (U L ⁻¹)	72	10.2	7.0	6.9	5	34	5.0–32.4	5.0–5.0	24.9–34.0
Albumin:Globulin ratio	74	0.57	0.55	0.18	0.21	1.01	0.27–1.01	0.21–0.32	0.90–1.01
Pre-albumin (mg dL ⁻¹)	74	0.25	0.25	0.07	0.09	0.42	0.12–0.40	0.09–0.15	0.36–0.42
Albumin (mg dL ⁻¹)	74	1.24	1.24	0.21	0.78	1.67	0.79–1.66	0.78–0.93	1.59–1.67
Alpha-1 globulin (mg dL ⁻¹)	74	0.14	0.13	0.03	0.09	0.27	0.09–0.23	0.09–0.09	0.18–0.27
Alpha-2 globulin (mg dL ⁻¹)	74	0.68	0.64	0.16	0.40	1.18	0.43–1.11	0.40–0.49	1.00–1.18
Beta globulin (mg dL ⁻¹) ^	74	1.18	1.10	0.37	0.47	2.24	0.61–2.22	0.47–0.74	1.84–2.24
Gamma globulin (mg dL ⁻¹) ^	74	0.85	0.70	0.50	0.24	2.13	0.24–2.02	0.24–0.32	1.79–2.13
CORT (mg dL ⁻¹) *	73	36.13	35.80	13.86	11.9	78.0	12.41–65.08	11.90–15.77	59.61–78.00

* outlier(s) removed

^required Box-Cox transformation

Note: Sample size (n), mean, median, standard deviation (SD), minimum (Min), and maximum (Max) values with reference intervals and 90% confidence intervals (CI) of reference limits.

7.1.1.3 Relationship of Planning Area with Blood Analytes of Adults

Among the independent variables we tested, coefficient estimates for the categorical variable “planning area” were most often strongly associated with the concentration of a blood analyte. The eastern planning area was set as the reference level because it had the lowest level of oil and gas activity in coastal and marine waters. Concentrations of analytes were lower in both the central and western planning area compared to the eastern planning area for creatine phosphokinase (CPK), lipase, total protein, beta globulin, and gamma globulin. Concentrations of analytes were lower in the central or western planning area when compared to the eastern planning area for calcium (west), alanine aminotransferase (ALT) (west), aspartate aminotransferase (AST) (central), albumin (west), and Alpha 1 globulin (A1G) (central). Concentrations of analytes were higher in both the central and western planning area compared to the eastern planning area for potassium and creatinine. Concentrations of analytes were higher in either the central or western planning area compared to the eastern planning area for calcium (central). Pelicans in the western planning area were likely to have lower levels of sodium than birds in either the eastern or central planning area ($\chi^2_2 = 29.0$, $P < 0.0001$).

7.1.1.4 Relationship of Body Condition Index, Sex, and Home Range with Blood Analytes of Adults

BCI was positively related to potassium and negatively related to creatinine, uric acid, and BUN:CRE. Differences in analytes by sex occurred for seven analytes. Levels of BUN, uric acid, and BUN:CRE were higher in females compared to males, but levels of calcium, lipase, cholesterol, and HDLc were higher in males compared to females. We found a negative relationship between home range size and creatine phosphokinase (CPK), lactate dehydrogenase, lipase, BUN, creatinine, and uric acid; and a positive relationship between home range size and HDLc.

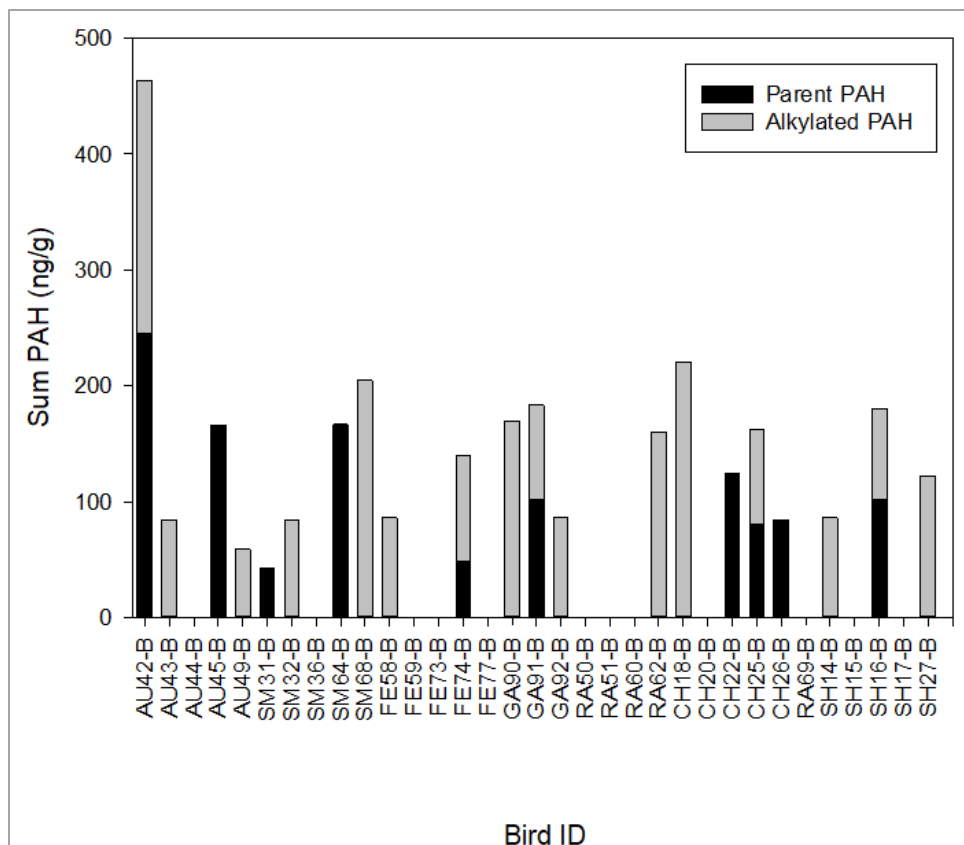


Figure 7.1. Summation of PAHs by individual from blood samples of adult brown pelicans breeding in the Northern GOM, 2013–2015: total PAH concentration (ng g⁻¹; wet weight) of parent and alkylated PAHs in blood samples

Notes: Bird ID = two letter abbreviation for the colony of origin of the sample (AU = Audubon Island, SM = Smith Island, FE = Felicity Island, GA = Gaillard Island, RA = Raccoon Island, CH = Chester Island, SH = Shamrock Island), a unique identification number, and B = blood sample).

8 Conclusions and Future Directions

To date, the research described in this report is one of the most spatially and temporally extensive research efforts conducted on the eastern brown pelican. Although the brown pelican has been a species of conservation concern in the GOM for decades, the species has been relatively understudied in the Northern GOM until research was initiated in approximately 2010 and after (e.g., Walter et al. 2013, 2014; this report). For example, before 2010, there were substantial data gaps on productivity, chick survival, and chick condition, and on the variability within each of those parameters both within and among colonies and years. Our understanding of movement patterns during breeding and non-breeding was limited to band return studies and anecdotal evidence of home range sizes, often from areas outside the GOM. These data gaps set the context for the research described herein. We focused on three primary areas of pelican ecology: (a) reproductive ecology, (b) spatial ecology and movement, and (c) health and exposure to contaminants.

8.1 Spatial Ecology: Home Range, Migration, and Movement

Tracking individual pelicans using GPS-equipped satellite tags allowed us to accumulate several locations per day per bird. Tag duration was typically sufficient to allow us to calculate home range sizes during the breeding season and migration patterns during the non-breeding season. We assessed characteristics of movement patterns during both breeding and non-breeding in relation to colony and individual characteristics, with a particular emphasis on the potential role that density of breeding birds might have on spatial ecology.

Data from GPS tracking revealed that colony characteristics more so than individual characteristics determined the foraging ranges of breeding pelicans, and that foraging locations were not unique or specific to a given colony. For example, 50% core areas for individuals ranged from $< 10 \text{ km}^2$ (6.21 mi^2) to ca. 500 km^2 (310.69 mi^2) and 95% use areas for individuals ranged from $< 10 \text{ km}^2$ (6.21 mi^2) to ca. $3,500 \text{ km}^2$ ($2,175 \text{ mi}^2$). The home range size of pelicans during the breeding was not consistent among colonies and we found a positive relationship between both 50% and 95% use areas and the abundance of breeding birds at a colony, suggesting a density-dependent effect on movement. We did not detect any effect of sex, body size, or body condition on home range size. We also found that individuals from different colonies overlapped in their space use during the breeding season in all three planning areas of the GOM. Therefore, in the event of an acute stress event that may occur off colony, such as an oil spill, the probability of an individual from a given colony interacting with that stressor will not be based strictly on the distance from the point-event to the nearest colony, but also will need to consider colony size and the distribution of colonies in the area. For example, our data demonstrated that individuals may forage $> 100 \text{ km}$ (62.1 mi) from their breeding colony and in a locale also frequented by birds from neighboring colonies.

Our data demonstrated that brown pelicans in the GOM are partially migratory. As a population, partial migration leads to migration probabilities and distances that are inconsistent even within a single colony. In our study we found that migration distance of individuals ranged from $< 50 \text{ km}$ (31 mi) to approximately $2,500 \text{ km}$ ($1,553 \text{ mi}$). At the colony level the proportion of migrants ranged from approximately 25% to approximately 75%. Both migration distance and migration probability were positively related to the abundance of breeding birds at a colony, suggesting a positive effect of density on migration. Migration strategies also varied with individual characteristics, including sex and body size. As with home ranges during the breeding season we also found that individuals from different colonies and even different planning areas of the GOM overlapped spatially and temporally during staging and migration. For example, pelicans from all three planning areas overlapped in the Mississippi River Delta region of Louisiana during staging, suggesting that this area presents a hot spot for migrating pelicans at a specific time of year (migration) when birds may be physically stressed. Birds from the western and

central GOM also overlapped on wintering grounds along the Yucatan Peninsula (an area not used by pelicans from the eastern GOM). As with the breeding season, therefore, our ability to predict the probability of birds from a specific colony being affected by a localized stressor event is complicated by the intra- and inter-colony differences observed in migration patterns. Furthermore, our data showed that pelicans breeding in Texas and Louisiana also winter along the Pacific coast of Mexico. Movement between ocean basins are uncommon for seabirds and, for pelicans, such migratory routes may facilitate genetic mixing and help distribute risk across populations.

We also documented some unique attributes of migration paths of pelicans during our study. Over-water migrations were not uncommon. These included north-south transits across the GOM from the Louisiana Delta region to the Yucatan Peninsula, and from the Florida Keys to Cuba. During these transits, individuals were using pelagic waters. Additional data on pelicans' use of GOM pelagic waters is being collected by vessel-based surveys of the Gulf of Mexico Marine Assessment Program for Protected Species (GoMMAPPS). Combining tracking data from our study with observation-based data from those vessel surveys may provide a unique opportunity to explore pelicans' use of GOM pelagic waters. The migration of GOM pelicans to Cuba also suggests that spatial overlap occurs with pelicans that breed along the Atlantic coast of the U.S. and also migrate to Cuban waters (Poli 2015). Whether pelicans that breed in other areas of the Caribbean also migrate to Cuban waters is not well known, but it appears that Cuba could represent an overlap in migration among multiple populations of pelicans.

The movement data we collected clearly demonstrates a wide range of patterns within and among colonies. Such variability presents numerous challenges with respect to our ability to link a specific colony to a specific location that may experience an acute stress event such as an oil spill. Our data demonstrate that proximity from a colony to a localized stress event cannot be used as the sole predictor of the probability of a bird encountering that stress event. The spatial and temporal overlap in use areas by birds from different colonies furthers that concept. Several factors related to colony and individual dynamics will also affect that probability. Our data suggest that the continued development of maps of use areas specific to colonies are an important step in our ability to assess risk or damage to specific colonies. In the context of linking environmental attributes or stress events to specific colonies, our data demonstrate that colonies and the individuals that occupy them are being affected by conditions that range spatially over three orders of magnitude during the breeding season (1s–100s km) and over four orders of magnitude during the non-breeding season (1s–1,000s km).

9 References

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Appendix A: Supplementary Methods for Analysis of Composition of Diet of Brown Pelican Chicks, 2013–2015

Processing: In the lab, we thawed samples in a hot water bath, separated and counted individual fish, and classified each fish according to its condition (Whole, W: completely intact; Partial-whole, PW: complete length, some skin and/or soft tissues and/or caudal fin missing; Partial, PA: incomplete length). We then identified each fish to species, individually weighed and measured (Total Length, TL, and/or Standard Length, SL; in mm) all W and PW fish, and collected a full sample weight. For PA and PW samples that contained more than 10 fish of the same species, we randomly selected a sample of 10 to weigh and measure.

Estimation of Total Length of Partial samples: In 2015, based on Whole (W: completely intact sample) regurgitates with recorded values for both TL and SL, we used a linear regression $TL \sim SL$ to populate the missing values in both parameters. Regression equations were as follows (**Table A.1**):

Table A.1. Regression values for total length of main prey species encountered in diet samples of juvenile brown pelicans, Northern Gulf of Mexico 2013–2015

Species	Regression equation	R ²	P
Brevoortia patronus	TL = 1.251 SL + 0.547	0.986	< 0.005
Micropogonias undulatus	TL = 1.167 SL + 6.531	0.989	< 0.005
Leiostomus xanthurus	TL = 1.259 SL - 1.118	0.997	< 0.005
Lagodon rhomboides	TL = 1.118 SL + 12.029	0.956	< 0.005
Anchoa mitchilli	TL = 1.117 SL + 4.387	0.945	< 0.005
Anchoa lyolepis	TL = 1.192 SL + 0.203	0.958	< 0.005
Anchoa hepsetus	TL = 1.114 SL + 4.060	0.970	< 0.005
Opisthonema oglinum	TL = 1.284 SL - 2.198	0.997	< 0.005

Estimation of mass of Partial-whole samples: based on TL and mass (in g) of W regurgitates and fresh bait bought near the breeding colonies, we calculated linear regressions ($\log(\text{mass}) \sim \log(\text{TL})$) for the main prey species of samples collected in 2014 and in 2015. We used the regression equations to correct the mass of PW samples. Regression equations were as follows (**Table A.2**).

Table A.2. Regression values for mass of main prey species encountered in diet samples of juvenile brown pelicans, Northern Gulf of Mexico 2013–2015

Year	Species	Regression equation	R ²	P
2014	Brevoortia patronus	mass = $e^{-12.233} \times TL^{3.138}$	0.988	< 0.005
2014	Micropogonias undulatus	mass = $e^{-11.298} \times TL^{2.926}$	0.826	< 0.005
2014	Leiostomus xanthurus	mass = $e^{-11.324} \times TL^{2.976}$	0.982	< 0.005
2014	Opisthonema oglinum	mass = $e^{-16.051} \times TL^{3.278}$	0.972	< 0.005
2015	Brevoortia patronus	mass = $e^{-12.060} \times TL^{3.100}$	0.978	< 0.005
2015	Micropogonias undulatus	mass = $e^{-9.862} \times TL^{2.630}$	0.989	< 0.005
2015	Lagodon rhomboides	mass = $e^{-9.980} \times TL^{2.763}$	0.974	< 0.005
2015	Anchoa mitchilli	mass = $e^{-10.470} \times TL^{2.641}$	0.858	< 0.005
2015	Anchoa hepsetus	mass = $e^{-8.603} \times TL^{2.223}$	0.876	< 0.005
2015	Opisthonema oglinum	mass = $e^{-16.051} \times TL^{3.278}$	0.972	< 0.005

Estimation of mass of Partial samples: If there were W or PW samples of the same species in the regurgitate, we calculated TL of PA samples as the average of observed TL of W and PW samples for that species in the same regurgitate. We then used the linear regressions to estimate the initial mass (in g) of the sample. If there were no W or PW samples of the same species in the same regurgitate, or if the species was not one of the main species, the initial mass of the sample was kept as the corrected mass. For *Leiostomus xanthurus* and *Anchoa lyolepis* the sample sizes were too small, and we used equations calculated from our 2014 samples.



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