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Research article

Blood biochemistry and hematology of adult and chick brown pelicans in the northern Gulf of Mexico: baseline health values and ecological relationships

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The northern Gulf of Mexico supports a diverse community of nearshore seabirds during both breeding and nonbreeding periods of the annual cycle and is also a highly industrialized marine ecosystem with substantial levels of oil and gas development particularly in the west and central regions. Stakeholders in the region often assess risk to species of interest based on these differing levels of development. We collected blood samples from 81 adult and 35 chick eastern brown pelicans (*Pelecanus occidentalis carolinensis*) from 10 colonies across the northern Gulf of Mexico and used these to establish baseline values for hematology and blood biochemistry. We assessed the potential influence of body condition, sex and home range size on hematology and blood biochemistry. We also assessed potential influences of oil and gas activity by considering differing levels of oil and gas development that occur regionally throughout the study area. Although blood analyte concentrations of adults and chicks were often associated with these regional differences, the pattern we observed was not entirely consistent with the differing levels of oil and gas activity across the Gulf, suggesting that regional levels of oil and gas activity around breeding sites may not be the primary drivers of hematology and blood biochemistry. We note that baseline values or reference intervals are not available for other nearshore seabirds that breed in the northern Gulf. Given that exposure and risk may differ among this suite of species based on diet, foraging strategies and life history strategies, similar assessments and monitoring may be warranted.

Key words: blood biochemistry, body condition index, brown pelican, Gulf of Mexico, health status, hematology, home range

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Introduction

Individual animals are regularly exposed to stressors in their environment that are acute and chronic both spatially and temporally and that originate from both anthropogenic and natural sources. Responses to these stressors can range from long-term and widespread effects to shorter-term and localized effects and can affect populations, sub-populations and/or individuals. At the individual level, focus is often placed on lethal effects, particularly following acute and extreme stressors such as oil spills, pollution events or severe weather events (Loss *et al.*, 2012; Haney *et al.*, 2014a). Sublethal effects are, however, also relevant and can include but are not limited to changes in behavior, movement or reproductive ecology (Peréz *et al.*, 2010; Eggert and Jodice, 2008; Wilkinson *et al.*, 2019). These changes are often driven by measurable effect to the physiology of an individual (Alonso-Alvarez *et al.*, 2007; Eggert *et al.*, 2010; Lewis *et al.*, 2013). Therefore, measures of species-specific traits such as physiological markers can be used to monitor changes in ecosystem condition, enhance our understanding of mechanisms underlying the status and trends of wildlife populations and inform conservation strategies (Maceda-Veiga *et al.*, 2015; Ottinger *et al.*, 2019; Polidoro *et al.*, 2021). The use of such data is strengthened when they are available from multiple locations throughout the range of a species to evaluate broad-scale patterns across environmental gradients, or from multiple taxa (Polidoro *et al.*, 2021).

The Gulf of Mexico (also Gulf throughout) is a subtropical ocean basin with natural and anthropogenic connections to the Caribbean and Gulf Stream ecoregions and to freshwater and terrestrial systems in North, Central and South America. The Gulf supports a rich assemblage of nearshore and pelagic seabirds, including local breeding populations as well as migratory and wandering individuals from interior and coastal North America, the eastern North Atlantic, the Caribbean Sea and the South Atlantic (Jodice *et al.*, 2019; Wilson *et al.*, 2019). Natural and anthropogenic stressors affecting seabirds in the system include, but are not limited to, severe weather events (e.g. tropical storms), pulses of freshwater input from inland flooding, pollution events (e.g. oil spills, hypoxic zones) and resource extraction (Coleman *et al.*, 2004; Houde *et al.*, 2006; Pollack *et al.*, 2011; Raynor *et al.*, 2013; Haney *et al.*, 2014b). Collectively, these stressors provide an impetus for understanding effects of environmental conditions on avian health across the northern Gulf (Ottinger *et al.*, 2019).

We used measures of blood biochemistry and hematology to assess the health of adults and chicks of the eastern brown pelican (*Pelecanus occidentalis carolinensis*). The species is widespread throughout the northern Gulf and common during all phases of the annual cycle (Lamb *et al.*, 2017a, 2020b). Based on its distribution patterns, behavior and sensitivity to chemical and oil contaminants (Blus, 1982; King *et al.*, 1985; Shields, 2020; Haney *et al.*, 2014a,b), brown pelicans are a high priority for monitoring and assessment in the Gulf (Jodice *et al.*, 2019). As a predator of schooling forage fish that occur in estuaries and nearshore habitats [particularly Gulf menhaden (*Brevoortia patronus*); Lamb *et al.*, 2017b], the species is also considered to be a good indicator of ecosystem conditions (Jodice *et al.*, 2019). Therefore, we sought to assess the health and condition of adult and chick pelicans during the breeding season. We selected colonies from across the northern Gulf that were located within administrative management units (i.e. planning areas) of the U.S. Bureau of Ocean Energy Management (BOEM; Fig. 1). The three planning areas within the Gulf coincide with varying levels of oil and gas development [Central Planning Area (CPA), most developed; Eastern Planning Area (EPA), least developed; and Western Planning Area (WPA), intermediate development between EPA and CPA; Fig. 1]. Stakeholders in the region have used the differing levels of oil and gas activity by region as a proxy for potential effects of acute and chronic exposure to wildlife, although the validity of this assumption has not been tested directly.

Our goal was therefore to assess blood biochemistry and hematology of pelican adults and chicks. Because it is unclear if individuals in our study area may have been exposed to oil either recently or formerly, or within their breeding range or migratory range, we refrain from defining these data as reference intervals, which assume the source population is fully healthy. We therefore refer to these data as baseline values or baseline intervals as they represent the first comprehensive assessment of health parameters for this species in this region. We examined these metrics in relation to the level of oil and gas development surrounding each colony (represented by administrative planning areas) and individual attributes (sex, body condition, foraging range). Our data provide an opportunity to assess the assumption that individual physiology is related to the level of oil and gas activity among the three planning areas and to establish a baseline for long-term monitoring in a region regularly exposed to acute and chronic stressors of natural and anthropogenic origins.

Materials and methods

Ethics statement

This study is one component of a broader research program that was focused on the ecology of brown pelicans in the northern Gulf of Mexico (Lamb *et al.*, 2020b). Research was authorized by permits from the Clemson University Animal Care and Use Committee (2013-026), U.S. Geological Survey Bird Banding Lab (#22408), Texas Parks and Wildlife (SPR-0113-005), Audubon Texas, The Nature Conservancy in Texas, Louisiana Department of Wildlife and Fisheries (LNHP-13-058 and LNHP-14-045) and the Florida Fish and Wildlife Conservation Commission (LSSC-13-00005).

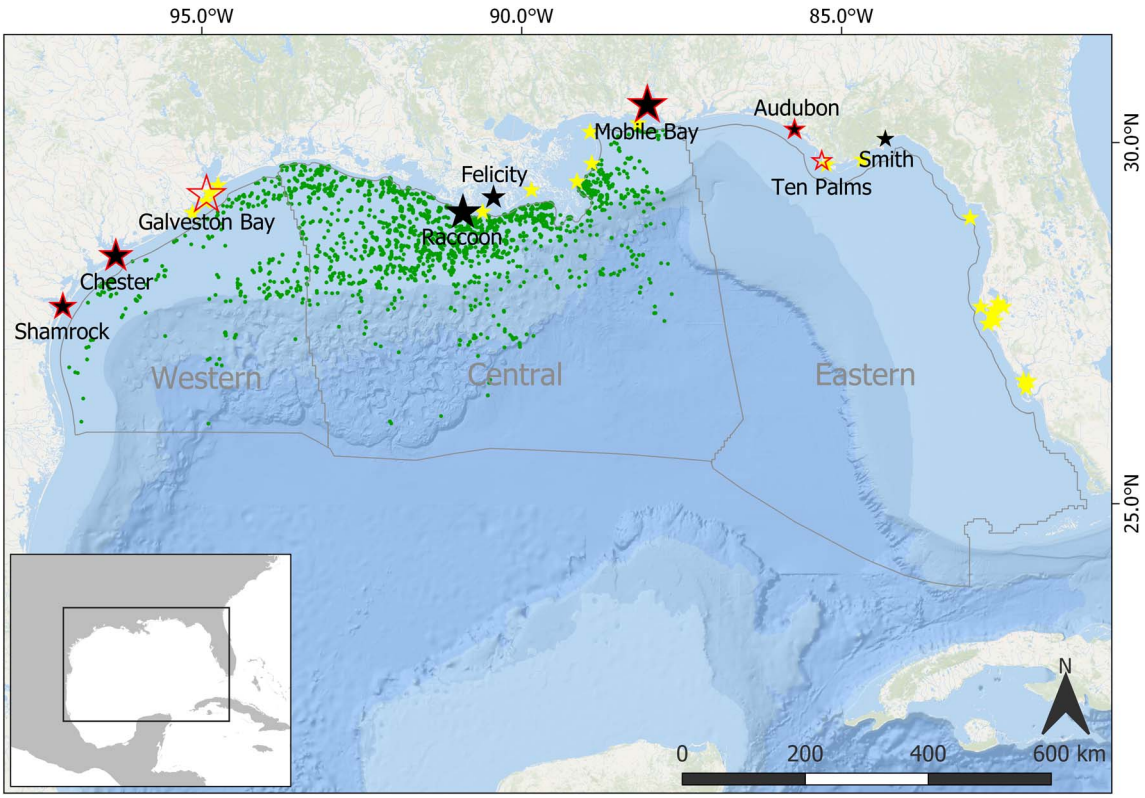

Figure 1: Location of brown pelican colonies in the northern Gulf of Mexico. Adults were sampled at colonies marked by a black symbol, and chicks were sampled at colonies marked by a red-outlined symbol. Yellow symbols represent colonies not sampled. Size of symbol is relative to colony size (75–5000 nesting pairs). The three planning areas of the BOEM are designated. Oil and gas infrastructure (green dots) is most common in the CPA, intermediate in the WPA and least in the EPA. (Base layer: ESRI, DeLorme, General Bathymetric Chart of the Oceans, National Oceanic and Atmospheric Administration, National Geophysical Data Center and other contributors).

Study area

Study sites extended from the Florida panhandle to the Texas coast. We sampled adults from six colonies: Audubon and Smith islands, Florida; Felicity and Raccoon islands, Louisiana; and Chester and Shamrock islands, Texas (Fig. 1). We sampled chicks from six colonies: Audubon and Ten Palms islands, Florida; Gaillard Island, Alabama; Marker 52 and North Deer (regrouped as Galveston Bay colonies), Chester; and Shamrock islands, Texas. Study colonies were all within a single marine ecoregion (Northern Gulf of Mexico within the Temperate North Atlantic Realm; Spalding *et al.*, 2007) and represent each of the three BOEM planning areas (EPA, CPA and WPA).

Sampling

Brown pelican adults and chicks were sampled from active nests during the breeding seasons of 2013–2015 (Lamb *et al.*, 2020b). We collected blood smears from adults ($n = 90$) and blood samples for complete blood counts (CBCs; $n =$ subset of 81 of the 90). Not all samples were suitable for complete analyses and so sample sizes vary among analytes and blood smears. We collected blood smears and blood samples from chicks (6–8 weeks post-hatch) for CBCs from 35 individuals.

For both adults and chicks, blood samples were collected during morning hours to avoid the potential for heat stress during research activities. We collected blood from the tarsometatarsal vein within 2 min of capture. After sterilizing the collection site, we collected 5 ml of 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), then spun down within 5–10 h and subsequently stored in a −80C freezer for ∼6 months until shipped for analysis.

For adults and chicks, we measured body mass (± 50 g), culmen length (± 1 mm), tarsus length (± 1 mm) and wing length (± 5 mm). From these we created a body condition index (BCI). The BCI provides an index for the mass of the bird in relation to its size and is calculated as the residual of the linear relationship between mass and culmen length (Lamb *et al.*, 2016). In brown pelicans, sex cannot be easily determined *in situ*. Therefore, the distribution of samples

between sexes is opportunistic. Sex of adults was determined from collected blood samples through PCR (Itoh *et al.*, 2001). We did not sex chicks.

Sample processing

To create blood smears from stored samples, we filled three capillary tubes with whole blood for hematocrit analysis and spun down both samples of whole blood 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 in a −80C freezer until analysis.

Biochemical, protein electrophoresis and serological tests were conducted by 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, 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, 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 corticosterone were measured by radioimmunoassay (MP Biomedicals Double Antibody Corticosterone radioimmunoassay, Santa Ana, California). We classified each analyte as 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 1000× magnification to determine differential counts of white blood cells (WBCs). We also measured the concentration ($*10^3$ mu L^{-1}) of all WBC and the concentration of heterophils, lymphocytes, monocytes, eosinophils and basophils. Because blood smears were not read via an automated process but instead read manually by technicians with no direct connection to the study, reliability with respect to differential counts of WBCs is considered high and the potential for bias considered low.

Statistical analyses

To create baseline values for parameters, we used the software package MedCalc for Windows, version 20.111 (MedCalc Software, Ostend, Belgium). We first assessed each analyte for outliers using the Tukey outlier test as performed in MedCalc. We then checked normality of the retained data using Q-Q plots and the D'Agostino-Pearson test as calculated in MedCalc, which computes a single *P*-value for the combination of the coefficients of skewness and kurtosis. For analytes that did not meet assumptions of normality, we conducted either a Box-Cox transformation (commonly used for data such as concentrations of blood analytes that are often heteroscedastic) or a logarithmic transformation.

Once outliers were removed and normality assessed, we created intervals for baseline values for each analyte following the National Committee for Clinical Laboratory Standards Committee (NCCLS) Clinical and Laboratory Standards Institute (CLSI) guidelines C28-A2 and C28-A3 for estimating percentiles and their 90% confidence intervals (CIs; CLSI 2008, NCCLS 2020). Because our sample sizes were <120, we used the robust method (CLSI Guidelines C28-A3), which uses bootstrapping to estimate the CIs on the baseline values. We report baseline values as either untransformed or back-transformed for all analytes.

We assessed the relationship between blood analytes and a suite of independent variables using Generalized Linear Models (GLMs) and composite scores from ordination analyses (described in detail below). Independent variables included the following: sex (adults; categorical, reference level = female), BCI (adults and chicks; continuous and standardized prior to analysis), BOEM planning area (adults and chicks; categorical, reference level = EPA) and home range size (adults; continuous and standardized prior to analysis). Home range was reported as the 50% core area for any individual that was equipped with a satellite transmitter ($n = 64$; see Lamb *et al.*, 2017a, 2020b for details on tagging and home range estimation). We selected the 50% core area as opposed to the 95% use area as it better represents the conditions encountered regularly by an individual particularly during chick-rearing.

We did not assess relationships for each blood analyte individually but rather grouped analytes (see below) as metabolites (cholesterol, triglycerides, high-density lipoprotein, uric acid and total protein), electrolytes (sodium, calcium, phosphorous), enzymes (amylase, lipase, aspartate aminotransferase, creatine phosphokinase, and gamma-glutamyl transferase) and plasma proteins (pre-albumin, albumin, alpha-1 globulin, alpha-2 globulin, beta globulin and gamma globulin). We included corticosterone for analysis but did not group it with any of the aforementioned categories. We opted not to model relationships for potassium and glucose because storage times prior to centrifugation may have resulted in spurious high (potassium) or low (glucose) values from cell leakage (potassium) or consumption (glucose). We also

Table 1: Models used in an information theoretic approach to assess relationships among blood analytes and independent variables for brown pelican adults and chicks sampled from breeding colonies in the northern Gulf of Mexico, 2013–2015

	Variables included	Adult model	Chick model	Comments
Model 1	Sex	Yes	No	Sex not available for chicks
Model 2	BCI	Yes	Yes	
Model 3	Planning area	Yes	Yes	East, Central, West (Fig. 1)
Model 4	Home range size	Yes	No	Home range of chicks not a relevant metric
Model 5	Home range size + sex	Yes	No	Neither variable available for chicks
Model 6	BCI + planning area	Yes	No	Terms are interrelated for chicks; not modeled together
Model 7	BCI + home range size	Yes	No	Home range irrelevant for chicks
Model 8	Null model	Yes	Yes	

omitted creatinine and the ratio of BUN to creatinine (B:C ratio) from subsequent analyses as creatinine is a metabolite present in very small amounts in birds and has uncertain significance in an ecological context.

For the remaining analytes we first assessed correlations among each. For correlated pairs ($|r| \geq 0.5$) we examined each analyte and dropped those with higher coefficients of variation, those that were correlated with a greater number of other analytes, and/or those for which ecological interpretations were likely to be less clear. For each of the four functional groups we then used a principal components analysis (PCA) to reduce each to one or two axes. To avoid over- or under-fitting, we chose the final number of axes by conducting a Bartlett's test followed by a broken-stick test of eigenvalues for each principal component (Peres-Neto *et al.*, 2005). Bartlett's test results were significant ($p < 0.05$) for all analyte groups, suggesting that at least one axis should be retained for each group. We retained each additional axis n for which the eigenvalue (variance explained) was greater than the nth-largest value resulting from a random partitioning of the observed variance. Using this method, we retained a single axis for all analyte groups except for metabolites (two axes). We used the composite scores in GLMs to assess ecological relationships (see below). When axes are loaded primarily positively, the interpretations of ecological relationships from subsequent GLMs are consistent with the sign of the coefficient estimate from each model. When axes are loaded primarily negatively, the interpretations of ecological relationships from subsequent GLMs are inverse to the sign of the coefficient estimate from each model. All subsequent figures use a raw scale for both dependent and independent variables for ease of interpretation.

We built eight GLMs (Table 1) to assess the relationships among analytes and independent variables. Correlated independent variables were not used within the same models, although all variables of interest were included in the overall suite of models (Table 1). We calculated coefficient estimates and 90% CIs from any model that was within 2 AIC points of the top-ranked model. Coefficients where 90% CIs do not overlap 0 are more likely to represent an ecological relationship between the analyte and the independent variable and are therefore treated as relationships of interest (although all coefficients and CIs from variables in highly supported models are provided). Interaction terms for the aforementioned models were developed and assessed post-hoc. No interactions of the independent variables returned coefficients likely to be ecologically relevant (i.e. SE > mean in all cases).

Hematology data did not fit a normal distribution (either as raw data or when transformed). We therefore performed nonparametric analyses (Kruskal–Wallis with Dunn's multiple comparison post-hoc test or Kendall correlation) to assess the relationships between hematology data [total WBC count, WBC count by type and the heterophil:lymphocyte (H:L) ratio] and sex, planning area, BCI and home range size. We also conducted a nonparametric Kendall correlation between H:L and corticosterone.

When box plots were used to display data, the median and quartiles defined the boxes, the whiskers defined the 10th and 90th percentiles and data beyond the 10th and 90th percentiles were shown as circles.

Results

Baseline values for biochemistry and hematology

Of the 30 analytes examined for adults (Table 2), 23 had outliers removed (Table S1) and 5 required transformation. Errant values were recorded for sodium for 43 individuals (>250 mEq L^{-1}). We examined all other data associated with these individuals to determine if there were patterns that might explain these high values but found none. We concluded that these were likely spurious and removed these values, but not these individuals, from subsequent analyses.

Hematology values for adults appear in Table 3. Outliers were removed from each parameter (Table S2). Heterophils

Table 2: Baseline values and summary statistics for serum chemistry for adult brown pelicans sampled from breeding colonies in the northern Gulf of Mexico, 2013–2015

Functional group/analyte (units)	*n*	*Mean*	*Median*	*SD*	*Min*	*Max*	*Baseline interval*	*Lower 90% CI*	*Upper 90% CI*
Electrolytes and minerals									
Calcium (mg dL^{-1})[a]	70	8.22	8.50	1.44	4.5	10.8	5.43–11.33	4.86–6.10	10.87–11.83
Phosphorus (mg dL^{-1})[a]	69	4.88	4.70	1.37	2.5	9.2	2.03–7.59	1.55–2.49	7.05–8.07
Potassium (mEq L^{-1})[a]	692	3.78	3.60	1.46	1.2	7.0	0.73–6.69	0.32–1.16	6.14–7.20
Sodium (mEq L^{-1})[a]	28	140.0	144.0	11.0	111	156	117.2–166.0	110.9–126.3	158.7–171.7
Enzymes									
Amylase (U L^{-1})[a]	66	1158.4	1165	104.4	922	1445	950.6–1372.0	911.6–992.7	1337.0–1407.2
Aspartate aminotransferase (U L^{-1})[a]	69	170.5	160.0	46.9	71	270	71.3–264.8	58.1–86.6	244.9–279.7
Alanine aminotransferase (U L^{-1})[a]	69	28.7	29.0	7.3	12	44	14.7–43.1	12.3–17.3	40.7–45.4
Creatine phosphokinase (U L^{-1})	71	974.9	943	436.4	48	1854	96.6–1857.3	0–232.8	1713.9–1983.4
Gamma-glutamyl transferase (U L^{-1})$*$ L^{-1})	63	8.1	7	3.2	5	17	0.6–14.5	0–1.9	12.6–15.9
Lactate dehydrogenase (U L^{-1})[a]	70	4644.8	4592.5	1359.2	1764	7428	1816.2–7286.0	11419.5–2244.3	6778.9–7789.3
Lipase (U L^{-1})[a,b]	68	19.2	19	n/a	1	55	2.9–55.1	1.7–4.4	46.8–63.6
Lipids									
High-density lipoprotein cholesterol (mg dL^{-1})	71	83.2	84	15.1	50	111	53.4–114.0	48.5–58.9	109.8–118.5
Low-density lipoprotein cholesterol (mg dL^{-1})[a,b]	66	9.3	10	n/a	5	21	5.0–22.7	4.7–5.5	18.7–26.4
Triglycerides (mg dL^{-1})[a,c]	66	48.2	48	n/a	27	107	23.0–97.5	20.5–25.5	84.9–110.7
Metabolites									
Blood urea nitrogen (mg dL^{-1})[a,b]	67	2.8	3	n/a	1	11	0.7–12.1	0.6–0.9	9.8–15.2
Cholesterol (mg dL^{-1})[a]	71	150.3	152	29.7	80	218	90.8–210.6	80.9–100.9	200.9–220.3
Creatinine (mg dL^{-1})[a]	67	0.67	0.7	0.2	0.2	1.2	0.16–1.14	0.08–0.23	1.04–1.23
Glucose (mg dL^{-1})[a]	71	205.9	210.0	36.3	111	291	133.2–280.2	120.7–146.9	268.2–292.1
Total protein (g dL^{-1})	72	4.31	4.20	0.88	2.4	6.7	2.47–6.05	2.15–2.74	5.72–6.37
Uric acid (mg dL^{-1})[a,b]	67	9.94	9.60	n/a	0.7	27.3	1.83–27.77	1.21–2.62	23.37–32.04
BUN:Creatinine ratio[a]	63	5.22	5.0	2.77	1.7	11.7	0–10.58	0–0.08	9.32–11.79

(Continued)

Table 2: Continued.

Functional group/analyte (units)	*n*	*Mean*	*Median*	*SD*	*Min*	*Max*	*Baseline interval*	*Lower 90% CI*	*Upper 90% CI*
Plasma protein									
Albumin (mg dL^{-1})	72	1.24	1.24	0.21	0.78	1.67	0.82–1.66	0.76–0.89	1.59–1.72
Alpha-1 globulin (mg dL^{-1})[a]	68	0.13	0.13	0.02	0.09	0.17	0.09–0.17	0.08–0.09	0.17–0.18
Alpha-2 globulin (mg dL^{-1})[a]	69	0.66	0.63	0.13	0.40	1.01	0.36–0.91	0.31–0.40	0.85–0.97
Beta globulin (mg dL^{-1})[a]	70	1.14	1.10	0.31	0.47	1.99	0.50–1.74	0.40–0.60	1.62–1.85
Gamma globulin (mg dL^{-1})[a]	71	0.82	0.69	0.48	0.24	2.01	0–1.73	0–0	1.51–1.93
Pre-albumin (mg dL^{-1})	72	0.25	0.25	0.07	0.13	0.42	0.12–0.38	0.10–0.14	0.36–0.41
Albumin:Globulin ratio	72	0.57	0.55	0.18	0.28	1.01	0.20–0.92	0.14–0.25	0.85–0.99
Other							–	–	–
Corticosterone (mg dL^{-1})[a]	70	35.48	35.75	13.20	11.90	62.80	8.50–61.61	4.79–12.66	56.91–65.94

Sample size (*n*), mean, median, standard deviation (SD), minimum (Min) and maximum (Max) values with baseline interval and 90% CI of baseline interval.
[a]Outlier(s) removed (see Table S1).
[b]Required Box-Cox transformation, values presented are backtransformed.
[c]Required log transformation, values presented are backtransformed.

Table 3: Baseline values and summary statistics for PCV (averaged over three readings) and leukocyte profiles for adult brown pelicans sampled from breeding colonies in the northern Gulf of Mexico, 2013–2015

Analyte	n	Mean	Median	SD	Min	Max	Baseline Interval	Lower	Upper
PCV average[a]	70	45.8	46.0	3.6	39.0	54.0	38.5–52.9	37.3–39.8	51.7–54.1
White blood cell count[a,b]	81	10.5	11.0	na	3.0	25.0	4.6–24.5	4.0–5.3	21.5–27.9
Heterophil (10^3/ml)[a]	82	8.1	7.7	3.2	1.0	15.0	1.5–14.3	0.5–2.4	13.2–15.4
Lymphocytes (10^3/ml)[a,b]	77	2.0	2.0	na	0.3	7.1	0.6–7.3	0.5–0.7	6.0–8.9
Monocyte (10^3/ml)[a]	80	0.24	0.18	0.25	0.0	0.90	0.0–0.72	0.0–0.0	0.61–0.81
Eosinophil(10^3/ml)[a]	81	0.13	0.09	0.18	0.0	0.75	0.0–0.45	0.0–0.0	0.0–0.53
Basophil(10^3/ml)[a]	81	0.029	0.0	0.07	0.0	0.30	0.0–0.0	0.0–0.0	0.0–0.0
Heterophil Lymphocyte ratio[a]	75	4.34	3.65	2.87	0.25	11.50	0.0–9.70	0.0–0.0	8.5–10.9

Sample size (*n*), mean, median, SD, minimum (min) and maximum (Max) values with baseline interval and 90% CI of baseline interval.
[a]Outlier(s) removed (see Table S2).
[b]Required log transformation, values presented are backtransformed.

were the most common WBC type and were observed in all samples. Samples frequently failed to include monocytes ($n = 24$), eosinophils ($n = 38$) and basophils ($n = 66$), and the modal value for each was 0.

Of the 30 analytes examined for chicks (Table 4), 15 had outliers removed (Table S3) but none required transformation. Hematology values for chicks appear in Table 5. Outliers were removed from 7 parameters (Table S4). Heterophils were the most common WBC type and were observed in all samples. Samples frequently failed to include monocytes ($n = 14$), eosinophils ($n = 20$) and basophils ($n = 23$), and the modal value for each was 0.

Individual attributes, hematology and plasma chemistry of adults

The BCI of males (43.1 ± 242.9) was higher ($F_{1,63} = 4.1$, $P = 0.04$) compared with females (-85.4 ± 255.4). BCI did not differ by planning area or sex * planning area ($P > 0.10$ for each). Home range size differed by planning area ($F_{2,61} = 2.5$, $P = 0.09$) but not by BCI, sex or sex * planning area ($P > 0.10$ for each). Home range size was least in the EPA (46.0 ± 50.7 km^2) compared with the CPA (161.4 ± 222.3 km^2) and WPA (122.7 ± 168.1 km^2). Related variables were not paired together in subsequent models of blood analytes (Table 1).

The five metabolites grouped into two principal components totaling 67% (PC 1: 37%; PC 2: 30%). Cholesterol and HDLP primarily loaded the first axis, while uric acid and triglycerides primarily loaded the second axis (Table 6). For both axes the primary loadings were negative. The model containing planning area carried 63% of the model weights, and the model containing BCI and planning area carried 24% of the model weights for axis 1 (Table S5). The model containing home range size and sex carried 97% of the model weights for axis 2 (Table S5). Levels of metabolites were lower in both the CPA and WPA compared with the EPA (Table 7). There was no ecological relationship with BCI (Table 7). Levels of metabolites along axis 2 declined with home range size and were lower in males compared with females (Table 7).

The two electrolytes grouped into a single principal component explaining 64% of variance that was loaded negatively by calcium and phosphorus (Table 6). The model including region carried 31% of the model weights. The null model and model including BCI also received some support (16 and 13% of model weights, respectively; Table S5). Levels of electrolytes along axis 1 were lower in both the CPA and WPA compared with the EPA, although there was no ecological relationship with BCI (Table 7).

The five enzymes grouped into a single principal component explaining 37% of variance that was primarily loaded negatively by CPK and AST (Table 6). The model including planning area carried 69% of the model weights (Table S5). Enzyme levels were higher in the EPA compared with the CPA (Table 7).

The six plasma proteins grouped into a single axis explaining 37% of variance that was primarily positively loaded by beta globulin (Table 6). The model including region carried 51% of the model weights, while the model including BCI and planning area carried 12% of the model weights (Table S5). Levels of plasma proteins were higher in the EPA compared with the CPA and WPA, although there was no ecological relationship with BCI (Table 7).

The top-ranked models for corticosterone included planning area, which carried 60% of the model weights, and BCI plus planning area, which carried 25% of the model weights (Table S5). While the coefficient estimates for BCI indicated that an ecological relationship was not likely (i.e. 90% CIs overlapped 0), corticosterone was lower in adults in the CPA compared with the EPA.

Table 4: Baseline values and summary statistics for serum chemistry for brown pelican chicks sampled from breeding colonies in the northern Gulf of Mexico, 2013–2015

Functional group/analyte (units)	n	Mean	Median	SD	Min	Max	Baseline interval	Lower 90% CI	Upper 90% CI
Electrolytes and minerals									
Calcium (mg dL^{-1})	34	10.7	10.7	0.8	8.9	12.1	9.1–12.4	8.7–9.6	12.0–12.7
Phosphorus (mg dL^{-1})[a]	33	7.1	7.0	1.1	5.4	9.6	4.6–9.2	4.2–5.2	8.6–9.9
Potassium (mEq L^{-1})[a]	33	3.5	3.4	0.5	2.4	4.9	2.3–4.6	2.0–2.6	4.3–4.8
Sodium (mEq L^{-1})[a]	33	146.1	147.0	4.3	136	154	137.8–156.2	135.0–140.2	153.7–158.0
Enzymes									
Amylase (U L^{-1})	34	1293.0	1268.0	209.1	919	1756	825.9–1694.6	732.4–923.4	1562.0–1825.0
Aspartate aminotransferase (U L^{-1})[a]	31	154.4	147.0	39.7	97	246	61.2–228.3	45.4–86.5	204.9–255.3
Alanine aminotransferase (U L^{-1})	34	31.0	30.0	8.3	19	51	12.6–47.0	8.7–17.0	42.3–51.9
Creatine phosphokinase (U L^{-1})[a]	32	1335.0	1361.5	216.1	769	1687	891.6–1795.9	774.9–1016.4	1686.6–1893.7
Gamma-glutamyl transferase (U L^{-1})[a]	32	7.3	7.0	1.6	5	10	3.8–10.7	2.8–4.6	9.9–11.4
Lactate dehydrogenase (U L^{-1})[a]	31	5532.2	5432.0	1043.1	3790	8130	3307.6–7663.8	2839.6–3876.6	7053.5–8221.5
Lipase (U L^{-1})[a]	33	26.5	28.0	6.6	13	41	12.5–40.6	9.5–16.5	37.1–43.8
Lipids									
High-density lipoprotein cholesterol (mg dL^{-1})	34	80.7	77.5	13.8	57	111	50.8–109.4	45.3–57.6	101.1–115.7
Low-density lipoprotein cholesterol (mg dL^{-1})	34	19.8	19.0	7.5	8	35	3.8–35.1	0.25–7.12	31.1–38.6
Triglycerides (mg dL^{-1})	34	99.3	95.5	37.3	42	177	20.0–175.6	2.6–36.3	155.2–193.0
Metabolites									
Blood urea nitrogen (mg dL^{-1})[a]	33	3.2	3.0	1.0	1	6	1.0–5.3	0.4–4.0	3.5–5.9
Cholesterol (mg dL^{-1})	34	203.1	201.5	35.1	140	284	127.9–273.3	112.7–145.8	253.8–291.5
Creatinine (mg dL^{-1})	34	0.48	0.40	0.19	0.10	0.90	0.001–0.868	0.0–0.130	0.740–0.974
Glucose (mg dL^{-1})[a]	33	180.4	182.0	28.4	100	238	124.1–241.5	108.9–140.7	223.2–256.6
Total protein (g dL^{-1})	34	4.2	4.2	0.6	3.3	5.6	3.0–5.3	2.8–3.3	5.0–5.6
Uric acid (mg dL^{-1})[a]	33	14.8	14.9	6.4	5.4	29.5	1.4–27.8	0.0–4.3	24.4–30.9
BUN:Creatinine ratio[a]	31	7.0	6.7	2.5	2.5	13.3	1.6–12.2	0.2–2.6	10.4–13.7

(Continued)

Table 4: Continued

Functional group/analyte (units)	n	Mean	Median	SD	Min	Max	Baseline interval	Lower 90% CI	Upper 90% CI
Plasma proteins									
Albumin (mg dL^{-1})[a]	33	1.55	1.55	0.19	1.13	1.29	1.18–1.94	1.08–1.27	1.85–2.03
Alpha-1 globulin (mg dL^{-1})	34	0.13	0.12	0.03	0.09	0.18	0.06–0.19	0.06–0.08	0.17–0.20
Alpha-2 globulin (mg dL^{-1})[a]	33	0.71	0.73	0.13	0.47	1.00	0.44–0.98	0.39–0.51	0.92–1.04
Beta globulin (mg dL^{-1})	34	0.98	0.96	0.15	0.76	1.26	0.66–1.30	0.60–0.72	1.22–1.37
Gamma globulin (mg dL^{-1})	34	0.58	0.51	0.18	0.34	1.02	0.11–0.91	0.04–0.23	0.79–1.04
Pre-albumin (mg dL^{-1})	34	0.27	0.26	0.06	0.18	0.39	0.13–0.41	0.10–0.16	0.36–0.43
Albumin:Globulin ratio	34	0.76	0.79	0.14	0.43	1.06	0.48–1.07	0.41–0.56	1.00–1.13
Other									
Corticosterone (mg dL^{-1})[a]	33	55.2	57.2	19.3	16.6	99.7	14.5–95.1	5.3–24.8	84.4–104.2

[a]Outlier(s) removed (see Table S3).
Sample size (*n*), mean, median, SD, minimum (Min) and maximum (Max) values with baseline interval and 90% CI of baseline interval. Untransformed robust values reported when available, otherwise untransformed standard data reported.

Table 5: Baseline values and summary statistics for PCV (averaged over three readings) and leukocyte profiles for brown pelican chicks sampled from breeding colonies in the northern Gulf of Mexico, 2013–2015

Analyte	*n*	Mean	Median	SD	Min	Max	Baseline Interval	Lower	Upper
PCV %	34	40.5	40.0	4.8	30	48.7	30.5–50.4	28.3–32.8	47.7–52.7
White blood cell count[a]	32	17.9	16.0	8.3	5	37	0–34.2	0–3.0	28.7–39.4
Heterophil (10^3/ml)[a]	31	8.9	8.1	4.0	2.3	17.9	0.0–16.5	0.0–1.9	13.9–19.1
Lymphocytes (10^3/ml)[a]	32	7.8	6.1	4.5	2.7	20.1	0.0–16.2	0.0–0.0	12.7–19.3
Monocyte (10^3/ml)[a]	33	0.21	0.11	0.25	0.0	0.75	0.0–0.67	0.0–0.0	0.0–0.86
Eosinophil (10^3/ml)[a]	32	0.11	0.0	0.19	0.0	0.69	0.0–0.0	0.0–0.0	0.0–0.53
Basophil (10^3/ml)[a]	33	0.09	0.0	0.17	0.0	0.58	0.0–0.0	0.0–0.0	0.0–0.0
Heterophil lymphocyte ratio[a]	27	1.16	1.08	0.42	0.46	2.13	0.20–1.99	0.02–0.43	1.70–2.28

[a]Outlier(s) removed (see Table S4).
Sample size (*n*), mean, median, SD, minimum (min) and maximum (Max) values with baseline interval and 90% CI of baseline interval.

Table 6: Axis loadings from PCA procedure for each blood analyte within each analyte grouping for adult and chick brown pelicans sampled from breeding colonies in the northern Gulf of Mexico, 2013–2015

Analyte	Adults axis 1	Adult axis 2	Chicks axis 1	Chicks axis 2
Metabolites				
Cholesterol	−**0.69**	0.00	−**0.58**	0.27
High-density lipoprotein	−**0.58**	0.30	−0.38	0.42
Triglycerides	−0.10	−**0.65**	−0.42	−**0.57**
Total protein	−0.42	−0.21	−**0.50**	0.21
Uric acid	−0.03	−**0.67**	−0.30	−**0.62**
Electrolytes				
Calcium	−**0.71**		−**0.65**	
Phosphorous	−**0.71**		−0.47	
Sodium	n/a		−**0.60**	
Enzymes				
Amylase	−0.33		0.35	
Aspartate aminotransferase	−**0.52**		−0.35	
Creatine phosphokinase	−**0.53**		**0.59**	
Gamma-glutamyl transferase	−0.36		0.61	
Lipase	−0.45		−0.17	
Plasma proteins				
Albumin	−0.39		0.25	
Alpha 1 globulin	−0.48		0.28	
Alpha 2 globulin	−0.29		**0.54**	
Beta globulin	−**0.53**		**0.50**	
Gamma globulin	−0.48		**0.52**	
Pre-albumin	−0.14		0.21	

Loading ≥ |0.5| are displayed in boldface.

Table 7: Summary of coefficient estimates (± 90% CIs) from model selection process assessing relationships between blood analytes of adult brown pelicans breeding in the northern Gulf of Mexico and BCI, home range (50% use area), sex and planning area, 2013–2015

Functional group	Primary loading variables	BCI	50% home range	Sex	Planning area
Metabolites axis 1	Cholesterol (−), HDLP (−)	−0.21(−0.33, 0.29)			**C = 1.54 (0.79, 2.28) W = 1.10 (0.46, 1.74)**
Metabolites axis 2	Uric acid (−), triglycerides (−)		**0.56 (0.34, 0.79)**	**1.08 (0.62, 1.54)**	
Electrolytes axis 1	Calcium (−), phosphorous (−)	0.12(−0.14, 0.38)			**C = 0.89 (0.23, 1.55) W = 0.64 (0.06, 1.22)**
Enzymes axis 1	Creatine phosphokinase (−), AST (−)				**C = 1.78 (0.97, 2.60)** W = 0.68 (−0.03, 1.40)
Plasma proteins axis 1	Beta globulin (−)	−0.27(−0.57, 0.03)			**C = 1.48 (0.70, 2.26) W = 1.48 (0.79, 2.17)**
Corticosterone		0.10 (−2.72, 2.93)			**C = −13.21 (−20.22, −6.19)** W = −4.42 (−10.56, 1.71)

Only coefficients where 90% CIs do not overlap 0 (i.e. an ecological relationship between the analyte and the independent variable is more likely) are reported. No interactions of the independent variables returned coefficients likely to be ecologically relevant (i.e. SE > mean in all cases). For sex, the reference level was female so the coefficient shows the difference in males. For planning area, the reference level was the EPA so the coefficient shows the difference in the central (C) and western (W) planning areas. Coefficient estimates for corticosterone are presented on their raw scales. For functional groups where the primary PCA loading was negative (see Table 6), the relationship between the dependent and independent variables is the inverse of the sign for that coefficient. Bolded values are CIs that do not overlap zero.

Across all response variables (five principal components representing four functional groups, plus corticosterone), the model that was most often highly supported included planning area ($n = 5$) followed by the model that included BCI and planning area ($n = 4$; Table S5).

Hematocrit [i.e. packed cell volume (PCV)] was higher ($F_{1,62} = 8.1$, $P = 0.006$) in males (median, 46.5; range, 39.0, 54.0) compared with females (median, 43.8; range, 32.5, 54.0). Monocytes were slightly less common (Kruskal–Wallis $H_1 = 2.8$, $P = 0.09$) in males (median, 0.18; range, 0, 1.65) compared with females (median, 0.30; range, 0, 1.19). There was a moderate negative correlation between monocyte counts and BCI in females (Kendall tau = −0.23) but no correlation between monocyte counts and BCI in males (Kendall tau = − 0.01; Fig 2a). No other relationships occurred among sex or BCI and WBC counts or PCV ($P > 0.1$ for each).

WBC counts were slightly higher in the WPA (Kruskal–Wallis $H_2 = 4.7$, $P = 0.09$; mean rank difference = 16.3, $P = 0.04$; Fig. 2b). Heterophils were higher in the EPA than in the CPA (Kruskal–Wallis $H_2 = 6.7$, $P = 0.03$; mean rank difference = 14.8, $P = 0.05$) and higher in the WPA than in the CPA (mean rank difference = 15.9, $P = 0.04$; Fig. 2c). The H:L ratio was higher in the EPA than in the CPA (Kruskal–Wallis $H_2 = 6.6$, $P = 0.04$; mean rank difference = 17.4, $P = 0.04$; Fig. 2d). No other relationships occurred between planning area and WBC counts or PCV ($P > 0.2$ for each). The H:L ratio increased with home range size (Kendall tau = 0.16, $P = 0.05$; Fig. 2e). No other relationships occurred between home range and WBC counts or PCV ($P > 0.13$ for each). No correlation occurred between H:L and corticosterone (Kendall $T = -0.02$, $P = 0.8$).

Individual attributes, hematology and plasma chemistry of chicks

The two independent variables (BCI and planning area) were interrelated and therefore not used within the same models. BCI ranged from −976.9 to 1148.2 (mean = −13.9 ± 584.5). BCI of chicks differed by planning area ($F_{2,32} = 11.4$, P = < 0.0002) and was least in the WPA (Fig. 3a).

The five metabolites grouped into two principal components explaining 43% and 33% of variation, respectively. Cholesterol and total protein had the highest loadings on the first axis (both negatively loaded), while the second axis was primarily loaded negatively by uric acid and triglycerides (Table 6). For axis 1 the null model carried 60% of the model weights (Table S6). For axis 2 the model containing BCI carried 77% of the model weights (Table S6). BCI was positively related to the level of metabolites (Table 8).

The three electrolytes were best described by a single principal component accounting for 59% of variation and were negatively loaded primarily by calcium and sodium (Table 6). The model including region carried 59% of the model weights although the null model also received support

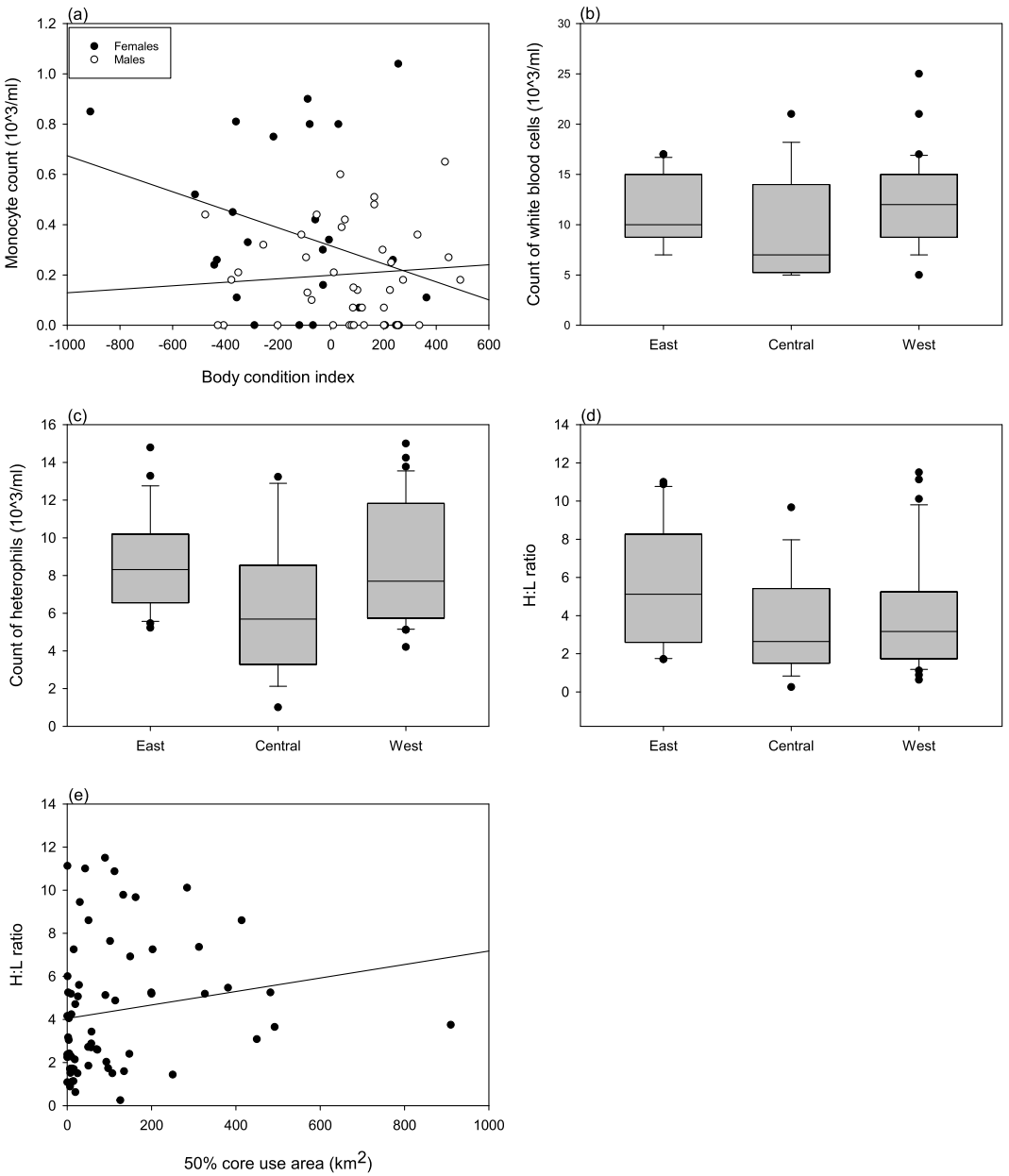

Figure 2: Hematology of adult brown pelicans sampled in the northern Gulf of Mexico in relation to (a) BCI, (b–d) planning area and (e) home range size.

and carried 23% of the model weights (Table S6). Electrolytes were slightly lower in the WPA compared with the EPA (Table 8).

The five enzymes reduced to a single principal component explaining 38% of variation. The first axis was primarily loaded positively by GGT and CPK (Table 6). The highest ranked model included BCI which carried 50% of the model weights. The null model received support and carried 28% of the model weights and planning area carried 22% (Table S6). BCI was positively associated with enzymes (Table 8). Enzymes were lower in the WPA compared with the EPA (Table 8).

The six plasma proteins reduced to a single principal component explaining 50% of variation. The first axis was positively and evenly loaded by alpha-2 globulin, beta globulin and gamma globulin (Table 6). The null model received the most support and carried 56% of the model weights (Table S6). Although the model containing planning area also

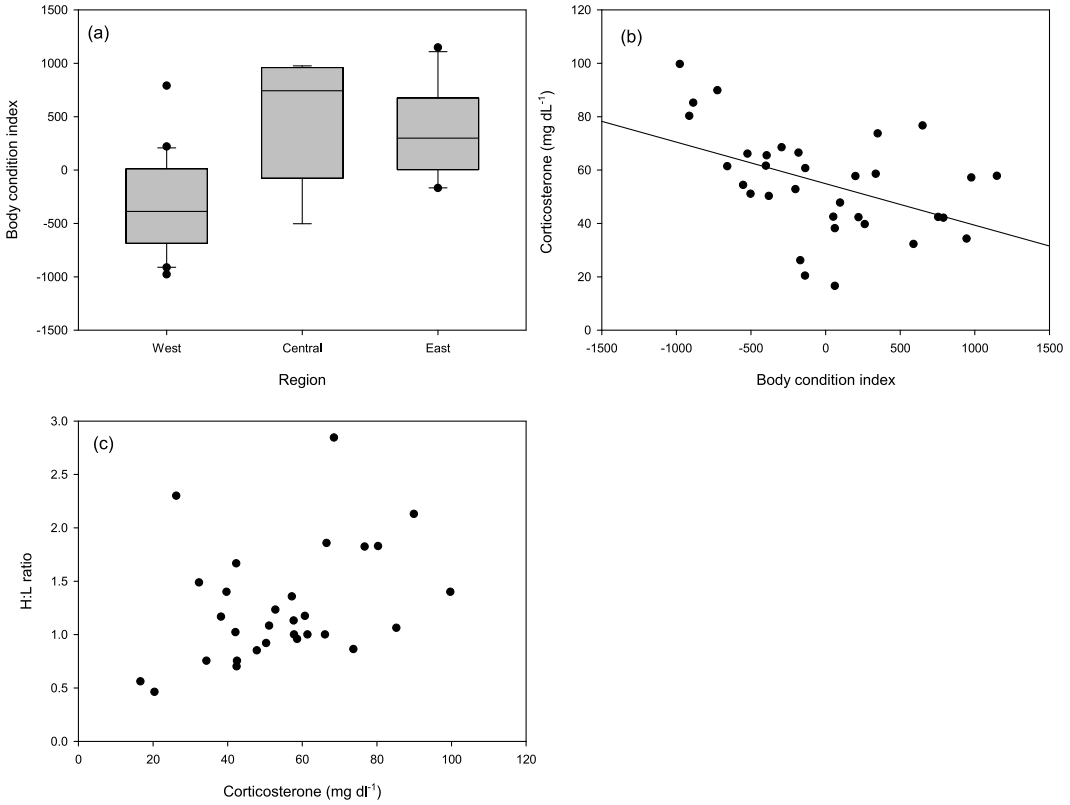

Figure 3: Condition of brown pelican chicks in the northern Gulf of Mexico as shown through BCI in relation to (a) the three BOEM planning areas in the northern Gulf of Mexico (oil and gas activity are highest in the Central region, intermediate in the West and least in the East; see Fig. 1 for boundaries of planning areas) and (b) levels of corticosterone in blood samples and (c) the correlation between corticosterone and the ratio of heterophils to lymphocytes.

Table 8: Summary of coefficient estimates from model selection process assessing relationships between blood analytes of brown pelican chicks in the northern Gulf of Mexico and two independent variables, 2013–2015

Functional group	Loading variables	BCI	Planning area
Metabolites axis 1	Cholesterol (−), Total Protein (−)		
Metabolites axis 2	Uric Acid (−), Triglycerides (−)	**−0.51 (−0.87, −0.15)**	
Electrolytes axis 1	Calcium (−), Sodium (−)		C = 0.69 (−0.56, 1.95) **W = 1.32 (0.47, 2.17)**
Enzymes axis 1	GGT (+), Creatine phosphokinase (+)	**0.39 (0.04, 0.75)**	C = 0.07 (−1.09, 1.24) **W = −0.91 (−1.75, −0.08)**
Plasma proteins axis 1	Beta globulin (+), Gamma globulin (+), Alpha-2 globulin (+)		C = −0.74 (−2.45, 0.98) W = 0.78 (−0.36, 1.92)
Corticosterone		**−11.39 (−17.53, −5.26)**	

BCI is a continuous variable and is defined in the methods. Planning area is a categorical variable (East = reference level); see Fig. 1. Only coefficients where 90% CIs do not overlap 0 (i.e. an ecological relationship between the analyte and the independent variable is more likely) are reported. No interactions of the independent variables returned coefficients likely to be ecologically relevant (i.e. SE > mean in all cases). For planning area, the reference level was the EPA so the coefficient shows the difference in the central (C) and western (W) planning areas. Coefficient estimates for corticosterone are presented on their raw scales. For functional groups where the primary PCA loading was negative (see Table 6), the relationship between the dependent and independent variables is the inverse of the sign for that coefficient. Bolded values are CIs that do not overlap zero.

received moderate support and carried 24% of the model weights (Table S6), the 90% CIs on the coefficient estimates for the CPA and WPA both overlapped zero, indicating there was no ecological relationship (Table 8).

The top-ranked model for corticosterone included BCI, which carried 94% of the model weights (Table S6). There was a negative relationship between BCI and corticosterone (Table 8, Fig. 3b).

Across all four functional groups (described by five principal components) plus corticosterone, the null model was supported in four cases and both planning area and BCI in three cases each (Table S6).

Because WBC data did not fit a normal distribution (either as raw data or when transformed), we performed nonparametric analyses (Kruskal–Wallis or Kendall correlation) to assess the relationships between WBC data and planning area and BCI. No significant relationships occurred for any WBC counts with either planning area (Kruskal–Wallis Chi-square ≤ 3.1, $P \geq 0.2$ for each) or BCI (|Kendall's tau r| < 0.19 for each). There was a significant positive, yet highly variable, relationship between H:L and corticosterone (Kendall $T = 0.36$, $P = 0.01$; Fig. 3c). Hematocrit also did not vary with planning area ($F_{2,31} = 1.1$, $P = 0.3$) or BCI ($F_{1,32} = 0.06$, $P = 0.8$).

Discussion

Two previously published data sets present measures of blood analytes in adult brown pelicans. These studies included captive individuals (Wolf *et al.,* 1985; Zaias *et al.,* 2000) and free-ranging individuals during the nonbreeding period (Zaias *et al.,* 2000). Our measures of two enzymes (ALT and AST) and three plasma proteins (albumin, A1G and A2G) appeared lower by ∼50% compared with those reported in Wolf *et al.* (1985) and Zaias *et al.* (2000) and mean values of these analytes from the aforementioned studies did not occur within our baseline values. Lower levels of ALT, AST and albumin in our sample may be indicative of nutritional or physiological stress due to breeding activities relative to captive birds or birds during the nonbreeding season (Dean *et al.,* 2017; Fiorello, 2019), or perhaps to sample degradation that may occur under field conditions or during sample storage prior to analysis. Higher values of A1G and A2G in the aforementioned studies compared with our data may be indicative of infection or parasitism, particularly in captive birds (Ferguson *et al.,* 2014).

The most relevant data set from pelican chicks that provides a comparison with ours appears in Ferguson *et al.* (2014); chicks were sampled at a similar age to our study from colonies along the Atlantic coast in South Carolina, USA. The upper end of the baseline value for triglycerides in the Gulf was above the reference interval for the South Carolina sample. Lipids are sensitive to nutritional state and may reflect time since feeding or food type (among other factors; Alonso-Alvarez *et al.,* 2007; Fiorello, 2019), neither of which were measured for either data set. Although baseline values for CPK and LDH appear lower in the Gulf compared with the reference intervals from South Carolina, high outliers from the Gulf data were within the upper end of the reference interval for the South Carolina data. Our data also suggest that the level of one enzyme (amylase) from the Gulf samples was higher than the reference interval of the sample from South Carolina. A lack of detailed studies on the role of amylase in birds (Fiorello, 2019) makes an interpretation of this comparison challenging, but the disparate data from these two samples suggests that measuring this analyte in future studies to establish a more robust reference interval for the species may be beneficial.

Baseline values from our study for PCV, total WBC count and differential were all within the reference intervals of previously published data for the species for adults and chicks (Ferguson *et al.,* 2014; Fiorello, 2019). In both adults and chicks, heterophils were the most common type of WBC, although in chicks the difference between heterophil and lymphocyte counts was less substantial. Fiorello (2019) notes that pelicans may be heterophilic and our data appear to support that observation.

H:L ratios can reflect disease, stress (including nutritional stress) or parasitism (Fiorello, 2019). Our estimate of the H:L ratio in adults (4.3) appears higher than that reported by Zaias *et al.* (2000) for a combined sample comprised of captive and free-ranging adult and juvenile pelicans of both sexes from southern Florida, USA (H:L range, 1.0–1.5). This comparison suggests that at some level adult pelicans from the Gulf may have been experiencing an enhanced level of physiological stress compared with those in the Florida sample, perhaps due in part to regional factors or behavior (e.g. Fig. 2). Our data suggest that reproductively active adult brown pelicans can present with a range of H:L ratios that are within the range for other Pelecaniformes (Work, 1996; Fiorello, 2019). The H:L ratio we measured in chicks appears similar to that estimated from Ferguson *et al.* (2014) in pelican chicks from South Carolina and Georgia, USA (∼1.2).

Blood profiles of adult pelicans

Blood analytes of adults during our study were frequently related to the three BOEM planning areas. We observed lower values of ordinated metabolites (along axis one), electrolytes, enzymes, plasma proteins and corticosterone in the WPA and CPA compared with the EPA. We posit that higher levels of corticosterone in the EPA may be due to increased disturbance at those colonies due to their proximity to human recreation and other activities (i.e. industrial activities, fishing). Globulins (the primary variables weighting this axis) can decrease with chronic stress or with mobilization of protein stores for energy (Awerman and Romero, 2010). Electrolytes and metabolites (along axis one) also can decline with nutritional stress or dehydration (Alonso-Alvarez *et al.,* 2007;

Maceda-Veiga *et al.*, 2015; Fiorello, 2019). Although BCI did not differ among regions, home range size was larger in the WPA and CPA compared with the EPA (also see below). If a larger size of home range is indicative of lower food quality (e.g. Ashmole's halo; see Lamb *et al.*, 2017a), then signs of nutritional stress such as lower electrolytes may occur.

Higher levels of some enzymes are often noted as being indicative of muscle and hepatic damage associated with oil exposure (Alonso-Alvarez *et al.*, 2007; Fiorello, 2019). The distribution of oil and gas activity in the region is, however, inconsistent with the pattern we observed (i.e. higher enzyme levels in the EPA). In a related study, however, the highest concentrations of parent and alkaline PAHs in blood from adult pelicans were detected in individuals nesting in the EPA adjacent to an area with substantial industrial development (same individuals as this study; Lamb *et al.*, 2020b). These results suggest that potential contaminant sources other than those related to regional levels of oil and gas activity may lead to higher enzyme levels, and that the factors that influence physiology of a species throughout a region may be complex and operate across multiple spatial scales. Harr (2002) notes that while these enzymes are sensitive to stressors such as oiling, they are not specific, and caution should therefore be applied when interpreting patterns.

The pattern we observed of a higher H:L ratio in the EPA compared with the WPA and CPA does not appear to be consistent with many of the other patterns we observed among planning areas that suggest some level of nutritional or physiologic stress in the WPA and CPA compared with the EPA. The exception, however, may be the higher levels of the ordinated enzymes in the EPA compared with the WPA and CPA. If elevated enzyme levels are in part attributable to high levels of PAHs (Lamb *et al.*, 2020b), then higher H:L ratios may also be expected. Caution should be applied when comparing WBC data among planning areas for adults as most values were within ranges reported by other studies for this species (H:L ratios being the exception).

Although home range size appeared in two of seven models of analytes with reasonable support, the only ecological relationship we observed was a positive relationship with the H:L ratio and a negative relationship with the metabolites that weighted axis two. Typically, smaller home ranges are associated with higher levels of resource availability compared with larger home ranges. Therefore, larger home ranges associated with higher H:L ratios would be consistent with lower resource availability which may lead to nutritional stress. Metabolites (e.g. uric acid and triglycerides that weighted this axis) often rise post-prandially. Individuals with smaller home ranges (i.e. had traveled shorter distances between foraging areas and return to nests where we sampled them) may have eaten more recently compared with birds with larger home ranges and hence presented with higher levels of metabolites.

Individuals experiencing muscle damage or other physiological stress (e.g. increased energetic effort) may also present with higher levels of metabolites such as uric acid (Maceda-Veiga *et al.*, 2015). This pattern would appear to be inconsistent with the negative relationship we observed between metabolites and home range size. Individuals experiencing muscle damage or other physiologic stress may, however, have smaller foraging ranges due to their reduced condition. Therefore, it may be that home range does not drive the pattern in metabolites but rather that the blood analytes drive the reduced size of the home range.

Data comparing home range size to blood profiles in birds is lacking. In our samples, home ranges are measured over a period of days to months following the collection of the blood sample. In contrast, the blood samples reference the time period before blood sampling over a period of days to weeks. Therefore, attempts to relate home range size to blood analyte levels assume that home range size is consistent over time and therefore the two can be linked despite the difference in the temporal frame of reference. While home range size changes with breeding stage, relative differences in home range sizes among individuals appeared relatively stable in our study (Lamb *et al.*, 2020b). However, because of the confounding nature of our data with respect to home range size, the lack of studies explicitly designed to examine the relationship between home range size and blood analytes and the potential temporal mismatch between blood samples and home range size, a study designed to specifically assess this relationship appears warranted.

Sex appeared in only one model with reasonable support and the only ecological relationship we observed was with the metabolites that weighted axis 2 (primarily uric acid and triglycerides). The patterns we observed may be consistent with requirements of egg-laying and subsequent changes to body condition. For example, Zaias *et al.* (2000) also found higher levels of triglycerides in females compared with males and attributed this difference to physiological changes related to egg-laying, as did Work (1996) who reported similar results in Great Frigatebirds (*Fregata minor*). In our study, females also had lower BCI compared with males suggesting these differences may be indicative of depressed condition in females compared with males at the time of sampling due perhaps to costs of egg development.

Although BCI often appeared in models with reasonable support, we did not detect any ecological relationships with metabolites, electrolytes, enzymes, plasma proteins or corticosterone in adults, suggesting that, overall, the individuals we sampled were relatively healthy and therefore provide useful reference values. Because body mass can fluctuate substantially within an individual over short periods of time (e.g. due to recent feeding), caution should be applied when interpreting BCIs. Within our data set BCI did not differ by planning area, suggesting that the condition of the adults we sampled, although variable, was not related to any region-wide conditions in the northern Gulf. Males did have higher BCI compared with females. A sex-based difference in BCI is not uncommon during early breeding due to the physiological

stress experienced by females from egg-laying (Kalmbach *et al.*, 2004). The negative relationship between monocyte counts in adults and BCI is consistent with an increase in monocyte activity with infection, inflammation or physiologic stress (Harr, 2002; Dean *et al.*, 2017), all of which may contribute to lower BCI.

Blood profiles of pelican chicks

We detected a positive relationship between BCI and the metabolite two axis, which is consistent with an increase in metabolites with nutritional state or hydration (Alonso-Alvarez *et al.*, 2007; Fiorello, 2019). We also observed a positive relationship between BCI and enzymes. The mechanism underlying this relationship is unclear but may be related to larger individuals producing higher levels of enzymes (e.g. CPK) post-capture as a stress response. Corticosterone increased with lower BCI, which is indicative of stress, and the H:L ratio also increased with lower BCI. Lamb *et al.* (2016) also observed a negative relationship between corticosterone in feathers of pelican chicks and BCI, and also documented a negative relationship between corticosterone in feathers of pelican chicks and fledging success. Planning region was related to electrolytes and enzymes, which were higher in the EPA. Given that electrolytes tend to decline with nutritional stress or dehydration (Maceda-Veiga *et al.*, 2015), lower levels of electrolytes in the WPA would be consistent with the lower BCI of chicks in that region.

Conclusion

Eastern Brown Pelicans are a species of conservation concern and a monitoring priority in the northern Gulf of Mexico (Jodice *et al.*, 2019) and the Atlantic portion of their range (Jodice *et al.*, 2013; Shields, 2020). Baseline values or reference intervals from chicks are now available from the northern Gulf (this study) and the South Atlantic Bight (Ferguson *et al.*, 2014), leaving data gaps for the subspecies in the southern and northern extents of their breeding range. Our data represent the most spatially extensive sample for breeding adult brown pelicans, and data gaps remain for free-ranging adults throughout the Atlantic and southern portion of their breeding range. The regional patterns we observed in blood analytes in adult pelicans suggest that environmental factors are an important driver of inter-individual differences in blood chemistry and health. Nonetheless, our results in total suggest that it may be difficult to use planning area and the underlying level of oil and gas activity across planning areas, as a single metric by which health of pelicans can be interpreted. For example, blood analytes may vary on time and space scales that are not relevant to the broader planning areas, particularly since adults may be sensitive to highly localized factors that vary within planning areas or may migrate to entirely different regions during non-breeding (Lamb *et al.*, 2017a; Lamb *et al.*, 2020a, 2020b). Planning area can also be confounded with other factors including, but not limited to, home range size and diet (Lamb *et al.*, 2017a,b). Therefore, it would be prudent to use caution when seeking to interpret relationships in analytes among planning areas particularly if no other explanatory variables were available. Health parameters in chicks appear to be more sensitive to individual condition than to environmental factors, suggesting that localized nutritional conditions may drive nestling health regardless of broader regional differences.

Biomarkers such as hematology and blood chemistry can be used to establish baselines of overall health for a population or to investigate specific aspects of a stressor on a population. Baseline values or reference intervals for hematology and plasma chemistry also can provide a tool for long-term monitoring of individual health (Ottinger *et al.*, 2019) as well as for monitoring changing relationships between blood analytes and environmental or ecological variables of interest (e.g. planning area, sex, BCI). Continued monitoring of brown pelicans is likely in the northern Gulf (Jodice *et al.*, 2019) and therefore opportunities to develop longitudinal reference levels may exist for this species. Exposure and risk may, however, differ among the suite of nearshore seabirds that breed in the northern Gulf (e.g. pelicans, terns, gulls) due to differences in diet, foraging strategies and life history strategies (Jodice *et al.*, 2019). Therefore, similar assessments and monitoring may be considered to develop baseline values or reference intervals across this suite of species in an ecosystem prone to natural and anthropogenic stressors.

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Author contributions

P.G.R.J.: conceptualization, methodology, formal analysis, resources, writing (original and reviews), visualization, supervision, project administration and funding acquisition. J.S.L.: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing (original and reviews), visualization, supervision and project administration. Y.G.S.: validation, investigation, data curation, writing (reviews) and visualization. CEF: writing (original and reviews).

Data availability

Data from this research are available as a USGS data release at https://doi.org/10.5066/P94GBJ4G (Lamb *et al.*, 2019).

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Supplementary material

Supplementary material is available at *Conservation Physiology* online.

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