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Assessing Passeriformes health in South Texas via select venous analytes

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ABSTRACT

The handheld point of care analyzer is a quick and feasible option to obtain hematology data from individuals. The iSTAT-1® was used to evaluate select venous blood analytes obtained via jugular venipuncture from 238 passerine birds from South Texas. These data were used to assess the health of birds in the area while taking into consideration life history (migratory or sedentary), locale, seasonality, sex, and age. We attributed increased values of pO_2 and hematocrit, in addition to hemoglobin and glucose concentrations of migratory birds compared to sedentary birds as the increased need of oxygen carrying capacity and energy for long duration flights. Increased glucose and lower ionized calcium concentrations were observed in migratory birds likely based on breakdown of fat deposits in the body to fuel the muscular endurance of migration. During the hotter months of the year, birds' responses to handling were exhibited by relative respiratory acidosis. When sedentary birds sampled from South Texas were compared to a previous study from Central Texas, venous blood analytes differed by locale but were within the ranges of healthy populations. These findings lead us to conclude that sedentary avian communities can be used as ecosystem bioindicators.

1. Introduction

Effects of habitat alteration or destruction are frequently investigated, but many studies only approach the issue from a habitat management perspective. To fully understand the effects that environmental changes have, especially on organisms such as birds, we must address their physiological response to the change (Albano, 2012). Environmental change can cause stress to the inhabitants of the area and has been linked to nutritional deficiencies, hormonal imbalance, inflammation, and chronic infection (Briggs et al., 1996). Focusing on the ecosystem level, the classic idiom of the canary in the coalmine holds true. Many passerine birds preferentially seek out human habitations or areas of heavy urbanization, such as Great-tailed Grackles (*Quiscalus mexicanus*), European Starlings (*Sturnus vulgaris*), and House Sparrows (*Passer domesticus*). Birds are more sensitive to environmental changes (Morrison, 1986) and can show a physiological response before humans; this is the basis of a sentinel population. Birds ingest insects and plants affected by human activity such as pollutants and habitat degradation (Goulson, 2014; Liang et al., 2016). Several studies assessed the effects of an altered (polluted or degraded) ecosystem via the hematological response of bird inhabitants (Llacuna et al., 1996; Ruiz et al., 2002; Elezaj et al., 2013). These studies found differences of certain hematological analytes that impact bird health associated with the conditions affecting health of the ecosystem. Passeriformes represent a good study organism for this type of evaluation of ecosystems because there is a cornucopia of them with similar physiology through family and genera that is relatively easy to capture and handle with sample techniques established.

Hematological assessment is a popular method to assess health with minimal negative impact on the individual bird (Fokidis et al., 2008; Sheldon et al., 2008; Deem et al., 2011; Maceda-Veiga et al., 2015). Point of care analyzers allow for assessment of respiratory and cardiovascular systems via measurement of avian acid-base status, biochemical fluid balance, electrolytes, and blood gases (Heatley et al., 2013). The iSTAT-1® analyzer requires small amounts of blood and provides results within a short time for up to 10 blood analytes per sample. The iSTAT-1® has been used to determine multiple analytes for avian species such as chickens, passerine birds, and parrots (Steinmetz et al., 2007; Paula et al., 2008; Martin et al., 2010; Harms and Harms, 2012). This study aims to assess the health of free living passerine birds in southern Texas via select venous blood analytes, and by including life history traits (migration), locale, seasonality, and other intrinsic variables as covariates. We also compare our results from southern Texas to similar data collected from birds occupying a distinctly different habitat

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Table 1
Summary of 238 passerine birds sampled for hematology on East Foundation ranches in South Texas.

Family or location	Total	Males	Females	Adult	First year
Cardinalidae	72	37	34	38	12
Emberizidae	20	8	5	11	2
Fringillidae	3	2	1	1	2
Icteridae	36	10	18	17	5
Mimidae	25	3	4	7	6
Paridae	5	2	2	4	1
Parulidae	51	14	22	28	9
Troglodytidae	4	2	1	3	0
Turdidae	2	0	2	2	0
Tyrannidae	13	3	4	8	1
Vireonidae	7	1	2	3	1
El Sauz Ranch	194	71	72	99	24
San Antonio Viejo Ranch	44	11	23	23	12
Total	238	82	95	122	36

in Central Texas.

Several recent studies have investigated bird hematology with respect to migration and were able to conclude that the strenuous activity of migration contributed to the fluctuating parameters of the blood (D'Amico et al., 2010; Vinkler et al., 2010; Minias et al., 2014). We hypothesize that sedentary and migratory bird blood analytes will differ based on the physiological needs of migration. We further hypothesize that differences amongst analytes of birds from our South Texas sampling sites and between South and Central Texas will be minimal and generally reflective of good health. Finding significant differences of these hematological analytes could be important to the larger goal of understanding free-living passerine bird health and their interaction with environmental conditions. Should venous blood analytes of sedentary avian species be altered by local factors, these species could represent good local bioindicators of their ecosystem's health.

2. Materials and methods

2.1. Field sampling

Passerine birds (Tables 1, 2) were sampled on the East Foundation lands of San Antonio Viejo Ranch (located inland, in Jim Hogg and Starr Counties) and, El Sauz Ranch (located coastally in Kenedy and Willacy Counties) (Fig. 1) from March 2014 to November 2015. During field sampling the average temperature during spring, summer, and fall were recorded as 22.7 °C, 30.6 °C, and 24.4 °C (https://beaumont.tamu.edu/ClimaticData) and average precipitation for these sample seasons were 3.5 in., 1.5 in., 3.3 in. (http://www.usclimatedata.com). All birds were captured via mist net and placed in cloth bags for about 5 min, allowing them to calm before sampling occurred (Heatley et al., 2013). All birds were handled according to protocol from Texas A & M University Institutional Animal Handling and Use Committee. Birds were restrained by hand for collection of 0.2–0.5 ml of blood via jugular venipuncture with needle and syringe. Blood samples obtained from birds that were subsequently released back to the environment was always < 1.0 ml/100 g of body weight. Blood samples were transferred to lithium heparin microtubes (Terumo America Inc., Elkton, MD, USA) to prevent clotting.

2.2. Sample analysis

Blood sample analysis occurred within 5 min of sample collection using a handheld point of care analyzer, the iSTAT-1® system (Abbott Laboratories, Abbott Park, IL, USA). Venous blood, 0.15–0.2 ml was placed in the cartridge and the results were provided within 2 min of analysis. Blood sample analysis was performed with the blood gas cartridge (CG4 + or CG8 +) first, followed by the Chem 8 cartridge. Each cartridge has fresh sensors and calibration fluid and the iSTAT-1® performs a quality check/self-calibration before each test (Groves, 2002). Venous blood values (iSTAT-1® system manual, 2012) were determined for the following analytes: pH, pCO_2 (carbon dioxide partial pressure), pO_2 (oxygen partial pressure), lactate, bicarbonate, total CO_2, base excess, sO_2 (dissolved oxygen), ionized calcium, glucose, blood urea nitrogen (BUN), hematocrit, hemoglobin, sodium, potassium, and chloride. These parameters were chosen for assessment based on availability in this analyzer's platform and usefulness for clinical assessment of avian health. Previous work suggested that some analytes are more useful for assessment of passerine birds as indicators of local environmental health (Heatley et al., 2013). The iSTAT-1® system measures most values directly, but total CO_2, hemoglobin, base excess, and sO_2 are calculated based on assumptions of blood and plasma characteristics of clinically normal humans. Temperature correction of the measured analytes was not applied in this study. Blood samples were loaded into ammonium heparin microhematocrit (Drummond Scientific Co., Broomall, PA, USA) capillary tubes and centrifuged (Clay-Adams, Inc., New York, USA) at 15,000*g* for 5 min within 24 h of collection to assess packed cell volume. A physical examination and assignment of body condition score (BCS) was performed on each bird after blood collection (Tully, 2009). The body condition was scored on a scale of 1–5 by assessing the mass of the pectoral muscle and fat located on the chest, with BCS 1 being the lowest condition score and BCS 5 the highest. Scoring was performed by using the thumb and fore finger to palpate muscle along the keel, examining the contour of the breast muscle. In this technique, mid-range body condition scores (3) are representative of optimum health. After sampling, some birds were humanely sacrificed via thoracic compression (Leary et al., 2013; Paul-Murphy et al., 2016) and prepared as voucher specimens for the Biodiversity Teaching and Research Collection at Texas A & M University, while others were placed in the cloth bag for 10 min and released. Intrinsic variables such as species, sex, and age were recorded in the field (Dunn and Alderfer, 2011), if possible, by external field markings as well as age and sex confirmed during specimen preparation (Nero, 1951). Migratory or sedentary status was assigned using life history information (Dunn and Alderfer, 2011).

2.3. Statistical methods

Analysis of the data was performed using Analyse-it for Microsoft Excel® statistical software (version 2.20 Microsoft Office 2010, Analyse-it® Software Ltd., http://www.analyse-it.com/, 2009). Normality for each analyte was assessed by histogram and Shapiro-Wilk test ($P > 0.05$); for all other statistical analyses significance was accepted at $P < 0.05$. The effects of migratory status, age, sex, and locality were evaluated using Student's *t*-test ($P < 0.05$) for parametric data and by Kruskal-Wallis test ($P < 0.05$) nonparametric data (Kruskal and Wallis, 1952). Season (fall, spring, or summer) and BCS were assessed using a one-way analysis of variance ($P < 0.05$) and Tukey-Kramer method was used for the post hoc analysis to verify results of these two variables. The effect of species was also measured using a one-way analysis of variance for five sedentary species from South and Central Texas that had a minimum of 10 individuals sampled, for a total of 108 birds (Table 3). To assess whether habitat drives variation in analytes, the data from select sedentary birds sampled in South Texas (East Foundation properties) were analyzed in conjunction with data from a previous study in which birds were sampled from the ecologically and elevationally different Edward's Plateau in Central Texas (Fig. 1: Heatley et al., 2013).

The use of the iSTAT-1® in determination for hematocrit seems to require a correction factor for use in passerine birds. The output from the point of care analyzer uses a formula derived from humans using normal mean hemoglobin concentrations of 34 mg/dl (Heatley et al., 2013).

Table 2
Migratory and sedentary birds from East Foundation ranches sampled for blood gas and electrolytes.

Migratory *N* = 128	Sedentary *N* = 110
Cardinalidae	Cardinalidae
Blue Grosbeak (*Passerina caerulea*)	Northern Cardinal (*Cardinalis cardinalis*)
Dickcissel (*Spiza americana*)	Pyrrhuloxia (*Cardinalis sinuatus*)
Indigo Bunting (*Passerina cyanea*)	
Painted Bunting (*Passerina ciris*)	Emberizidae
Scarlet Tanager (*Piranga olivacea*)	Lark Sparrow (*Chondestes grammacus*)
Summer Tanager (*Piranga rubra*)	Olive Sparrow (*Arremenops rufivirgatus*)
Emberizidae	Fringillidae
Clay-colored Sparrow (*Spizella pallida*)	Lesser Goldfinch (*Spinus psaltria*)
Lincoln's Sparrow (*Melospiza lincolnii*)	
	Icteridae
Icteridae	Audubon's Oriole (*Icterus graduacauda*)
Baltimore Oriole (*Icterus galbula*)	Bronzed Cowbird (*Molothrus aeneus*)
Hooded Oriole (*Icterus cucullatus*)	Brown-headed Cowbird (*Molothrus ater*)
	Red-winged Blackbird (*Agelaius phoeniceus*)
Mimidae	
Gray Catbird (*Dumaetella carolinensis*)	Mimidae
	Curve-billed Thrasher (*Toxostoma curvirostre*)
Parulidae	Long-billed Thrasher (*Toxostoma longirostre*)
Bay-breasted Warbler (*Setophaga castanea*)	Northern Mockingbird (*Mimus polyglottos*)
Black and White Warbler (*Mniotilta varia*)	
Black-throated Green Warbler (*Setophaga virens*)	Paridae
Canada Warbler (*Cardellina canadensis*)	Black-crested Titmouse (*Baeolophus atricristatus*)
Golden-winged Warbler (*Vermivora chrysoptera*)	
Louisiana Waterthrush (*Parkesia motacilla*)	Parulidae
Magnolia Warbler (*Setophaga magnolia*)	Common Yellowthroat (*Geothlypis trichas*)
Nashville Warbler (*Leiothlypis ruficapilla*)	
Northern Waterthrush (*Parkesia noveboracensis*)	Troglodytidae
Orange-crowned Warbler (*Leiothlypis celata*)	Bewick's Wren (*Thryomanes bewickii*)
Tennessee Warbler (*Leiothlypis peregrina*)	
Worm-eating Warbler (*Helmitheros vermivorum*)	Tyrannidae
Yellow-breasted Chat (*Ictera virens*)	Couch's Kingbird (*Tyrannus couchii*)
Yellow Warbler (*Seophaga petechia*)	Great Kiskadee (*Pitangus sulphuratus*)
Troglodytidae	Vireonidae
House Wren (*Troglodytes aedon*)	White-eyed Vireo (*Vireo griseus*)
Turdidae	
Gray-cheeked Thrush (*Catharus minimus*)	
Swainson's Thrush (*Catharus ustulatus*)	
Tyrannidae	
Brown-crested Flycatcher (*Myiarchus tyrannulus*)	
Eastern Phoebe (*Sayornis phoebe*)	
Great-crested Flycatcher (*Myiarchus crinitus*)	
Scissor-tailed Flycatcher (*Tyrannus forficatus*)	
Willow Flycatcher (*Empidonax traillii*)	
Vireonidae	
Blue-headed Vireo (*Vireo solitarius*)	
Warbling Vireo (*Vireo gilvus*)	

$$\text{Hemoglobin (g/dl)} = \text{hematocrit (HCT\%)} \times 0.34$$

For comparison of hemoglobin values measured from the iSTAT-1® a correction formula was used on packed cell volume (Velguth et al., 2010).

$$\text{Hgb} = 0.33 \times \text{PCV} + 0.11$$

A Bland-Altman plot (Bland and Altman, 1986) was constructed to assess the agreement between measurement of packed cell volume by centrifugation and hematocrit by the iSTAT-1®. A separate Bland-Altman was performed to analyse the agreement of hemoglobin measured from the analyzer compared to hemoglobin obtained from corrected packed cell volume.

3. Results

3.1. East Foundation Passeriformes

A total of 238 passerine birds were sampled from East Foundation Lands, comprising 12 families, 52 species, and representing both migratory and sedentary life histories (Tables 1 and 2). Analyte data from birds captured on both ranches from South Texas had parametric distribution for nine analytes and, non-parametric distribution for eight analytes (Table 4). Bird sex was determined for only 177 individuals (82 males, 95 females) based on sexually dimorphic field markings. We were unable to determine the sex of 61 individuals due to lack of sexual dimorphism in the species in combination with release of the remaining individuals post blood collection. Female birds had a trending increase concentration (all values given as mean ± standard deviation) of ionized calcium (0.964 ± 0.017 mg/dl) compared to males

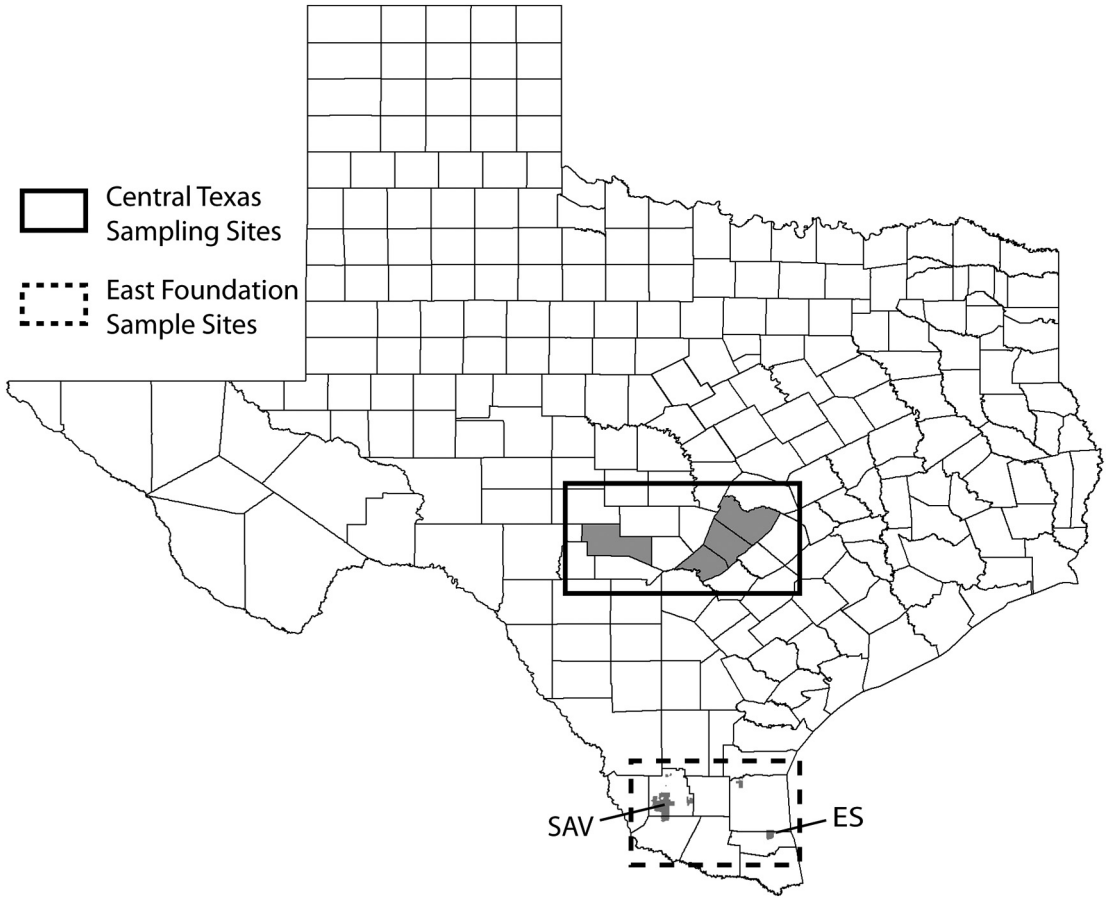

Fig. 1. Sampling localities for birds included in this study. The dashed rectangle outlines the East Foundation properties with San Antonio Viejo (SAV) and El Sauz (ES) ranches noted. The solid rectangle outlines counties in Central Texas, from which birds from a previous study (Heatley et al., 2013) are used for analyte comparisons to South Texas.

(0.918 ± 0.018 mg/dl) ($t_{127} = -1.97$, $P = 0.0515$). Age classifications (adult or first year) were determined for 145 birds based on field markings and assessment of skull ossification during museum preparation. Total carbon dioxide concentrations were significantly decreased in first year birds (22.5 ± 17.5 mmol/l) compared to adults (24.5 ± 20.9 mmol/l) ($t_{139} = -2.13$, $P = 0.0352$).

Migratory birds had increased concentrations of pO_2, hematocrit, and hemoglobin compared to sedentary birds, whereas sodium and ionized calcium concentrations were decreased in migratory birds (Table 5). For all birds, samples collected in the fall had relatively increased values for pH, pO_2, sO_2, and lower concentrations of pCO_2 and lactate than those obtained in spring or summer (Table 6). Additionally, chloride concentrations were higher ($t_{50} = -2.11$, $P = 0.04$) in fall than in spring. Ionized calcium concentrations were increased in summer compared to spring and potassium concentrations were relatively decreased during the summer (Table 6). All birds were issued a body condition score (BCS; range from 1 to 4), with most birds assigned a BCS of 2 or 3. For BCS 3 birds we found an increase in pCO_2 ($t_{198} = 2.84$, $P = 0.0050$) but a trending decrease in pO_2 ($H_1 = 3.73$, $P = 0.0534$), % sO_2 ($H_1 = 5.78$, $P = 0.0162$), chloride ($t_{70} = -2.38$, $P = 0.0202$), and ionized calcium, compared to BCS 2 birds.

The Bland-Altman plot showed fair agreement between the measurement of packed cell volume (PCV) by centrifugation and hematocrit (HCT) measured through the iSTAT-1®. We defined a fair agreement for a Bland-Altman plot as a lack of outliers, most within the limits of agreement, and small clinically acceptable bias between two tests (Rettenmund et al., 2014). Packed cell volume differed by mean average of 7% lower than the iSTAT-1® HCT measurement (Fig. 2). Hemoglobin was compared from the iSTAT-1® and hemoglobin obtained from correction formula of packed cell volume. The Bland-Altman plot shows that the iSTAT-1® is clinically acceptable with a mean average of 2.23 g/dl lower than the corrected PCV (Fig. 3).

3.2. Sedentary Passeriformes

When migrants were excluded from analysis, few hematologic analytes differed in samples obtained from coastal versus inland properties in southern Texas. Blood samples obtained from birds on El Sauz Ranch (coastal) had lower decreased concentrations for TCO_2 ($t_{200} = 3.06$, $P = 0.0025$) and ionized calcium ($t_{167} = 4.10$, $P < 0.0001$), while blood samples of birds captured on the San Antonio Viejo Ranch (inland) had relatively increased glucose concentrations ($t_{198} = 2.13$, $P = 0.0346$).

3.3. South Texas versus Central Texas comparisons

Sex was determined for 74 individuals: 39 males and 35 females. Within the sedentary species' blood analytes collectively, sex effect was not found for any of the tested analytes or hematology values. Seasonality was evaluated for spring and summer, with fall being excluded based on low sample size ($n = 3$). Electrolytes, blood gases, lactate and hematology analytes were not affected by season. A decrease of pH ($H_1 = 4.40$, $P = 0.0359$) and base excess ($H_1 = 5.81$, $P = 0.0159$) values occurred in summer compared to spring. Species affected base excess, sO_2, glucose, sodium, and ionized calcium. Notably, many analytes differed based on locality. As compared to Central Texas birds, samples collected in South Texas showed decreased pH values and decreased concentrations of HCO_3, pCO_2, TCO_2, glucose, ionized calcium, hematocrit, and hemoglobin. South Texas birds also showed an increased pO_2 and sO_2 (Table 7).

Table 3
Total samples from the five sedentary species selected from Central Texas and South Texas.

Species	Central Texas	South Texas	Total
Bewick's Wren	7	3	10
Black-crested Titmouse	14	5	19
Northern Cardinal	7	39	46
Northern Mockingbird	1	20	21
White-eyed Vireo	5	8	13

Table 4
Blood gas and electrolyte intervals from Passeriformes sampled in South Texas using Shapiro-Wilk test of normality ($P > 0.05$).

Analyte	Units	Birds sampled	Mean	95% CI	*P* value
pH*	pH	222	7.659	7.644–7.673	0.0187
pCO_2	mm Hg	222	20.7	20.0–21.5	0.5626
pO_2*	mm Hg	225	54.1	51.2–57.0	< 0.0001
Base excess	mmol/l	226	2.6	1.9–3.2	0.1475
Bicarbonate	mmol/l	225	23.1	22.5–23.7	0.7881
TCO_2	mmol/l	202	23.7	23.1–24.3	0.3259
sO_2*	%	226	90.4	89.5–91.3	< 0.0001
Lactate*	mmol/l	83	4.47	4.12–4.82	< 0.0001
Glucose	mg/dl	200	330.2	319.6–340.8	0.0728
BUN*	mg/dl	9	3.9	2.8–4.9	0.0032
Sodium	mmol/l	203	155.7	155.1–156.3	0.0651
Potassium*	mmol/l	202	4.2	4.0–4.3	< 0.0001
Chloride	mmol/l	80	122.6	121.6–123.6	0.3084
iCa	mg/dl	169	0.956	0.937–0.975	0.343
Hct*	%	203	39.9	39.3–40.7	0.0133
Hgb*	g/dl	203	13.6	13.3–13.8	< 0.0001
PCV*	%	231	47.0	46.1–47.8	0.0002

* Denotes non-normal distribution of values.

Table 5
Venous blood analytes of passerine birds that differ based on life history strategy (migratory v sedentary) collected from East Foundation properties.

Analyte	Units	*N*	Migratory	Sedentary	*P* value
pO_2*	mm Hg	225	56.8, 49.0, (52.4–61.1), 123	51.0, 44.0, (47.3–54.6), 102	0.0506
Sodium	mmol/l	203	155.2, 155.0, (154.4–156.0), 109	156.4, 156.0, (155.6–157.2), 94	0.0370
iCa	mg/dl	169	0.922, 0.930, (0.894–0.949), 79	0.986, 1.010, (0.960–1.013), 90	0.0008
Hct*	%	203	40.9, 41.0, (40.0–41.9), 109	38.8, 39.0, (37.8–39.9), 94	0.0011
Hgb*	g/dl	203	13.9, 13.9, (13.5–14.3), 109	13.2, 13.3, (12.8–13.6), 94	0.0011
Glucose	mg/dl	200	342.8, 342.0, (327.3–358.2), 107	315.8, 318.0, (301.8–329.8), 93	0.0119

All values given as mean, median, (95% confidence interval), individuals sampled.
* Denotes non-normal distribution of values.

4. Discussion

4.1. East Foundation Passeriformes

Several other studies have assessed venous blood gases in free living birds using a point of care analyzer (Heatley et al., 2013; Harms et al., 2016). The majority of blood gas analytes measured from Passeriformes in South Texas (Fig. 3) resemble values recorded from previous studies, with few exceptions. Blood pH, PO_2, and PCO_2 concentrations reported from passerine and non-passerine birds are similar, but birds sampled from South Texas have higher pH, PO_2, and lower PCO_2 than the range provided in the literature (Harms and Harms, 2012; Heatley et al., 2013; Montesinos and Ardiaca, 2013; Gardhouse et al., 2016; Harms et al., 2016). Passerine birds have increased HCO_3, chloride and sodium concentrations, while also having decreased lactate concentrations compared to non-passerine species (Paula et al., 2008; Burgdorf-moisuk et al., 2012; Harms and Harms, 2012; Heatley et al., 2013, 2015; Montesinos and Ardiaca, 2013; Harms et al., 2016; Schaal et al., 2016). Many similar studies have used a temperature correction formula to represent their data. The necessity of performing this temperature correction is controversial (Cowley et al., 2013). Within the clinicopathologic and medical field, some authors believe that temperature correction provides a more realistic evaluation of the human body's physiological processes and the seriousness of acid base derangements (Ashwood et al., 1983). However, the theory has not been widely evaluated in non-domestic species (Lewbart et al., 2014). Furthermore, we did not take the temperature of each bird sampled for use in the correction formula. Thus, any “correction” created would simply be a transformation of data based on literature values for Passeriformes (Prinzinger et al., 1991).

The measurement method of hematocrit and hemoglobin by the iSTAT-1® or packed cell volume by centrifugation can be interchangeable because most points fall within the 95% limits of agreement. Although we do not suggest the iSTAT-1® analyzer as the only measurement for hematocrit because it does not agree with the clinical gold standard, PCV (Heatley et al., 2015), we do consider the point of care analyzer as a good measurement of hemoglobin. We attribute the differences that occur to human error when processing and measuring the microhematocrit tubes, as well as the analyzers' measurement designed for humans.

Sex and age had minimal impact on most analytes we assessed in this study. Female birds had increased blood concentrations of ionized calcium compared to males, which is in agreement with previous research (Howard et al., 2004). Ionized calcium plays an important role in many physiological processes, to include eggshell calcification. Therefore, the increased demands for ionized calcium expected during ovulation (de Matos, 2008) could explain the relatively increased concentrations we observed in female birds. First year birds exhibited lower total CO_2 than adults possibly based on a lack of endurance and increased effort to maintain flight in inexperienced juveniles (Heatley et al., 2013).

Table 6
Effect of seasonality on venous blood analytes from passerine birds sampled on East Foundation properties using ANOVA ($P < 0.05$).

Analyte	Units	*N*	Fall	Spring	Summer	*P* value
pH*	pH	222	*7.747, 7.741, (7.708–7.788), 24*	7.648, 7.651, (7.631–7.665), 166	7.647, 7.642 (7.618–7.678), 32	< 0.0001
pCO_2	mm Hg	225	*15.14, 15.60, (13.36–16.91), 26*	21.47, 21.10, (20.61–22.34), 167	21.34, 22.05, (19.60–23.09), 32	< 0.0001
pO_2*	mm Hg	225	*77.6, 75.0, (66.2–89.0), 26*	52.2, 44.0, (49.0–55.3), 167	45.3, 44.0, (42.2–48.4), 32	< 0.0001
sO_2*	%	226	*97.0, 98.0, (95.8–98.3), 27*	89.6, 90.0, (88.6–90.7), 167	88.8, 90.0, (86.8–90.8), 32	< 0.0001
Lactate*	mmol/l	83	*3.89, 3.70, (3.47–4.30), 26*	4.93, 4.65, (4.24–5.63), 25	4.57, 4.26, (3.92–5.23), 32	0.008
Potassium*	mmol/l	202	4.37, 4.15, (4.05–4.68), 28	4.20, 4.10, (4.07–4.33), 146	*3.61, 3.40, (3.34–3.88), 28*	0.0055
iCa	mg/dl	169	–	0.941, (0.920–0.963), 141	1.047, (1.008–1.086), 28	0.0007

All values given as mean, median, (95% confidence interval), seasonal individuals sampled.
Values that are *italicized* represent the statistically significant group selected by Tukey-Kramer method during post-hoc analysis.
* Denotes non-normal distribution of values.

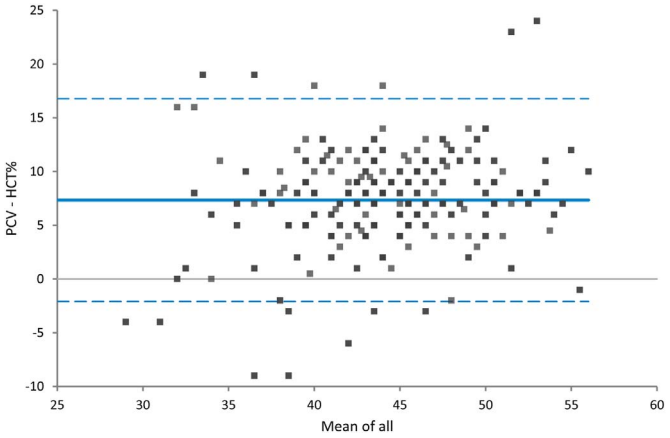

Fig. 2. Bland-Altman plot showing a fair agreement of the two methods of measuring red blood cells, hematocrit from the iSTAT-1 and packed cell volume by centrifugation.

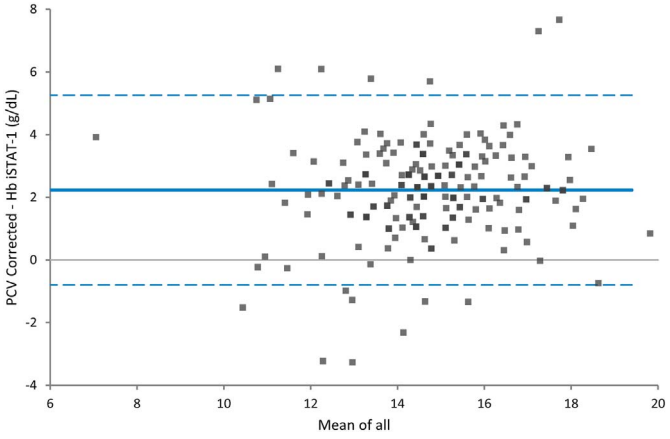

Fig. 3. Bland-Altman plot comparing hemoglobin measured from the iSTAT-1® and the corrected hemoglobin obtained from centrifugation of microhematocrit tubes (PCV).

The increased hematocrit and hemoglobin concentrations found in migratory birds were expected based on reports in other passerine species (Barlein and Totzke, 1992; Morton, 1994; Piersma and Everaarts, 1996). Migration is an energetically taxing activity for birds which increases the need for oxygen, subsequently increasing hematocrit and hemoglobin (Barlein and Totzke, 1992; Hõrak et al., 1998; Swanson, 1990). The need for increased blood oxygen during migration was also associated with increased oxygen saturation and partial pressures of oxygen (pO_2). Several factors can influence pO_2 values such as cardiac function, tissue metabolic rate, hematocrit, or cellular oxygen use (Swanson, 1990; Barlein and Totzke, 1992; Heatley et al., 2013).

Decreased sodium concentrations might be explained by the birds' water consumption. While at stop over sites or reaching the final destination of their migration (i.e. when they were sampled), birds could be consuming large quantities of water which dilutes blood plasma and decreases sodium concentrations (Pierce and McWilliams, 2004). Lower ionized calcium concentrations from migratory species may stem from the role calcium has in the muscular work of respiration and prolonged flight. Expected glucose concentrations for passerine birds via the iSTAT-1® have been recorded to be around 300 mg/dl (Fokidis et al., 2011; Heatley et al., 2013), which is similar to glucose concentrations determined in this study. During migration, birds must rely on fat reserves for energy. Increases of glucagon facilitate metabolism of fat deposits and increases the amount of blood glucose concentrations (Barlein and Totzke, 1992). This appears to be a seasonal change that occurs before migration and can still be observed even after prolonged flight. This suggests migratory birds captured at the South Texas sites have not exhausted all their nutrients and have adequate glucose reserves. This process might partially explain the relatively increased glucose concentrations we observed in migratory birds.

Birds with a BCS of 2 may have experienced greater physical exertion than BCS 3 birds. Decreased BCS score in birds has been associated with smaller pectoralis muscle and fat reserves (Gregory and Robins, 1998). The blood oxygen and carbon dioxide interaction values could be indicating an acute tissue hypoxia that reduces pectoral muscle mass and justifies the original body score.

Both South and Central Texas commonly experience extreme weather condition variation during the year. One of these noticeable changes is seen during the spring and summer months, where there is a high increase in ambient temperatures as compared to fall months. In the spring and summer months, temperatures in South and Central Texas routinely exceed 40 °C and 35 °C respectively. During this transition to higher temperatures, it takes approximately two weeks of heat exposure and conditioning for birds to regulate their bodies' acid-base homeostasis (Marder, 1990). Increased PCO_2, blood lactate concentration, and a more acidic venous blood pH can be associated with higher ambient temperatures and the birds' response to environmental stress (Levine, 1975). Handling stress in our study may have resulted in relative respiratory acidosis of birds sampled during the hotter summer months. Nutrient variability is also likely to vary seasonally. Nutrients from the fruit produced by cacti and other woody plants from South Texas contain the potassium necessary for the dietary needs for avian activity during the harsh environmental conditions in the summer months (Everitt and Alaniz, 1981). Since potassium appears available in the environment and accessible through dietary intake, the relatively lower concentrations of potassium, chloride, and ionized calcium in birds from South Texas ranches might indicate relative euhydration/hyperhydration (Heatley et al., 2013). There are multiple methods to assess hydration status such as measuring total solids, packed cell volume, total protein, and skin turgor, but these objectives were beyond the scope of this study. However, none of the analytes we recorded were clinically abnormal or lower than expected in a healthy bird (Scanes, 2014).

Table 7
Venous blood analyte differences in passerine birds captured in Central Texas and in South Texas.

Analyte	Units	*N*	Central Texas	South Texas	*P* value
pH	pH	94	7.593, 7.580, (7.560–7.626), 28	7.672, 7.670, (7.646–7.698), 66	< 0.0001
pCO_2	mm Hg	94	25.11, 26.05, (23.15–27.08), 28	19.54, 19.00, (18.19–20.89), 66	< 0.0001
pO_2*	mm Hg	93	38.1, 38.0, (35.7–40.4), 28	51.3, 44.0, (46.4–56.2), 65	< 0.0001
Bicarbonate	mmol/l	94	23.95, 24.30, (22.69–25.21), 28	21.79, 22.40, (20.69–22.89), 66	0.024
TCO_2	mmol/l	97	24.7, 25.0, (23.4–26.0), 28	22.7, 23.0, (21.8–23.6), 69	0.0261
sO_2*	%	93	81.3, 82.5, (78.5–84.0), 28	90.2, 90.0, (88.6–91.8), 65	< 0.0001
iCa	mg/dl	65	1.074, 1.120, (0.977–1.172), 9	0.970, 0.970, (0.937–1.003), 56	0.0237
Glucose	mg/dl	86	374.8, 369.0, (350.0–399.6), 28	299.6, 306.5, (282.9–316.2), 58	< 0.0001
Hgb*	g/dl	90	14.2, 13.9, (13.7–14.6), 31	12.8, 12.9, (12.4–13.2), 59	< 0.0001
Hct*	%	90	41.7, 41.0, (40.4–42.9), 31	37.6, 38.0, (36.5–38.8), 59	< 0.0001

All values given as mean, median, (95% confidence interval), individuals sampled.
* Denotes non-normal distribution of values.

Throughout the year, Passeriformes may exhibit differing needs of oxygen demands based on changing environmental conditions. During times of the year when ambient temperature is cooler, increased oxygen need may be based on metabolic processes such as shivering to stay warm. Decreased concentrations of lactate from birds sampled in fall suggest decreased anaerobic metabolic processes (Swanson, 1991). Hematocrit and hemoglobin of blood samples of birds in fall did not differ from those obtained in the spring and summer suggesting a functional change of oxygen affinity at the tissue level or lack of need in sea level non-migratory species in relatively warm ambient temperatures (Swanson, 1990). The differences seen in oxygen saturation and partial pressure oxygen are involved directly as part of the parameters that describe oxygen affinity. A mechanism to satisfy the oxygen needs of hypoxic tissue might involve an accumulation of localized hemoglobin acting as a larger molecule and increasing oxygen saturation. The hemoglobin will aggregate together to form a larger cohesive molecule delivering and transporting oxygen as opposed to many smaller molecules with less carrying capacity. These larger hemoglobin molecules are traveling to tissues with high oxygen demand or if many hemoglobin molecules aggregate at hypoxic tissue areas, they can adequately provide the tissue with oxygen (Lapennas and Reeves, 1983). This process possibly explains why there is increased pO_2 and sO_2 without a statistically significant difference in hematocrit or hemoglobin in the colder months compared to the warmer months. Birds sampled in fall appear to handle changes in oxygen requirements in a manner similar to those previously recorded in other passerine birds (Swanson, 1990).

The differences between East Foundation ranches were minimal and may be explained as birds adapting to their specific ecosystem. With one coastal ranch and the other more inland, the changes in electrolytes observed could indicate differing ecosystem acclimation at the same latitude. Overall, the blood gas data suggests that the values observed within the sampling of the East Foundation properties may be reasonable indicators of passerine bird health in the ecoregion.

4.2. Sedentary Passeriformes

Assessment of blood analytes from sedentary birds from the East Foundation and Central Texas failed to demonstrate expected changes in hematocrit and hemoglobin based on sex (Archawaranon, 2005; Norte et al., 2009; Heatley et al., 2013). The decrease of pH and base excess in sedentary species sampled during summer months may represent metabolic acidosis, most likely based on the birds' response to handling stress during high ambient temperatures. The results that were seen when combining the Central Texas and South Texas sedentary bird species were similar to those previously recorded on versus off the Edward's Plateau. Glucose, ionized calcium, pH, hematocrit, and hemoglobin were values that were not changed and may exhibit normal levels for Central Texas (Heatley et al., 2015).

When select sedentary species were evaluated based on geography (South versus Central Texas), most blood gas and hematology comparisons were not different. The significant differences were likely based on differences in diet or other physiological adaptations (Heatley et al., 2013). Each ecosystem may drive many unique physiological adaptations in resident sedentary bird species. Each locality, though different, harbors healthy bird communities. Our data suggests that rather than relying on a single bird species, there is potential to use a community of birds as bioindicators of ecosystem health. An assessment of a bird community in a less stable (or degraded) habitat is clearly needed to assess this further.

5. Conclusions

This study was designed to assess the health of birds in South Texas using multiple venous blood analytes and compare them to other ecoregions. We also investigated the differences in blood physiology associated with migration. Migratory birds' venous blood analytes differed from sedentary species, particularly those associated with oxygen transport and capacity. We suggest that the venous blood analytes of nonmigratory Passeriformes best suited for use as bioindicators include lactate, electrolyte concentrations and blood gas values. Sedentary species are advantageous as bioindicators as they directly reflect the year-round adaptations needed to survive in their ecosystem. As many analytes we studied appear similar in the sedentary species from Central Texas and South Texas, we suggest that sedentary birds, on a local level, are similar and adapted to the local environmental stresses of their habitat. We find on an ecosystem scale that sedentary birds differ in hematology based on location.

Although the iSTAT-1® was a useful field tool to assess venous blood analytes from birds at the time of sampling, it does have limitations. Overfill, underfill and other reasons for cartridge failure were not uncommon, and has been documented in other studies. We observed that a 32% cartridge failure rate is a result higher than previously recorded (Rettenmund et al., 2014). The most common failures we encountered dealt with poor contact between the analyzer and cartridge, dirty contact pads on cartridges or dirty connector in the analyzer, and temperature (Table 8). The poor contact and dirty components of the cartridge and analyzer are likely associated with sand and other debris in the air, which are common to South Texas habitats. Further, the analyzer is sensitive to ambient temperature and would fail if not maintained within 26–30 °C, although it's operating manual indicates that the machine's operating range is 16–30 °C. This problem allowed for a limited working window for our studies in South Texas where temperatures over 40 °C are common for much of the day. Based on our findings we suggest that future research areas involving passerine birds and ecosystem health would be best focused on sedentary avian communities to assess local ecosystem health and to develop a more comprehensive baseline of hematology analytes across regions.

Table 8
Summary of the 203 error codes produced by the iSTAT-1® and the explanation of each error.

Error code	Number of errors	Explanation
Electronic simulator, #23, #27	99	Poor contact of analyzer pins and the cartridge chip
#2	30	Analyzer temperature outside of operating range
#21, #43	43	Mishandling cartridges, dirty contact pads or dirty connector in analyzer
#36, #38	9	Cartridge overfull or underfill
#66	22	Analyzer error, mechanical or electronic failure

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