DOI: 10.7589/52.2S.S54 *Journal of Wildlife Diseases*, 52(2) Supplement 2016, pp. S54–S64

BLOOD GAS, LACTATE, AND HEMATOLOGY EFFECTS OF VENIPUNCTURE TIMING AND LOCATION AFTER MIST-NET CAPTURE OF MOURNING DOVES (*ZENAIDA MACROURA*), BOAT-TAILED GRACKLES (*QUISCALUS MAJOR*), AND HOUSE SPARROWS (*PASSER DOMESTICUS*)

Craig A. Harms,[1,4] Maggie R. Jinks,[2] and Ronald V. Harms[3]

[1] Center for Marine Sciences and Technology and Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, 303 College Circle, Morehead City, North Carolina 28557, USA
[2] College of Veterinary Medicine, North Carolina State University, 1060 William Moore Drive, Raleigh, North Carolina 27607, USA
[3] 814 South Yaupon Terrace, Morehead City, North Carolina 28557, USA
[4] Corresponding author (email: craig_harms@ncsu.edu)

ABSTRACT: Venous blood gas partial pressures, pH, bicarbonate and lactate concentrations, packed cell volume, white blood cell differential counts, and heterophil/lymphocyte ratios were measured from Mourning Doves (*Zenaida macroura*), Boat-tailed Grackles (*Quiscalus major*), and House Sparrows (*Passer domesticus*). Birds were bled promptly after mist-net capture and banding or following a targeted delay of 45–60 min, in order to assess the impacts of a brief holding period commonly practiced in large-scale bird banding operations. Additionally, effects of venipuncture location (basilic [=ulnar] vein versus jugular vein) were evaluated in male Boat-tailed Grackles sampled promptly after capture and banding. All comparisons were with unpaired samples; no birds were subjected to more than one venipuncture. All three species exhibited moderate improvements in blood gas and acid-base status after the delay, with reductions in lactate concentrations with or without concurrent increases in pH and bicarbonate. Boat-tailed Grackles exhibited an increased proportion of heterophils in the differential white blood cell count following a delay in sampling, suggestive of a stress leukogram. There were no significant differences between basilic and jugular venipuncture results from male Boat-tailed Grackles. Most metabolic, respiratory, and acid-base alterations were minor, but a small number of birds exhibited values (e.g., temperature-corrected pH <7.3, lactate >10 mmol/L) that could be of concern if combined with other adverse conditions. For such birds, a short delay between capture and processing could benefit their blood gas and acid-base status, although loss of time foraging or feeding young and greater activation of the hypophyseal-pituitary-adrenal axis are additional considerations.

Key words: Animal welfare, banding, blood gas, columbiform, lactate, mist net, passerine, stress leukogram.

INTRODUCTION

Mist netting and banding (ringing) are essential tools for study of wild bird demographics, migration, and population biology, and venipuncture of birds handled during mist-netting operations enables studies of avian genetics, health, and physiology. Minimizing adverse impacts of these procedures is important both for ensuring quality results and for responsible animal use in research. A mortality rate greater than 1% is considered unacceptable, and a recent review of large-scale banding operations indicates that this benchmark is readily achievable, reporting an average injury rate of 0.59% and mortality rate of 0.23% (Spotswood et al. 2012). Still, with over 1 million birds banded in the United States every year (Spotswood et al. 2012), a mortality rate of 0.23% translates to over 2,300 bird deaths. Thus, ongoing efforts to minimize adverse effects of capture are important for investigators engaged in mist netting and banding (Spotswood et al. 2012; Mackenzie and Gahbauer 2014).

Procedures in addition to basic capture and handling for banding, such as venipuncture, may increase the risk of morbidity or mortality. Blood collection from wild birds is generally considered a minor procedure, with only transient effects when

performed proficiently and without exceeding guidelines for safe volumes (Gaunt and Oring 1999; Sheldon et al. 2008). However, a report of reduced survival in Cliff Swallows (*Petrochelidon pyrrhonota*) undergoing venipuncture following mist netting, compared with those that were not bled, raised concerns about this common procedure (Brown and Brown 2009). Factors proposed as potentially contributing to reduced survival of wild birds undergoing venipuncture include synergistic effects of environmental conditions, diet, ectoparasitism, foraging strategy, hypovolemia and shock, reduced oxygen carrying capacity, lactic acidemia, body condition, and hematoma formation (Voss et al. 2010). Some of these factors could also occur in the absence of venipuncture. Stress was identified as the most common cause of mortality associated with mist netting and handling in a review of large-scale banding operations (Spotswood et al. 2012). Stress was defined behaviorally as panting, lethargy, closed eyes, raised feathers, or requiring a period of recovery in a closed box prior to release. The presence of underlying blood gas and acid-base disturbances resulting in behavior meeting that definition of stress is a distinct possibility.

A study undertaken to evaluate physiologic impacts resulting from mist netting and banding leading up to venipuncture in one species of Columbiformes (Mourning Dove, *Zenaida macroura*) and two species of Passeriformes (Boat-tailed Grackles, *Quiscalus major*, and House Sparrow, *Passer domesticus*) found some degree of lactic acidemia in all three species, greatest in Mourning Doves and least in House Sparrows, and a mild relative respiratory acidosis in House Sparrows (Harms and Harms 2012). The metabolic, respiratory, and acid-base alterations observed in that study were minor in most cases, indicating general safety of the field procedures, but a few birds exhibited values (e.g., temperature-corrected venous blood pH <7.3, lactate >10 mmol/L) that could potentially be cause for concern if accompanied by other adverse conditions. A later study of 110 Passeriformes of 23 species in Texas found somewhat less impact on blood gas and acid-base status (Heatley et al. 2013). Intranasal midazolam at 5.6 ± 2.7 mg/kg prior to sampling, euthanasia, and specimen preparation in Texas Passeriformes effectively sedated the birds and further reduced lactate concentrations, but slightly increased partial pressure of CO_2 (pCO_2) and decreased partial pressure of O_2 (pO_2), suggesting relative hypoventilation (Heatley et al. 2015). Besides differences in species composition, size of birds, and degree of handling prior to venipuncture, a noteworthy difference between Harms and Harms (2012) and Heatley et al. (2013) was timing of venipuncture. In the former, blood collection was as soon as possible after disentanglement from the net and banding, and in the latter it was after a delay of up to 30 min (Harms and Harms 2012; Heatley et al. 2013). In fact, processing birds following a brief delay is the more common practice, particularly in large banding operations when many birds are caught at once. General recommendations for banding operations are holding nonbreeding birds for no more than 1 hr and breeding birds for no more than 30 min (North American Banding Council 2001; Mackenzie and Gahbauer 2014). Depending on how a bird reacts to bag restraint, a short recovery period from the initial mist-net impact and disentanglement could be either helpful or harmful. Evaluating physiologic and metabolic effects of different handling procedures, including postcapture holding times, can inform strategies to minimize adverse impacts on study animals.

Blood gases and acid-base status have been evaluated uncommonly in wild-caught birds, but capture stress has been evaluated frequently by using indicators of hypothalamus-pituitary-adrenal (HPA) axis activation including plasma corticosterone (Nicholson et al. 2000; Romero and Romero 2002; Newman et al. 2005) and

heterophil/lymphocyte (H/L) ratio (Newman et al. 2005; Davis et al. 2008; Laiolo et al. 2009). The corticosterone response is more rapid than shifts in the leukocyte profile (Davis et al. 2008), necessitating rapid sampling if baseline endocrine values are desired (Romero and Romero 2002). Increases in H/L ratios may require over an hour to hours in vertebrates responding to stressors (Davis et al. 2008), but increased H/L ratios have been observed within 30 min in Xantus's Murrelets (*Synthliboramphus hypoleucus*) when isoflurane anesthesia and radiomarking by subcutaneous anchor attachment were conducted prior to venipuncture (Newman et al. 2005). Multiple simultaneous measures of capture effects on physiology can be hampered by limits on safe blood sample volume in small individuals. Available portable handheld point-of-care analyzers require only about 100 μL for blood gas analysis, however, and blood smears can be made with minimal additional volume, so that field assessments of respiratory and metabolic acid-base status and activation of the HPA axis are feasible even for small passerines.

Use of a central vein for venipuncture is recommended for measuring venous blood gases and pH, and the right jugular is the central vein typically used for birds (Heatley et al. 2013; Montesinos and Ardiaca 2013). Presence of a cervical vascular plexus and lack of cervical apteria in Columbiformes necessitate use of other vascular access points in pigeons and doves, however. Cross-species comparisons could be complicated when different venipuncture locations are used (Harms and Harms 2012). Venipuncture site can affect hematology results in a wide range of species, including reptiles (López-Olvera et al. 2003; Stewart et al. 2012), elasmobranchs (Mylniczenko et al. 2006; Naples et al. 2012), and birds (Kern and De Graw 1978; Sheldon et al. 2008). Venous blood gas and acid-base parameters could be especially prone to differences depending on the mass and metabolic state of tissues draining to the selected vascular access site. Lower venous pH and bicarbonate and higher lactate concentrations that were previously identified in Mourning Doves compared with Boat-tailed Grackles and House Sparrows immediately following mist-net capture and banding could have resulted in part from basilic (ulnar) venipuncture in Mourning Doves and jugular venipuncture in the other two species, rather than from species differences in physiologic response (Harms and Harms 2012). Mourning Dove anatomy precludes jugular access for comparing effects of venipuncture location, and the small size of House Sparrows makes basilic venipuncture impractical. Boat-tailed Grackles are sufficiently large, however, to be suitable subjects for comparing jugular and basilic venipuncture sites with respect to blood gas, lactate, and hematology effects.

The objective of this study was to build on a previous study (Harms and Harms 2012) by measuring venous blood gas partial pressures, pH, bicarbonate and lactate concentrations, packed cell volume, white blood cell differential counts, and H/L ratios in three bird species captured by mist net and handled for banding, and comparing the effects of processing birds immediately versus following a 45–60-min delay. An additional objective was to compare the effects of venipuncture location (jugular versus basilic vein) on all measured values in one species (Boat-tailed Grackle).

MATERIALS AND METHODS

The study site was a residential backyard in Morehead City, North Carolina, USA, adjacent to a salt marsh, Calico Creek (34°43.722′N, 76°43.915′W). Birds were captured by mist net (black nylon, 38-mm mesh, four- to five-shelf, 2.6×6–9-m mist nets, Avinet, Inc., Dryden, New York, USA) between 21 March 2010 and 30 July 2014, with temperatures ranging from 4 to 32 C. Removal from the mist net was initiated as soon as any bird was captured. Birds were carried in a solid-bottom/mesh-top cloth holding bag (Avinet). They were examined for external abnormalities, and body condition was subjectively assessed by palpation of

pectoral muscles and keel. Birds were weighed (Pesola® spring scales, 100×1 g and 300×2 g, Avinet), measured (wing cord, tail length), aged and sexed (Pyle 1997), and banded with a US Geological Survey numbered aluminum leg band. After banding, 0.10–0.25 mL of blood was collected from the right jugular vein (House Sparrows, most Boat-tailed Grackles) or left basilic vein (Mourning Doves, subset of male Boat-tailed Grackles) using a heparinized (heparin sodium USP, 1,000 units/mL, APP Pharmaceuticals, Schaumburg, Illinois, USA) 1-mL insulin syringe with an integral 28-gauge×1.27-cm needle and negligible dead space, which reduces potential for blood gas alterations ex vivo. The venipuncture site was prepared with minimal ethanol prior to needle insertion, and after sampling, direct pressure was applied for hemostasis and to minimize hematoma formation. Blood volume did not exceed 0.5% (5 μL/g) of body mass. Birds were handled and restrained by a bird bander with over 30 yr of banding experience (R.V.H.), and blood was collected by a veterinarian with over 20 yr of nondomestic species experience (C.A.H.).

Times at which a bird hit the mist net and was removed from the net, banded, bled, and released were recorded. Time spans were calculated to the nearest minute. Venipuncture was performed either as soon as possible ("immediate" sample) after capture and banding or with a targeted delay ("delayed" sample) of 45–60 min after mist-net capture. During the delay, birds were kept individually in a mesh/cloth holding bag hung in a quiet location with minimal stimulation. The range of sampling times was based primarily on sequential processing of birds captured at approximately the same time, not on greater or lesser handling times. Venous blood gas analysis was performed promptly after blood collection using an iStat Portable Clinical Analyzer (Abaxis, Union City, California, USA) with CG4+ cartridges (Abaxis). Analytes measured were pH, pCO_2, pO_2, and lactate. The iStat instrument performs analysis of samples at 37 C and corrects pH, pCO_2, and pO_2 for patient temperature using human-based algorithms. Cloacal temperatures were not measured because the procedure was judged to be an unnecessary additional stressor. For reference and comparison with other studies, 41 C was used for iStat temperature corrections of pH, pCO_2, and pO_2. This temperature was selected based on mean reported temperatures for awake Mourning Doves (Bartholomew and Dawson 1954), Red-winged Blackbirds (*Agelaius phoeniceus*; Lustick et al. 1970), and House Sparrows (Murakami et al. 2001) approximating 41 C, and assuming this would be a normal body temperature for birds in the current study. Results for pH, pCO_2, and pO_2 are presented as directly measured values at 37 C and as temperature-corrected values, recognizing that a variety of temperature corrections could be employed other than those of the iStat (Ashwood et al. 1983). Packed cell volume was determined by centrifugation of heparinized 32×0.8 mm capillary hematocrit tubes (Drummond Scientific Co., Broomali, Pennsylvania, USA). Blood smears were made promptly after collection, and stained later the same day with a rapid hematology stain (Hemacolor®, Harleco, EMD Chemicals, Gibbstown, New Jersey, USA). Differential white blood cell counts were performed according to established criteria (Campbell and Ellis 2007) by a single observer (M.R.J.) and H/L ratios were calculated.

Because some data were not normally distributed (Shapiro-Wilk W-test, $P<0.05$), nonparametric methods were used for all statistical analysis, and summary statistics are presented as median and minimum–maximum. Statistical analysis was performed using a commercial program (JMP® Pro 11.0, SAS Institute, Inc., Cary, North Carolina, USA). Effects of venipuncture timing (immediate versus delayed) within species and effects of venipuncture location (jugular versus basilic) in male Boat-tailed Grackles were compared using the Wilcoxon rank sums test. Associations between ambient temperature and blood gas and lactate values were tested within species for both immediate and delayed venipuncture samples using the Kendall tau test. A sequential Bonferroni test was applied within blood gas and hematology sets of analyses to correct for multiple simultaneous inferences (Rice 1989). A value of $P<0.05$ was accepted as statistically significant.

Research was conducted under required state and federal bird banding permits to two authors (C.A.H., R.V.H.), and with approval of the North Carolina State University Institutional Animal Care and Use Committee. A large subset of the initial sample time data used for comparison with delayed sampling in the current study has been previously reported with a focus on differences among species (Harms and Harms 2012).

RESULTS

The study included 48 Mourning Doves (median, minimum–maximum weight: 122, 84–157 g), 54 Boat-tailed Grackles (147, 97–228 g), and 36 House Sparrows (27, 22–30 g). Captures of Boat-tailed Grackles included 25 females (103, 97–116 g) and

TABLE 1. Median (minimum–maximum) venous blood gas and lactate values for Mourning Doves sampled from the basilic vein promptly following capture by mist net and banding ($n=24$, except for lactate where $n=23$; median 9 min, range 5–17 min after contacting mist net), or sampled following a delay ($n=20$, except for lactate and pO_2 where $n=19$; median 52 min, range 41–68 min). Wilcoxon rank sums tests; **bold** and * indicate that significant differences remain following Bonferroni correction for multiple simultaneous inferences. TC = temperature corrected to 41 C.

Analyte	Immediate	Delayed	*P*
$pH_{37\ C}$	7.453 (7.385–7.558)	7.492 (7.363–7.562)	0.052
pH_{TC}	7.394 (7.230–7.496)	7.432 (7.306–7.500)	0.052
$pCO_{2\ 37\ C}$ (mm Hg)	28.6 (21.0–45.9)	29.6 (24.8–38.7)	0.340
$pCO_{2\ TC}$ (mm Hg)	34.1 (25.0–54.6)	35.2 (29.5–46.1)	0.340
$pO_{2\ 37\ C}$ (mm Hg)	49 (22–63)	40 (34–64)	0.117
$pO_{2\ TC}$ (mm Hg)	65 (29–83)	53 (45–84)	0.117
HCO_3^- (mmol/L)	21.1 (14.4–30.1)	22.5 (18.6–29.6)	**0.004***
Lactate (mmol/L)	7.72 (3.94–14.1)	4.83 (1.60–10.6)	**0.001***

29 males (178, 134–228 g). Sample sizes for particular analyses (Tables 1–8) may not add up to the species total because of different venipuncture locations and timing under consideration (Boat-tailed Grackles) and missing data from occasional instrument error, operator error, damaged blood smears, or sample leakage in the hematocrit centrifuge.

Venous blood gases, pH, and lactate results were compared between immediate and delayed venipuncture for Mourning Doves (Table 1), Boat-tailed Grackles (Table 2), and House Sparrows (Table 3). Lactate concentrations were significantly lower in all three species when venipuncture was delayed. Additionally, bicarbonate was significantly higher when venipuncture was delayed in Mourning Doves and Boat-tailed Grackles, and pH was higher in Boat-tailed Grackles following a delay. Higher venous pO_2 and pCO_2 values in Boat-tailed Grackles following a delay were not significantly different from corresponding immediate venipuncture values after Bonferroni correction. No significant associations with ambient temperature were detected for any blood gas analyte for either immediate or delayed sample times.

Packed cell volumes, differential white blood cell counts, and H/L ratios were compared between immediate and delayed venipuncture for Mourning Doves (Table 4), Boat-tailed Grackles (Table 5), and House Sparrows (Table 6). Only Boat-tailed Grackle percentage heterophils differed significantly between immediate (33.5, 10–51%) and delayed (50, 20–67%) sample

TABLE 2. Median (minimum–maximum) venous blood gas and lactate values for Boat-tailed Grackles sampled from the jugular vein promptly following capture by mist net and banding ($n=22$, except for lactate where $n=18$; median 9 min, range 9–18 min after contacting mist net), or sampled following a delay ($n=20$; median 54 min, range 45–63 min). Wilcoxon rank sums tests; **bold** and * indicate that significant differences remain following Bonferroni correction for multiple simultaneous inferences. TC = temperature corrected to 41 C.

Analyte	Immediate	Delayed	*P*
$pH_{37\ C}$	7.500 (7.411–7.672)	7.541 (7.473–7.684)	**0.009***
pH_{TC}	7.439 (7.353–7.609)	7.480 (7.413–7.619)	**0.009***
$pCO_{2\ 37\ C}$ (mm Hg)	29.1 (21.8–36.6)	32.1 (23.8–37.7)	0.024
$pCO_{2\ TC}$ (mm Hg)	34.7 (25.9–43.6)	38.2 (28.3–44.9)	0.024
$pO_{2\ 37\ C}$ (mm Hg)	46 (36–56)	44 (40–52)	0.050
$pO_{2\ TC}$ (mm Hg)	61 (48–74)	58 (53–69)	0.050
HCO_3^- (mmol/L)	23.5 (15.8–30.9)	28.2 (24.6–35)	**<0.001***
Lactate (mmol/L)	5.61 (3.09–8.75)	2.62 (1.24–5.32)	**<0.001***

TABLE 3. Median (minimum–maximum) venous blood gas and lactate values for House Sparrows sampled from the jugular vein promptly following capture by mist net and banding ($n=18$, except for lactate where $n=17$; median 12 min, range 5–23 min after contacting mist net), or sampled following a delay ($n=18$; median 54 min, range 50–69 min). Wilcoxon rank sums tests; **bold** and * indicate that a significant difference remains following Bonferroni correction for multiple simultaneous inferences. TC = temperature-corrected to 41 C.

Analyte	Immediate	Delayed	*P*
$pH_{37\ C}$	7.454 (7.304–7.519)	7.452 (7.382–7.512)	0.635
pH_{TC}	7.395 (7.248–7.458)	7.392 (7.324–7.451)	0.624
$pCO_{2\ 37\ C}$ (mm Hg)	37.5 (31.8–49.6)	36.9 (29.2–42.4)	0.223
$pCO_{2\ TC}$ (mm Hg)	44.7 (37.8–59.0)	43.9 (34.7–50.5)	0.223
$pO_{2\ 37\ C}$ (mm Hg)	40 (27–49)	38 (32–44)	0.187
$pO_{2\ TC}$ (mm Hg)	52 (36–60)	50 (42–58)	0.187
HCO_3^- (mmol/L)	25.8 (16.9–30.1)	25.3 (20.9–27.6)	0.334
Lactate (mmol/L)	4.77 (2.66–12.0)	3.14 (1.26–5.50)	**0.004***

times ($P=0.002$, Table 5). In addition, the Boat-tailed Grackle eosinophil percentage was lower (5, 0–26% vs. 11, 2–48%) and the H/L ratio was higher (1.73, 0.34–3.25 vs. 1.02, 0.18–2.0) in the delayed samples, but the difference did not remain significant following Bonferroni correction.

Jugular and basilic venipuncture sites were compared for pH, blood gases, and lactate (Table 7) and also for packed cell volumes, differential white blood cell counts, and H/L ratios (Table 8) in male Boat-tailed Grackles sampled immediately after mist-net capture and banding. There were no significant differences in any analyte between venipuncture sites. A trend toward higher pH in blood collected from the basilic vein (7.465, 7.419–7.615) than from the jugular vein (7.417, 7.353–7.500) was not significant following Bonferroni correction.

DISCUSSION

Portable point-of-care analyzers are increasingly used in wildlife field studies for analysis of blood samples because of their convenience, rapid results, small sample volume requirements, and generally acceptable agreement with standard diagnostic laboratory methods (Stoot et al. 2014). Use of the iStat point-of-care analyzer in evaluating acid-base status in birds has been reviewed, and pH and blood gas measurements and temperature corrections are considered reliable when compared with benchtop analyzers (Montesinos and Ardiaca 2013). Venous blood pCO_2, pH, and bicarbonate values are considered broadly representative of arterial values when draining the same source, whereas venous pO_2 is a poor indicator of arterial values (Malatesha et al. 2007).

All three species in the present study exhibited modest but general improvement in blood gas and acid-base status when venipuncture was performed following a delay, compared with immediately following capture and banding. Lactate was lower for all three species following a delay, and there were associated increases in bicarbonate in Mourning Doves and Boat-tailed Grackles and pH in Boat-tailed Grackles. In a previous report, Mourning Dove lactate concentrations decreased and Boat-tailed Grackle bicarbonate concentrations increased even within the brief time span of immediate sampling (Harms and Harms 2012), and on visual inspection of scatter plots, nonsignificant trends within the immediate sampling time span were consistent with differences between immediate and delayed sampling, with no inflections at the earliest time points. Even earlier blood sampling might yield more favorable blood gas values, but the objective of the study was to evaluate blood gas status after complete timely processing (including banding, weighing, and measuring), not at the earliest possible

TABLE 4. Median (minimum–maximum) hematology values for Mourning Doves sampled from the basilic vein promptly following capture by mist net and banding (n=17, except for PCV where n=22; median 9 min, range 5–13 min after contacting mist net), or sampled following a delay (n=18, except for PCV where n=19; median 51 min, range 41–68 min). Wilcoxon rank sums tests.

Analyte	Immediate	Delayed	*P*
PCV (%)	48 (41–58)	50 (41–60)	0.283
Heterophils (%)	19 (1–62)	27 (6–51)	0.409
Lymphocytes (%)	41 (11–72)	43 (30–64)	0.620
Monocytes (%)	9 (5–23)	16 (5–46)	0.124
Eosinophils (%)	10 (1–77)	5 (1–37)	0.0877
Basophils (%)	0 (1–1)	0 (1–1)	1.000
Heterophil/lymphocyte ratio	0.45 (0.02–2.6)	0.63 (0.14–1.4)	0.766

sampling time either while still in the net or as the first procedure after extraction from the net. No birds in the delayed venipuncture group had a temperature-corrected blood pH less than 7.3, and only one Mourning Dove had a lactate >10 mmol/L. Reference intervals for blood gases, pH, and lactate in unrestrained wild birds of any species are not available, to our knowledge, and would be challenging to acquire. Values reported here are not intended as reference intervals. As a frame of reference, in avian anesthesia, arterial blood pH of 7.4–7.6 is considered adequate, with values <7.2 potentially detrimental (Edling et al. 2001; Desmarchelier et al. 2007). None of the mist-netted birds in the present study had lactate values as low as unrestrained domestic geese (0.94 mmol/L; Le Maho 1992), nor as high as postflight values for unconditioned raptors (22.2 mmol/L; Chaplin et al. 1993). The highest lactate values were comparable to postflight values of conditioned prerelease rehabilitated raptors (11.1–13.2 mmol/L; Chaplin et al. 1993). The aggregate mean lactate value (4.55 mmol/L) for 23 species of Texas passerines sampled after a delay of up to 30 min (Heatley et al. 2013) was lower than values for Mourning Doves and Boat-tailed Grackles sampled immediately after mist-net capture and banding, but higher than values for Boat-tailed Grackles and house sparrows following a delay of 41–69 min in the present study. In combination, that study and the present study indicate that a brief delay between mist-net capture and processing may be helpful in normalizing acid-base status. For the few birds that become clinically acidotic, this could be an important consideration if they are also in marginal health at the time of capture. Benefits in acid-base

TABLE 5. Median (minimum–maximum) hematology values for Boat-tailed Grackles sampled from the jugular vein promptly following capture by mist net and banding (n=12, except for PCV where n=22; median 9 min, range 6–15 min after contacting mist net), or sampled following a delay (n=22; median 54 min, range 45–63 min). Wilcoxon rank sums tests, **bold** and * indicate that a significant difference remains following Bonferroni correction for multiple simultaneous inferences.

Analyte	Immediate	Delayed	*P*
PCV (%)	47 (40–56)	48 (41–60)	0.676
Heterophils (%)	33.5 (10–51)	50 (20–67)	**0.0018***
Lymphocytes (%)	34 (21–60)	30 (18–58)	0.370
Monocytes (%)	15 (6–30)	9 (4–23)	0.412
Eosinophils (%)	11 (2–48)	5 (0–26)	0.00956
Basophils (%)	0 (0–1)	0 (0–3)	0.698
Heterophil/lymphocyte ratio	1.02 (0.18–2.0)	1.73 (0.34–3.25)	0.0265

TABLE 6. Median (minimum–maximum) hematology values for House Sparrows sampled from the jugular vein promptly following capture by mist net and banding (n=5, except for PCV where n=18; median 10 min, range 6–22 min after contacting mist net), or sampled following a delay (n=14, except for PCV where n=18; median 54 min, range 50–69 min). Wilcoxon rank sums tests, no significant differences remain following Bonferroni correction for multiple comparisons.

Analyte	Immediate	Delayed	*P*
PCV (%)	48 (41–58)	48 (29–56)	1.000
Heterophils (%)	26 (15–50)	28 (9–49)	0.889
Lymphocytes (%)	65 (42–66)	53 (8–70)	0.577
Monocytes (%)	8 (5–12)	14 (6–24)	0.0291
Eosinophils (%)	3 (1–13)	6 (1–26)	0.176
Basophils (%)	0 (0–0)	0 (0–1)	0.434
Heterophil/lymphocyte ratio	0.39 (0.23–1.2)	0.58 (0.13–6.0)	0.746

status resulting from delayed processing could be outweighed by other factors, however, such as time lost foraging or feeding young, if the delay were unduly extended.

An indication in Heatley et al. (2013) that lactate ranges were smaller for the aggregate 23 species than those previously reported for these three species without a delay (Harms and Harms 2012) probably appears exaggerated because of how results are reported, as mean and 95% confidence interval of the mean (Heatley et al. 2013) versus median and minimum–maximum (Harms and Harms 2012; present study). Another important factor in lactate differences between the two reports is size of species studied; none of the 23 species in Heatley et al. (2013) were as large or well muscled as Boat-tailed Grackles or Mourning Doves. Exertional rhabdomyolysis, triggered by anaerobic metabolism and lactic acidemia, is noted as a particular problem in well-muscled and long-legged birds (Williams and Thorne 1996; Rogers et al. 2004; Marco et al. 2006), generally larger or longer-legged than the species under consideration here. Exertional myopathy has also been reported in captive White-headed Pigeons (*Columba leucomela*; Hill and Miller 2013), a columbiform species larger than Mourning Doves. Although bird species of a size commonly captured by mist net apparently are not at high risk for overt exertional myopathy, well-muscled birds with explosive takeoffs like Mourning Doves may at least be more prone to lactic acidosis.

Mist-netted birds classified as stressed based on external behavioral criteria such as lethargy, closed eyes, and panting

TABLE 7. Median (minimum–maximum) venous blood gas and lactate values for male Boat-tailed Grackles sampled promptly following capture by mist net and banding, from the jugular vein (n=10, except for lactate where n=9; median 9 min, range 6–17 min after contacting mist net) or from the basilic vein (n=10; median 10 min, range 7–22 min). Wilcoxon rank sums tests; no significant differences remain for any analyte following Bonferroni correction for multiple simultaneous inferences. TC = temperature corrected to 41 C.

Analyte	Jugular	Basilic	*P*
$pH_{37\ C}$	7.476 (7.411–7.561)	7.526 (7.479–7.680)	0.041
pH_{TC}	7.417 (7.353–7.500)	7.465 (7.419–7.615)	0.041
$pCO_{2\ 37\ C}$ (mm Hg)	28.8 (23.1–36.6)	28.0 (16.9–32.2)	0.257
$pCO_{2\ TC}$ (mm Hg)	34.3 (27.5–43.6)	33.3 (20.1–38.3)	0.257
$pO_{2\ 37\ C}$ (mm Hg)	47 (36–55)	45 (37–60)	0.880
$pO_{2\ TC}$ (mm Hg)	62 (48–73)	60 (49–79)	0.880
HCO_3^- (mmol/L)	22.2 (15.8–30.9)	22.5 (19.6–25.8)	0.570
Lactate (mmol/L)	5.94 (4.05–8.75)	6.05 (4.05–7.02)	0.850

TABLE 8. Median (minimum–maximum) hematology values for Boat-tailed Grackles sampled from the jugular vein promptly following capture by mist net and banding (n=6, except for PCV where n=10; median 9 min, range 6–17 min after contacting mist net), or from the basilic vein (n=11, except for PCV where n=10; median 10 min, range 7–22 min). Wilcoxon rank sums tests.

Analyte	Jugular	Basilic	*P*
PCV (%)	47 (40–52)	44 (37–60)	0.210
Heterophils (%)	40 (25–47)	40 (24–56)	1.000
Lymphocytes (%)	35 (22–50)	40 (11–58)	0.479
Monocytes (%)	19 (6–30)	16 (12–26)	0.612
Eosinophils (%)	8 (3–16)	4 (1–17)	0.264
Basophils (%)	0 (0–1)	0 (0–0)	0.310
Heterophil/lymphocyte ratio	1.23 (0.5–2.0)	1.00 (0.41–4.18)	0.763

(Spotswood et al. 2012) may be experiencing pronounced blood gas and acid-base disturbances. In our small-scale mist netting operation we did not have opportunity to test that possibility directly. In fact, birds exhibiting such behaviors would have been excluded from venipuncture precisely to help ensure their survival. Ambient temperature could reasonably be expected to affect morbidity and mortality risk in avian captures (Gaunt and Oring 1999). For the temperature range, species, handling times, and sample size of the current study, no significant associations were found between ambient temperature and venous blood gases, pH, or lactate.

A response that could be exacerbated with longer duration confinement of birds prior to processing is activation of the HPA axis, or the general stress response. Differential white blood cell counts were used as a surrogate for plasma corticosterone concentrations (Davis et al. 2008) in the current study. Within the delayed time frame of approximately 1 hr, Boat-tailed Grackles displayed shifts in differential counts suggestive of a stress leukogram, with a significantly increased percentage of heterophils plus a decreased percentage of eosinophils and an increased H/L ratio that did not remain significant following Bonferroni correction. Mourning Doves and House Sparrows did not exhibit a shift in the leukocyte profile. One hour may not have been sufficient time for a stress leukogram to develop, however (Davis et al. 2008), and differential count data were highly variable, diminishing discrimination power. Without resampling birds some hours later, which would require either excessive holding time or a reliable means to recapture them, a late-developing stress leukogram cannot be detected or compared between immediate versus delayed processing protocols.

In male Boat-tailed Grackles sampled immediately after mist-net capture and banding, no significant differences were detected for blood gases, lactate, or hematology values between jugular and basilic venipuncture sites. A trend towards lower blood pH from jugular samples suggests that a previously identified relative acidemia of Mourning Doves sampled from the basilic vein compared with Boat-tailed Grackles sampled from the jugular vein following mist-net capture and banding (Harms and Harms 2012) could have been slightly greater if jugular venipuncture were feasible in Mourning Doves. Although a larger sample size might yield statistically significant differences between venipuncture sites, such differences are unlikely to be of clinical relevance for the analytes measured.

As previously reported (Harms and Harms 2012; Heatley et al. 2013, 2015), metabolic, respiratory, and acid-base alterations in columbiform and passerine birds captured by mist net are relatively minor in most cases. Values for a small number of birds sampled promptly after mist-net disentanglement and banding approached

levels considered concerning in anesthetized birds, and in the presence of adverse conditions (e.g., malnutrition, ectoparasitism, thermal stress; Voss et al. 2010), additive impacts could occur. For such birds, a delay after capture and before processing, while held quietly in a loose cloth bag as is commonly practiced in banding operations, may be beneficial for their blood gas and acid-base status. Other factors such as reduced foraging time or greater activation of the HPA axis could eventually outweigh that benefit however, and for many birds such factors may be more important. Thus, published guidelines to hold birds for less than 1 hr, and breeding birds less than 30 min (Mackenzie and Gahbauer 2014; North American Banding Council 2001), remain sensible even if processing is delayed briefly for logistical or blood gas and acid-base considerations.

ACKNOWLEDGMENTS

We thank J. Barnes for guidance on blood smear interpretation and P. Harms for logistical support. Funding came from the Merial Veterinary Scholars Program (M.R.J.) and the State of North Carolina.

LITERATURE CITED

Ashwood ER, Kost G, Kenney M. 1983. Temperature correction of blood-gas and pH measurements. *Clin Chem* 29:1877–1885.

Bartholomew GA, Dawson WR. 1954. Body temperature and water requirements in the mourning dove, *Zenaidura macroura marginella*. *Ecology* 35:181–187.

Brown MB, Brown CR. 2009. Blood sampling reduces annual survival in cliff swallows (*Petrochelidon pyrrhonota*). *Auk* 126:853–861.

Campbell TW, Ellis CK. 2007. *Avian and exotic animal hematology and cytology*, 3rd Ed. Blackwell Publishing, Ames, Iowa, pp. 3–50.

Chaplin SB, Mueller LR, Degernes LA. 1993. Physiologic assessment of rehabilitated raptors prior to release. In: *Raptor biomedicine*, Redig PT, Cooper JE, Remple JD, Hunter DB, Hahn T, editors. University of Minnesota Press, Minneapolis, Minnesota, pp. 167–173.

Davis AK, Maney DL, Maerz JC. 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. *Funct Ecol* 22:760–772.

Desmarchelier M, Rondenay Y, Fitzgerald G, Lair S. 2007. Monitoring of the ventilator status of anesthetized birds of prey using end-tidal carbon dioxide measured with a microstream capnometer. *J Zoo Wildl Med* 38:1–6.

Edling TM, Degernes LA, Flammer K, Worne WA. 2001. Capnographic monitoring of anesthetized African grey parrots receiving intermittent positive pressure ventilation. *J Am Vet Med Assoc* 219:1714–1718.

Gaunt AS, Oring LW. 1999. *Guidelines to the use of wild birds in research*, 2nd Ed. The Ornithological Council, Washington, DC. 56 pp.

Harms CA, Harms RV. 2012. Venous blood gas and lactate values of mourning doves (*Zenaida macroura*), boat-tailed grackles (*Quiscalus major*), and house sparrows (*Passer domesticus*) after capture by mist net, banding, and venipuncture. *J Zoo Wildl Med* 43:77–84.

Heatley JJ, Cary J, Kingsley L, Beaufrere H, Russell KE, Voelker G. 2015. Midazolam sedates Passeriformes for field sampling but affects multiple venous blood analytes. *Vet Med Res Rep* 6:61–69.

Heatley JJ, Cary J, Russell KE, Voelker G. 2013. Clinicopathologic analysis of passeriform venous blood reflects transitions in elevation and habitat. *Vet Med Res Rep* 4:21–29.

Hill AG, Miller R. 2013. Exercise-induced deep pectoral necrosis in white-headed pigeons (*Columba leucomela*). *J Zoo Wildl Med* 44:990–995.

Kern MD, De Graw WA. 1978. Differences in the composition of venous and cardiac blood from white-crowned sparrows. *Condor* 80:230–234.

Laiolo P, Banda E, Lemus JA, Aguirre JI, Blanco G. 2009. Behaviour and stress response during capture and handling of the red-billed chough *Pyrrhocorax pyrrhocorax* (Aves: Corvidae). *Biol J Linn Soc* 96:846–855.

Le Maho Y, Karman H, Briot D, Handrich Y, Robin JP, Mioskowski E, Cherel Y, Farni J. 1992. Stress in birds due to routine handling and a technique to avoid it. *Am J Physiol* 263:R755–R781.

López-Olvera JR, Montané J, Marco I, Martinez-Silvestre A. Soler J, Lavín S. 2003. Effect of venipuncture site on hematologic and serum biochemical parameters in marginated tortoise (*Testudo marginata*). *J Wildl Dis* 39:830–836.

Lustick S, Talbot S, Fox EL. 1970. Absorption of radiant energy in redwinged blackbirds (*Agelaius phoeniceus*). *Condor* 72:471–473.

Mackenzie SA, Gahbauer M. 2014. Guidelines for prioritizing bird safety during high capture events. *N Am Bird Bander* 39:61–65.

Malatesha G, Singh NK, Bhariza A, Rehani B, Goel A. 2007. Comparison of arterial and venous pH, bicarbonate, pCO_2 and pO_2 in initial emergency department assessment. *Emerg Med J* 24:569–571.

Marco I, Mentaberre G, Ponjoan A, Bota G, Mañosa S, Lavin S. 2006. Capture myopathy in little bustards after trapping and marking. *J Wildl Dis* 42:889–891.

Montesinos A, Ardiaca M. 2013. Acid-base status in the avian patient using a portable point-of-care analyzer. *Vet Clin Exot Anim* 16:47–69.

Murakami N, Kawano T, Nakahara K, Nasu T, Shiota K. 2001. Effect of melatonin on circadian rhythm, locomotor activity and body temperature in the intact house sparrow, Japanese quail and owl. *Brain Res* 889:220–224.

Mylniczenko ND, Curtis EW, Wilborn RE, Young FA. 2006. Difference in hematocrit of blood samples obtained from two venipuncture sites in sharks. *Am J Vet Res* 67:1861–1864.

Naples LM, Mylniczenko ND, Zachariah TT, Wilborn RE, Young FA. 2012. Evaluation of critical care blood analytes assessed with a point-of-care portable blood analyzer in wild and aquarium-housed elasmobranchs and the influence of phlebotomy site on results. *J Am Vet Med Assoc* 214:117–125.

Newman SH, Carter HR, Whitworth DL, Zinkl JG. 2005. Health assessments and stress response of Xantus's murrelets to capture, handling and radio-marking. *Mar Ornithol* 33:147–154.

Nicholson D, Lochmiller SRL, Stewart MD, Masters RE, Leslie DM. 2000. Risk factors associated with capture-related death in eastern wild turkey hens. *J Wildl Dis* 36:308–315.

North American Banding Council. 2001. *The North American bander's study guide.* 66 pp. http://www.nabanding.net/wp-content/uploads/2012/04/STUDYGUIDE1.pdf. Accessed 30 December 2014.

Pyle P. 1997. *Identification guide to North American birds, part 1: Columbidae to Ploceidae.* Slate Creek Press, Bolinas, California, 732 pp.

Rice WR. 1989. Analyzing tables of statistical tests. *Evolution* 43:223–225.

Rogers RI, Battley PF, Sparrow J, Koolhaas A, Hassell CJ. 2004. Treatment of capture myopathy in shorebirds: a successful trial in northwestern Australia. *J Field Ornithol* 75:157–164.

Romero LM, Romero RC. 2002. Corticosterone responses in wild birds: the importance of rapid initial sampling. *Condor* 104:129–135.

Sheldon LD, Chin EH, Gill SA, Schmaltz G, Newman AEM, Soma KK. 2008. Effects of blood collection on wild birds: an update. *J Avian Biol* 39: 369–378.

Spotswood EN, Goodman KR, Carlisle J, Cormier JL, Humple DL, Rousseau J, Guers SL, Barton GG. 2012. How safe is mist netting? Evaluating the risk of injury and mortality to birds. *Methods Ecol Evol* 3:29–38.

Stewart K, Mitchell MA, Norton T, Krecek RC. 2012. Measuring the level of agreement in hematologic and biochemical values between blood sampling sites in leatherback sea turtles (*Dermochelys coriacea*). *J Zoo Wildl Med* 43:719–725.

Stoot LJ, Cairns NA, Cull F, Taylor JJ, Jeffrey JD, Morin F, Mandelman JW, Clark TD, Cooke SJ. 2014. Use of portable blood physiology point-of-care devices for basic and applied research on vertebrates: a review. *Conserv Physiol* 2: doi:10.1093/conphys/cou011.

Voss M, Shutler D, Werner J. 2010. A hard look at blood sampling of birds. *Auk* 127:704–708.

Williams ES, Thorrne ET. 1996. Exertional myopathy (capture myopathy). In: *Noninfectious diseases of wildlife*, 2nd Ed., Fairbrother A, Locke LN, Hoff GL, editors. Iowa State University Press, Ames, Iowa, pp. 181–193.