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PLASMA METABOLITES AND CREATINE KINASE LEVELS OF SHOREBIRDS DURING FALL MIGRATION IN THE PRAIRIE POTHOLE REGION

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ABSTRACT.—Wetland habitats in the Prairie Pothole Region of North America have been greatly reduced since European settlement, but the availability of managed wetlands has increased. The relative efficacy of these two habitats for meeting energetic demands of migrating shorebirds is unknown. To assess the relative suitability of stopover sites, we measured plasma metabolites and creatine kinase in Least Sandpipers (*Calidris minutilla*), Semipalmated Sandpipers (*C. pusilla*), and Pectoral Sandpipers (*C. melanotos*) at natural and managed wetland sites during fall migration in northeastern South Dakota and west-central Minnesota. We used stepwise multiple regression to identify significant effectors of plasma metabolite levels, followed by analysis of covariance to compare metabolite values between birds in the two habitat types. Plasma metabolite levels generally did not differ significantly between birds in the two habitat types, with two exceptions. Plasma triglycerides of Pectoral Sandpipers were 2.6× higher at managed than at natural wetlands, suggesting higher rates of fattening at managed sites, but this was not supported by plasma glycerol levels, which did not differ significantly between birds in the two habitat types, or by body mass, which was greater for adult males in natural wetlands. Plasma creatine kinase levels of Least Sandpipers were 75% higher at managed than at natural wetlands, which suggests that repair of flight-induced muscle damage may be slower for this species at managed wetlands. The general absence of significant differences in plasma metabolites among shorebirds in the two wetland types suggests that natural and managed wetlands serve as similarly effective stopover habitat for fall-migrating shorebirds in the Prairie Pothole Region. *Received 14 September 2012, accepted 17 June 2013.*

Key words: *Calidris*, creatine kinase, habitat quality, plasma metabolite, shorebird, stopover biology.

Metabolitos del Plasma y Niveles de Creatina Quinasa en Aves Playeras durante la Migración de Otoño en la Región de Prairie Pothole

RESUMEN.—Los humedales de la región de Prairie Pothole en Norte América se han visto fuertemente reducidos desde el establecimiento europeo, pero la disponibilidad de humedales manejados se ha incrementado. La eficacia relativa de estos dos hábitats para cumplir las demandas energéticas de las aves playeras migratorias se desconoce. Para determinar la idoneidad relativa de los sitios de parada, medimos los metabolitos del plasma y los niveles de creatina quinasa en *Calidris minutilla*, *C. pusilla* y *C. melanotos* en humedales naturales y manejados durante la migración de otoño en el noreste de Dakota del Sur y el centro-oeste de Minnesota. Usamos regresiones múltiples escalonadas para identificar los determinantes de los niveles de metabolitos en el plasma, seguidas de análisis de covarianza para comparar los valores de metabolitos entre aves en los dos tipos de hábitat. Los niveles de metabolitos en el plasma generalmente no fueron significativamente diferentes entre las aves de los dos tipos de hábitat, con dos excepciones. Los triglicéridos en el plasma de *C. melanotos* fueron 2.6 veces más altos en los humedales manejados que en los naturales, lo que sugiere tasas más altas de engorde en los sitios manejados; sin embargo, esto no fue apoyado por los niveles plasmáticos de glicerol, que no fueron significativamente diferentes entre las aves de los dos tipos de hábitat, ni por la masa corporal, que fue mayor para los machos adultos en los humedales naturales. Los niveles plasmáticos de creatina quinasa de *C. minutilla* fueron 75% más altos en los humedales manejados que en los naturales, lo que sugiere que la reparación del daño muscular inducido por el vuelo podría ser más lenta para esta especie en los humedales manejados. La ausencia general de diferencias significativas en los metabolitos del plasma entre aves en los dos tipos de hábitat sugiere que los humedales naturales y manejados podrían servir como sitios de parada igualmente efectivos para las aves playeras migrantes de otoño en la región de Prairie Pothole.

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MIGRATING SHOREBIRDS ARE among the longest-distance migrants of all birds and generally cannot carry enough fuel (primarily fat) to complete the entire migration, so they must stop over at locations along the migratory route to rest and replenish fuel stores (Moore et al. 1995). During migratory preparation and stopover, birds become hyperphagic and are able to rapidly deposit fat (Blem 1990, Berthold and Terrill 1991, Deviche 1995, Kvist and Lindström 2003), which is the preferred fuel for migration because of its high energy density, and birds routinely increase fat stores during migration (McWilliams et al. 2004). Most studies of fattening rates of birds during migration have relied on recaptures to assess changes in both body mass and subcutaneous fat stores. More recently, measures of plasma metabolites have been used to estimate relative rates of fattening and to assess differences in habitat quality (Jenni-Eiermann and Jenni 1994; Williams et al. 1999; Seaman 2004; Guglielmo et al. 2005; Cerasale and Guglielmo 2006b; Anteau and Afton 2008, 2011). Plasma metabolites provide an integrated index of relative fattening rates and changes in energetic condition in the period immediately prior to capture from a single blood sample (Guglielmo et al. 2002, Cerasale and Guglielmo 2006a, Seaman et al. 2006). These measures allow determination of differences in relative fattening rates between sites or between groups of individuals within a site. This is particularly beneficial for birds, such as shorebirds, that have low recapture rates at stopover sites (Thomas 2008).

Rates of fat deposition depend on the quantity and quality of food at a stopover site (Schaub and Jenni 2001, Bairlein 2002). Because some metabolites, such as triglycerides, increase during fat deposition, whereas others, such as β -hydroxybutyrate, increase during fat catabolism, these metabolites can be used to assess between-habitat differences in fattening (Jenni-Eiermann and Jenni 1994, Guglielmo et al. 2005, Anteau and Afton 2011). Guglielmo et al. (2002) demonstrated that plasma triglyceride levels are more variable during stopover than measures of fat catabolism and, therefore, may provide a more precise indicator of differences in relative fattening rates. These measures are now routinely used to measure fattening rates of birds during migration (Guglielmo et al. 2002, 2005; Cerasale and Guglielmo 2006b; Smith and McWilliams 2010).

In addition to the fat stores used to fuel migratory flights, birds also catabolize protein from a variety of sources, including flight muscles and digestive organs (Ramenofsky 1990; Battley 1999; Piersma et al. 1999a, b; Battley et al. 2000, 2001; Bauchinger and Biebach 2001; McWilliams and Karasov 2005; Bauchinger and McWilliams 2010). Birds may also use protein as a source of water during flight, and protein catabolism may also function to eliminate excess muscle as fat stores are reduced during migratory flights (Jenni and Jenni-Eiermann 1998, Pennycuik 1998, McWilliams et al. 2004). In addition to protein mobilization as a fuel source, migrants also incur muscle damage during migratory flights, as indicated by levels of plasma creatine kinase, which is a muscle enzyme usually found only in very small amounts in the blood (Guglielmo et al. 2001). High levels of plasma creatine kinase activity, therefore, are an indicator of muscle damage, such as would occur after a long migratory flight (Guglielmo et al. 2001). Juvenile birds may incur more muscle damage than adults because they lack training prior to their first fall migration (Guglielmo et al. 2001). Repair of such muscle damage may be slower at low-quality stopover sites, thus prolonging residency

periods, so we hypothesize that high plasma creatine kinase levels may indicate relatively slow repair of muscle damage and serve as an indicator of poor habitat quality.

We focused our study of plasma metabolites and creatine kinase levels on midcontinental shorebird migrants because these birds have received little study, especially during fall migration. In eastern South Dakota and western Minnesota, most of the available fall stopover habitat for shorebirds consists of relatively small natural wetlands, the water levels of which can vary markedly within and among years, depending on annual variations in weather conditions (Euliss et al. 1999, Tallman et al. 2002). Historically, wetlands in this region were abundant and widespread, numbering >12 million (van der Valk and Pederson 2003). Losses of these wetlands have been drastic, with more than half now gone, primarily because they were drained for agriculture (Tiner 1984, Dahl 1990, Dahl and Johnson 1991). This loss of habitat has most severely affected the critical ephemeral and seasonal wetlands that make up a large percentage of the total number of wetlands used by migrating shorebirds (Skagen and Knopf 1993, Skagen and Thompson 2000). However, several larger wetland complexes also occur in this region, and these are sometimes managed to serve as stopover habitat for shorebirds, so the potential exists for managed wetlands to at least partially substitute for lost natural wetlands. Birds utilize both types of wetlands during migration, but little work has focused on differences in habitat quality that may occur between natural and managed wetlands (Roush et al. 1997, Elphick 2000, Cole et al. 2002, Thomas 2008).

Ydenberg et al. (2002) demonstrated differences in body mass and plasma metabolites associated with fattening between shorebirds stopping over at large and small mudflats in coastal British Columbia, suggesting that differences that affect stopover ecology may occur between large and small wetland sites. Such differences might also occur along midcontinental migration routes, although both natural and managed sites in our study area occurred largely in an open, agricultural (row crops and pastures) landscape matrix, so that perches for avian predators, which were an important factor in the differences between large and small wetlands in Ydenberg et al.'s (2002) study, are likely much less of a factor at our study sites. We investigated three plasma metabolites of shorebirds during fall stopover in the Prairie Pothole Region of North America. We measured plasma levels of triglycerides (TRIG), glycerol (GLYC), and β -hydroxybutyrate (BUTY). Additionally, we measured plasma creatine kinase (CK) activity as an indicator of muscle damage. Because TRIG levels are higher, BUTY levels lower, and CK activity potentially lower (if muscle damage is repaired more rapidly at high-quality sites) in migrating birds at high-quality than at low-quality stopover sites (e.g., Guglielmo et al. 2001, 2005), we compared these metabolite levels in shorebirds at natural and managed wetlands to assess the relative quality of these habitats for migration stopover. We also expected higher plasma CK levels in juveniles than in adults.

METHODS

We studied Least Sandpipers (*Calidris minutilla*), Semipalmated Sandpipers (*C. pusilla*), and Pectoral Sandpipers (*C. melanotos*) in the Prairie Pothole Region of northeastern South Dakota and west-central Minnesota during fall migration. We used managed wetlands at Big Stone National Wildlife Refuge (BSNWR) in Big

Stone and Lac Qui Parle counties, Minnesota (45°14'N, 96°20'W), as the center of our study area and utilized available natural wetlands within a 50-km radius of BSNWR (Fig. 1). This refuge is managed by the U.S. Fish and Wildlife Service and consists of 8,908 ha, with marsh and water habitat comprising almost 10% (809 ha) of the refuge and separated into nine management pools. BSNWR was established in 1975, and intentional water-level manipulation with the purpose of managing for shorebirds began in 2000. Natural sites included wetlands in Traverse, Big Stone, Stevens, Swift, Chippewa, Lac Qui Parle, and Yellow Medicine counties in Minnesota and Deuel, Grant, Codington, and Roberts counties in South Dakota. Natural wetlands were located using National Wetlands Inventory (see Acknowledgments) data in conjunction with ARCGIS (ESRI, Redlands, California), and suitable wetlands were determined with subsequent ground truthing. Both natural and managed wetlands in our study area occurred in an open, agricultural matrix dominated by row crops and pastures.

We captured birds during 23 July–6 September in 2002, 14 July–9 September in 2003, 19 July–2 September in 2004, and 13 July–30 August in 2005, using 9 × 2.6 m (38-mm mesh) mist nets arranged in “L” and “W” shapes on suitable mudflat foraging habitat. Netting occurred from 30 min prior to sunrise until 0900–1100 hours CST, depending on weather conditions, and again from 1600–1700 hours until 30 min following sunset. Mist nets were used only on days with winds <25 kph and without rain. Blood samples were obtained from birds as soon after capture as possible, but within 5 min of capture for most birds, with some samples collected up to 30 min after capture. The time from

capture until bleeding (hereafter “bleed time”) was recorded, and plasma levels at different times after capture were considered in analyses to determine whether there was an effect of time until bleeding on metabolite levels.

We obtained blood samples by pricking the brachial vein on the underside of the wing with a 26-gauge needle and collecting blood in a 50- μ L heparinized microhematocrit capillary tube (Smith 2003). A blood sample of 50–200 μ L, depending on the size of the individual, was collected from each individual (McGuill and Rowan 1989). Once blood was drawn, isopropyl alcohol and pressure were applied to the pricked vein to stop the bleeding and disinfect the wound. This sampling method and the quantity of blood collected are consistent with guidelines presented in the Ornithological Council’s *Guidelines to the Use of Wild Birds in Research* (Gaunt and Oring 1999) and were approved by the University of South Dakota IACUC. After blood was collected, it was immediately transferred to labeled microcentrifuge tubes and stored on ice while in the field. Upon return from the field location, whole blood was centrifuged for 10 min at 2,000 g and 4°C and the plasma was drawn off and frozen at –20°C for later assays. The maximum period between collection of blood samples and freezing of plasma was 5 h.

Following collection of the initial blood sample, we banded birds with federal bands (U.S. Geological Survey Bird Banding Laboratory, Laurel, Maryland) and measured body mass (M_b ; ± 0.01 g), unflattened wing chord (± 1 mm), narina (distance between proximal end of nares and tip of bill, ± 0.1 mm), tarsus (± 0.1 mm), and fat score (on a scale from 0 to 5; Helms and Drury 1960). Pectoral Sandpipers were sexed by wing measurements, with birds having a wing chord >140 mm assigned as males and those with a wing chord ≤ 140 mm assigned as females (Johnsgard 1981). Measurements were conducted using a portable electronic scale (Ohaus, Pine Brook, New Jersey) and digital calipers (Mitutoyo American, Aurora, Illinois). All three species were aged as hatch-year (juvenile) or after-hatch-year (adult) using plumage characteristics (Hayman et al. 1986, Farmer et al. 2013).

We employed spectrophotometric (Beckman DU 7400) assays using commercially available assay kits to measure plasma metabolite levels. We ran all samples in duplicate and used the mean of the two samples in subsequent analyses. Plasma TRIG and GLYC were measured using Sigma Kit TR0100 (Sigma-Aldrich, St. Louis, Missouri), which is a sequential end-point assay. Free glycerol (GLYC) was measured first, followed by a second reading of glycerol, after digestion of triglycerides, to provide the measure for TRIG ($GLYC_{final} - GLYC_{initial}$). BUTY was measured with a kinetic assay, Autokit 3-HB (Wako Diagnostics, Richmond, Virginia). CK was measured using Wako CK Kit 20R (Wako Diagnostics) or Pointe Scientific CK Kit (Pointe Scientific, Canton, Michigan), which are identical N-acetyl-L-cysteine (NAC)-activated kits. We conducted all assays in 1.5 mL polystyrene UV/Vis semi-micro cuvettes (United Laboratory Plastics, St. Louis, Missouri) according to the manufacturer’s instructions for each kit, using wavelengths of 540 nm for TRIG/GLYC, 405 nm for BUTY, and 340 nm for CK (Swanson and Thomas 2007).

Statistical analyses were performed using SPSS, version 10.0 (SPSS, Chicago, Illinois), or SIGMASTAT, version 3.5 (Systat, Port Richmond, California), with significance accepted as $P < 0.05$. We used ANOVA with Tukey’s HSD post hoc tests to

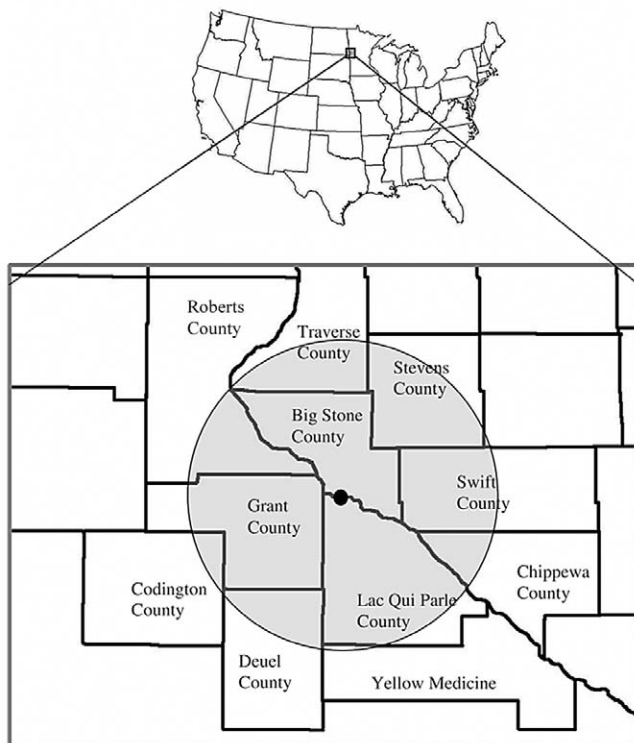


FIG. 1. Detailed location of the 50-km study area (gray circle) and Big Stone National Wildlife Refuge (dark circle in center). The South Dakota–Minnesota border runs diagonally through the study area

compare M_b and fat scores among years, with wetland types and ages analyzed separately for each species. We used least squares regression of M_b and energetic condition (M_b /wing chord) against time of day to determine whether M_b or energetic condition varied temporally over the course of the day. We also applied multivariate analyses for comparisons of M_b , energetic condition, and plasma metabolites, using multiple backward stepwise regression with M_b , energetic condition, and each metabolite as dependent variables. We \log_{10} -transformed values prior to analyses if data were not normally distributed. We conducted separate multiple regression analyses for each species. Independent variables in the original model included capture time, bleed time, Julian date, year, age (0 = adult, 1 = juvenile), habitat type (0 = managed, 1 = natural), energetic condition (defined as body mass/wing chord), and sex (only for Pectoral Sandpipers; 0 = male, 1 = female), and we retained independent variables in the model at $P < 0.10$ (Guglielmo et al. 2005). The variables retained after this regression were maintained as covariates in an analysis of covariance (ANCOVA) to obtain estimated marginal means for plasma metabolites to graphically represent the data and test for differences between habitat types. We also used a multivariate procedure to combine metabolites into a fattening index using principal component analysis followed by multivariate analysis of variance (MANOVA) of PC1 and PC2 scores to test for between-habitat differences among the three species (Guglielmo et al. 2005, Seewagen et al. 2011). We used only TRIG and BUTY for these analyses, because GLYC was not linearly related to TRIG levels (see below). Results are reported as means \pm SE.

RESULTS

We captured a total of 3,077 birds for within-species comparisons of body mass and fat between wetland types (Table 1). We collected blood samples from 440 of these birds (205 Least Sandpipers, 179 Semipalmated Sandpipers, and 56 Pectoral Sandpipers). The distribution of blood-sample collections in relation to time of day was similar in natural (65% morning, 35% evening) and managed (68% morning, 32% evening) wetlands. Plasma samples were analyzed for as many plasma metabolites as possible, but because of low plasma volumes in some samples, not all individuals could be analyzed for all metabolites.

Body mass and condition.—Mean M_b of adult Least Sandpipers (natural: 26.46 ± 0.25 , $n = 296$; managed: 26.48 ± 0.26 , $n = 284$; Table 1) differed from that of juveniles (natural: 24.10 ± 0.20 , $n = 377$; managed: 24.92 ± 0.17 , $n = 667$; Table 1) at both natural ($F = 53.895$, $df = 1$ and 671 , $P < 0.001$) and managed ($F = 25.633$, $df = 1$ and 949 , $P < 0.001$) wetlands. Within adult birds, there was not a mass difference between habitat types ($P = 0.981$); but among juveniles, M_b was heavier for birds at managed sites than for those at natural sites ($F = 9.216$, $df = 1$ and $1,042$, $P = 0.002$). Fat scores of Least Sandpipers were also significantly higher ($P < 0.001$) for birds captured at managed wetlands (2.36 ± 0.06 , $n = 601$) than for those captured at natural wetlands (1.75 ± 0.05 , $n = 671$). Semipalmated Sandpipers exhibited a similar pattern between habitat types, with adult birds (natural 31.22 ± 0.20 , $n = 659$; managed 33.60 ± 0.38 , $n = 214$; Table 1) significantly heavier than juveniles (natural 30.21 ± 0.41 , $n = 161$; managed 31.86 ± 0.34 , $n = 251$; Table 1) at both natural ($F = 5.04$, $df = 1$ and 818 , $P = 0.025$) and managed ($F = 11.55$, $df = 1$ and 463 , $P = 0.001$) wetlands. Differences between habitat types occurred for both age classes in Semipalmated Sandpipers: both adults ($F = 33.73$, $df = 1$ and 871 , $P < 0.001$) and juveniles ($F = 9.46$, $df = 1$ and 410 , $P = 0.002$) were significantly heavier at managed than at natural wetlands. Fat scores of Semipalmated Sandpipers were also significantly higher ($P < 0.001$) for birds at managed wetlands (3.6 ± 0.08 , $n = 363$) than for birds at natural wetlands (2.4 ± 0.05 , $n = 823$). Pectoral Sandpiper mass was analyzed as for the previous species, but also between sexes. Adult male birds were significantly ($F = 4.225$, $df = 1$ and 39 , $P = 0.047$) heavier at natural (102.79 ± 2.96 , $n = 31$; Table 1) than at managed (95.92 ± 5.47 , $n = 8$; Table 1) wetlands, but adult female mass did not differ ($F = 1.019$, $df = 1$ and 125 , $P = 0.31$) between natural (72.04 ± 1.31 , $n = 77$; Table 1) and managed (70.79 ± 1.84 , $n = 39$; Table 1) wetlands. Neither males ($P = 0.59$) nor females ($P = 0.07$) had significantly different fat scores between wetland types. Juvenile birds were captured in low numbers ($n = 13$), which did not allow for a thorough comparison, but no significant difference was found between sexes ($P = 0.91$) in juvenile birds.

For simple linear regression of body mass and energetic condition against time of day, both M_b and energetic condition were significantly positively correlated with time of day in Semipalmated Sandpipers (M_b : $r = 0.193$, $P = 0.004$; energetic condition: $r = 0.217$, $P = 0.001$). However, neither M_b nor

TABLE 1. Mean (\pm SE) body mass (g) by age, wetland type, and sex for Least, Semipalmated, and Pectoral sandpipers captured in Minnesota and South Dakota during autumn migration, 2002–2005.

| | | Adult | | Juvenile | |
|------------------------|--------|-------------------------------|-----------------------------------|-----------------------------|-------------------------------|
| | | Natural | Managed | Natural | Managed |
| Least Sandpiper | | 26.5 ± 0.2^a $n = 296$ | 26.5 ± 0.3^a $n = 284$ | 24.1 ± 0.2 $n = 377$ | 24.9 ± 0.2^b $n = 667$ |
| Semipalmated Sandpiper | | 31.2 ± 0.2^a $n = 659$ | $33.6 \pm 0.4^{a,b}$ $n = 214$ | 30.2 ± 0.4 $n = 161$ | 31.9 ± 0.3^b $n = 251$ |
| Pectoral Sandpiper | Male | 102.8 ± 3.0^c $n = 31$ | 95.9 ± 5.5 $n = 8$ | – | 66.9 ± 1.7 $n = 2$ |
| | Female | 72.0 ± 1.3 $n = 77$ | 70.8 ± 1.8 $n = 39$ | 83.0 ± 0 $n = 1$ | 66.5 ± 3.5 $n = 10$ |

^a Adults significantly ($P < 0.05$) heavier than juveniles within habitats.

^b Birds significantly ($P < 0.05$) heavier at managed than at natural sites.

^c Birds significantly ($P < 0.05$) heavier at natural than at managed sites.

energetic condition varied significantly with time of day for Least or Pectoral sandpipers (M_b : Least Sandpiper, $r = 0.070$, $P = 0.231$; Pectoral Sandpiper, $r = 0.029$, $P = 0.813$; energetic condition: Least Sandpiper, $r = 0.074$, $P = 0.208$; Pectoral Sandpiper, $r = 0.049$, $P = 0.685$).

Multiple regression analyses of M_b in Least Sandpipers identified capture date, age (lower M_b in juvenile birds), and size (both tarsus and wing) as significant effectors of M_b . Date and age (lower in juvenile birds) significantly influenced energetic condition for Least Sandpipers (Table 2). M_b in Semipalmated Sandpipers was significantly related to capture time, capture date, age (lower in juvenile birds), and size (both tarsus and wing). Similarly, energetic condition of Semipalmated Sandpipers was significantly related to capture time, capture date, and age (lower in juvenile birds; Table 2). Only sex significantly influenced M_b and energetic condition in Pectoral Sandpipers, with males having both higher M_b and higher energetic condition than females (Table 2). Habitat type was not a significant effector of either M_b or energetic condition in multiple regression analyses, which suggests that between-habitat differences documented by univariate analyses are explained by variation in other effectors of M_b and energetic condition.

Fat deposition.—In multiple regression analyses, date and energetic condition were significant effectors of plasma TRIG in Least Sandpipers (Table 2). Age, date, bleed time, energetic condition, and year were significant effectors of plasma TRIG in Semipalmated Sandpipers (Table 2). In Pectoral Sandpipers, year and habitat (higher at managed wetlands) significantly influenced plasma TRIG (Table 2). When these effectors were retained in subsequent ANCOVAs, no significant difference between plasma TRIG levels for birds at natural and managed wetland types was evident for Least Sandpipers ($F = 1.81$, $df = 1$ and 155 , $P = 0.180$) or Semipalmated Sandpipers ($F = 0.23$, $df = 1$ and 137 , $P = 0.631$; Fig. 2). Pectoral Sandpipers showed a significant difference in plasma TRIG levels between birds in natural and managed wetlands using ANCOVA with year as a covariate ($F = 8.59$, $df = 1$ and 26 , $P = 0.007$), with birds from managed wetlands having 2.6× higher values (Fig. 2).

Fat catabolism.—For multiple regressions, date and year were significant effectors of plasma GLYC in Least Sandpipers (Table 2). Date of capture, capture time, bleed time, and habitat significantly influenced plasma GLYC for Semipalmated Sandpipers,

but for Pectoral Sandpipers no variables significantly influenced plasma GLYC (Table 2). ANCOVA models revealed no significant between-habitat differences in plasma GLYC for any species (Least Sandpiper, $F = 0.66$, $df = 1$ and 162 , $P = 0.417$; Semipalmated Sandpiper, $F = 2.01$, $df = 1$ and 143 , $P = 0.251$; Pectoral Sandpiper, $F = 1.13$, $df = 1$ and 27 , $P = 0.297$) (Fig. 3).

Multiple regression analyses of plasma BUTY levels revealed energetic condition and bleed time as significant effectors for Least Sandpipers (Table 2). For Semipalmated Sandpipers, capture time, capture date, year, and age were all significant effectors (Table 2). No variables significantly affected plasma BUTY levels in Pectoral Sandpipers (Table 2). ANCOVA detected no significant differences in plasma BUTY between birds in the two wetland types for Least Sandpipers ($F = 1.20$, $df = 1$ and 90 , $P = 0.277$) or Semipalmated Sandpipers ($F = 1.52$, $df = 1$ and 148 , $P = 0.219$) (Fig. 4). There were not enough Pectoral Sandpipers from managed wetlands to include them in analyses.

Principal component analyses and MANOVAs that combined TRIG and BUTY levels into a common index of fattening performance gave generally similar results to individual plasma metabolites, with no between-habitat differences detected for any species (all $P > 0.28$).

Relationships among plasma metabolites.—Plasma TRIG and BUTY levels were significantly negatively correlated for Semipalmated Sandpipers ($r = 0.227$, $P = 0.016$), but not for Least ($r = 0.263$, $P = 0.052$) or Pectoral ($r = 0.254$, $P = 0.479$) sandpipers, although the Least Sandpiper correlation was nearly significant and the sample size was small for Pectoral Sandpipers (Fig. 5). Linear regressions showed that plasma levels of TRIG and GLYC were significantly positively correlated for Semipalmated Sandpipers ($r = 0.272$, $P = 0.002$), but not for Least ($r = 0.088$, $P = 0.282$) or Pectoral sandpipers ($r = 0.345$, $P = 0.078$). However, quadratic (U-shaped) equations fit the data better for all three species (Least Sandpiper, $r = 0.318$, $P < 0.001$; Semipalmated Sandpiper, $r = 0.333$, $P < 0.001$; Pectoral Sandpiper, $r = 0.687$, $P < 0.001$) (Fig. 6).

Creatine kinase.—For Least Sandpipers, plasma CK levels were significantly influenced by bleed time, capture time, and habitat (higher levels in managed habitats) (Table 2). Semipalmated Sandpipers demonstrated significant effects of bleed time, energetic condition, and year on plasma CK levels (Table 2). In Pectoral Sandpipers, plasma CK was not significantly correlated with any variables. Interestingly, age did not significantly

TABLE 2. Variables retained in the multiple regression models following backward stepwise regression at the $P < 0.10$ level for each species and metabolite. Abbreviations: LESA = Least Sandpiper, SESA = Semipalmated Sandpiper, PESA = Pectoral Sandpiper, TRIG = triglycerides, GLYC = glycerol, BUTY = β -hydroxybutyrate, CK = creatine kinase, Time = time of day of capture, Bleed = bleed time following capture, Habitat = type of wetland habitat (0 = managed, 1 = natural), EC = energetic condition, Wing = wing chord, Sex (0 = male, 1 = female), Age (0 = adult, 1 = juvenile). Direction of effect, if applicable, is indicated by (+) for positive effect and (–) for negative effect.

| Variable | LESA | SESA | PESA |
|---------------------|-------------------------------|--|------------------|
| TRIG | Date(+), EC(+) | Age(–), Date(+), EC(+), Year, Bleed(–) | Habitat(–), Year |
| GLYC | Date(+), Year | Date(+), Bleed(+), Time(–), Habitat(–) | – |
| BUTY | EC(–), Bleed(+) | Age(+), Date(–), Time(–), Year | – |
| CK | Bleed(+), Time(–), Habitat(–) | Year, Bleed(+), EC(–) | – |
| M_b | Date(+), Age(–), Wing(+) | Date(+), Age, Wing(+), Time(+) | Sex(–) |
| Energetic condition | Date(+), Age(–) | Date(+), Age(–), Time(+) | Sex(–) |

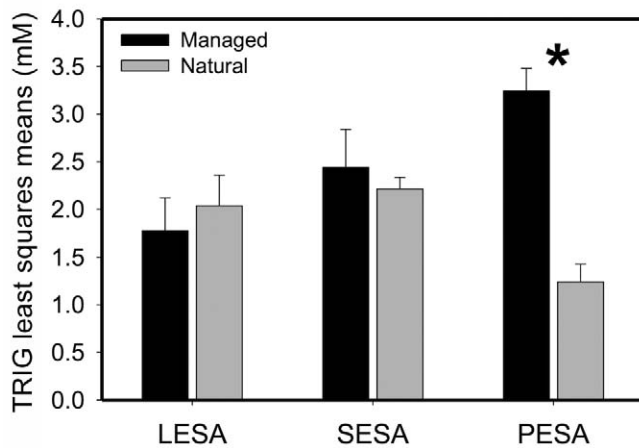


FIG. 2. Least square means of plasma triglycerides from ANCOVA (with covariates as in Table 2) for three species of shorebirds captured in Minnesota and South Dakota during autumn migration, 2002–2005. Abbreviations: LESA = Least Sandpiper, SESA = Semipalmated Sandpiper, and PESA = Pectoral Sandpiper. A significant difference ($P < 0.05$) is indicated by an asterisk above the difference.

influence plasma CK levels in any of the three species. In Least Sandpipers, a significant difference was found between birds in the two habitat types by ANCOVA ($F = 13.39$, $df = 1$ and 117 , $P < 0.001$; Fig. 7), with 75% higher levels at managed wetlands. Neither Semipalmated Sandpipers ($F = 2.38$, $df = 1$ and 89 , $P = 0.127$; Fig. 7) nor Pectoral Sandpipers ($F = 1.59$, $df = 1$ and 38 , $P = 0.216$; Fig. 7) showed a significant difference in plasma CK levels between natural and managed wetlands.

DISCUSSION

We found few significant differences in plasma metabolites between birds in natural and managed habitats. The only significant differences detected were for plasma triglycerides in Pectoral

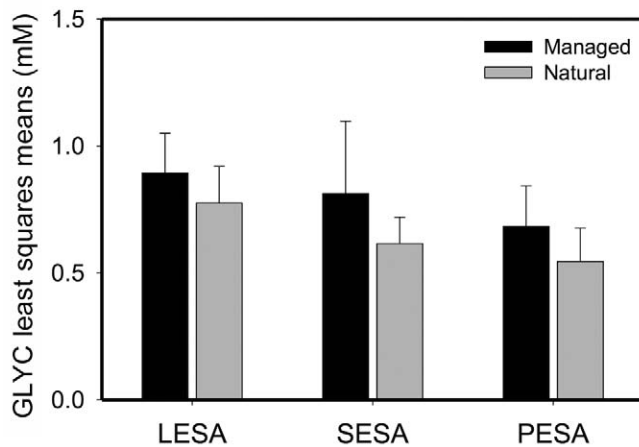


FIG. 3. Least square means of plasma glycerol from ANCOVA (with covariates as in Table 2) for three species of shorebirds captured in Minnesota and South Dakota during autumn migration, 2002–2005. For abbreviations, see Figure 2.

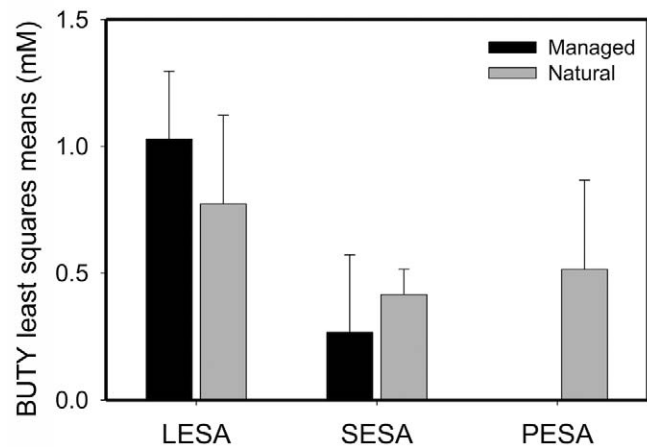


FIG. 4. Least square means of plasma β -hydroxybutyrate from ANCOVA (with covariates as in Table 2) for three species of shorebirds captured in Minnesota and South Dakota during autumn migration, 2002–2005. Sample sizes for Pectoral Sandpipers from managed wetlands were not sufficient to calculate mean values. For abbreviations, see Figure 2.

Sandpipers (higher in birds from managed than from natural sites) and plasma creatine kinase levels in Least Sandpipers (higher at managed wetlands). Because other studies have shown that plasma TRIG is the best overall indicator of fattening rates (Guglielmo et al. 2002, Seaman et al. 2006, Smith and McWilliams 2010), elevated triglyceride levels at managed wetlands suggest that this habitat type may result in higher fattening rates for Pectoral Sandpipers. However, this increase was not accompanied by lower rates of fat catabolism (i.e., lower plasma GLYC), as often occurs for differences in habitat suitability (Guglielmo et al. 2005, Lyons et al. 2008, Seewagen et al. 2011), and habitat did not significantly influence M_p or energetic condition in multiple regression analyses, so these data only partly support the idea that managed wetlands serve as better stopover habitat for Pectoral Sandpipers. The higher levels of plasma creatine kinase at managed wetlands for Least Sandpipers suggest that flight-induced muscle damage is repaired more slowly at managed than at natural sites, which could be interpreted as showing that natural sites serve as better stopover habitat for this species (Guglielmo et al. 2001). However, fat deposition and catabolism data from Least Sandpipers in our study do not support this conclusion, so these data provide little consistent evidence for differences in the suitability of stopover sites. In summary, the general absence of between-habitat differences in plasma metabolite levels and the lack of a consistent direction for the few differences detected for three species of shorebirds and four plasma metabolites suggest that natural and managed wetlands provide similar stopover-site quality for these birds during fall migration. Moreover, energetic condition was significantly positively correlated with plasma TRIG in both Least and Semipalmated sandpipers in our study, which suggests that birds increase their energetic condition by fattening at both wetland habitat types and that both habitats provide suitable habitat for replenishing fat stores in these species (Guglielmo et al. 2002).

Previous work suggests that of the fat catabolites measured here, BUTY is a better indicator than GLYC of fat catabolism in

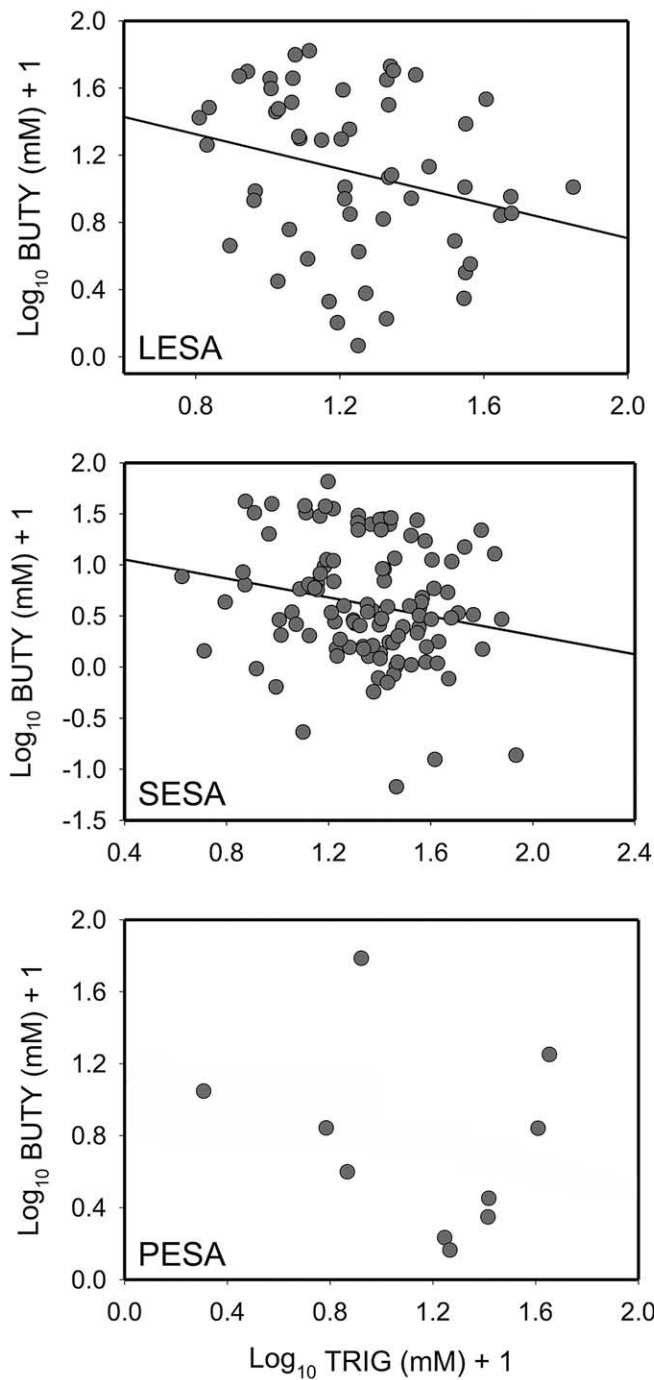


FIG. 5. Linear regressions of plasma β -hydroxybutyrate versus plasma triglycerides (both transformed as Log_{10} mM + 1) for three species of shorebirds captured in Minnesota and South Dakota during autumn migration, 2002–2005. All three species showed negative relationships, but the relationship was significant only for Semipalmated Sandpipers ($P = 0.016$), although nearly significant ($P = 0.052$) for Least Sandpipers. For abbreviations, see Figure 2.

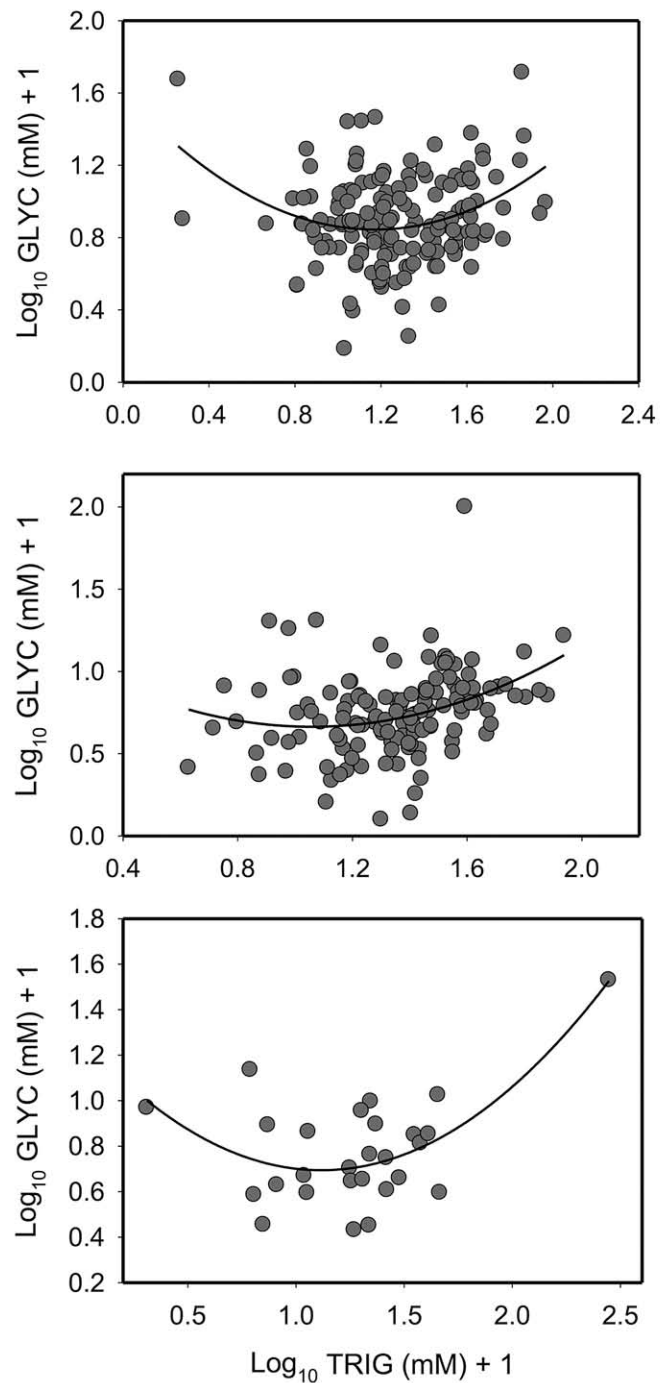


FIG. 6. Nonlinear regressions of plasma glycerol versus plasma triglycerides (both transformed as Log_{10} mM + 1) for three species of shorebirds captured in Minnesota and South Dakota during autumn migration, 2002–2005. All three species showed significant U-shaped relationships. Predictive equations for the three species were as follows: LESA, $y = -0.199 - 0.125x + 0.527x^2$; SESA, $y = -0.344 - 0.169x + 0.683x^2$; and PESA, $y = -0.348 - 0.062x + 0.465x^2$. For abbreviations, see Figure 2.

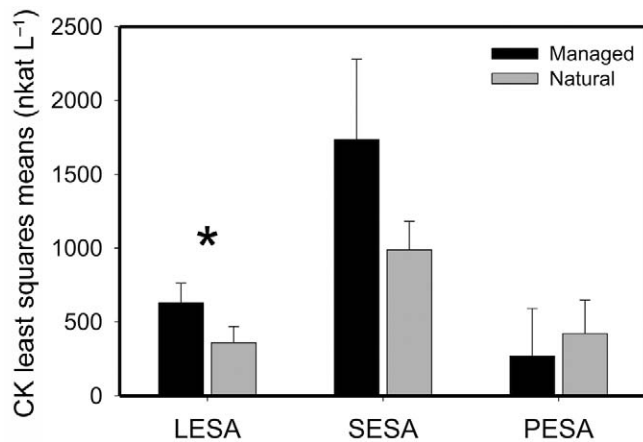


FIG. 7. Least square means of plasma creatine kinase (CK) activity from ANCOVA (with covariates as in Table 2) for three species of shorebirds captured in Minnesota and South Dakota during autumn migration, 2002–2005. For abbreviations, see Figure 2. A significant difference ($P < 0.05$) is indicated by an asterisk above the difference.

migrant birds (Guglielmo et al. 2005, Cerasale and Guglielmo 2006b, Seaman et al. 2006). In our study, as in Guglielmo et al. (2005), BUTY was consistently negatively correlated with TRIG, whereas GLYC showed U-shaped relationships with TRIG (Figs. 5 and 6). We found that no fat catabolites differed significantly within any species between the two habitats. The absence of between-habitat differences in plasma fat catabolites corroborates our results for plasma TRIG and, collectively, these data strongly suggest that natural and managed wetlands in the Prairie Pothole Region provide similarly suitable stopover habitat for migrating shorebirds.

The time required to increase fat stores prior to migratory departure can be viewed as a determinant of stopover-site quality. If fat deposition rates are low, birds may move among available habitats to seek better-quality habitat. Avian predators may also influence stopover habitat quality and habitat use for shorebirds, especially in areas where perch sites adjacent to wetland areas are abundant (Warnock and Bishop 1998; Ydenberg et al. 2002, 2004; Lank et al. 2003; Pomeroy 2006). Additionally, shorebirds may move among wetland stopover sites to balance tradeoffs of predation danger and fattening rate as their energetic condition changes, with fat birds moving to low-quality and/or low-danger sites before departure (Ydenberg et al. 2002). In our study region, however, woodlands are scarce, comprising <2.5% of the total landscape (Castonguay 1982), so perches for avian predators near wetlands were uncommon, and we observed few raptors at either wetland type during our study (N. E. Thomas pers. obs.). In addition, radiotracking and color-banding studies of birds using our study sites showed very limited movement among wetlands, and birds generally did not move from one wetland type to another prior to departure (Thomas 2008). Because many interior-migrating shorebirds typically migrate in short hops between successive stopovers, they spend less time refueling and may carry smaller fat stores than coastally migrating species that engage in longer migratory flights (Piersma 1987, Skagen 2006). Consequently, balancing tradeoffs between fattening rates and danger from avian predators seems likely to play a lesser role in shaping habitat-use patterns in the Prairie Pothole Region

than in other regions where woodlands form a greater component of the landscape.

The general absence of between-habitat differences in plasma metabolite levels contrasts with differences in raw M_b and fat scores between the two wetland types within sex or age classes for all three sandpiper species. This discrepancy suggests that birds are fattening at similar rates in the two habitats, but nevertheless maintain M_b differences. However, no between-habitat differences in body mass or energetic condition persisted after multiple regression analyses, which indicates that these differences can be attributed to variation in other factors influencing M_b and energetic condition (Table 2).

Age was a prominent effector of metabolite levels, M_b , and energetic condition in Least and Semipalmated sandpipers, with adult birds generally showing higher M_b and energetic condition in both species, and higher levels of metabolites associated with fat deposition and lower levels of metabolites associated with fat catabolism in Semipalmated Sandpipers. Adult birds precede juvenile birds at our study sites for these species, so higher levels of fattening in adults than in juveniles could result from greater prey availability earlier in the season (Adamus 1995), and/or increased foraging efficiency because of previous migratory experience (Groves 1978, Jones et al. 2002, Heise and Moore 2003).

Age was not a significant effector of plasma creatine kinase levels for any of the three study species. In a previous study of coastally migrating Western Sandpipers (*Calidris mauri*), plasma CK was significantly higher in juvenile birds than in adults even though they did not differ in body mass, fat load, or muscle size (Guglielmo et al. 2001). Guglielmo et al. (2001) suggested that these differences may occur because of increased muscle damage in juvenile birds on their first migration. In our study, juvenile birds were lighter than adults, which could have reduced any flight-induced muscle damage from long-distance flights. In addition, because of the short-hop migratory strategy of most midcontinental shorebirds (Piersma 1987, Warnock 2010), shorter migratory flights may have dampened any age-related differences in muscle damage compared with coastally migrating species.

The positive effect of date on plasma TRIG levels, M_b , and energetic condition in multiple regressions for Least and Semipalmated sandpipers (along with the negative effect of date on plasma BUTY in Semipalmated Sandpipers), suggests that, after accounting for age effects, later-migrating birds were fattening at higher rates than birds earlier in the fall migration season. Peak numbers of birds at our study sites occur in August and decline thereafter (Thomas 2008), so reduced competition for food resources could contribute to the positive effect of date on fattening and energetic condition. Year was also a common effector of plasma metabolite levels, suggesting some variation in fattening rates among years, potentially resulting from differences in the amount of available habitat or prey among years (Thomas 2008).

Bleed time was positively correlated with plasma CK levels in both Least and Semipalmated sandpipers in our study, with higher CK levels at longer bleed times. This positive relationship of plasma CK with bleed time is consistent with results from previous studies (Guglielmo et al. 2001). TRIG (negatively) and BUTY (positively) also often vary with bleed time in migrants (Guglielmo et al. 2002). Such trends are also generally consistent with our data (Table 2), despite the limited amount of variation in the timing of sample collection in our study (most samples were collected within 5 min of capture).

Natural wetlands undergo annual and long-term cyclic patterns of filling and drying, and such cycles act to increase productivity and provide high-quality habitat (Mitsch and Gosselink 1986, Weller 1987, Euliss et al. 1999), but similar cycling does not occur at managed wetlands. Thus, we expected that if a difference in stopover habitat quality existed between natural and managed wetlands, greater rates of fat deposition would occur at natural wetlands as a function of their higher productivity. BSNWR is a relatively young refuge (1975), and wetlands on the refuge had been undergoing intentional water fluctuations for 2 years prior to our study. However, because control of the water upstream of the refuge was limited, periodic changes in water level occurred before the period when intentional shorebird management began. Our data suggest that the managed wetlands at BSNWR serve as high-quality stopover sites during fall migration for Calidrine sandpipers and provide sufficient habitat to allow fattening rates similar to those for nearby natural wetlands.

Because of the drastic declines of natural wetlands from anthropogenic alterations over the past century, the likely loss of additional wetlands with climate change, and the impacts of other anthropogenic factors such as contaminants in runoff recharging these wetlands, information regarding the suitability of stopover sites is critical for successful conservation of midcontinent shorebird migrants (Dahl 1990, Poiani and Johnson 1991, Johnson et al. 2005). Our data demonstrate that anthropogenic wetlands provide effective stopover habitat that can serve to supplement natural wetlands for migrating shorebirds in the midcontinent. Because shorebirds are strongly dependent on habitat availability during stopover, drawdowns of managed wetlands that coincide with peak migration dates would maximize available habitat area and, potentially, improve habitat quality. Additionally, appropriately timed drawdowns would provide additional stopover sites in a landscape that is losing wetland complexity and where distances between high-quality stopover sites are increasing, so such a practice could be greatly beneficial to midcontinent shorebird migrants (Skagen and Knopf 1993, Farmer and Parent 1997, Johnson et al. 2005).

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