ORNITOLOGIA NEOTROPICAL 19 (Suppl.): 641–651, 2008

PLASMA CHOLINESTERASES FOR MONITORING PESTICIDE EXPOSURE IN NEARCTIC-NEOTROPICAL MIGRATORY SHOREBIRDS

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Resumen. – Colinesterasas en el plasma para monitorear la exposición a pesticidas en aves playeras migratorias. – Los plaguicidas organofosfatados (OP) y carbamatados (CB) son productos agroquímicos de uso común en el hemisferio occidental. Estos plaguicidas han causado mortalidad en aves migratorias y producido efectos fisiológicos adversos en pruebas realizadas con especies de aves cautivas. Los chorlos y playeros migratorios utilizan una variedad de hábitats cuando pasan el invierno en la zona templada de Sudamérica y durante su migración a través de las Grandes Llanuras de los Estados Unidos. Los hábitats con alto riesgo de exposición incluyen arrozales y campos de cultivo de césped en los que se utilizan productos agroquímicos. La colinesterasa (ChE) es un indicador biológico específico para monitorear la exposición a OP y CB y se puede medir usando simples procedimientos de laboratorio. La actividad de ChE en el plasma es útil como método no letal de monitorear la exposición aviar a los plaguicidas OP y CB. Muchas variables pueden afectar la actividad enzimática y no siempre es posible realizar ensayos de reactivación; por lo tanto, los valores de referencia de ChE son un componente necesario del monitoreo de

la exposición. Durante la migración hacia el norte del 2006, tomamos muestras de cuatro especies de aves playeras de altiplanicie y cinco especies de aves playeras de humedales en tres estados de Norteamérica, caracterizando y midiendo los niveles de ChE en el plasma en todas las especies. Las especies de cuerpo pequeño tienen niveles más altos de actividad de ChE en el plasma que las especies de cuerpo grande. La acetilcolinesterasa (AChE), la enzima cuya inhibición lleva a los efectos de envenenamiento, muestra menos variación entre las especies que la butirilocolinesterasa (BChE). La actividad de ChE en el plasma mostró variación según la fecha de captura en tres de cinco especies. Diferencias por sexo fueron significativas en una de las dos especies testeadas. Nuestra investigación presenta valores referenciales de colinesterasa para aves playeras migratorias y provee el marco para futuros estudios ecotoxicológicos en especies de aves playeras Neotropicales y Neárticas.

Abstract. – Organophosphorus (OP) and carbamate (CB) pesticides are commonly used agrochemicals throughout the Western Hemisphere. These pesticides have caused mortalities in migratory birds and adverse physiological effects in trials with captive birds. Migratory shorebirds use a variety of habitats during the austral summer in temperate South America and during migration through the Great Plains of the United States. Habitats where risk of exposure is high include rice fields and turf grass farms where agrochemicals are used. Cholinesterase (ChE) is a specific biomarker for monitoring OP and CB exposure and can be measured using standard laboratory procedures. Plasma ChE activity is useful as a non-lethal means of monitoring avian exposure to OP and CB pesticides. Many variables can affect enzyme activity and reactivation assays are not always possible, thus reference ChE values are a necessary component of monitoring exposure. During northbound migration in 2006, we sampled four upland and five wetland shorebird species at four pesticide-free sites in North America, characterizing and measuring plasma ChEs in all shorebird species. Small-bodied species had higher levels of ChE activity in plasma than large-bodied species. Acetylcholinesterase (AChE), the enzyme whose inhibition leads to poisoning symptoms, showed less inter-specific variation than butyrylcholinesterase (BChE). Plasma ChE activities varied with date of capture in three of five species. Sex differences were significant in one of two species tested. Our baseline ChE values for migratory shorebirds provide a framework for future ecotoxicological studies of Nearctic-Neotropical migrant shorebirds. *Accepted 20 December 2007*.

Key words: Carbamate, cholinesterase, ecotoxicology, organophosphate, waders.

INTRODUCTION

Organophosphates (OPs) and carbamates (CBs) averaged 68% of insecticide active ingredients used in the United States from 1980 through 2001 (Kiely *et al.* 2004). The use of OPs and CBs increased in the 1970's after organochlorine pesticides (e.g., DDT) were banned due to health and environmental hazards (e.g., Henny & Bennett 1990). OPs and CBs provide an alternative to the environmental persistence and bioaccumulation of organo-chlorines (Blus 2003). In spite of their limited persistence in the environment, many of these chemicals are highly toxic to avian species and incidental kills of migratory birds are well documented (Basili & Temple 1995, Goldstein *et al.* 1999a). Mass mortality incidents have resulted in public awareness campaigns that emphasized the toxicity of OP and CB pesticides, and in some countries, lead to laws against the use and manufacture of some of these pesticides (Hooper *et al.* 1999, Hooper *et al.* 2003).

Although many highly toxic OPs and CBs are prohibited or highly regulated in the Americas (Anonymous 2004, USEPA 2007), instances of mortalities and high level exposures have been reported recently (Pain *et al.* 2004, Wobeser *et al.* 2004). Furthermore, less toxic OPs and CBs continue to be used in agriculture throughout North and South America. For example, chemicals that inhibit cholinesterase (ChE) are part of the rice culti-

vation industry in Uruguay and Argentina (Garamma *et al. fide* Blanco *et al.* 2006, MEZ pers. comm.). In the United States, OPs and CBs are recommended for pest control on a variety of crops including rice and turf grass (Fagerness *et al.* 2001, Merchant 2005, Way & Cockrell 2007).

As part of their annual journey between breeding and non-breeding ranges, migratory shorebirds cross international boundaries in search of available stop-over habitat. With the loss of natural wetlands and grasslands (Knopf 1994, Skagen 2006), shorebirds are forced into human-altered habitats. Rice fields and turf grass farms provide important alternative wintering and migratory stopover habitats for shorebirds (Twedt *et al.* 1998, Corder 2005, Blanco *et al.* 2006, Robbins 2007), but also represent potential exposure to ChE-inhibiting chemicals (Flickinger *et al.* 1986).

Although ChE activity has traditionally been measured by destructive sampling of brain tissue, bird populations can be effectively monitored for OP and CB exposure using non-lethal methods by measuring ChE activities in blood plasma (Hooper *et al.* 1989, Thompson 1991).

Acetylcholinesterase (AChE), an important enzyme in the central and peripheral nervous systems, is responsible for the hydrolysis of the neurotransmitter acetylcholine (ACh), at the nerve–nerve or nerve–effector interface. Without hydrolysis, ACh accumulates in the synapse, disrupting neurotransmission, impairing behavior and physiology, and eventually leading to death (Grue *et al.* 1997, Goldstein *et al.* 1999a). Plasma ChE activity can demonstrate exposure levels consistent with intoxication and death in subsets of a population (Hooper *et al.* 1989, Goldstein *et al.* 1999a), as well as a lack of exposure (Goldstein *et al.* 1999b).

Comparison of ChE activity from field samples to reference values can be used alone or in conjunction with reactivation assays. Poisoning by OPs and CBs produces similar physiological effects but reactivation assays allow for differentiation between these two types of poisonings. Reactivation assays also address potential concerns associated with inter-species or inter-individual ChE variation (Grue 1982, Hill 1989, Fossi *et al.* 1996). However, reference values of ChE activity are especially important if reactivation assays cannot be used because sample volumes are too small or because OP aging results in chemically stable OP–enzyme bonds (Wilson *et al.* 1992).

Here, we present reference values of plasma ChE activity for apparently healthy, free-living individuals of nine shorebird species that use upland and wetland habitats. To describe ChE activity within and among shorebird species, we tested five factors that are known to affect ChE activity in other birds: interspecific variation with regard to body mass and intraspecific variation with regard to sex, body condition and date and time of capture. Our estimates of plasma ChE activity are among the first values published for shorebirds and will be useful as reference values in future toxicological studies of Nearctic-Neotropical migratory shorebirds.

METHODS

Shorebird capture. Shorebird capture occurred in three states (Texas, Kansas, and Nebraska) in the United States, and three countries (Paraguay, Argentina, and Uruguay) in South America from April through December 2006. The subset of data used for baseline plasma ChE analysis included individuals captured between 22 April and 1 June 2006 during northbound migration in the United States at protected wetlands and grasslands. Data from individuals captured in South America were not included in plasma ChE analyses but contributed to mean mass calculations. Northbound migration capture sites included

Anahuac National Wildlife Refuge, Chambers County, TX (29°34'N, 94°32'W), Quivira National Wildlife Refuge, Stafford County, KS (38°08'N, 98°29'W), Konza Prairie Biological Station, Riley County, KS (39° 04'N, 96°33'W), and Kissinger Wildlife Management Area, Clay County, NE (40°26'N, 98°06'W). In 2006, rice production at Anahuac National Wildlife Refuge was strictly organic, and there were restrictions on pesticide application around Quivira National Wildlife Refuge boundaries (M. Whitbeck pers. com., USEPA 2006). Konza Prairie and Kissinger Wildlife Management Area are natural preserves that were also pesticide free (E. Horne and R. Souerdyke pers. com.). Shorebirds were live-captured using mist nets, night-lighting, and drop nets, under applicable state and federal research permits.

Sample collection and preparation. Mass of live-captured birds was measured using a Pesola spring scale (± 1.0 g). Wing length was measured with a wing rule (± 0.5 mm). Total head, culmen and tarsus length were measured using vernier calipers (± 0.1 mm). All birds were fitted with a USFWS metal band with a unique number. When possible, shorebirds were sexed in the field according to Prater *et al.* (1977). Upland Sandpipers (*Bartramia longicauda*) were sexed using molecular markers based on the CHD gene (Baker *et al.* 1999, A. E. Casey unpubl.).

Blood was collected using a 27-gauge needle and heparinized capillary tubes (70 µL) from the brachial vein of the wing. Total blood collected per bird ranged between two to six capillary tubes (140–420 µL) and was < 1% of the bird's body mass (Gaunt *et al.* 1999). Blood samples were transferred to 0.5 mL screw cap cryovials, stored on wet ice in the field, and centrifuged within 8 hours to separate plasma from red blood cells. Plasma samples were stored at –20°C for less than one month and transferred to –80°C until laboratory analysis could be conducted. All samples were assayed within one year of collection.

Laboratory analysis. Samples were thawed immediately before ChE activity determination. As a first step, six plasma samples from each species were pooled for characterization of optimal enzyme dilution and reagent (acetylthiocholine-iodide [AThCh] and tetra-isopropyl pyrophosphoramide [iso-OMPA]) concentrations. ChE activity was determined using the method of Ellman *et al.* (1961) as modified by Gard & Hooper (1993) for use in a 96-well spectrophotometric plate reader (Molecular Devices, Palo Alto, CA) with Softmax Pro software (Molecular Devices, Palo Alto, CA). Final volume of each assay was 250 µL and contained the following components: 0.05 M final concentration (FC) of Trizma buffer (pH 8.0), 3.23×10^{-4} M FC of 5,5-dithio[bis-2-nitrobenzoic acid] (DTNB), diluted enzyme sample, and 1.00×10^{-3} M FC of AThCh. To separate butyrylcholinesterase (BChE) from AChE, samples were incubated with the BChE-specific inhibitor iso-OMPA at FCs between 1.0×10^{-4} M and 1.0×10^{-5} M according to the characterization of each species. BChE was calculated as the difference between total cholinesterase (TChE) and AChE activity in the presence of iso-OMPA. All samples were run in triplicate at 25°C with the spectrophotometer set in kinetic mode. Absorbance was measured at 412 nm at 15 s intervals for 180 s with 0 s lag time. ChE activities were converted from absorbance units per min to µmoles AThCh hydrolyzed per min (units) per milliliter of plasma using an extinction coefficient of 13,600 $(\text{cm} \times \text{M})^{-1}$.

Statistical analysis. All statistical analyses were conducted using procedures of SAS (ver 9.1, SAS Institute, Cary, NC, USA). All ChE activ-

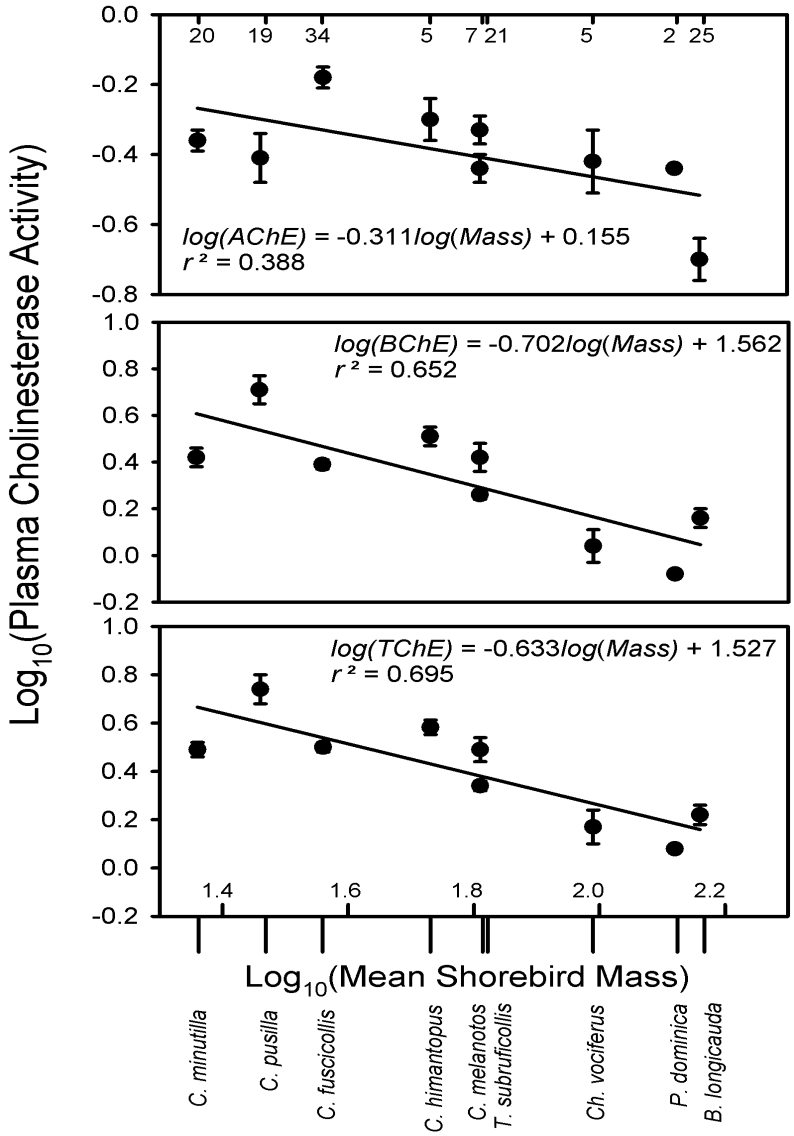

FIG. 1. Log_{10}-log_{10} plot showing the relationship between mean body mass and plasma ChE activity in nine species of shorebirds captured during migration in the Great Plains of the United States. Sample sizes are inside the uppermost x-axis and error bars represent ± SE. Pectoral Sandpiper (*Calidris melanotos*) is the higher of the two log_{10}(ChE) values where log_{10}(body mass) = 1.81.

ities fell within ± 3 SD of the mean except for two TChE and BChE values for the Least Sandpiper (*Calidris minutilla*) which were over 4.5 times the mean for this species. These two outliers were removed from subsequent analysis. All data presented are in raw form but statistical results are based on log_{10}-transformed data to correct for allometric scaling. General

TABLE 1: Descriptive statistics of ChE activity (μmol AThCh hydrolysed/min per mL plasma) for nine shorebird species sampled during northbound migration in the Great Plains of the United States including sample size of individuals (n)†, mean, standard deviation (SD), minimum (min) and maximum (max) values.

Species	n	TChE				AChE				BChE			
		Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
American Golden-Plover	2	1.20	0.01	1.20	1.21	0.37	0.00	0.37	0.37	0.83	0.01	0.83	0.84
Killdeer	5	1.56	0.55	0.91	2.42	0.41	0.16	0.21	0.59	1.15	0.45	0.69	1.89
Upland Sandpiper	25	1.81	0.70	0.69	3.29	0.24	0.14	0.02	0.65	1.57	0.67	0.51	3.11
Buff-breasted Sandpiper	21	2.22	0.43	1.71	3.22	0.40	0.19	0.16	0.89	1.83	0.31	1.43	2.56
Pectoral Sandpiper	7	3.22	0.80	1.53	4.01	0.48	0.12	0.32	0.66	2.74	0.72	1.21	3.35
White-rumped Sandpiper	34	3.29	0.80	1.95	5.05	0.72	0.38	0.30	2.31	2.56	0.69	1.53	3.99
Stilt Sandpiper	5	3.82	0.67	2.89	4.72	0.52	0.16	0.37	0.69	3.31	0.60	2.45	4.03
Least Sandpiper	20	3.25	0.94	1.20	4.67	0.45	0.13	0.27	0.81	2.80	0.89	0.81	4.16
Semipalmated Sandpiper	19	6.38	2.95	1.28	10.78	0.46	0.22	0.07	0.89	5.92	2.85	1.21	10.11

†Total sample size. Estimates of TChE and BChE exclude two outliers from Least Sandpipers.

linear models (Proc GLM) were used to determine the relationship of plasma ChEs among species using a single factor fixed effects ANOVA. Regression models (Proc REG) were calculated for plasma ChEs and time of capture, date of capture, and body condition for species with ≥ 15 samples. For those species where sex could be reliably determined, sex differences in plasma ChEs were compared using a Student's t-test (Proc TTEST). Time of capture was divided into four time periods of 6-h blocks each according to the following criteria: 1 = 0–05:59 h, 2 = 06:00–11:59 h, 3 = 12:00–17:59 h, and 4 = 18:00–23:59 h. A multivariate index of body condition was computed by regressing the mass of each individual at capture on PC1 from principal components analysis (PCA), using the residuals as an index of body condition. PCA analyses were based on four morphological measurements, total head, culmen, wing, and tarsus, and were calculated separately for each species. PC1 explained between 34% and 66% of the variation in the four morphometrics. PC1 was an index of body size because all eigenvectors were positive in seven of nine species; in the remaining two species one eigenvector was negative (K. M. Strum unpubl.). Average mass for each species was calculated using a larger dataset of captured birds that included the subset used in ChE analysis. All tests were two-tailed and considered significant at an α–level ± 0.05 after Bonferroni correction for the number of tests (Rice 1989).

RESULTS

During northbound migration, we captured 174 individuals from 16 shorebird species, and obtained sufficient plasma for ChE analysis from 138 individuals of nine species. All samples were used in analysis of AChE activity and after removing two outliers from Least Sandpiper BChE and TChE activity, 136 samples were used. We calculated average body mass for these nine species from captures of 511 individuals at migratory and nonbreeding sites throughout the Western Hemisphere. Our study species included: American Golden-Plover (*Pluvialis dominica*), Killdeer (*Charadrius vociferus*), Upland Sandpiper, Buff-breasted Sandpiper (*Tryngites subruficollis*), Pectoral Sandpiper (*Calidris melanotos*), White-

TABLE 2: Trends in plasma ChE's of five shorebird species as a function of date of capture, time of capture and an index of body condition using $\log_{10}$ transformed ChE activity. After sequential Bonferroni correction for number of tests, test statistics were considered significant at an α–level of 0.05 if $P < 0.002$.

Species	ChE type	Date of capture			Time of capture			Index of body condition		
		df	*F*	*P* ≤	df	*F*	*P* ≤	df	*F*	*P* ≤
Upland Sandpiper	log(AChE)	1,23	0.0	0.956	1,23	0.9	0.359	1,23	1.8	0.193
	log(BChE)	1,23	8.8	0.007	1,23	0.0	0.836	1,23	0.2	0.640
Buff-breasted Sandpiper	log(AChE)	1,19	0.0	0.891	1,19	0.3	0.858	1,19	0.2	0.692
	log(BChE)	1,19	2.2	0.152	1,19	0.8	0.373	1,19	0.4	0.550
White-rumped Sandpiper	log(AChE)	1,32	4.2	0.048	1,32	0.1	0.750	1,32	0.0	0.876
	log(BChE)	1,32	2.5	0.123	1,32	0.2	0.653	1,32	0.3	0.599
Least Sandpiper	log(AChE)	1,18	0.1	0.817	1,16	0.6	0.452	1,18	0.5	0.498
	log(BChE)	1,16	6.8	0.019	1,14	0.0	0.886	1,16	0.0	0.977
Semipalmated Sandpiper	log(AChE)	1,17	0.1	0.736	1,17	0.3	0.615	1,16	0.0	0.926
	log(BChE)	1,17	3.1	0.097	1,17	0.1	0.715	1,16	0.7	0.419

rumped Sandpiper (*Calidris fuscicollis*), Stilt Sandpiper (*Calidris himantopus*), Least Sandpiper, and Semipalmated Sandpiper (*Calidris pusilla*).

TChE and BChE were highly correlated ($r^2 = 0.984$, $P < 0.001$, n = 136). TChE and AChE were also significantly correlated ($r^2 = 0.533$, $P < 0.001$, n = 136) though less variation in TChE could be explained by AChE. Results are reported for BChE and AChE only. TChE values for comparisons to other studies can be obtained by combining our AChE and BChE values provided that substrate, substrate concentration, and assay temperature are identical. Plasma BChE activity varied negatively with body size ($F_{8,127} = 20.3$, $P < 0.001$) as did AChE ($F_{8,129} = 11.0$, $P < 0.001$, Fig. 1). Mean AChE ranged from 0.24 units/mL (± 0.14 SD, n = 25) in Upland Sandpipers, to 0.72 (± 0.38 SD, n = 34) in White-rumped Sandpipers, whereas mean BChE ranged from 0.83 (± 0.01 SD, n = 2) in American Golden-Plovers to 5.92 (± 2.85 SD, n = 19) in Semipalmated Sandpipers (Table 1). Values for Least Sandpiper outliers were BChE: 15.68 and 19.66, TChE: 16.04 and 20.17. Both of these individuals were females and had longer than average wing chord (≥ 100 mm).

Sex differences in plasma ChEs were evaluated in two species, Semipalmated and Upland sandpipers. Mean BChE was lower in male Upland Sandpipers (1.28 ± 0.54 SD, n = 13) than females (1.89 ± 0.67 SD, n = 12, $t_{23} = 2.60$, $P = 0.016$). However, mean AChE was not significantly different between male (0.20 ± 0.07 SD, n = 13) and female Upland Sandpipers (0.28 ± 0.19 SD, n = 12, $t_{14.9} = 0.50$, $P = 0.615$ [unequal variance]). Similarly, mean plasma ChEs did not differ between male (AChE: 0.55 ± 0.25 SD, n = 7; BChE: 5.70 ± 2.95 SD, n = 7) and female Semipalmated Sandpipers (AChE: 0.41 ± 0.19 SD, n = 12; BChE: 6.04 ± 2.92 SD, n = 12, AChE: $t_{17} = –1.02$, $P = 0.324$, BChE: $t_{17} = –0.06$, $P = 0.956$).

In four species, the relationship between plasma ChEs and date of capture, time of capture and body condition were analyzed. Three species showed trends in ChE activity as a function of capture date. Levels of BChE activity increased throughout the capture period in Upland Sandpipers ($r^2 = 0.276$, $F_{1,23} = 8.8$, $P = 0.007$) and Least Sandpipers ($r^2 =$

0.298, $F_{1,16} = 6.79$, $P = 0.019$), whereas levels of AChE increased throughout the capture period in White-rumped Sandpipers ($r^2 = 0.117$, $F_{1,32} = 4.24$, $P = 0.048$). Trends were marginally significant in all three species after Bonferroni corrections for the number of tests (Rice 1989). Other components of plasma ChE did not vary with capture period in any of these species (Table 2). There was no significant relationship between time of capture or body condition for any species tested (Table 2).

DISCUSSION

Interspecific variation in plasma BChE activity decreased with increasing shorebird mass similar to results from a study of plasma ChEs in European raptors (Roy *et al.* 2005). Mass-specific metabolic demands decrease as shorebird body size increases (Kvist & Lindström 2001), which may be a partial explanation for the inverse relationship between shorebird plasma ChE activity and body mass. Based on the high correlation between TChE and BChE, most of the variation in shorebird TChE can be attributed to BChE activity. BChE has been shown to successfully buffer AChE inhibition from some OP chemicals (Leopold 1996, Parker & Goldstein 2000). Birds lack A-esterases which hydrolyze OP and CB pesticides (Aldridge 1953) and higher levels of BChE activity may provide some protection against poisoning and information about exposure.

Inclusion of all ChE activity results is important when presenting baseline ChE values, however extreme outliers may influence statistical tests. For this reason, we removed two outliers from our dataset before analysis. The causes of extreme BChE activity were unknown but the two individuals with outlier values could have had liver damage or unusual levels of lipid metabolism during migration (Rattner & Fairbrother 1991, Valle *et al.* 2006).

Due to interspecific variation in ChE activity with regard to body size, our data can be used to estimate normal plasma ChEs of species without reference values for field sample comparison. While there is no substitute for species-specific reference values, patterns of mass-specific variation in plasma ChE activity provide an initial framework for assessing exposure in other shorebird species.

We found sex differences in mean plasma BChE in one species, the Upland Sandpiper. At the time of capture, females were heavier than males (female mean mass = 166 g, n = 12; male mean mass = 136 g, n = 13) and would be expected to have lower plasma ChE activities based on the interspecific results of this study. However, female Upland Sandpipers had higher mean BChE activity than males. Our results may be related to breeding condition because Upland Sandpipers evaluated in this study had recently arrived on the breeding grounds and many females were at an egg-laying stage (B. K. Sandercock unpubl.). An increase in plasma ChEs during egg-laying has been reported in other avian species (Rattner & Fairbrother 1991). Samples of Upland Sandpipers during the nonbreeding season as well as samples from males and females of other shorebird species on the breeding grounds are needed to further investigate this idea.

The condition of individual shorebirds was not related to plasma ChE activity in our study. This is an important result since the physiological stress of migration can result in inter-individual variation in body condition depending on the time since arrival at a stopover site and the distance traveled prior to capture. Individuals in better condition presumably have more fat and muscle translating into larger relative mass (Schulte-Hostedde *et al.* 2005), unrelated to ChE activities. However, birds that died from anti-ChE exposure had lower fat and muscle scores due to reduced food intake after poisoning (Grue

1982). In our study, body condition was not used as an indicator of chemical exposure but it might be in another study.

Increases in plasma ChEs were marginally significant in three species throughout the capture period. In each case, the variation explained in plasma BChE was fairly low ($r^2 < 0.3$). Seasonal variation in mean plasma ChEs has been detected in other migratory birds and has been attributed to changes in diet (Goldstein *et al.* 1999b). In shorebirds, variation in plasma ChEs during the capture season could be due to changes in diet or to changes in physiological condition caused by changes in organ size during migration (Piersma & Gill 1998). Further investigation of the relationship between ChEs and date will be conducted using data from individuals sampled in South America. With larger datasets from additional sites, seasonal patterns in ChE activity may be more apparent.

The new data presented here provide a starting point for understanding variation in plasma ChEs in Nearctic-Neotropical shorebirds. Future analyses should be conducted with samples collected at non-breeding sites in South America as well as the breeding grounds. The relationship of plasma ChEs to environmental covariates should be further explored to provide a more complete picture of shorebird plasma ChEs throughout the annual cycle. Data on ChE activity could then be used to assess shorebird exposure to ChE-inhibiting pesticides at any time of year. Once exposure is determined, efforts could be focused on affected species to evaluate if the level of exposure poses a population threat. If so, efforts could begin on developing regulations for OP and CB pesticides through partnerships with local and international governments. If future studies demonstrate that shorebird exposure to ChE-inhibitors is limited, this information will be used to redirect research efforts into other possible causes of shorebird population declines.

ACKNOWLEDGMENTS

For access to field sites and logistical support, we thank Matt Whitbeck and the staff of Anahuac National Wildlife Refuge; Dave Hilley and Jim Sellers and the staff of Quivira National Wildlife Refuge; Joel Jorgensen from Nebraska Game and Parks Commission and John McCarty and LaReesa Wolfenbarger at the University of Nebraska at Omaha. Ashley Casey, Tara Conkling, Kyle Gerstner, Kate Goodenough, Karl Kosciuch and USDA Wildlife Services Office of Manhattan, Kansas provided field assistance in North America. Laura Addy, Natalia Bossel, Noelia Calamari, Julieta Decarre, Andrea Goijman, Benito Jaubert, Leandro Macchi, Laura Solari, and Romina Suarez aided with field sampling in South America. Chuck Otte from Kansas State University Extension Office provided assistance with pesticide use information. Stephanie Jones and Suzanne Fellows of the U.S. Fish and Wildlife Service provided logistical support for the project. Funding for field work and lab analyses was provided by grants from the Neotropical Migratory Bird Conservation Act (NMBCA) and Migratory Bird Management (MBM) programs of the U.S. Fish and Wildlife Service.

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