

January 24, 2008

SUP Application for St. Vincent's NWR

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Project Title: “Assessing the importance of horseshoe crab (*Limulus polyphemus*) eggs in the diets of migrating red knots (*Calidris canutus rufa*) and sanderlings (*Calidris alba*) during refueling stops on selected Florida beaches”

Project Duration: 2008-2010

Project Season: Feb 15-May 31

Project Summary: The project's main goals are to look at the diet content, habitat use, and body condition of migratory red knots and sanderlings and to examine these factors' relationship to horseshoe crab egg availability and abundance. Please see attached SWG grant proposal. This grant was successfully funded and all research objectives are based on this grant.

During each season (late Feb-May) for all three study regions combined (Panhandle, Cedar Key and Tampa Bay) we propose to band up to 500 birds, sample blood from up to 200 birds for plasma metabolite analysis, and collect diet samples from up to 100 birds. St. Vincent's NWR would possibly comprise between 5-10% of these total numbers. These sample sizes were selected based on our ability to gain statistical significance from our data and the feasibility of capturing birds. We would also be conducting shorebird surveys on a regular basis, but this would not interfere with the normal behaviors of the birds. Additionally we would be collecting sediment core samples from areas where birds are foraging/captured.

Methodology: We will capture birds using cannon nets (and possibly mist nets). Birds will be banded with a standard USFWS band on the lower leg, a individual-specific flag on the upper leg, and a blue Darvic band on the opposite upper leg (to identify it as being banded in FL). We will bleed birds from the brachial vein using a small lance and capillary tube. We will sample diet using a warm-water crop flush. All birds will be released alive and as quickly as possible.

Possible benefits to St. Vincent's NWR: This project will provide accurate and thorough biological information about migratory shorebird diet and habitat use on St. Vincent's. It could provide managers additional information when making land-use decisions. It should not place a significant burden on refuge staff as I have all the equipment necessary to conduct field activities (including a boat for transportation). Additionally if there are any activities (such as posting or other short term refuge maintenance) that my technician and I can help with please let me know as we would be happy to assist.

Application Cover Page 2006

PROJECT TITLE: Assessing the importance of horseshoe crab (*Limulus polyphemus*) eggs in the diets of migrating red knots (*Calidris canutus rufa*) and sanderlings (*Calidris alba*) during refueling stops on selected Florida beaches

Principal Investigator	Authorizing Official
Name: Dr. Anne McMillen-Jackson Department: Fish and Wildlife Research Institute (FWRI) Institution: Fish & Wildlife Conservation Commission Address: 100 8 th Ave. SE Address: City: St. Petersburg State: FL Zip: 33701 Telephone: (727) 896-8626 E-mail: anne.jackson@myfwc.com	Name: Gil McRae Department: Director, FWRI Institution: Fish & Wildlife Conservation Commission Address: 100 8 th Ave. SE Address: City: St. Petersburg State: FL Zip: 33701 Telephone: (727) 896-8626 E-mail: gil.mcrae@myfwc.com

COOPERATORS:

Name	Affiliation
Janell Brush	FWC/FWRI/Avian Research Program
Susan Gerhart	FWC/FWRI/Crustacean Fisheries Research
Amy Schwartzer	Univ. of Florida/Cooperative Fish & Wildlife Research Unit
Nancy Douglass	FWC/Habitat and Species Conservation
Jim Rodgers	FWC/ FWRI/Avian Research Program
Franklin Percival	Univ. of Florida/Cooperative Fish & Wildlife Research Unit
Jaime Collazo	North Carolina State Univ./Cooperative Fish & Wildlife Research Unit

In the space provided, demonstrate how this project addresses the criteria included in the Grants Cycle Notice for Florida's State Wildlife Grants Program (Cite the specific location in the Comprehensive Wildlife Conservation Strategy where this topic or action is discussed)

1. The Strategy cites as a priority the collection of accurate and up-to-date biological and ecological information for Species of Greatest Conservation Need (SGCN) to fill data gaps (Florida's Comprehensive Wildlife Conservation Strategy [CWCS], pg. 48, Priority Data Gaps). This project directly addresses this issue for multiple SGCN: red knot (*Calidris canutus rufa*), sanderling (*Calidris alba*), and horseshoe crab (*Limulus polyphemus*). Trends for these three species are declining or unknown. The beach/surf zone is essential habitat for these species – shorebird foraging and horseshoe crab nesting – but subject to considerable stress and habitat loss from coastal development (CWCS, pg. 128, Beach/Surf Zone). Migrating shorebirds rely almost solely on horseshoe crab eggs for nutrition at refueling beaches in other regions of the eastern U.S., and conservation and management regulations are based on this ecological dependence. In this study, we also will collect data on parameters related to migratory shorebird fitness with regard to habitat use and body condition. Understanding the inter-relationship among these parameters yields a tractable, quantifiable basis to define habitat quality for migratory shorebirds in Florida, and thus, a better foundation upon which to formulate management recommendations.
2. We will provide a 50:50 non-federal match to grant funds (see budget for match details).
3. Cooperators on this project are from the following institutions: FWC/FWRI Crustacean Fisheries and Avian Research groups; FWC Habitat and Species Conservation section, University of Florida/Cooperative Fish & Wildlife Research Unit, North Carolina State University/Cooperative Fish & Wildlife Research Unit
4. This project will follow the state's fiscal year, and will not exceed three years in duration.
5. This project is feasible, logical, and has a high probability of success. The field and lab work is straightforward and uses standard procedures. The personnel involved are experienced in shorebird and horseshoe crab research. The cooperators have conducted related research in other species or locations, and are eager to contribute their expertise.

PROGRAM:

Are you submitting this project for funding to Florida's Nongame Wildlife Grants Program? (type YES or NO): Yes

PROJECT SUMMARY

PROJECT TITLE: Assessing of the importance of horseshoe crab (*Limulus polyphemus*) eggs in the diets of migrating red knots (*Calidris canutus rufa*) and sanderlings (*Calidris alba*) during refueling stops on selected Florida beaches

GRANT PERIOD: (July 1 – June 30) Start date (MM/DD/YYYY): End date (MM/DD/YYYY):
(maximum 3 years) 07/01/2007 06/30/2010

NEED:

Shorebirds constitute an ecologically and economically valuable segment of Florida's avifauna, and many are listed as Species of Greatest Conservation-Need in Florida's Comprehensive Wildlife Conservation Strategy, including the red knot and sanderling. Successful migration, and subsequent reproduction, in these species depends heavily on food availability at refueling stops. In other areas of the U.S. east coast, horseshoe crab eggs are the major food item at refueling stops for red knots, sanderlings, and other migrating shorebirds, and this relationship is a core issue in federal regulations on horseshoe crab harvests. Anecdotal evidence suggests that the shorebird/horseshoe crab association is also important on Florida beaches. Empirical validation of this relationship is essential for the successful conservation of these species in Florida because habitat loss due to coastal development affects both shorebirds and horseshoe crabs.

OBJECTIVE(s):

1. Conduct shorebird surveys to determine size and species composition of the shorebird flocks on selected beaches.
2. Capture and color-band red knots and sanderlings to determine intra- and inter-annual beach usage.
3. Determine what proportion of the diet of captured red knots and sanderlings is composed of horseshoe crab eggs.
4. Estimate density of horseshoe crab eggs and other shorebird prey items on selected beaches.
5. Assess the refueling value of selected Florida beaches to red knots and sanderlings by measuring levels of plasma metabolites in captured birds.

EXPECTED BENEFITS:

The project proposed herein is designed to quantify how levels of prey base (i.e., horseshoe crab eggs), influence two sets of parameters intimately related to shorebird fitness: habitat use, defined here as intra-annual and inter-annual patterns (e.g., residency rates, apparent annual survival rates); and patterns of fat deposition and its influence on body condition. Understanding the inter-relationship among these parameters yields a tractable, quantifiable basis to define habitat quality for migratory shorebirds in Florida, and thus, a better foundation upon which to formulate management recommendations. The proposed multi-species approach is the essence of ecosystem management and conservation, a focus of Florida's CWCS. Finally, the findings of this project provide an opportunity to gain insights of migratory shorebird ecology and conservation at larger geographic scales by comparing study results with similar studies conducted in other areas of the U.S. and on other shorebird species.

APPROACH:

To determine the use of horseshoe crab spawning beaches as shorebird foraging habitat, we will conduct surveys at two-week intervals over the migration period to identify and quantify shorebirds. Human activity will also be quantified because shorebird habitat use is influenced by levels of activity. For each survey period, red knots and sanderlings will be captured using mist nets, and color-banded. Within-season habitat use will be determined by estimating residency rates, or the probability that a bird marked in the beaches of interest remain over time. Examination of stomach contents will be used to estimate what proportion of the diet of red knots and sanderlings is composed of horseshoe crab eggs, and this data will be compared to densities of horseshoe crab eggs and other prey derived from cores of beach sediments. We will assess the refueling value of selected Florida beaches to red knots and sanderlings by measuring plasma metabolites levels from blood samples of captured birds. Surveys will occur at three locations over three years, to account for intra- and inter-annual variation in bird and horseshoe crab egg densities.

LOCATION (Statewide, County, local, etc.): Gulf Coast of Florida

AREA OF INTEREST OR TARGET SPECIES: red knot (*Calidris canutus rufa*), sanderling (*Calidris alba*), horseshoe crab (*Limulus polyphemus*)

ESTIMATED COST:

Requested Grant: \$ _112,158_____
Match (50% of total): \$ _114,933_____
Total: \$ _227,091_____

Sources of match: Salary, equipment, equipment-operation expenses

PROJECT DESCRIPTION

INTRODUCTION

Shorebirds constitute an ecologically and economically valuable segment of Florida's avifauna, and many are listed as Species of Greatest Conservation Need [SGCN] in Florida's Comprehensive Wildlife Conservation Strategy (CWCS; <http://myfwc.com/wildlifelegacy/strategy.html>), including the red knot (*Calidris camutus rufa*; Status: medium, Trend: declining; a candidate for federal Endangered Species status) and sanderling (*Calidris alba*; Status: unknown, Trend: unknown). These two species have both winter-resident and transient populations that follow the Atlantic Flyway and stop on Florida beaches to feed *en route* to their breeding grounds.

Successful migration, and subsequent reproduction, depend heavily on food availability at refueling stops (Krapu et al. 2006). In other areas of the U.S. east coast, horseshoe crab (*Limulus polyphemus*) eggs are the major food item at refueling stops for red knots, sanderlings, and other migrating shorebirds (Tsipoura and Burger 1999). This relationship was a core issue in the development of federal regulations on horseshoe crab harvests (<http://www.asmf.org/>). Anecdotal evidence suggests that the shorebird/horseshoe crab association is also important on Florida Gulf coast beaches. Empirical validation is essential for the successful conservation of these species in Florida because habitat loss due to coastal development affects both shorebirds and horseshoe crabs (SGCN with a declining trend).

The project proposed herein is designed to quantify how levels of prey base (i.e., horseshoe crab eggs), influence two sets of parameters intimately related to shorebird fitness. The first set pertains to habitat use, defined here as intra-annual and inter-annual patterns (e.g., residency rates, apparent annual survival rates). The second is patterns of fat deposition and its influence on body condition. Understanding the inter-relationship among these parameters yields a tractable, quantifiable basis to define habitat quality for migratory shorebirds in Florida, and thus, a better foundation upon which to formulate management recommendations. The proposed multi-species approach is the essence of ecosystem management and conservation, a focus of Florida's CWCS. Finally, the findings of this project provide an opportunity to gain insights of migratory shorebird ecology and conservation at larger

geographic scales by comparing study results with similar studies conducted in other areas of the U.S. (Karpanty et al. in press) and on other shorebird species (Lyons et al. in review).

OBJECTIVES

1. Conduct shorebird surveys to determine size and species composition of the shorebird flocks on selected beaches.
2. Capture and color-band red knots and sanderlings to determine intra- and inter-annual beach usage.
3. Determine what proportion of the diet of captured red knots and sanderlings is composed of horseshoe crab eggs.
4. Estimate density of horseshoe crab eggs and other shorebird prey items on selected beaches.
5. Assess the refueling value of selected Florida beaches to red knots and sanderlings by measuring levels of plasma metabolites in captured birds.

METHODS

Survey and field sampling of shorebirds

Study beaches will be located near the eastern Panhandle, Cedar Key, and Tampa Bay. Chosen areas will have high densities of migratory shorebirds and horseshoe crab spawning sites. We will survey shorebirds at two-week intervals over the migration period at low tides. Surveys will be conducted by boat and/or land, depending on beach topography and accessibility, using a double-observer approach to adjust counts by detection probability (Nichols et al. 2000). All shorebird species will be identified and counted. Human activity will also be quantified because waterbird habitat use, including shorebirds, is influenced by levels of activity (e.g., Stolen 2002). Data will be coded to reflect levels of human activity (e.g., low, medium, high). Data will be analyzed using a repeated-measures ANOVA where beach and week will be treated as class variables. Beach will be treated as a random effect and beach*week the repeated measure. Also at two-week intervals, red knots and sanderlings (up to 100/species/sampling occasion) will be captured using mist nets (12 m x 2.6 m, 32-mm mesh size). Each bird will be weighed and aged, and color-banded according to the protocols of the Patuxent Wildlife Research Center's Bird Banding Laboratory (<http://www.pwrc.usgs.gov/bbl/>). Birds will be held no longer than two hours and

released alive. During scheduled surveys, all color-marked birds will be tallied by location. Within-season habitat use will be determined by estimating residency rates, or the probability that a bird marked in the beaches of interest remain over time. Depending on data characteristics, data could be analyzed treating all beaches as one sampling unit (e.g., global residency rate), or as different strata and analyzed using a multi-state approach (e.g., probability of being on one beach and moving to another, remaining on the same beach, or moving and returning between sampling occasions). Prey levels and a code for human density on the beaches will be used as covariates. At the end of three seasons, data will be used to estimate apparent annual survival rates, that is, the probability that a bird banded in any of the study beaches study will survive, return, and be available for detection. Data analyses will be conducted using program MARK (White 1999).

Food habits and Plasma Metabolites

During banding occasions, a subset of birds will be selected to ascertain food habits and plasma metabolites. Only birds weighing 40 g or greater will be used to minimize any energetic drawback caused by our work on the short-term energetic balance of the bird. To determine food habits, we will obtain the stomach contents from up to five individuals of each species using a warm water crop flush (Rosenberg and Cooper, 1990, Tsioura and Burger 1999). Samples will be preserved in 10% ethanol. Each prey type will be counted and volumes estimated. From the same birds, we will collect blood samples for plasma metabolite work. Plasma metabolite levels (triglycerides, glycerol, and β -hydroxybutyrate) indicate whether a bird is gaining or losing mass at the time of sampling, and thus are an indication of the refueling value of the location. Blood samples (up to 200 μ L) will be collected in heparinized capillary tubes and stored on ice in the field, centrifuged at 5,000 rpm for 10 min., and the plasma stored at -20°C until analysis. Plasma metabolite levels will be analyzed by staff in the Department of Pathobiology, College of Veterinary Medicine, University of Florida, using manufacturers' protocols with modifications for small volumes of plasma and 96-well microplates (Williams et al. 1999). Data analyses will follow procedures used by Lyons et al. (in review).

Determination of densities of horseshoe crab eggs and other potential shorebird prey

After each capture and sampling of shorebirds, we will collect core samples to analyze densities of horseshoe crab eggs and other potential prey in beach sediments (James-Pirri et al. 2004, Karpanty et al. in press). We will collect ten cores (5-cm diameter, 0-20-cm depth) evenly spaced across the feeding area of the flock. Each of these cores will be divided into surface (0-5 cm) and deep (5-20 cm) portions and all ten samples of each portion will be combined. Three 80-ml aliquots will be removed from both shallow and deep portions and preserved in 95% ethanol. We will quantify and estimate volumes for horseshoe crab eggs and other shorebird prey species in the shallow samples as representative of readily available food sources. Only horseshoe crab eggs will be quantified from the deep samples, as representative of potential food source; horseshoe crabs lay their eggs 15-20 mm below the surface of the sand, and eggs work their way higher in the sand column as a result of wave action and subsequent horseshoe crab spawning activity. Data will be analyzed using a repeated measures ANOVA where beach and week will be treated as class variables. Sampling location will be treated as a random effect, and beach*week the repeated measure.

References

- James-Pirri MJ, Tuxbury K, Marino S, Koch S. 2005. Spawning densities, egg densities, size structure, and movement patterns of spawning horseshoe crabs, *Limulus polyphemus*, within four coastal embayments on Cape Cod, Massachusetts. *Estuaries* 28: 296-313.
- Karpanty, SM, Fraser JD, Berkson J, Niles LJ, Dey A, Smith EP. In press. Horseshoe crab eggs determine red knot distribution in Delaware Bay. *Journal of Wildlife Management*.
- Krapu GL, Eldridge JL, Gratto-Trevor CL, Buhl DA. 2006. Fat dynamics in arctic-nesting sandpipers during spring and mid-continental North America. *Auk* 123: 323-334.
- Lyons JE, Collazo, JA, Guglielmo CG. In review. Plasma metabolites and migration physiology of semiplumated sandpipers: refueling performance at five latitudes.
- Nichols JD, Hines JE, Sauer JR, Fallon FW, Fallon JE, Heglund PJ. 2000. A double-observer approach for estimating detection probability and abundance from point counts. *Auk* 117: 393-408.

BIOGRAPHICAL SKETCHES

Anne McMillen-Jackson, Ph.D. (Principal Investigator): Dr. McMillen-Jackson has a B.S. in Biology (Simmons College Boston, MA, 1978), a M.S. in Biology (University of Houston, Houston, TX, 1989), and a Ph.D. in Biology (University of South Florida, Tampa, FL, 2003). She is leader of the FWC Fish and Wildlife Research Institute's Crustacean Fisheries research group, and has 15 years experience conducting crustacean-related research and consulting on management-related issues for crustacean fisheries. She has conducted horseshoe crab research since 2000, and coauthored a poster entitled 'Identification of potential horseshoe crab (*Limulus polyphemus*) spawning sites in four Florida estuaries using GIS' at the 2004 Benthic Ecology Meeting. She represented FWC on the Atlantic States Marine Fisheries Commission Horseshoe Crab Technical Committee from 2000 to 2005, and currently is the FWRI representative to the Gulf States and Atlantic States Marine Fisheries Commissions' crustacean work groups. Dr. McMillen-Jackson manages two federal and state grants, and is co-principal investigator on a third grant.

Janell M. Brush (Co-Project Director): Ms. Brush has a B.S. in Biology (University of Nebraska, 1998) and a M.S. in Wildlife Ecology and Conservation (University of Florida, FL, 2006). As a Project Manager/Research Assistant for the University of Florida Cooperative Fish and Wildlife Research Unit (2000-2006), she managed research projects in the Florida Everglades, Central Florida, and the Savannah River Delta, Georgia. These projects included research on demography and nesting of Snail Kites; neotropical migratory bird utilization along a salinity gradient; and the effects of management activities on the use patterns and abundance of wetland dependent avifauna. She joined the Florida Fish and Wildlife Conservation Commission in 2006 as an Avian Research Biologist. She has experience in designing research projects, managing budgets, hiring and training personnel, supervising field work, organizing and analyzing data, writing reports and technical papers, and presenting data at international conferences.

Susan D. Gerhart (Co-Project Director): Mrs. Gerhart has a B.A. in Zoology (University of Florida, 1984) and a M.S. in Marine Science (University of South Florida, 1990). She is a Biological Scientist II with the FWC Fish and Wildlife Research Institute's Crustacean Fisheries research group. She has 11 years of experience in crustacean research, including projects on horseshoe crabs, stone crabs, and blue crabs. She became coordinator of the FWRI Horseshoe Crab Spawning Survey in 2003 and represents FWC on the Atlantic States Marine Fisheries Commission Horseshoe Crab Technical Committee. She has a FWRI Technical Report in review entitled "A Review of the Biology and Management of Horseshoe Crabs, with Emphasis on Florida Populations." Current research studies include: timing of spawning and mating behavior of horseshoe crabs in Tampa Bay; distribution and characteristics of horseshoe crab populations in Florida; population dynamics and fisheries biology of stone crabs in Tampa Bay; size at sexual maturity of stone crabs.

Amy C. Schwarzer (Graduate Research Assistant): Ms. Schwarzer has a B.A. in Environmental Science (University of Florida, 2003). Since 2000, she has conducted field research on a variety of birds and other wildlife. During the period April 2004 to January 2005, she participated in studies of shorebirds and horseshoe crabs at Monomoy National Wildlife Refuge in Massachusetts, acquiring skills and knowledge directly applicable to the research proposed herein. Since May 2006, she has been employed as a Biological Technician with the University of Florida's Cooperative Fish and Wildlife Cooperative Unit. Her research experience includes coordinating and conducting field activities; training and supervising personnel; managing data; writing reports; and handling, banding, and bleeding birds.

BUDGET

Salaries and Wages

We are requesting two OPS positions for this project: a graduate research assistant and a field technician. Amy Schwarzer will fill the graduate research assistant position; she will be a master's degree graduate student under Franklin Percival of the University of Florida and Jaime Collazo of the North Carolina State, and will conduct the work under supervision of Janell Bush at the FWRI office in Gainesville. She will work full time during the spring and summer (Jan. 1 – Aug. 31) to plan and conduct the field sampling; analyze stomach content and sediment samples collected in the field; input and proof all data; analyze data as required; and write progress reports. The field technician is yet to be identified; that person will work full time only during the spring sampling season (Mar.1 – May 31). These two people will participate in all sampling trips, and will be responsible for collecting, preserving, and storing all samples. Ms. Schwarzer will also be responsible for processing the samples, analyzing data, and assisting in writing reports. The salaries for these two positions are appropriate for these types of scientific positions. Other agency personnel, including Dr. Anne McMillen-Jackson, Janell Brush, Susan Gerhart, and James Rodgers will assist with field work as necessary, particularly during bird capture and sampling.

Fringe Benefits

Fringe benefits have been calculated for A. McMillen-Jackson, J. Brush, and J. Rodgers according to the FWRI fringe benefits spreadsheet for 2005-2006. Fringe benefits for OPS are based on the social security rate of 7.65%.

Equipment

The spotting scope and laptop computer will be purchased by the Avian Research group for use during the project. The match dollars for these items were based on rental cost for the time needed (as per Brian Banciforte); computer rental was based on Dell leasing program of \$86/month.

Travel

Beaches at three sites will be sampled: the Panhandle, Cedar Key, and Tampa Bay. Hotel costs are based on those customary to the area; meal per diem is based on the state rate of \$36/day. The last day of each trip is paid by \$80 per diem. On the budget sheet, the Cost noted for each travel period includes hotel, meal, and per diem costs for one person for the entire trip (e.g. \$472). The Quantity listed is the number of person-days (e.g., 12: four travelers multiplied by three trips each). The initial sampling period will require four people and four days for each site (plus one extra day travel time for personnel from St. Petersburg to reach the Panhandle). All four people must travel to the Panhandle, but only one will need to travel to Cedar Key and three to Tampa Bay because other personnel are already located in those areas. Subsequent sampling will only require two people (graduate research assistant and field technician) and 2-3 days for each site. No travel expenses will be incurred for Cedar Key sampling as these personnel will be located in Gainesville.

Equipment Operation

A state truck will be used for preliminary identification of beaches before the first sampling period. State trucks and boats will be used for travel and sampling. Match amounts were based on rental of each vehicle (SUV and 21-foot boat) for the time needed. Gas was estimated at \$2.50 per gallon multiplied by the number of gallons estimated for each vehicle per year. Maintenance and repairs will be covered by state funds.

Materials and Supplies

Supplies to obtain core samples, blood samples, and stomach contents include supplies for collecting, storing, and processing samples, such as cores, buckets, syringes, ethanol, and plastic bags. Banding tools and color leg bands will be purchased in bulk at a rate of \$0.23/band in the first year and used throughout the project. Binoculars and GPS units will be purchased by the Avian Research group for use

during the project; the match dollars for these items were based on rental cost for the time needed (as per Brian Banciforte).

Other Costs

Plasma metabolite analysis will be conducted at the University of Florida by trained personnel. Cost is approximately \$15 per sample. The indirect costs were originally estimated at 16.6%; the difference from the new rate will be used for expenses.

Indirect Costs

Indirect costs were calculated at the FWC-negotiated rate of 13.95% of total salaries.