1. Title: Role of Free-ranging Wildlife in the Deposition of *Escherichia coli* into a Texas River Floodplain

2. Focus Category: Non-Point Pollution, Management and Planning, Water Quality

3. Key Words: Wildlife, *Escherichia coli*, Population Estimation, Fecal Deposition Rate, Concentrations

4. Duration: March 1, 2009 through February 28, 2010

5. Federal Funds Requested: $4,997

6. Non-Federal (matching) Funds Pledged: Please see associated letter of confirmation

7. Principal Investigator (graduate student): Israel David Parker, Texas A&M University, Department of Wildlife and Fisheries Sciences, Ph.D. Candidate, Email: iparker@tamu.edu, Telephone: 979-739-0679, Postal Address: 006B Nagle Hall, 2258 TAMU, College Station, TX 77843-2258

8. Co-Principal Investigator (faculty advisor): Roel R. Lopez, Ph.D., Texas A&M University Department of Wildlife and Fisheries Sciences, Associate Professor, Email: roel@tamu.edu, Telephone: 703-604-1820, Postal Address: 319 Nagle Hall, 2258 TAMU, College Station, TX 77843-2258

9. Congressional District(s) where project will occur: Congressional District 17

10. Abstract: Current Environmental Protection Agency standards depend upon *Escherichia coli* content as a determinant of fecal contamination into water bodies. Previous studies have demonstrated that *E. coli* has a variety of potential sources including city effluent and agricultural operations. More recent studies also suggests *E. coli* can be transmitted by a variety of familiar wildlife populations including mammals (Sargeant et al. 1999, Olsen et al. 2002), reptiles/amphibians (Souza et al. 1999, Tobe et al. 2006), and birds (Brittingham et al. 1988, Makino et al. 2000, McLellan 2004). Although previous *E. coli* research has investigated the role of traditional sources of fecal pollution, further studies are needed to understand the role of free-ranging wildlife populations in the deposition of *E. coli* in order to accurately describe the sources of fecal contamination (Brittingham et al. 1988, Dobson and Foufopolous 2001, Renter et al. 2001, Solomon et al. 2001). Land managers and natural resource decision-makers need to understand the role of wildlife in the deposition of *E. coli* into Texas watersheds in order to successfully manage water supplies in the state and to implement effective pollution control strategies. We will quantify the role of free-ranging wildlife in the deposition of *E. coli* through population density estimation, fresh fecal collection, and fecal deposition rate estimation.

11. Statement of Critical Regional Water Problems: The population of Texas continues to grow with some estimates placing it well over 30 million people by 2025. Central Texas is showing regional population growth as well. Additionally, water resources are increasingly polluted from point and non-point sources. Agricultural operations dominate the Central Texas rural landscape both contributing and suffering from water contamination issues. However, urban settings continue to negatively impact both urban and rural water quality. Without improvements in both water use efficiency and water quality standards, Texas may fail to meet the minimum allotment of water necessary for the expected population growth.

12: Nature, Scope, and Objectives of the Research: Our study objectives are to identify, characterize, and quantify *E. coli* deposition from free-ranging wildlife populations into a floodplain of an impaired water body. This project will seek to clarify the spatial distribution of fecal sources, subsequent fecal deposition, and *E. coli* locations. Target species are exclusively mammalian (medium to large; e.g., raccoons [*Procyon lotor*], white-tailed deer [*Odocoileus virginianus*], feral hogs [*Sus scrofa*]). Specific objectives are as follows: 1) identify and estimate relative densities of major wildlife contributors of fecal material in the study floodplain. Focal species are limited to those in direct contact to water course, 2) evaluate the presence and persistence of *E. coli* levels in fecal samples from identified major wildlife contributors, 3) estimate the approximate amount of fecal material deposited by major contributors into the watershed on a weekly/seasonal basis.

Study Area

Cedar Creek is approximately 40 minutes drive time from Texas A&M University, College Station. Cedar Creek flows southeast for a total of approximately 27 miles through Robertson County and the northern part of Brazos County before emptying into the Navasota River on the eastern border of Brazos County. We will conduct our research on 2 large private ranches (460 ha, 430 ha) that straddle Cedar Creek.

Objective 1 – Identify major contributors

First, we will use infrared triggered cameras to aid in determining relative abundances of mid-size to large mammals present within the floodplain (Riley et al. 1998, Hagedorn et al. 1999, Trolle 2003, Acevedo et al. 2006). We will select approximately 40 grid-based points to place remotely-operated infrared digital cameras for 21 consecutive days once during the winter, summer, and fall seasons for the 2 years of the study (Watts et al. 2008). We will rank species presence by camera images and determine relative abundances using mark-resight methods (Karanth and Nichols 1998, Jacobson et al. 1997, Main and Richardson 2002, Watts et al. 2008). Second, we will determine medium-sized mammal relative abundance by analyzing trapping numbers in live-trap grids (Main and Richardson 2002). We will use a grid-design (42 traps total for each property) that has been shown to adequately sample animals that are highly attracted to aromatic baits (e.g., raccoons and opossums). We will use randomly located trap arrays in order to capture armadillos, rabbits, and skunks (i.e., species less attracted to baits; 43 traps total for each property; Faulhaber 2003). Relative densities of trapped species will be calculated based on mark-recapture methodology. For both capture methods, captured animals will be temporarily marked and released safely (Krebs 1999).

Objective 2 – Evaluate the presence and concentration of *E. coli* in fecal samples.

We will collect fecal material of major contributing species during transects and during medium-sized mammal trapping (see Objective 1). Only fresh samples collected during the live-trapping will be sent to laboratories at Texas A&M University (College Station, TX) for analysis of *E. coli* presence and density. In order to identify potential seasonal *E. coli* fluctuations, we will collect fecal samples of identified sources during the winter and summer seasons for 2 consecutive years. Technicians will collect all fecal material from the traps. After releasing animals from the trap and collecting a fecal sample, the trap will be cleaned (bleach water) and moved to prevent possible cross contamination of subsequent fecal samples. We will utilize hunting practices in the area to collect fresh hog and deer fecal samples. We will request samples of feral hog fecal samples during the summer and fall months from multiple landowners in the watershed through flyers. We will also conduct feral hog trapping on one of the cooperating properties to collect fecal samples. This will occur in concert with another

researcher analyzing bait effectiveness for feral hogs. We will depend on landowners (through flyers) in the watershed to provide fecal samples of hunted white-tailed deer during the fall hunting season.

Objective 3 – Determine amount of fecal material deposited into the watershed.
We will initially conduct a comprehensive literature review to determine fecal shedding rate for species analyzed in Objective 1, and to validate results from our study (McCleery et al. 2005). In addition, we will then use a random design to place 70-80 individual 600 m^2 transects within each study property (180 transects total). These plots will be recorded with handheld Global Positioning Systems (GPS) and entered into a GIS. Floodplain-scale estimates of the amount of species-specific fecal material will be extrapolated (Morrison et al. 2001). We will further our understanding of the major contributors to fecal deposition by ranking the number of fecal depositions per species and determining relative abundances. We will conduct these surveys once during the summer and winter seasons for the 2 years of the project.

Procedures for Fecal Collection
E. coli numbers from characterized waste streams for all dominant identified sources will be quantified using appropriate methods (TSSWCB Project Number 07-06, unpublished report). All sample bags will be stored in a cooler at 4°C and transported to the laboratory for *E. coli* analysis. Samples will only be collected from trapped or hunted animals, ensuring fresh fecal material and minimizing environmental contamination. All collection and handling of fecal specimens will be performed using protective gear (e.g., latex gloves). We will handle specimens aseptically to ensure sample quality and minimize exposure of personnel to pathogens and parasites. All feces collected will be placed in sterile Whirl-Pak containers (Nasco, Fort Atkinson, WI). Fecal specimens will be placed in an insulated cooler on ice during transport to the College Station lab. All fecal material will be cultured within 24 hours of reaching the lab.

Data Analyses
We will use a variety of statistics including maximum likelihood tests and likelihood ratio tests to determine the relationships for seasonal and annual analyses (Krebs 1999). We will use Statistical Applications Software (SAS, Cary, NC) and program MARK (White and Burnham 1999) to create spatially and temporally-based models for wildlife fecal deposition. Determination of appropriate statistics will be further clarified with preliminary study data collection designed to test methods prior to research.

13. Results Expected from this Project: This study will provide a comprehensive analysis on the role of free-ranging wildlife in the deposition of *E. coli* into Texas watersheds. Expected benefits include the following: 1) more precise parameter data for use in pollution dynamics study of watersheds. We will provide data that improves the understanding in the relationship between wildlife contributions to watershed impairment, 2) a model of the contribution of wildlife to *E. coli* problems in impaired watersheds. We will construct a model to predict trends to the floodplain contamination using data collected in this study. While the exact form of the model will depend on the data collected, we anticipate a model that will incorporate these data and present a comprehensive assessment of the wildlife contribution of *E. coli* to the system, and 3) public dissemination of results via publications. Education and dissemination of our work is an important component of a successful research project. We will publish our findings in a series of scientific, peer-reviewed outlets and extension publications appropriate for land managers and other natural resource decision-makers in the state.

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