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Role of free-ranging mammals in the deposition of *Escherichia coli* into a Texas floodplain

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Abstract

Context. The role of wildlife in faecal pollution of water bodies (deposition of *Escherichia coli* (*E. coli*)) is not well understood. Current water-quality and land-use planning research largely relies on unreliable wildlife data (e.g. poor sourcing of abundance estimates, population density estimates applied to multiple fundamentally different areas, suspect or insufficiently described data collection techniques)

Aims. Our goal for the present research was to investigate deposition of *E. coli* into a floodplain by free-ranging mammals. Objectives of the research were to determine the density of important free-ranging meso- and large mammals in the study area, determine faecal *E. coli* loads for each species, and evaluate spatial data on species-specific faecal deposition.

Methods. We conducted our research in south-eastern Texas, USA, on two cattle ranches bisected by Cedar Creek (44-km long). Cedar Creek has elevated *E. coli* concentrations. We conducted mark–recapture and mark–resight population density estimates (2008/09) for meso- and large mammals in the study areas. We collected faecal samples from all captured wildlife. We also conducted transects through the study area to determine faecal-deposition patterns.

Key results. We found that raccoons (*Procyon lotor*), wild pigs (*Sus scrofa*), Virginia opossums (*Didelphis virginiana*) and white-tailed deer (*Odocoileus virginianus*) all had substantial faecal *E. coli* loads and population densities, thus implying an important role in *E. coli* deposition into the study floodplain. All species were widely distributed through the floodplain.

Conclusions. Free-ranging mammals contribute *E. coli* to floodplains and potentially affect water quality. We determined that four species commonly found in floodplains throughout North America all contributed *E. coli* to the study floodplain, thus implying mammal *E. coli* contributions in many locations and this is potentially important for *E. coli* management.

Implications. Improved locally specific mammal population estimates and estimates of locally derived *E. coli* concentration will improve floodplain and water-quality models that often depend on data of various quality. Additionally, our analyses demonstrated the need for continued research into the role of wildlife in *E. coli* deposition.

Additional keywords: faecal contamination, floodplain, impaired, mammals, water quality.

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Introduction

Escherichia coli (*E. coli*) is a group of enteric bacteria symbiotic with warm-blooded animal species. Current USA Environmental Protection Agency (EPA) standards depend on the concentration of non-pathogenic *E. coli* as a determinant of faecal contamination into water bodies. These determinations can be complicated by nebulous understanding of wildlife contributions of *E. coli* (Teague *et al*. 2009). This is especially important as faecal material is becoming a more common contaminant of water bodies used by humans for food, irrigation, drinking water and recreation (Fisher *et al*. 2000; Mallin *et al*. 2000). Previous studies have shown that *E. coli* can originate from a variety of sources, including municipal wastewater-treatment plants, agricultural operations and direct deposition from wild and domestic animals (Hagedorn *et al*. 1999; Booth *et al*. 2003; Kaller and Kelso 2006; Puri *et al*. 2009).

Although previous *E. coli* research has investigated the role of traditional sources of faecal pollution, little research has evaluated wildlife *E. coli* loads (Brittingham *et al*. 1988; Dobson and Foufopoulos 2001). Further studies are needed to understand the role of free-ranging wildlife populations in the deposition of *E. coli* so as to accurately describe the sources of contamination (Renter *et al*. 2001; Solomon *et al*. 2002). Land managers and natural resource decision-makers need to understand the role of wildlife in the deposition of *E. coli* into Texas watersheds so as to successfully manage water supplies in the state and to implement effective pollution-management strategies. Furthermore, information concerning the contribution of *E. coli* by free-ranging wildlife populations is needed to improve watershed-level contamination models and reliability of model results.

Although we found in our literature review that wildlife data in water-quality studies often originates from state natural resource conservation agencies, the methodology used to attain density estimates and the results vary significantly. Many water-quality studies and models incorporate wildlife data with foci on individual or few species, variable accuracy, unclear data sources, divergent methodologies, terminologies and interpretations, or unknown study-area applicability (e.g. Lawson 2001; Culver *et al*. 2002; Cox *et al*. 2005; Rice 2005; Teague *et al*. 2009). No known studies have attempted to determine the important species in the watershed, provide density estimates of those species, and comprehensively collect faecal samples from them. For instance, Parajuli *et al*. (2008) evaluated the effects of best management practices on water quality and relied on summer roadkill indices and expert opinions to determine wildlife densities. Kaller *et al*. (2007) compared water quality in watersheds to determine the impact of wild pigs (*Sus scrofa*). They relied on harvest data to determine population trends. In their model of microbial contaminants from grazed fields, Tian *et al*. (2002) used stock units that covered expected wildlife contribution without getting wildlife data.

Our study was a subset of a larger water-quality project conducted in Texas that included water and sediment sampling, microbiological analyses, livestock faecal sampling and hydrological research (e.g. Padia *et al*. 2011). Our study objectives were to identify, characterise and quantify *E. coli* deposition from free-ranging wildlife populations into a floodplain of an impaired water body. Target species were exclusively mammalian (medium to large; e.g. Virginia opossums (*Didelphis virginiana*), raccoons (*Procyon lotor*), white-tailed deer (*Odocoileus virginianus*) and wild pigs). Our specific objectives were to (1) identify and estimate population densities of major wildlife contributors of faecal material in the study floodplain, (2) determine the *E. coli* levels in faecal samples from wildlife contributors and (3) evaluate locations of species-specific faecal deposition.

Materials and methods

Study area

We evaluated the role of wildlife in *E. coli* contribution in the Cedar Creek watershed (Brazos County, Texas; Fig. 1). Brazos County is located in south-eastern Texas in the Post Oak Savannah ecotone. Cedar Creek flows south-east for ~44 km through Robertson County and the northern part of Brazos County, before emptying into the Navasota River on the eastern border of Brazos County. The Navasota River ultimately merges with the Brazos River at the southern tip of the county. Cedar Creek intersects both agricultural (ranches and farms) and urban areas (City of Bryan) and is classified as impaired because of high bacterial loads (USA Environmental Protection Agency 2008). Although impaired, little is known about the sources of pollution into Cedar Creek. We conducted our research on two private ranches (Property A, 518 ha; Property B, 660 ha) bisected by Cedar Creek. Each ranch stocked cattle (Property A, 1 cow per 10.36 ha; Property B, 1 cow per 2.2 ha) on post oak savanna habitat of mixed upland and bottomland grasslands, with scattered post oak woodlands located both in the upland and bottomland zones. Both properties exhibited impacts from grazing, although Property B had shorter grasses and more affected soils likely because of a higher cattle stocking rate. Each property had ample available water from Cedar Creek and numerous stock tanks located throughout the properties. Property B had several active oil wells, with concomitant truck traffic and habitat alteration.

Species densities

We used multiple monitoring and capture techniques to determine which species were present in the study area and their respective densities. We used remotely activated infrared-triggered cameras (Non-Typical, Park Falls, WI, USA) to determine densities of white-tailed deer and wild pigs present within the Cedar Creek floodplain (Trolle 2003; Acevedo *et al*. 2007). We selected 30 grid-based points on Property A (1 camera per 14.3 ha; cameras not allowed on Property B) to place remotely operated infrared digital cameras for 25–50 consecutive days once during the winter, summer and fall seasons (winter, 22 December–21 March; summer, 22 June–21 September; fall, 22 September–December 21) for the 2-year study (Jacobson *et al*. 1997; Watts *et al*. 2008). Cameras were placed at observed wildlife trails or openings suitable for camera placement near each pre-determined grid point (Jeganathan *et al*. 2002; Claridge *et al*. 2004; Trolle and Kéry 2005; Roberts *et al*. 2006). Each camera (1 gigabyte flash card) was capable of storing ~1000 still images and short video clips (10 s). We applied 2 L of apple and persimmon-scented gel on nearby substrate (e.g. thick branches, stumps) every 5 days as attractant. We determined density using mark–resight methods (Jacobson *et al*. 1997; Karanth and Nichols 1998; Main and Richardson 2002; Watts *et al*. 2008). We individually identified white-tailed deer and wild pigs, using unique antler and skin patterns (i.e. spots, scars), respectively (Jacobson *et al*. 1997). These were classified as marked. Antler patterns are the only consistent natural marking available for white-tailed deer but are deciduous and limited to males, thereby limiting initial abundance estimates to late and early year male populations. We then relied on the observed ratio between males and females to determine female abundance and overall population estimates as recommended by Jacobson *et al*. (1997). Second, we determined meso-mammal density by analysing trapping numbers in live-trap grids (Main and Richardson 2002) by using a mark–recapture approach (Krebs 1999). We trapped on both properties using a grid-design (42 traps total for each property; raccoon and feral

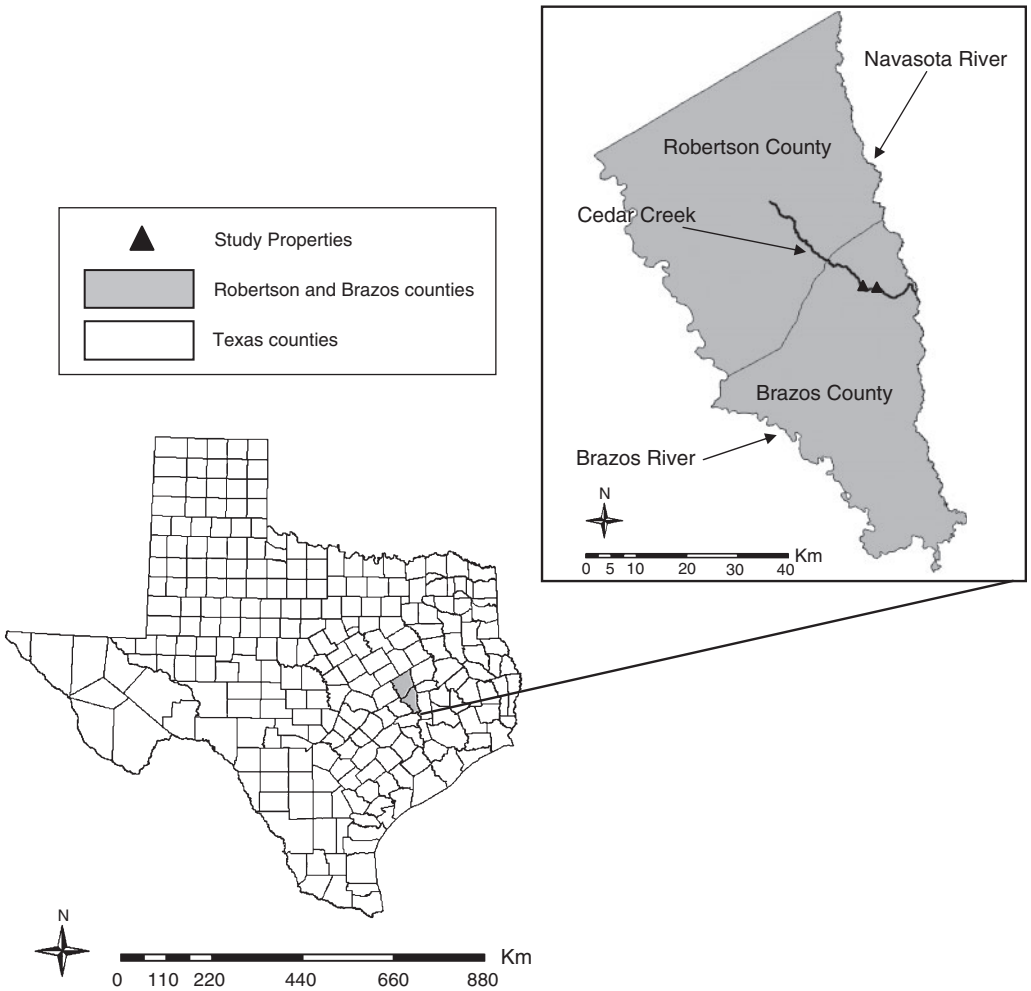

Fig. 1. Location of Cedar Creek study area, Brazos County, Texas, USA, 2008–2010.

cat-sized traps, 81 cm × 25 cm × 30 cm; Tomahawk Live Trap, Tomahawk, Wisconsin, USA), with 250-m spacing between traps that had been shown to adequately sample animals that were highly attracted to baits (e.g. raccoons, Virginia opossums). Trapping locations were trapped for 12 consecutive days, using Tomahawk box traps baited with canned dog food, apples, bananas, and fish scent. All traps received the same amount and ratio of baits. We uniquely marked captured animals by using non-toxic hair dye and released them 5–7 min later. We immobilised animals by using a Tomahawk squeeze cage to obviate the need for sedatives or tranquilisers (Parker *et al.* 2012). Sex, age, species and unique natural marks were recorded. All information was recorded in a database and within a geographical information system. We estimated effective sample area (ESA) for both meso-mammals (trap grids) and large mammals (camera grids) by adding a buffer (mean maximum movement between captures of individually identified animals) around the trap and camera-grid areas (Krebs 1999). Finally, we attempted to trap nine-banded armadillos (*Dasypus novemcinctus*), eastern cottontails (*Sylvilagus floridanus*) and striped skunks (*Mephitis mephitis*) on both properties by using trap arrays because each species was less attracted or only seasonally attracted to baits. We fabricated the arrays from 61-cm-tall chicken fencing with 61-cm-long wooden stakes. Each array had 8–12 double-door raccoon and rabbit-sized traps (43 traps total for each property; 48 cm × 15 cm × 15 cm; Tomahawk Live Trap, Tomahawk, Wisconsin, USA) with variable array setups designed to take advantage of the local vegetative community and topography. Previous studies have successfully conducted research on mammals using this technique (Faulhaber 2003; Perry 2004).

Escherichia coli *data*

We collected faecal material of major contributing species while mammal live-trapping during the summer (2008 and 2009) spring (2008), fall (2008–2009) and winter (2008) seasons (McCleery *et al.* 2005). On animal release, we collected all faecal material from the traps. We cleaned the trap thoroughly using bleach water and scrub brush and moved the trap (5-m radius) to prevent possible contamination of subsequent faecal samples (Rutala *et al.* 2008). Fresh faecal samples were kept in ice coolers and transported to the laboratory at Texas A&M University within 2 h for enumerating of *E. coli*

concentration. Fresh samples directly from the source animal helped reduce the risk of environmental contamination of the faecal samples.

We trapped and euthanised wild pigs during Summer 2008 (9 traps) and Summer 2009 (6 traps) for 7 days and 6 days, respectively, using three-panel corral-style traps on Property A (not allowed on Property B). Traps were baited daily with soured corn (max of 7.8 L) and locked open for 7 days before trapping, to increase the potential success rate. We checked the traps daily and rebaited with corn as necessary during trapping. Wild pigs were euthanised with a single gunshot to the head and faecal samples were collected from euthanised individuals. We supplemented sampling on Property A, by accompanying wild-pig hunters in watersheds near (at <3-km distance) the Brazos River (April 2009).

We live-captured white-tailed deer during Spring 2009 and Summer 2009 using drop nets (Lopez *et al*. 1998) on Property A. Drop nets were pre-baited for 7–10 days before capture with apple-scented corn. All deer were restrained with rope (legs bound) and a hood was placed over each animal's head. Average handling time was 5–10 min (no drugs administered). We recorded sex, age and capture location (Lopez *et al*. 2003). Each animal received an ear tattoo as a permanent marker (Silvy 1975). Faecal samples were collected directly from the immobilised deer. All capture, handling and euthanasia was conducted in compliance with approved permits issued by Texas Parks and Wildlife Department and Institutional Animal Use and Care Committee (AUP #2008-123) at Texas A&M University.

We used protective gear when collecting and handling faecal specimens (i.e. latex or nitrile gloves, eye protection). All faeces collected were placed in sterile Whirl-Pak containers (Nasco, Fort Atkinson, Wisconsin, USA). Faecal specimens were placed in an insulated cooler on ice and transported to the to the Biological and Agricultural Engineering laboratory at Texas A&M University within 2 h of collection. These samples were then frozen and analysis commenced within 24–72 h of delivery. We quantified *E. coli* numbers from characterised waste streams for focal species by using a standard membrane-filtration method on vortexed faecal samples described in a complementary study (Padia *et al*. 2011). The *E. coli* count (colony-forming units per g of wet faecal material) was derived from the observed colony development on the filter placed on the selective nutrient medium (modified membrane thermotolerant *Escherichia coli* agar). These results were then confirmed through replication (from same faecal sample) on a separate medium (Lurian–Burtani agar).

Species-specific deposition and spatial analysis

We determined locations of faecal deposition and source species by using transects. Proximity of faecal deposition to water body is directly correlated with the probability that it will be transported to the stream system (Collins and Rutherford 2004). We determined spatial deposition behaviour by using a random design to place 70–80 individual 600-m^2 (200 m × 3 m) transects within each study property (375 transects in total). All faecal material found within these transects (Property A: Summer 2008, Winter 2008, Summer 2009; Property B: Summer 2008, Winter 2009) was identified to species using identification guides, and location recorded was via hand-held global positioning system unit.

Data analyses

We compared faecal-deposition distance from water body by species and season by using ANOVA. We conducted Schumacher–Eschmeyer population-density tests on all subject species. The Schumacher–Eschmeyer (S–E) estimator is a variation of the Schnabel and Lincoln–Peterson estimators that assumes that the resight sample ratios of marked to unmarked animals accurately reflect the entire population (Pierce *et al*. 2012). The S–E estimator is conservative and was the primary estimator for all species densities. So as to compare estimates against conservative numbers, we generated minimum densities for all species on the basis of minimum number known alive (MNKA) from capture histories. We used AMOVA and Tukey's honestly significant difference to analyse species population densities. Finally, we compared the medians of *E. coli* concentrations among species using a Kruskal–Wallis *H* test.

Results

Species density

We gathered white-tailed deer data ($n = 1025$ total pictures) concurrently with wild pigs ($n = 1487$ total pictures). We grid-trapped 2328 trap-nights during the study (2008/09). Additional array trapping totalled 1680 trap-nights. Although we found insufficient numbers of naturally marked pigs during any season to determine density, we collected sufficient data to calculate densities for raccoons (all seasons), Virginia opossums (all seasons) and white-tailed deer (all seasons except Summer 2009). We were able to estimate ESA (pooled across seasons) for wild pigs (ESA = 452.3 ha), thus allowing us to calculate conservative population densities based on MNKA (Table 1). We captured negligible numbers of rabbits, armadillos and skunks using arrays. We pooled data across seasons and properties because of insufficient data, similar land-use patterns of properties and relatively short distance between properties (<3 km). We found that raccoons had significantly higher population densities than did white-tailed deer ($P = 0.035$), Virginia opossums ($P = 0.004$) and wild pigs ($P = 0.001$), but we found no significant density differences among these species when we removed raccoons from the analysis ($F = 1.748$, d.f. = 2, $P = 0.242$, see Table 1).

Escherichia coli *data*

During the study, we collected 338 faecal samples from 182 individuals, including raccoons (Property A: $n = 115$ samples; Property B: $n = 62$ samples), Virginia opossums (Property A: $n = 43$ samples; Property B: $n = 45$ samples), white-tailed deer (Property A: $n = 6$ samples; other properties (2 ranches <3 km from 2 primary study properties): $n = 4$ samples), wild pigs (Property A: $n = 39$ samples; other properties: $n = 6$ samples) and other species ($n = 18$ samples). We eliminated 29 samples because of contamination, insufficient quantity or omission of species, leaving a total of 309 samples available for analysis. Because of low sample numbers, we omitted nine-banded armadillos, eastern cottontails and striped skunks from further

Table 1. Compilation of density estimates for Property A and Property B, Brazos County, Texas 2008–2009

Property	Species	Season	Density (km^2)	CI – low (95%)	CI – high (95%)	Minimum density (km^2)[A]
A	Raccoon	Summer 2008	84.0	66.0	101	49.0
		Winter 2008	55.0	42.0	68.0	31.0
		Summer 2009	37.0	31.0	43.0	33.0
	Virginia opossum	Summer 2008	12.0	10.0	13.0	12.0
	White-tailed deer	Summer 2008	16.0	12.0	21.0	16.0
		Winter 2008	19.0	11.0	27.0	14.0
		Summer 2009[A]	–	–	–	21.0
	Wild pig	Summer 2008[A]	–	–	–	8.0
		Fall 2008[A]	–	–	–	5.0
		Winter-Spring 2008/09[A]	–	–	–	5.0
		Summer 2009[A]	–	–	–	3.0
B	Raccoon	Summer 2008	52.0	38.0	66.0	33.0
		Winter 2008	34.0	30.0	38.0	27.0
	Virginia opossum	Summer 2008	11.0	9.0	13.0	11.0
		Winter 2008	4.0	3.0	5.0	4.0

[A]Derived from minimum number known alive.

analysis. We pooled species across properties and seasons because some seasons and properties had relatively few samples. We found a significant difference in median *E. coli* concentration between white-tailed deer and the other focal species ($H = 10.409$, d.f. = 3, $P = 0.015$), whereas wild pigs, Virginia opossums and raccoons did not differ significantly from each other ($H = 2.977$, d.f. = 2, $P = 0.226$). We found that Virginia opossums had the widest observed ranges in faecal *E. coli* concentrations, followed by raccoons, wild pigs and white-tailed deer (Table 2).

Table 2. Summary across all seasons and properties ($n = 309$) of *Escherichia coli* concentrations in faecal material sampled from wildlife species, Brazos County, Texas, 2008–2009

CFU, colony-forming units

Species	Median (CFU g^{-1})	Range – low	Range – high
White-tailed deer	3.75×10^5	1.40×10^4	5.60×10^7
Wild pig	2.56×10^7	2.40×10^5	4.10×10^9
Raccoon	1.00×10^7	5.00×10^2	4.30×10^{11}
Virginia opossum	5.4×10^7	1.00×10^2	1.21×10^{11}

Species-specific deposition and spatial analysis

We collected 147 faecal samples during transects. Faecal samples were grouped by species and seasons, whereas property data were pooled and included only white-tailed deer, wild pigs and raccoons because of low sample sizes. White-tailed deer, on average, deposited faecal material closer to Cedar Creek ($n = 77$; $\bar{x} = 264$ m, s.d. = 204 m) than did wild pigs ($n = 25$; $\bar{x} = 279$ m, s.d. = 106.1 m) or raccons ($n = 18$; $\bar{x} = 335$ m, s.d. = 235 m), although these differences were not statistically significant for species ($F = 1.98$, d.f. = 2, $P = 0.14$) or season ($F = 2.85$, d.f. = 2, $P = 0.06$). We could not accurately determine the age of the faecal material, making attempts at standardising mass measurements impossible and preventing temporal analyses.

Discussion

Our analyses found that four species of free-ranging mammals deposited *E. coli* in the study area. We found varying concentrations of *E. coli* in faecal depositions from focal species, and wide-spread faecal depositions by all species within the study area. For comparison, the median *E. coli* concentrations in our study species were far higher than those found by Padia *et al.* (2011) in cattle in the same areas (median = 1.61×10^5, range = 3.35×10^2 – 1.74×10^7), although the highest observed *E. coli* concentrations in cattle overlapped with the lowest observed concentrations in our study species.

We found that the estimated density of observed species was similar to that in other reported studies (e.g. Michael 1965; Gehrt *et al.* 1997; Riley *et al.* 1998; Sweitzer *et al.* 2000; Blackwell *et al.* 2004). However, observed raccoon density was higher than that in many studies; it fell within population density estimates for suburban areas and suburban–rural interfaces (Riley *et al.* 1998). Our study area was intensively, although variably, managed for ranching interests (e.g. rotational grazing, fertiliser application and food provisions for domestic animals) and also subdivided in other areas of the watershed for smaller properties (2.0–10.1-ha parcels). Declines in raccoon populations were observed on both properties over the course of the study. Seasonal variation was expected; however, highly divergent rain patterns from Summer 2008 (dry) to Summer 2009 (wet) likely affected populations and population estimates (Connor *et al.* 1983). Wild-pig estimates were certainly affected by our concomitant lethal wild-pig sampling efforts. MNKA estimates of the wild-pig population reflected a generally declining population over the course of the study. The population estimates also may have been a product of differing rain patterns that allowed wild pigs to move further away from the bottomlands (location of several wild pig traps) on the study property (Dexter 1998; Mersinger and Silvy 2007). Some results were potentially attributable to vegetational differences (e.g. grass species present, height of grazed vegetation) between properties because of variable grazing intensity.

There is a paucity of available data on wildlife faecal deposition rates; however, defecation rates are an important

consideration when investigating potential deposition of *E. coli* by wildlife. According to literature, wild pigs have the highest mean individual daily defecation rate of any of the study species (wild pigs: 1.1 kg day^{-1} (Ohio State University Extension 2006; Mapston 2007), followed by white-tailed deer: 500–772 g day^{-1} (McCullough 1982; Sawyer *et al*. 1990; Wittman Hydro Planning Associates Inc. 2004), raccoons: 180–450 g day^{-1} (Sorvillo *et al*. 2002; Wittman Hydro Planning Associates Inc. 2004), and Virginia opossums: 75–108 g day^{-1} (Hopkins and Forbes 1979; Atwill *et al*. 2003)). We can cautiously infer from defecation rates, population densities and *E. coli* loads that these species were *E. coli* contributors that should be included in potential future water-quality research. For comparison, adult cattle are documented to defecate 16–28 kg day^{-1} (Atwill *et al*. 2003; Ministry of Forests Lands and Natural Resource Operations 2011), but with relatively low *E. coli* concentrations (as mentioned earlier).

Our evaluation of location of faecal deposition indicated that the study species used the entire floodplain without large differences among species. This lends some evidence to the conclusion that faecal deposits from study species have equal chance of reaching the water body during flood events. We urge caution as we were unable to age faecal deposits in the spatial analysis. This prevented us from assessing potential temporal associations of faecal deposition and flood season. It also disallowed analysis of the amount of faecal material likely to reach the water body because we were unable to determine the amount of decay. Additionally, spatial data were likely biased by wildlife behaviour. Raccoons, wild pigs and other species have been documented defecating directly into water bodies or very near water bodies (Lotze and Anderson 1979; Bracke 2011). These defecations are not discoverable through our methods, thereby biasing the results.

Free-ranging meso- and large mammals in our study area contributed *E. coli* into floodplains. Raccoons were larger potential contributors than were mammals such as wild pigs and white-tailed deer because of higher densities and high *E. coli* loads in faecal material (compared with other study species). Although undetected in this research, this is potentially exacerbated by the fact that raccoons stay near water and are known to defecate in water sources (Lotze and Anderson 1979). Wild pigs largely remain near water sources (Mapston 2007); however, the abundance of water on the properties (water tanks) probably allowed unrestricted movement away from bottomland streams. Wild pig coprophagy and quick deterioration of pig faeces likely further reduced their apparent faecal contribution (Copado *et al*. 2004; Mapston 2007). White-tailed deer defecated frequently and pellet groups were found more often than faecal contributions from other study species; however, they had relatively low *E. coli* concentration in their faecal material (compared with other study species).

Management of meso-mammal populations is possible; however, reduction of meso-mammal populations might have unintended collateral consequences (Naiman 1988; Kasparian *et al*. 2004). White-tailed deer are arguably the most important game species in Texas; thus, population management towards reducing their impact on water quality is complex. Wild pigs are potential candidates for mammal management because of their acknowledged role as an invasive exotic, with a plethora of documented ecological damage. The present research adds to the already compelling evidence supporting wild-pig control efforts (Singer 1981; Hone *et al*. 1992; Kaller and Kelso 2006; Kaller *et al*. 2007).

Few studies have attempted to determine the important contributing species in the watershed, provide density estimates of those species, and comprehensively collect faecal samples from them. We attempted to find all of these data for a specific water body so that the methodology could be tested and the results applied to other similar areas. Further research must be undertaken to determine faecal deposition rates, faecal degradation rates and *E. coli* viability in different faecal morphologies. It was impractical for us to conduct experiments to estimate faecal deposition rates because of time, manpower and financial constraints. Projects in the future should budget these necessities to increase the precision and usefulness of the research.

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