

Feral hog research in western Louisiana: expanding populations and unforeseen consequences

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Abstract:

Hunter harvest data suggest that feral hog (*Sus scrofa*) populations in western Louisiana are increasing, and population control in this region is complicated by the mixing of feral and domestic free-ranged hogs. Aggressive management may be warranted as feral and domestic hogs appear to be having unexpected effects on their ecosystem. We present the results of 3 recent investigations of genetic source-tracking to link waterborne bacteria with bacteria from feral hogs. We integrate our most recent findings with data regarding: (1) water quality in a watershed without hog management and (2) aquatic biota of the same watershed. Hog activity substantially increased waterborne bacteria, which often exceeded state and federal surface water guidelines. Aquatic biota, specifically freshwater mussels and aquatic insects of the collector and scraper feeding guilds, declined in stream reaches with hog activity. Finally, PCR (polymerase chain reaction)-based DNA source-tracking revealed a >95% similarity between coliform bacteria isolated from water and bacteria isolated from a feral hog harvested within the sample watershed. Further, when the isolated bacteria from the feral hog and water were compared with 900 other bacteria samples from a variety of domestic animals and wildlife, the bacteria isolated from the feral hog and water differed from the 900 other samples. These data suggest that the increasingly abundant hogs of western Louisiana are not only causing detriment to terrestrial flora and fauna, but are negatively impacting native freshwater mussels (*Bivalvia unionacea*) and insects, as indicated by genetic source-tracking methods.

Key words: DNA fingerprinting, *Escherichia coli*, feral hogs, freshwater mussels (*Bivalvia unionacea*), human–wildlife conflict, *Sus scrofa*

SINCE ARRIVING in North America with the earliest European colonists (Conover 2007), feral hogs (*Sus scrofa*) have expanded their range throughout the South and Southwest (Adkins and Harveson 2007; Engeman 2007a, 2007b; Mersinger and Silvy 2007). They inhabit parishes throughout southern and western Louisiana; in some wards of some parishes, domestic hogs are legally permitted to free-range on public lands. It is currently unclear whether domestic and feral hog groups intermingle, which would complicate management options for state and federal biologists and land managers. Feral hogs are not considered game animals by Louisiana, and unmarked hogs may be harvested year round on private lands and during open hunting seasons on public lands

with legally approved methods. Harvest is typically prohibited in areas with free-ranged domestic hogs to prevent loss of domestic animals due to hunter confusion over unclearly marked hogs. Thus, Louisiana contains areas with the potential for feral hog management and areas where feral hog management may require changes or modifications to local or state laws.

Since 1986, the Fort Polk Wildlife Management Area, Vernon Parish, Louisiana, has recorded hunter harvest at mandatory check stations, and these data suggest that hog populations are generally increasing in western Louisiana (Figure 1). Unlike other areas of western Louisiana, the Fort Polk Wildlife Management Area and neighboring Peason

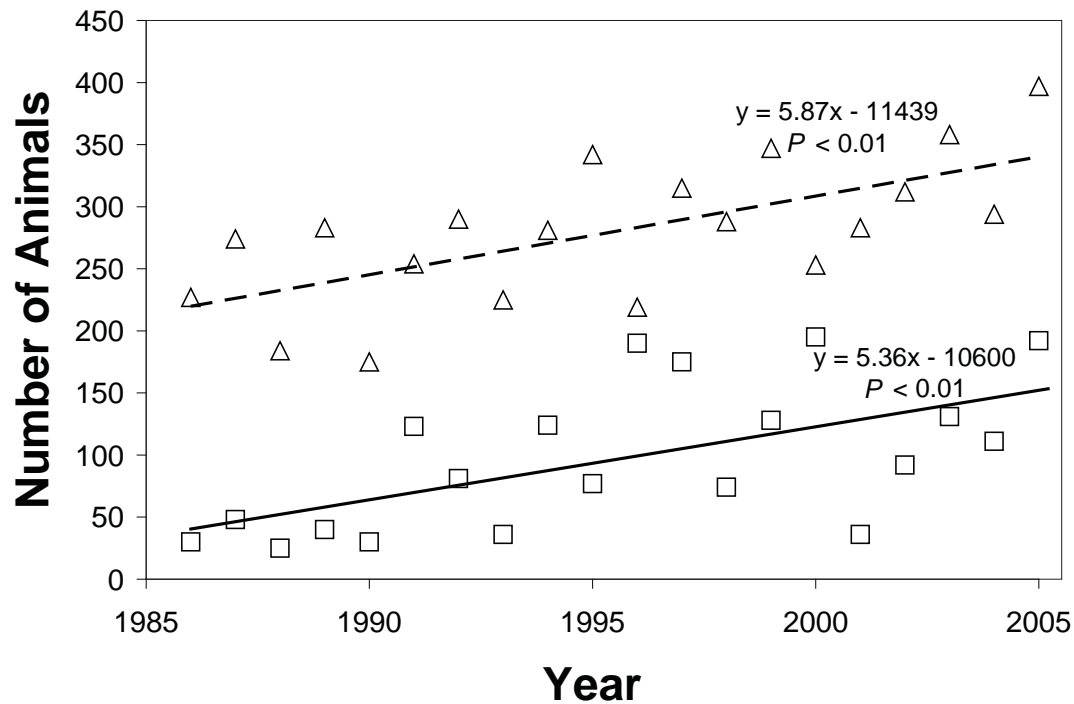


FIGURE 1. Hunter harvest data collected from 1986 to 2005 indicate increasing number of harvested feral hogs (squares) and white-tailed deer (triangles). Regression lines and equations are given for feral hogs (solid line) and white-tailed deer (dashed line).

Ridge Training Area, which are owned or leased by the U.S. Army, are occupied entirely by feral hogs, with no intermingling of domestic free-ranging hogs. This is the result of Congressional action and subsequent removal of all domestic animals from U.S. Army lands by 1985 (Fort Polk) and 1992 (Peason Ridge).

Competition with other wildlife, disease, as well as vegetative damage from feral hogs, are well-documented (Wolf and Conover 2003); however, data on the potential effects on aquatic biota are less available (Belden and Pelton 1975, 1976; Kaller and Kelso 2003, 2006). Previously, in western Louisiana, feral and free-ranging hogs were linked to increased fecal coliform and potentially pathogenic bacteria in water samples collected from Mill Creek, Allen Parish, Louisiana, which is located in a watershed where hog hunting does not occur (Kaller and Kelso 2003). The levels of fecal coliforms in Mill Creek at times exceeded human health standards (Kaller 2005). Further, the presence of hogs was negatively correlated with freshwater mussels (*Bivalvia unionacea*) and many types of aquatic insects in Mill Creek (Kaller and Kelso 2006).

However, these associations were based on comparisons of aquatic biota between sampling locations with and without evidence of recent hog activity, and direct links between feral hogs and aquatic communities were lacking. In this paper, we present data on the expansion of the feral hog populations in western Louisiana and its consequences. In conjunction with previous studies, these data offer some serious predictions regarding watershed health in the absence of hog management. We believe that these data justify more aggressive feral and free-ranging hog management in Louisiana.

Methods

Harvest data

We analyzed hunter harvest data collected at the Fort Polk Wildlife Management Area to examine trends in feral hog populations. Of its 42,712 ha, 68–94% are open to feral hog hunting, depending on hunting season and U.S. Army training. We used PROC MIXED (SAS 9.1.2, SAS Institute, Cary, N.C.) to model the total harvest and effort (big-game hunter trips) per harvest, of feral hogs and white-tailed deer (*Odocoileus*

virginianus) from 1986–2005. Time was a random effect in the mixed model.

Feral hog DNA fingerprinting

During August 2004, we harvested 1 feral hog approximately 300 m from west bank of the West Fork of Six Mile Creek (UTM, 15R, 499649S, 3433735W) within Fort Polk Wildlife Management Area, Vernon Parish, Louisiana. We removed the large intestine and rectum and immediately froze the sample. The sample was collected from Fort Polk along with 3 whole water column samples from West Fork of Six Mile Creek, taken according to guidelines of the American Public Health Association (1998), and returned to Baton Rouge, Louisiana, USA. We isolated fecal coliform colonies from the water samples using Millipore filters and type HC fecal coliform isolating media (both Millipore Incorporated, Billerica, Mass.) following 24-hour incubation at 38°C in a water bath. We isolated fecal coliform colonies from the sample hog feces using 2 enrichment broths followed by identification with streak plates of Levine's Eosin Methylene Blue agar. From both water samples and hog feces, 100 fecal coliform colonies, which were exclusively *Escherichia coli*, were selected. They were further cultured to enable us to place a Polymer Chain Reaction (PCR) product from the hog sample on multiple gels with water samples to reduce gel-to-gel error from comparing hog samples on 1 gel to water samples on another gel. We were able to compare the hog *E. coli* with fecal coliform colonies isolated from 900 known wildlife and domestic sources, including beavers (*Castor canadensis*), beef cattle (*Bos taurus*), coyotes (*Canis latrans*), white-tailed deer, domestic hogs, domestic and feral goats (*Capra aegagrus hircus*), hunter-harvested hogs, domestic and feral horses (*Equus caballus*), domestic and wild rabbits (*Sylvilagus* spp.), and raccoons (*Procyon lotor*). The samples were obtained throughout Vernon Parish during 2003 to 2004 by trained student volunteers from Pitkin High School, Pitkin, Louisiana. Volunteers used sterile swabs and plastic bags to collect scat samples directly from animals, or from scat directly observed by the student to have originated from specific animals. The scat generated a library of fecal coliform DNA following the same isolation methodology.

From the isolated August 2004 fecal coliform

water and hog feces colonies, we streaked 100 randomly-selected *E. coli* colonies, each from water and hog feces samples, onto Eosin Methylene Blue agar plates and incubated them for 24 hours at 37°C. The colonies were further incubated, with shaking, in 2-ml Luria broth for another 24 hours at 37°C. We standardized cell counts by dilution using a spectrophotometer at a wavelength of 600 nm (visible light) such that all samples contained 10 µl of *E. coli*. Cells were mechanically lysed to release DNA by alternate heating at 94°C and cooling in ice in 5-minute increments. Next, we mixed water, MgCl₂, a PCR bead (Puretaq Ready To Go, GE Healthcare, General Electric Company, Fairfield, Connecticut), and 1 of 2 10-mer arbitrary primers, either 01290 (GTGGATGCCGA) or 01254 (CCGCAGCCAA; prepared by the Genelab, LSU Department of Veterinary Medicine, Baton Rouge, La.) to a total volume of 25 µl. We produced and amplified DNA for PCR Random Amplified Polymorphic DNA (RAPD) using a Bio-Rad (Bio-Rad Laboratories, Inc., Hercules, Calif.) thermocycler with the following 2-part program: (1) 4 repetitions of 4 minutes at 94°C, 4 minutes at 37°C, and 4 minutes at 72°C; and (2) 32 repetitions of 1 minute at 94°C, 1 minute at 37°C, and 1 minute at 72°C. Then, for all samples, we added 12 µl of PCR product to 1 of 10 lanes in an 1.8% 3:1 agarose gel (AMRESCO Bulk Fine Chemicals, Solon, Ohio) in trisborate EDTA buffer (TBE; AMRESCO) solution. In the other 2 lanes, we added a 6 fragment marker, BSTN (digest of pBR433DNA). Next, we applied 80-V to the gel in a Bio-Rad electrophoresis tray for 70 minutes, removed the gel from the tray, and



Feral hog rooting damage in Allen Parish, Louisiana.

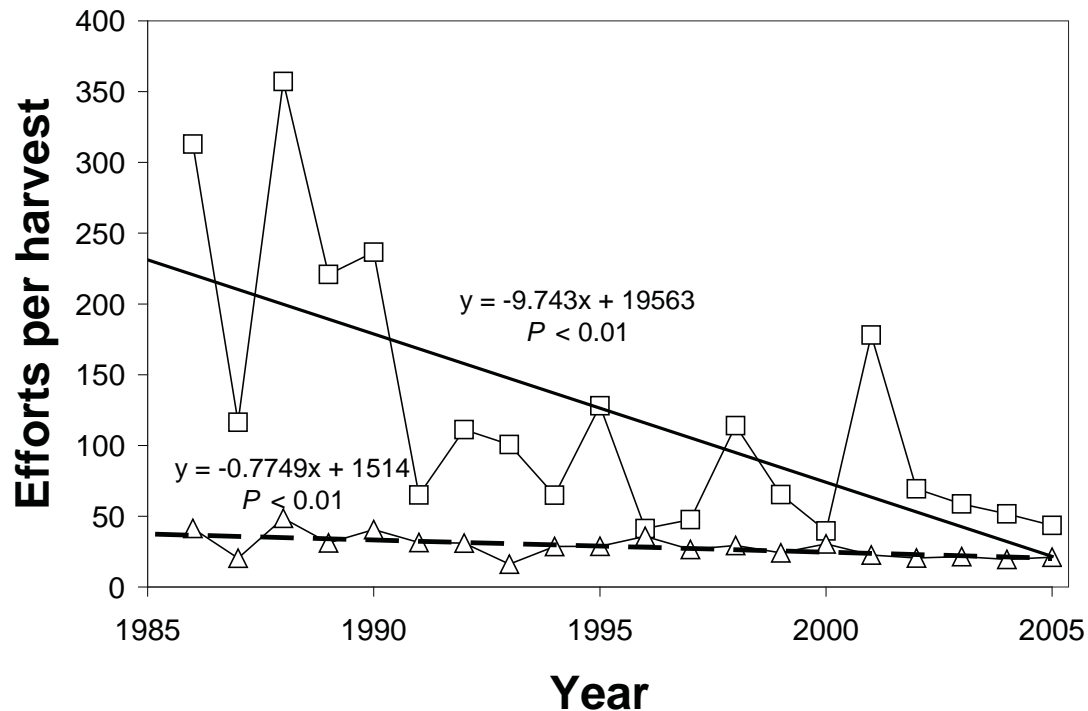


FIGURE 2. Hunter effort estimated as trips per harvest from 1986 to 2005 indicates stable effort for white-tailed deer (triangles) but decreasing effort for feral hogs (squares), suggesting increasing hog harvest opportunities from increasing hog populations. Regression lines and equations are given for feral hogs (solid line) and white-tailed deer (dashed line).

stained it for 45 minutes with 500 μ l of Ethidium bromide (AMRESCO). The gel was destained for another 45 minutes in TBE solution, rinsed, and imaged with an Eagle Eye II (Stratagene, La Jolla, Calif.) to photograph the patterns of polymorphism, which are multiple alleles, as expressed by bands at varying distances along the gels.

We input photographic images from the DNA library into Gel Compar II for comparison with our hog feces and water samples. Although the majority of genetic information coded in DNA is shared within a species, bacteria, such as *E. coli*, exhibit tremendous variability in genetic expression because *E. coli* consists of the multiple genetically-distinct strains that have high mutation rates and short generation times (E.C. Achberger, unpublished data). Therefore, based on both personal experiences comparing *E. coli* of known origins and guidelines from the literature (Wang and Trost 2001, Rodrigues et al. 2002, Shannon et al. 2002), we selected 90% Pearson correlation as the minimum to suggest similar genetic origin and $\geq 95\%$ Pearson correlation to indicate a common genetic origin

(i.e. same strain of *E. coli* from a single animal species). When Pearson correlations suggested strong (>95%) similarity based upon guidelines formulated by Rodrigues et al. (2002), we subsequently used Mantel tests (Mantel 1967; PROC IML macro modified from E. B. Moser, Louisiana State University Department of Experimental Statistics, SAS 9.1.2, SAS Institute, Cary, N.C.) following Wang and Trost (2001), Rodrigues et al. (2002), and Shannon et al. (2002) on the presence or absence of bands to test hypotheses that fecal coliform colonies in the water belonged to same genetic source as the hog feces fecal coliform colonies. We compared matrices from the RAPD PCR to 9,999 randomly generated model matrices constructed to indicate the same genetic source for expressed polymorphisms.

Results

Harvest data

Feral hogs constituted approximately 11% of big game animals taken in 1986 at Fort Polk and averaged 11.7% of harvest from 1984 to 1993 (Figures 1 and 2). Yet, feral hogs constituted 43%

of big game harvest in 2002 and averaged 27% of harvest from 1994 to 2005. Although effort (number of hunting trips) expended per harvest decreased by 9.7 (2.6 SE, $t_{18} = 3.64$, $P < 0.01$) efforts per year for feral hogs and 0.8 (0.3 SE, $t_{18} = 3.08$, $P < 0.01$) efforts per year for whitetail deer, harvest increased by 5.4 (1.8 SE, $t_{18} = 3.55$, $P < 0.01$) and 5.9 (1.7 SE, $t_{18} = 3.02$, $P < 0.01$) feral hogs and whitetail deer/year, respectively.

Feral hog DNA fingerprinting

Polymorphic band expression was similar with both arbitrary primers. However, gene expression on primer 01254 gels was clearer and easier to interpret than expression on primer 01290, so we restricted our interpretations to gels produced with primer 01254. In these gels, 87% of the isolated waterborne *E. coli* colonies were not genetically similar to our harvested hog *E. coli* or any *E. coli* from the mammal sample library. However, 9 colonies derived from our 3 water samples demonstrated similarity (> 90% Pearson correlation) with our harvested hog, and feral hog (hereafter fh) sample isolated colony 39 exhibited remarkable similarity to several water sample (hereafter w) isolate colonies, specifically w02, w09, w29, w47, and w49 (Figure 3). Pearson correlations for these isolated colonies were 95% or greater. A subsequent Mantel test suggested no significant differences (Mantel $r = 0.984$, $P = 0.54$) between gene expression in fh39 and w02,

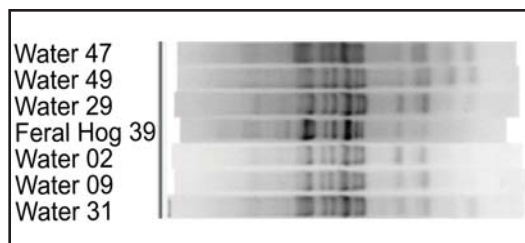


FIGURE 3. Polymorphic expression (dark bands) of water and hog feces *Escherichia coli* isolated colonies from the harvested hog and waterborne samples from West Fork of Six Mile Creek, Louisiana, USA, demonstrates strong similarity and common genetic origin using primer 01254. If images were from different gels, Gel Compar II software aligned gels using the 6 fragment BSTN marker (not shown).

w09, w29, w47, and w49. Further, high levels of similarity (>90%) occurred between w31 and w82 and fh13, fh16, fh46, and fh49; between w14 and w21 with fh94; and between w96 and fh26.

However, these relationships did not satisfy the hypothesis-testing guidelines of Rodrigues et al. (2002), and, therefore, we did not test these relationships with Mantel tests.

Comparisons between our water and feral hog isolated colonies with the 900 sample DNA library indicated our feral hog samples were more similar to the water samples, and both were generally less similar to samples in the mammal library. Feral hog *E. coli* was similar (>90% Pearson correlation) to 3 colonies from domestic cow samples and 5 colonies from previously harvested feral hog samples in the sample library. One water sample colony was similar (>90% Pearson correlation) to 2 previously collected beaver sample colonies. Again, these relationships failed testing guidelines of Rodrigues et al. (2002). One water sample (w07) colony was similar (>95% Pearson correlation; Mantel $r = 0.18$, $P = 0.28$) to 6 domestic cattle sample colonies. Generally, sample colonies from 1 mammal species exhibited little similarity (usually far less than 80% Pearson correlation) with other mammal species.

Discussion

Harvest data

In the absence of feral hog density data, hunter harvest data are the best information available to assess hog population trends. Statistical analyses of the hunter harvest data support our contention that hunters are harvesting increasing numbers of feral hogs. Numbers of feral hogs shot by hunters increased 3-fold from 1985 to 2005. Concomitantly, hunter effort/feral hog was decreasing, although hunter effort for white-tailed deer was fairly stable. The proportion of feral hogs in the overall harvest is higher in the second decade of data, and we believe this reflects hunters taking advantage of greater harvest opportunities from an increasing feral hog population. Whereas these data also may be interpreted to suggest hunters are increasingly willing to harvest hogs, we believe these data reflect increased sightings and subsequent successful harvesting opportunities. Our conclusion is supported by observations from local wildlife and fisheries biologists and land managers that suggest increasing and spreading populations of feral hogs.

Feral hog DNA fingerprinting

As a hazard to humans and other wildlife, *E. coli* itself may or may not be pathogenic depending on the introduced strain and those native to the gut of the consuming human or wildlife (Holt 2000, Conover and Vail 2007). Rather, evidence of *E. coli* is used as an indicator of disturbance and as indicative of a suite of contaminants, including organic matter enrichment and the introduction of other nonnative microorganisms (see Edberg et al. 2000, Field et al. 2003, Javorek et al. 2003). Therefore, we suggest that our *E. coli* data may be interpreted not only as an introduction of a potential human and wildlife pathogen, but also potentially as an indication of organic enrichment and the introduction of nonnative microorganisms by feral hogs.

We believe that our findings offer evidence that feral hogs contribute detectable *E. coli* into aquatic ecosystems, and that feral hog inputs may be differentiated from other sources. It is not surprising that most of waterborne *E. coli* were more similar to each other than to that of our harvested hog or sample library because coliforms may persist in sediments for lengthy periods of time (Craig et al. 2004), and they may mutate and become quite different from the source population. Therefore, it is important to emphasize that 5 waterborne colonies were highly similar to colonies from our harvested hog, which suggested recent and ongoing contamination of the water by feral hogs.

Rodrigues et al. (2002) suggested that gels produced by RAPD PCR were candidates for hypothesis tests, and correlations $\geq 95\%$ between 2 different gels were indicative of common genetic origin when substantiated by a Mantel test. Therefore, we believe that detecting 5 waterborne *E. coli* colonies with high degrees of similarity ($>95\%$) to a colony isolated from the feces of a feral hog is highly suggestive of direct feral hog contributions to *E. coli* populations in the water. Further, evidence of nonsignificant, but still notable, similarity (90–95%) between 6 colonies isolated from hog feces with 4 colonies isolated from water suggests that, with additional sampling of feral hogs, more matches may be detected because these values are quite high, although they are below the guidelines of Rodrigues et al. (2002). Therefore, 9% (9 of 100) of the fecal colonies isolated from water samples shared a large amount of genetic information with

the harvested hog, which was numerically more substantial than the 1% of colonies potentially attributable to beaver or domestic cattle. Further, the 9 colonies shared little genetic material with other potential sources of *E. coli*. However, we caution that our methodology does not allow us to quantify the contribution of *E. coli* from feral hogs based on sampling a single hog, and in no way do we suggest that 9% of all *E. coli* in the stream were derived from feral hogs. Instead, we suggest that hogs are contributing *E. coli* into this stream and, based on the prevalence of feral hogs throughout Louisiana, that this is very likely not an isolated case (see Kaller and Kelso 2003, Kaller 2005, Kaller and Kelso 2006).

Synthesis

Whereas documenting animal contribution of *E. coli* into aquatic ecosystems is not novel (see Savageau 1983 and references therein), our research differs by pointing out the missing connection between feral hogs and documented aquatic degradation. Given that hogs spend considerable time in aquatic habitat (Mersinger and Silvy 2007) and appear to contribute *E. coli* into streams, we believe that it is logical that the previously measured high fecal coliform counts in the Mill Creek watershed (Kaller and Kelso 2003, Kaller 2005) were probably the result of the large numbers of feral and free-ranging hogs rather than deer, turkeys, beavers, horses, or other potential sources. Further, the DNA data potentially implicate feral hogs as the primary source of fecal coliforms that were negatively associated with freshwater mussels and important nutrient processing insects in the Mill Creek watershed (Kaller and Kelso 2006). Currently, no scientifically-based estimates of the Mill Creek hog population exist. However, using conservative values of southeastern U.S. hog densities (Singer 1981) and data on the intensities of rooting and wallowing, we estimate feral hog density to be approximately 2 feral hogs/km², with an additional 1.6 to 2.7 free-ranged domestic hogs/km² based on Louisiana hog production (Louisiana State University Agricultural Center 2005) for a total hog density of 3.6 to 4.7 hogs/km². In light of these rough estimates, we cannot suggest a threshold density of hogs that would affect an aquatic ecosystem similar to Mill Creek. However, we suggest that the hog density at Fort Polk Wildlife Management Area is lower than at

Mill Creek because of the lack of free-ranging hogs, hog mortality from hunting, lower fecal coliform counts (Kaller 2005), and presence of hog-sensitive insects and mussels (Kaller and Kelso 2006). Yet, even at this lower density, feral hogs affect the water quality of West Fork of Six Mile Creek by introducing fecal coliforms.

Ultimately, the importance of these data depends on their relevance to the problem of aquatic resource degradation from feral hogs. If our interpretations of the hunter harvest data and the observations of Fort Polk Wildlife Management Area and Kisatchie National Forest personnel (S. Shively, U.S. Forest Service, personal communication) are correct, feral hog populations are expanding on the Fort Polk Wildlife Management Area, adjacent public lands, and probably adjacent private lands as well. Further, if the degradation of the aquatic resources of Mill Creek is indicative of a watershed where hog density overwhelms inherent resiliency of aquatic biota to disturbance, Louisiana watersheds may face significant problems in the future. Fecal coliforms (Louisiana Department of Environmental Quality 2004) and aquatic macroinvertebrates (Barbour et al. 1999), including insects demonstrated to be sensitive to feral hogs (Kaller and Kelso 2006), are components of surface water quality monitoring. Waters with fecal coliform colony counts in excess of 200 colonies/100 ml or that are deficient in expected diversity or abundance of aquatic insects may face listing on Louisiana's Clean Water Act, Section 303 (d) or 305 (b) lists. This is already the case for Whiskey Chitto Creek in Fort Polk Wildlife Management Area, which is on the new 305 (b) list (Al Hindrichs, Louisiana Department of Environmental Quality, personal communication), and Mill Creek, which regularly exceeded this standard during our 2002–2004 studies (Kaller and Kelso 2003, Kaller 2005), and is currently listed on Louisiana's 303 (d) list.

Perhaps more seriously, feral hogs appear to decrease freshwater mussel (members of the family *Unionidae* commonly known as pearly mussels) diversity and abundance by creating organic enrichment and changes in microbial community composition (Kaller and Kelso 2006). Hogs also may consume freshwater mussels in shallow water, headwater streams (S. Parris, U.S. Fish and Wildlife Service, personal communication). Southeastern states are home to

greater than 90% of the described species within the mussel family *Unionidae*, and 98% of the threatened or endangered *Unionid* spp. (Williams et al. 1993). Mussel populations appear to be declining throughout their range due to a variety of factors, including urbanization and other changes in land use that increase sedimentation and alter hydrology (Strayer 1993, Brim-Box and Mossa 1999, Brim-Box et al. 2002). Feral hogs may compound existing perturbations leading to further declines or localized extirpation.

We suggest that aggressive feral hog management is necessary, even if it requires changing state and local laws, to reduce feral hog density through promotion of hunter harvest, and possibly through increased trapping and hog euthanasia programs to prevent aquatic degradation, such as that observed in Mill Creek. This, in turn, will require cooperation among various federal, state, and local agencies (Hartin et al. 2007), and public education (Rollins et al. 2007). In the absence of control, feral hog populations will continue to grow, ranges will expand, and aquatic resources, specifically water quality, aquatic insect diversity and abundance and freshwater mussel diversity and abundance, will likely decline at local, if not watershed, scales. Without a change in state and local laws, we cannot prescribe specific measures for free-ranged domestic hogs because these animals are managed by local governments with state-level legislative oversight. However, without coincidental restrictions on free-ranging domestic hogs, feral hog management measures may ultimately fail. Nearly 35,000 water bodies are in poor or marginal condition throughout the United States (U.S. Environmental Protection Agency 2006) from historic and current pollution. Mill Creek is one of those water bodies, and hog activity may have tipped the scales in an already stressed watershed, leading to declines in aquatic insects and freshwater mussels (Kaller and Kelso 2006). Our data indicate feral hog populations are growing and contributing *E. coli* to water systems. We suggest that a potential exists for feral hogs to negatively impact other aquatic resources, particularly in already stressed ecosystems, throughout the southern United States.

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JAMES D. HUDSON III currently serves as the natural resources coordinator for the U.S. Army at Fort Polk. He holds a B.S. degree in biology from the University of Tennessee at Martin. He has 8 years of experience as a wildlife biologist, specializing in stream ecology and integrated natural resources management. He has worked extensively in southwest Louisiana streams, focusing on the role of macroinvertebrate communities in determining watershed management applications.



ERIC C. ACHBERGER has been on the faculty at Louisiana State University since 1983 as a microbiologist and molecular biologist in the Department of Biological Sciences. He received his Ph.D. from The Pennsylvania State University and completed postdoctoral research at the University of Washington School of Medicine. His current research interests include the use of DNA technologies to study microbial contaminants and dissemination of exotic species in water.



WILLIAM E. KELSO has been in the School of Renewable Natural Resources at Louisiana State University since 1984 and is currently serving as interim director of the school. He received a B.S. degree in biology at Florida State University, an M.S. degree in fisheries at the University of Massachusetts, and a Ph.D. in fisheries at Virginia Tech in 1983. His teaching responsibilities include lower level courses in natural resource conservation and fisheries, as well as senior level courses in freshwater and marine fisheries, and a graduate course in fish ecology. His research program has focused on organism-habitat relationships in streams, as well as the Atchafalaya River Basin (ARB), a bottomland floodplain swamp in south central Louisiana. Although several of his research projects have focused on fishes, much of his recent research has dealt with zooplankton and macroinvertebrate ecology, and most recently, the abundance and environmental relationships of exotic macrophytes such as hydrilla and water hyacinth, which currently dominate littoral habitats in the ARB.