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THE EFFECTS OF PRESCRIBED FIRE ON AMPHIBIAN AND REPTILE DIVERSITY IN AN OAK-GRASSLAND RESTORATION AREA.

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Historically, natural wild fires often swept through forests and grasslands, reducing plant biomass and affecting surrounding faunal communities. Prescribed fire management is a frequent tool in habitat restoration, yet the effects of such management on herpetofauna need to be better understood, because herpetofauna are a significant but underappreciated component of forest communities. We predicted that species adapted to drier and warmer environments would be more abundant in fire-managed habitats, outcompeting and filling niches at a higher rate than water dependent species, due to higher light levels, lower leaf litter, and less course woody debris (CWD) in such areas. Our study focused on eight wildlife ponds within a restoration burn area and eight similar ponds in an adjacent non-restored forest within Land Between The Lakes National Recreation Area. All ponds were sampled using dip-nets and minnow traps during June-August 2011, and drift fences were checked daily for captured animals from September through October. Amphibian larvae were captured and identified to species, and all herpetofauna found at fences were recorded. Data analysis is ongoing and our preliminary results will be discussed.

NOTES

RESULTS OF A DATA EVALUATION TO ESTABLISH PRIORITY REMEDIATION AREAS FOR DRY WEATHER FECAL CONTAMINATION IN A KARST INFLUENCED WATERSHED

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The goal of this study was to utilize a simple, cost effective means of identifying the land areas with the highest rate of sanitary sewer exfiltration to prioritize sanitary sewer investigations and point repairs. While wet weather problems including sanitary sewer overflows (SSOs) are generally well characterized by wastewater managers, sources of dry weather exfiltration are more difficult to identify, particularly in karst systems. This study was conducted in the Wolf Run watershed in Lexington, KY and analyzed stream flow and *E. coli* concentration data in conjunction with prior microbial source tracking assessments collected under low flow conditions in order to identify catchments with the highest incremental fecal load yields.

Two data sources were utilized in this analysis, each governed by an approved quality assurance project plan. A microbial source tracking study conducted in 2010 by the University of Kentucky Environmental Research and Training Laboratory (Brion et al 2011) was utilized to indicate the fecal load source. To calculate the fecal loading, dry weather *E. coli* sampling and stream flow measurements conducted under an EPA 319(h) grant for development of a Wolf Run watershed plan were utilized. *E. coli* samples were collected by Friends of Wolf Run and analyzed by the LFUCG Town Branch Laboratory and flow was measured by Third Rock Consultants.

The total loading and incremental loading were calculated in twelve catchment areas by utilizing the geometric mean of loading results from dry weather events and a simple spreadsheet model. In order to determine incremental yields, drainage areas were adjusted to account for karst flow.

As a result of this analysis, several areas within the Wolf Run watershed have emerged as priority areas to focus sanitary sewer remediation efforts. In order of priority, these areas include: 1) Wolf Run between Faircrest Drive and Lafayette Drive (W09), 2) Big Elm Tributary upstream of Harrodsburg Road (W11), and 3) Vaughn's Branch upstream of Tazwell Drive (W07). Correspondence between the microbial source tracking results and the highest loading sources indicates that human sewage is also the primary source of fecal pollution in the watershed.

As a result of this analysis, the LFUCG Division of Water Quality is conducting follow-up investigations, in addition to the remedial measures plan, to determine locations for point repair and remediation.

NOTES

DEVELOPMENT OF IN-HOUSE METHODS FOR HIGH-THROUGHPUT DNA EXTRACTION

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Given the high-throughput nature of many current biological studies, in particular field-based or applied environmental studies, optimization of cost-effective, efficient methods for molecular analysis of large numbers of samples is a critical first step. Existing methods are either based on costly kits purchased from the biotech industry or are low-throughput, time consuming and/or not optimized for environmental analyses. Here we describe the development and optimization of a 96 well, high-throughput method for extraction of DNA from enrichment broths targeting pathogens and indicators. The DNA extraction method that is being developed is derived from classic methods which involve microbial cell lysis, deprotenation, alcohol precipitation, washing of nucleic acid extract and silica matrix-based DNA capture.

The evaluation of extraction methods was carried out using overnight soil enrichments from samples collected from an on-going study established to evaluate survival of pathogens in conventional or no-till fescue plots with applied poultry litter or dairy manure. Samples (0.3 mL) from each broth type or from pooled broth samples were used for analyses. DNA concentration, DNA purity, PCR inhibition and amplification of targeted populations (particularly the *Salmonella sp. ttr* gene which is required for tetrathionate respiration) were measured.

The baseline for comparison was the FastDNA® Spin Kit for soil (MP Biomedical, Solan OH). The developed methods were modified from three previously published methods Petric et al., 2011; Li et al, 2010; and Boyle and Lew, 1995. Methods used variations of SDS lysis [100mM Tris (pH 8.0), 100 mM EDTA (pH 8.0), 100mM NaCl, and 2% (w/v) SDS] in conjunction with deprotenation (3M sodium acetate), DNA cleanup/alcohol precipitation and DNA recovery (precipitation or silica matrix). DNA concentrations (Table 1) were compared for diverse methods. However, to obtain consistent results in 96 Well plates, the final method (fRPE) involved mechanical beating using the FastPrep BeadBeater but without beads, realizing a significant cost savings.

Table 1. DNA Concentration per broth in ng/ul

Broth[a]	Fast DNA	PLB1[b]	PLB2[b]	mPLB[b] (tubes)	mPLB[b] (96 wells)
Bolton	32.78 ± 9.10	14.54 ± 7.86	69.89 ± 23.14	57.75 ± 2.57	10.52 ± 0.59
BPW	77.69 ± 11.12	35.37 ± 5.95	49.54 ± 6.85	27.53 ± 14.96	13.10 ± 4.21
RV10	6.90 ± 1.85	45.86 ± 8.12	55.78 ± 25.18	43.08 ± 33.98	11.41 ± 4.32
TTH	72.26 ± 29.26	25.07 ± 0.66	90.84 ± 40.00	77.72 ± 44.39	12.73 ± 0.83
UVM	32.60 ± 13.43	17.14 ± 8.71	12.5 ± 10.92	12.5 ± 10.92	12.36 ± 3.01

[a] Broth indicates media used for enrichment of *C. jejuni* (Bolton), All Cells (BPW), *Salmonella* (RV10 & TTH), *L. monocytegenes* (UVM)
[b] PLB or mPLB refers to modification of methods described by Petric,2011; Li, 2010; Boyle, 1995
Values represent the average± standard devation of triplicate samples quantified by the NanoDrop 100 Spectrophometer

Ultimately, the most significant factors in the method development were using a mechanical lysis and optimizing the type of silica matrix used to bind the extracted DNA (Table 2). The silica matrix binds DNA selectively in the presence of a chaotropic agent i.e. a chemical which disturbs the structure of water (Carter and Milton, 1993). A variety of chaotropic agents have been used for such purifications including sodium iodide (NaI), sodium perchlorate and guanidinium thiocyanate (GITC). Initial protocols utilized silica matrix resuspended in NaI but results were inconsistent, likely due to a slight change in the pH of NaI from 6.4 to more than 7.4. The binding of DNA to the silica is strongly pH dependent, above pH 7.5 binding is poor and rapidly becomes better below 7.5. We developed a HCl-based re-suspension solution for the glass milk (AGM) by combining 4M-6M solution of a chaotropic solution (NaI or GITC) with the silica solution brought to pH 2 with 30% HCl. In this way, pH is maintained between pH 6.0 and pH 7.4.

Table 2. Evaluation of *Salmonella* concentration in enrichment broth using modifications of extraction methods

Extraction method	Lysis Method	Silica Matrix Acidified	Chaotrophic Agent	*Salmonella* Conc. (Cells g^{-1})	Method Notable
Fast DNA Extractions	Beating w/Beads	Proprietary	Proprietary	0.15 ± 0.03 X 10^4	Standard Method
mPLB	Heat	No	NaI	4.79 ± 0.71 X 10^4	Best Method but inconsistent result
mPLB-2	Heat	No	NaI	1.77 ± 0.52 X 10^4	Lower Chaotrophic Agent
mPLB-3	Heat	No	NaI	3.39 ± 0.44 X 10^4	Higher Chaotrophic Agent
RPE-1	Heat	YES	NaI	2.64 ± 0.17 X 10^4	Acidified Silica
RPE-2	Beating w/Beads	YES	NaI	7.52 ± 1.02 X 10^4	Acidified, Beating & Added Beads
RPE-3	Beating no Beads	YES	NaI	9.55 ± 0.71 X 10^4	Acidified, Beating & No Beads
RPE-4	Beating no Beads	YES	GITC	4.11 ± 0.80 X 10^4	New Chaotrophic Agent-More stable
fRPE	**Beating no Beads**	**YES**	**GITC**	**7.67 ± 0.32 X 10^4**	**Acidified, GITC, Beating & No Beads**

This method is performed in a 96 well format using lab-based solutions for extraction and DNA binding. The high-throughput format permits preparation of up to 192 samples in a 4 hour time frame. By eliminating the use of purchased extraction solutions, extraction tubes and silica matrix, the cost per sample has been estimated to be as low at 10 cents.

References:

Boyle, J.S., Lew, A.M.1995. An inexpensive alternative to glassmilk for DNA purification. Trends in Genetics Vol. 11 No.1 p. 8.

Carter, M.J., Milton, I.D. 1993. An Inexpensive simple method for DNA purification on Silica Particles. Nucleic Acids Research 21:1044. Protocol 4.24. http://www.med.unc.edu/pmbb/MBT2000/DNA-ISOL.htm

Li, Jian-Feng; Li Li, Sheen, Jen. 2010. Protocol: rapid and economical procedure for purification of plasmid of plant DNA with diverse applications in plant biology. Plant Methods 6:1.

Petric, I, Philippot, L., Abbate, C., Bispo, A., et al. 2011. Inter-laboratory evaluation of the ISO standard 11063 “Soil quality — Method to directly extract DNA from soil samples”. Journal of Microbial Methods 84 p. 454-460.

VALIDATION OF THE SANITARY CATEGORY VALUE (SCV) MODEL FOR THE IDENTIFICATION OF LEAKING SEWER LINES: A STUDY OF THE WEST HICKMAN WATERSHED OF LEXINGTON, KY

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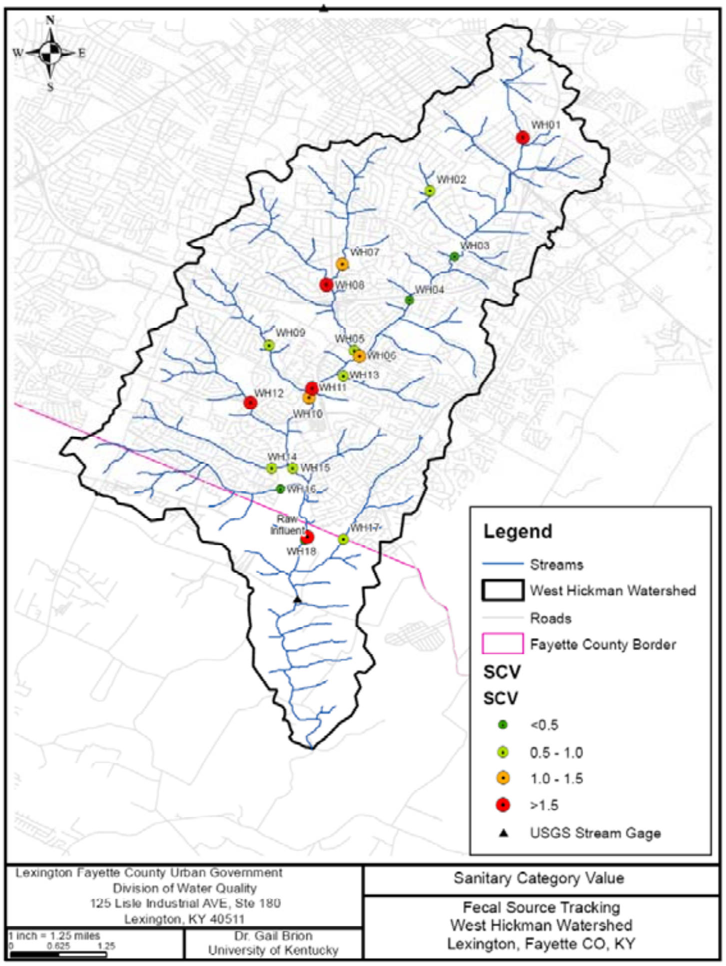
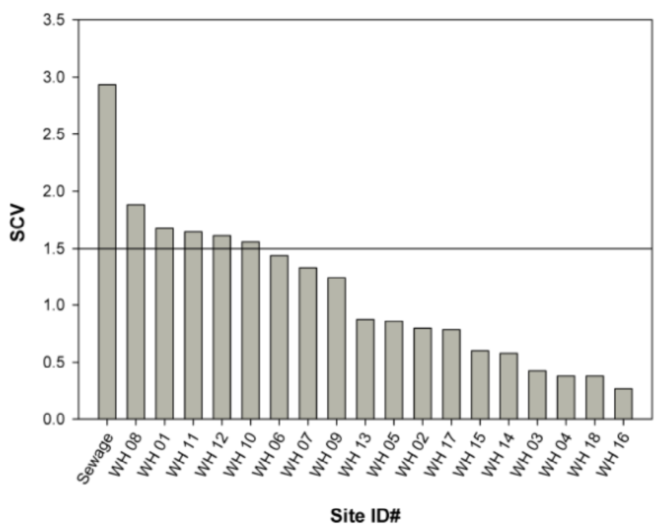
A study of the West Hickman watershed was conducted to validate an approach for relative ranking of sewage impacted locations within Lexington's watersheds for future remediation. A simple modeling system was created by researchers at the University of Kentucky and evaluated in a prior Lexington Fayette Urban County Government (LFUCG) sponsored project in the Wolf Run Watershed. The approach included indicators of fecal load, age, and source as well as a sampling plan that included a wide spatial and temporal range. This system was applicable for ranking regions within the watershed with respects to the degree of sewage intrusion based upon a Sanitary Category Value (SCV). The SCV could be used to pinpoint hotspots of sewage intrusion into the watershed and prioritize areas for remediation. The same approach and modeling system were applied to water quality samples obtained from the West Hickman Watershed under dry weather conditions in a recent study supported by LFUCG. Samples were collected by the employees of Third Rock Consulting from eighteen locations on four dates between August and October of 2011. The samples were analyzed at the ERTL facility at UK for viable *E.coli* bacteria, the ratio of atypical colonies (AC) to total coliforms (TC), and the concentrations of general and human specific *Bacteriodes* DNA markers. The results from each indicator analysis were then combined to generate a single SCV for each sample location. The overall results of this study indicate that while West Hickman does not have sites that are as severely impacted by fresh human sewage as were found in Wolf Run, there are some sample sites that show the continuous presence of human sewage. These hotspots are prioritized to receive further investigation and determination of source so that remediation can be undertaken and these potential sources of disease eliminated from the watershed. As was shown previously in the Wolf Run watershed, the SCV model approach provided identification of the highest priority sites and relative ranking of all sites in the West Hickman watershed without the need for more expensive engineering efforts.



Average Sanitary Category Values for West Hickman Watershed under Dry Weather Conditions



COMPARISON OF SOIL-MOISTURE BASED IRRIGATION SCHEDULING TO POTENTIAL EVAPOTRANSPIRATION IN TOMATO GROWN USING PLASTIC MULCH

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Irrigation scheduling has traditionally been weather or soil based. In weather-based scheduling, the decision to irrigate relies on the soil water balance. The water balance technique involves determining changes in soil moisture over time based on estimating evapotranspiration (Et) adjusted with a crop coefficient (Penman, 1948). This method takes environmental variables into account along with crop coefficients that are adjusted for growth stage and canopy coverage (Hartz, 1996). However, variability in production systems has made Et-based irrigations less common in vegetable crop production. (Amayreh and Al-Abed, 2005; Burman et al., 1980). Soil-based methods are often employed to manage irrigations in vegetable production. Typically these methods involve maintaining soil moisture between predetermined thresholds regardless of weather and crop conditions. A purpose of this trial was to determine water usage between several soil-moisture based irrigation regimes and potential evapotranspiration in plasticulture-grown tomatoes.

This study was conducted during 2009 and 2010 at the University of Kentucky Horticulture Research Farm in Lexington, KY. Tomato (*Lycopersicon esculentum*) 'Mountain Fresh' was grown using standard procedures for plasticulture production in KY (Coolong et al., 2009a). Automated irrigation was managed using paired switching tensiometers (model RA 12-inch; Irrometer, Riverside, CA). In paired treatments, one tensiometer functioned to turn on irrigation while the other turned it off. Tensiometers were placed approximately 20 cm from the tomato plants and 10cm from the edge of the raised beds, at a depth of 20cm from the upper surface of the bed. On/off set points for the four, two-tensiometer treatments were as follows: on/off -30/-10, -30/-25, -45/-10, -45/-40 kPa. These set points were based on previously reported thresholds (Coolong et al., 2009b; Wang et al., 2007). The frequency and duration of the automated and manual irrigation events were recorded with data loggers (Hobo U9 State Data Logger; Onset, Cape Cod, MA). There were four replications of irrigation treatments. Treatment plots consisted of 20 plants (measurements were taken on 16 plants in the center of each plot) arranged in a completely randomized design for a total of 20 experimental plots. Weather data were obtained from an on-farm weather station that recorded environmental variables every minute and provided hourly averages [Kentucky Mesonet, Fayette County Station, Lexington, KY (University of Kentucky, 2011)].

Despite hotter and drier growing conditions, water usage was generally lower in 2010 compared to 2009. The -45/-40 kPa treatment used the least amount of water in both years with 98,496 gallons/acre and 85,147 gallons per acre in 2009 and 2010, respectively. The soil-moisture based treatment using the most water differed from the -30/-25 kPa treatment in 2009 to the -30/-10 kPa treatment in 2010. Nonetheless, all soil-moisture based treatments used substantially less water than estimated potential Et. In 2009 potential Et was determined to be 353,997 gallons/acre using FAO-based crop coefficients (Allen et al., 1998) for field-grown tomato or 235,367 gallons/acre using a crop coefficient determined for plasticulture-grown tomato (Amayreh and Al-Abed, 2005). Due to differences in spacing, soil coverage and localized drip irrigation the potential Et calculated using FAO crop coefficients were always greater than those using coefficients developed specifically for plasticulture production. In 2010, conditions were drier and hotter and potential Et was determined to be 422,136 and 638,578 gallons/per acre for plasticulture-based and FAO crop coefficients, respectively. Total marketable yields among irrigation treatments were not significantly different in 2009, but did differ in 2010; however, there were no clear trends between yield and water use in 2010. Yields were commercially satisfactory in both years suggesting that soil moisture was not limiting production. These results suggest that soil-moisture based irrigation can significantly reduce water use compared to using Et-based methods. In addition, for plasticulture-grown tomatoes, Et-based methods may significantly overestimate water usage as differences in microclimate and production methods may not be accounted for in calculations using standardized crop coefficients.

References

Allen, R., L.S. Pereira, D. Raes, and M. Smith. 1998. Crop evapotranspiration – guidelines for computing crop water requirements. FAO Irrigation and drainage paper 56. Rome, Italy.

Amayreh, J. and N. Al-Abed. 2005. Developing crop coefficients for field-grown tomato (*Lycopersicon esculentum* Mill.) under drip irrigation with black plastic mulch. Agr. Water Mgt. 73:247-254.

Burman, R.D., P. Nixon, J. Wright, and W. Pruitt. 1980. Water requirements, p. 189-234. In: M. Jensen (ed.). Design and operation of farm irrigation systems. Amer. Soc. Agr. Eng., St. Joseph, MI.

Coolong, T.C., R. Bessin, K. Seebold, J. Strang, and R.T. Jones. 2009a. Vegetable production guide for commercial growers 2010-11. University of Kentucky Coop Ext. Serv. Bul. ID-36.

Coolong, T.C., J. Strang, and A. Lentz. 2009b. The effects of pulsing drip irrigation on tomato yield and quality in Kentucky. 2009 Fruit and vegetable research report. University of Kentucky Coop Ext. Serv. Bul. PR-603. 39-40.

Hartz, T.K. 1996. Water management in drip-irrigated vegetable production. HortTechnology 6:165-167.

Penman, H.L., 1948. Natural evaporation from open water, bare soil, and grass. Proc. Royal Soc. London Ser. A-Mathematical Physical Sci. 193:120-145.

Wang, D., Y. Kang, and S. Wan. 2007. Effect of soil matric potential on tomato yield and water use under drip irrigation condition. Agr. Water Mgt. 87:180-186.