Oklahoma Water Resources Research Institute
Annual Technical Report
FY 2005

Introduction

The Environmental Institute (EI) at Oklahoma State University promotes interdisciplinary environmental research, graduate education, and public outreach leading to better understanding, protecting, and sustainably developing the natural environment. The Oklahoma Water Resources Research Institute, located within the EI, is responsible for developing and coordinating water research funding to address the needs of Oklahoma. To assist in meeting this objective, input is obtained from discussions with state regulators, policymakers, and other water resources professionals about pressing water research needs in the State.

Research Program

In 2005, proposals were solicited from all comprehensive universities in Oklahoma. Proposals were received from three of these institutions: Oklahoma State University, University of Oklahoma, and East Central University. Eleven proposals were submitted and from these four projects were selected for funding for one year each.

Optimal Selection of Management Practices, Policies, and Technological Alternatives for Phosphorus Abatement: Using GIS and Economic Methodology to Model a Watershed is an evaluation of the economic efficiency of a set of policies designed to remedy phosphorus pollution problems in the Eucha-Spavinaw watershed in Eastern Oklahoma and Western Arkansas. Estimating the Orientation and Intensity of Fractures in Sedimentary Rocks Using Multicomponent 3-D Ground-Penetrating Radar is a feasibility study to determine if multicomponent 3-D ground-penetrating radar (GPR) technique can be used effectively to map the fracture orientation and intensity in fractured rocks. Science, Development and Public Opinion: The Adjudication of Groundwater Policy for the Arbuckle-Simpson Aquifer is a multi-year investigation that will assess the impact on public opinion and water policy of another scientific study being conducted by the Oklahoma Water Resources Board. Protocol to Determine the Optimal Placement of Riparian/Buffer Strips in Watersheds is a project to develop a protocol to determine the optimal placement of riparian/buffer strips in a watershed to maximize its efficiency in removal of sediment and nutrients load and improve its impact on water quality.

In addition, two 2004 projects which were extended into 2005 are reported on here. Evaluation of Chemical and Biological Loading to Blue River is an investigation of bacterial and nutrient loading to a tributary of the Red River in south-central Oklahoma. Springs in time: Comparison of Present and Historical Flows is an evaluation of groundwater elevation changes across the state.

Optimal Selection of Management Practices, Policies, and Technological Alternatives for Phosphorus Abatement: Using GIS and Economic Methodology to Model a Watershed

Basic Information

Title:	Optimal Selection of Management Practices, Policies, and Technological Alternatives for Phosphorus Abatement: Using GIS and Economic Methodology to Model a Watershed
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Principal Investigators:	Brian D. Adam, Franklin Bailey Norwood, Arthur Stoecker, Daniel E. Storm

Publication

1. Chala, Zelalem Teklu 2005, "Least Cost Solutions for Removing Poultry Litter from the Eucha-Spavinaw Watershed under Phosphorus Restrictions," M.S. Thesis, Department of Agricultural Economics, Oklahoma State University, Stillwater, Oklahoma, 77 pages.
2. Sadhu, J. 2005, "Valuation of Economic Gains to Broiler Producers by Modulating Ventilation and Using Alum for Ammonia Control," M.S. Thesis, Department of Agricultural Economics, Oklahoma State University, Stillwater, Oklahoma, 84 pages.
3. Othomi, Aya. "Long-Term Effects of Management Practices on Sustainability of Water Quality and Land Productivity in the Eucha/Spavinaw Watershed," Senior Honors Thesis, Submitted to the Honors College, Oklahoma State University, Stillwater, Oklahoma, 30 pages.

Annual Report

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State Date: 3/1/05
End Date: 2/28/06

Problem and Research Objectives:

In eastern Oklahoma and western Arkansas, poultry litter from broiler producing operations has saturated the land, causing nitrate leaching and runoff of potassium and phosphorus, harming water supplies. In response to this problem, our research had three objectives:

Objectives:
The overall objective of the study was to evaluate the efficiency of a set of policies designed to remedy phosphorus pollution problems in the Eucha-Spavinaw watershed in Eastern Oklahoma and Western Arkansas. Specific objectives of the study were to:

1. Determine the economic viability and best location for poultry litter-to-energy facilities.
2. Determine the most economically effective set of poultry litter management practices and/or STP regulations that meet specified limits on soluble phosphorus runoff.
3. Determine the most efficient pattern of litter transportation for use within the watershed and for removal of excess litter from the watershed.

Methodology:
The most efficient overall policy is expected to be a set of individual interdependent complementary policies. For example the economic viability of a litter-to-energy plan and the litter transportation will be affected by a soil test phosphorus level (STP) that limits litter application at various locations within the watershed. Implementation of uniform STP limits over the watershed are expected to be more costly and create different transportation patterns than would STP phosphorus limits based on the P-Loss Index concept.

The composition of the best policy set is highly dependent upon the total amount of allowable discharge from the watershed. It is necessary to not only determine the optimal level of annual phosphorus given by our model but also to estimate the benefits (reduced environmental damages) and costs associated with the most efficient policy set for meeting each of several possible discharge limits. This allows users to make adjustments for qualitative factors not addressed by the model.

The first step was the construction of a basin level mathematical programming model. The model is capable of simultaneously determining: a) the optimal location of processing facilities for and the quantity of poultry litter to be converted to energy, b) the quantity of litter to be transported from poultry houses to locations within and out of the watershed, and c) the best management practices for applying poultry litter in each HRU within the watershed so that the total cost of meeting specific phosphorus emission targets is minimized.

Information for the litter-to-energy plant and transportation network from poultry houses in the basin was obtained by completing Objective 1. This information is reported in greater detail in Chala. The information (farm income, soil phosphorus levels, and phosphorus runoff) on alternative poultry litter management practices and P application rules was obtained from a series of SWAT simulations. Software was developed to "read" the numerous SWAT output files for each hydraulic response unit (an area of common soil type and land use) from each simulation run and directly incorporate the results into a programming model. The programming model is a necessary step to determine the most economically efficient set of management practices. This is because the uniform regulations easily analyzed by simulation models are not the most cost effective. The programming model is a mechanism to accumulate the SWAT simulations. Solution of the programming model allows different management practices to be used in different HRUs. The concept is similar to that of targeting soil conservation practices to "Highly Erodible" land where more erosion can be prevented at lower cost.

The second step was to conduct a series of policy analysis scenarios with the completed programming model. The effectiveness of the possible abatement policies was determined by parametrically varying the annual limit on annual phosphorus discharges. The allowable range of annual phosphorus loading from the watershed was be varied by five-ton increments from the minimum attainable value upward to the annual current load of 50 tons. The solutions indicate the most economically efficient mix of policy methods to achieve each level of phosphorus abatement. Estimates of the amount of water treatment cost avoided and the amount of lake recreation obtained provide policy makers with the tradeoff between cost of further reducing P loads against the value of environmental damages avoided.

Findings:

Objective 1:

The purpose of this objective was to determine the economic viability and best location for poultry litter-to-energy facilities. The complete project has been reported in a thesis by Chala, but the results are summarized here. Three scenarios were examined. Scenario I analyzed reduction of poultry litter applied to land in eastern Oklahoma with an assumption that no processing plant is established. In this situation, continuous variables represented the quantity of litter transported from each poultry grower to each selected wheat and/or forage-growing county outside the watershed. A linear programming model was used to determine the optimum solution.

Scenario II examined the combined alternatives of establishing processing plants and transporting some amount of Oklahoma poultry litter outside the watershed. In this case, binary variables were used to select or not select a particular location and processing plant capacity. Mixed integer programming was used to find the optimum solution. Scenario III was like Scenario II, but included poultry litter from western Arkansas.

Since the assumptions about profitability of processing plants were projections and have not been tested, the model results were tested for sensitivity to an alternative assumption. The alternative assumption was that, rather than achieving its expected profitability, the processing plant achieved only half that amount. This might happen, for example, if electricity or fertilizer yields were lower than predicted, or if wholesale electricity and fertilizer prices were lower than predicted, or if the "green energy" tax credit (currently 1.8¢/kwh) were not available.

The model choice variables were quantities of litter transported from each farm to each processing plant and processed, capacity of each processing plant that is built, and quantity of litter transported from each farm to each of several phosphorous-deficit counties.

The model selected one of five plant capacities for each of five prospective plant locations: 100,000, 200,000, 300,000, 400,000, and 500,000 tons per year of litter processing capacity. Alternatively, the model chose to build no plant at a particular location. Binary variables were used to model these choices. Continuous variables were used to determine the quantity of litter transported from each farm.

Key Results

- Using previous projections of processing profitability (determined under the previous year's OWRRI study), the model's optimum solution is to build one 400,000-ton capacity plant and one 500,000-ton capacity plant. Operating at 90% capacity, the 400K-ton plant processes 289,560 tons of Arkansas litter and 70,440 tons of Oklahoma litter, while the 500K-ton plant processes 340,920 tons of Arkansas litter and 109,080 tons of Oklahoma litter. Profit reported by Chala is $8.88/ton of litter. Updating those results with increased transportation costs results in an expected profit of $6.81/ton of litter.
- If processing is as profitable as projections indicate, processing is an effective way of removing litter from the region, and no mandates are necessary. Transporting litter out of the region is more costly.

- If processing is 50% less profitable than projections indicate, it is still less costly than transporting the litter out of the region, and is thus an effective way of removing litter from the region. However, neither processing nor transporting litter out of the region will occur without incentives or mandates, such as a constraint on minimum amount of litter to be removed. In this case, raising the minimum amount of litter that must be removed reduces per-ton cost of removing litter because it forces larger amounts to be processed, taking advantage of economies of size in processing.
- There is a tradeoff between reducing cost (increasing profitability) of removing litter, and amount of litter removed from Oklahoma. Profitability is increased by allowing Arkansas litter to be processed, but this reduces the amount of litter removed from Oklahoma.

Findings:

Objectives 2 and 3:

Objectives 2 and 3 were to determine the most economically effective set of poultry litter management practices and/or STP regulations that meet specified limits on soluble phosphorus runoff, and to determine the most efficient pattern of litter transportation for use within the watershed and for removal of excess litter from the watershed. These objectives were to include a poultry litter-to-energy processing plant as one of the alternative uses for the litter if the plant had the potential to be economically viable. The results from Objective 1 found that with quite conservative assumptions a litter-to-energy plant could be economically viable, so it is included in the following analysis.

The procedures used to accomplish objectives 2 and 3, and a detailed summary of the findings, are described below.

An Approach to Efficient Watershed Pollution Abatement

The recent explosive growth in geographical information systems (GIS) with environmental databases has been accompanied by growth and improvement in watershed level simulation models. The latter have found a ready audience with those particularly concerned with nonpoint source pollution problems. The main problem treated in the paper is the use of these new developments to assign management practices to particular areas within the watershed to effectively control nonpoint source pollution at least cost. This approach illustrates a relatively simple method of using GIS based simulation models to estimate nonpoint source coefficients that can be input into a conventional mathematical programming model. The programming model is then used to select most efficient management practices for each location in the watershed so that an overall pollution target is reached at least cost. The motivation for this process can be better understood by first reviewing the conceptual framework for determining optimal abatement levels and the inherent problem with using only a simulation model to accomplish the task.

Conceptual Framework

The conceptual framework is based on this concept of minimizing the sum of total pollution abatement cost and total environmental damage cost (Freeman, Haveman and Kneese, 1973). Assume there is a watershed with two heterogeneous sources of pollution (each emitting 100 tons per year) that cause environmental damage. For each source i, total unregulated pollution (TP_i) is equal to pollution removed or abated (R_i) and pollution remaining (P_i). The optimal social welfare pollution abatement problem can be expressed as,

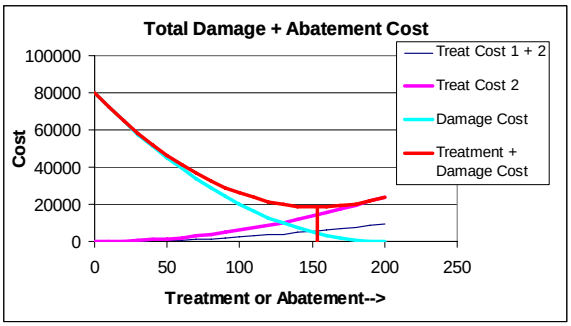
(1) Min $W(P) = a_1R_1^2 + a_2R_2^2 + d(P_1 + P_2)^2 + \lambda_1(TP_1-P_1-R_1) + \lambda_2(TP_2-P_2-R_2)$,

where W(p) is a pollutant dependent welfare function, M* is the maximum amount of market goods produced in a economy without any abatement, E* is the maximum potential value of environmental services obtained from a pristine environment, $a_iR_i^2$ is the total abatement or treatment cost at source i for removing R_i units of pollution, $d(\Sigma Pi)^2$ is the monetary value of environmental damage caused by the remaining pollution from each source i. Since M* and E* can be treated as endowments that are fixed in the short run, the total social well being can be maximized by minimizing the sum of pollution abatement costs and environmental damages costs. The first order conditions with respect to Ri, Pi are,

$\partial W/\partial R_1 = 2a_1R_1 - \lambda_1 = 0$, $\partial W/\partial R_2 = 2a_2R_2 - \lambda_2 = 0$,

$\partial W/\partial P_1 = 2d(P_1 + P_2) - \lambda_1 = 0$, $\partial W/\partial P_2 = 2d(P_1+P_2) - \lambda_2 = 0$.

If all P_i, $R_i > 0$ are in the optimal solution, then the last equation indicates that $\lambda_1 = \lambda_2$ and that $2a_1R_1 = 2a_2R_2 = 2d(P_1+P_2)$. In a watershed with n sources of pollution, optimal abatement occurs where $MAC_1 = MAC_2 = \ldots = MACn = MDC$, where MAC_i is the marginal abatement cost for source i and MDC is the marginal damage cost at the point of measurement. If for the same amount of abatement ($R_1 = R_2$) we have $MAC(R_2) > MAC(R_1)$, then at the optimum, $R_2 < R_1$. That is, most of the abatement should be accomplished by R_1, the source with the lower marginal abatement cost.



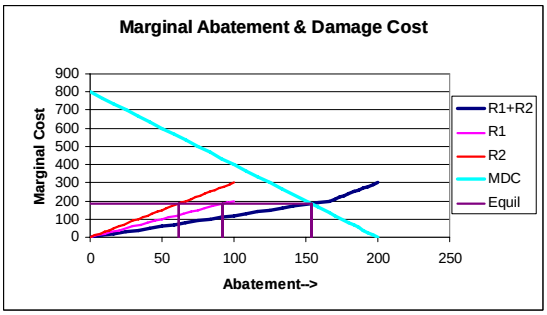


Figure 1. Total and Marginal Abatement and Damage Costs for the Example Problem.

This concept is illustrated in Figure 1 above where a_1=\$1, a_2 = \$1.5 and d=\$2. The optimum level of pollution abatement occurs where sum of total Treatment + Damage cost is a minimum. The optimal solution calls for the abatement of 154 tons with source one removing 92 tons and source two removing 62 tons. This level of removal equates the marginal abatement costs for each source to each other and to the aggregate marginal damage cost curve at \$185 per ton. Note the solution requires an equation of marginal abatement costs across sources and does not assume equal or proportional abatement across sources. For this approach to be operational in a watershed, empirical estimation of both abatement and environmental damage costs for the selected pollutant or pollutants is needed.

Geographical Information Systems and Simulation Models.

Large efforts are underway to develop high quality Geographical Land Use data sets in many countries of the world. At a minimum these data sets typically contain layers for land use and characteristics for soil type. At the same time many GIS based simulation models have been developed to help environmental planners deal with pollution problems at the watershed level. Several authors (Gurnell and Montgomery, 2000), (Arnold *et al.*, 1998), discuss recent advances in biophysical modeling due to the advent of GIS data use as well as other dramatic improvements in computing capabilities. These advances create an opportunity for more precise modeling of the environmental-economic processes relevant for the problem of point and

nonpoint pollution abatement at the watershed and river basin level. These advances could be used in designing environmentally and economically effective policies. However these are simulation models while efficient solutions to the above problem require optimization as illustrated in the example below.

The example below in Figure 2 illustrates a relatively simple two-step procedure for combining simulation and mathematical programming to determine a set of phosphorus abatement practices for a watershed. In the example, the objective is to determine how much poultry litter can be applied to each HRU if total producer income from the watershed is maximized while total phosphorus emissions are held below a target level.

The first step is to conduct a series of multiyear simulations where alternative management practices are tested in each HRU of the watershed. Assume the management practices consist of applying from 1 to 6 tons of poultry litter per hectare on land used for hay. A 10-year simulation is run in which 1 ton of litter is applied to each HRU. From the simulation output, the yield averaged over 10 years is multiplied by the price of hay ($70/ton). Then the variable costs of $15 for materials and labor per hectare and $5 per ton of litter applied are subtracted. For HRU_1, the net revenue calculation, ($70/mt)(.59 mt)- $15 - $5 /mt = $21) is entered in the objective function. The average P loss of 2.46 kg per hectare is entered in the PLoss row of the model. From the same simulation run, calculations for applying one ton of poultry litter in each of the otherHRUs are made and the coefficients are entered into the programming model. Another 10 year simulation is conducted with two tons of poultry litter applied to each HRU. The average net

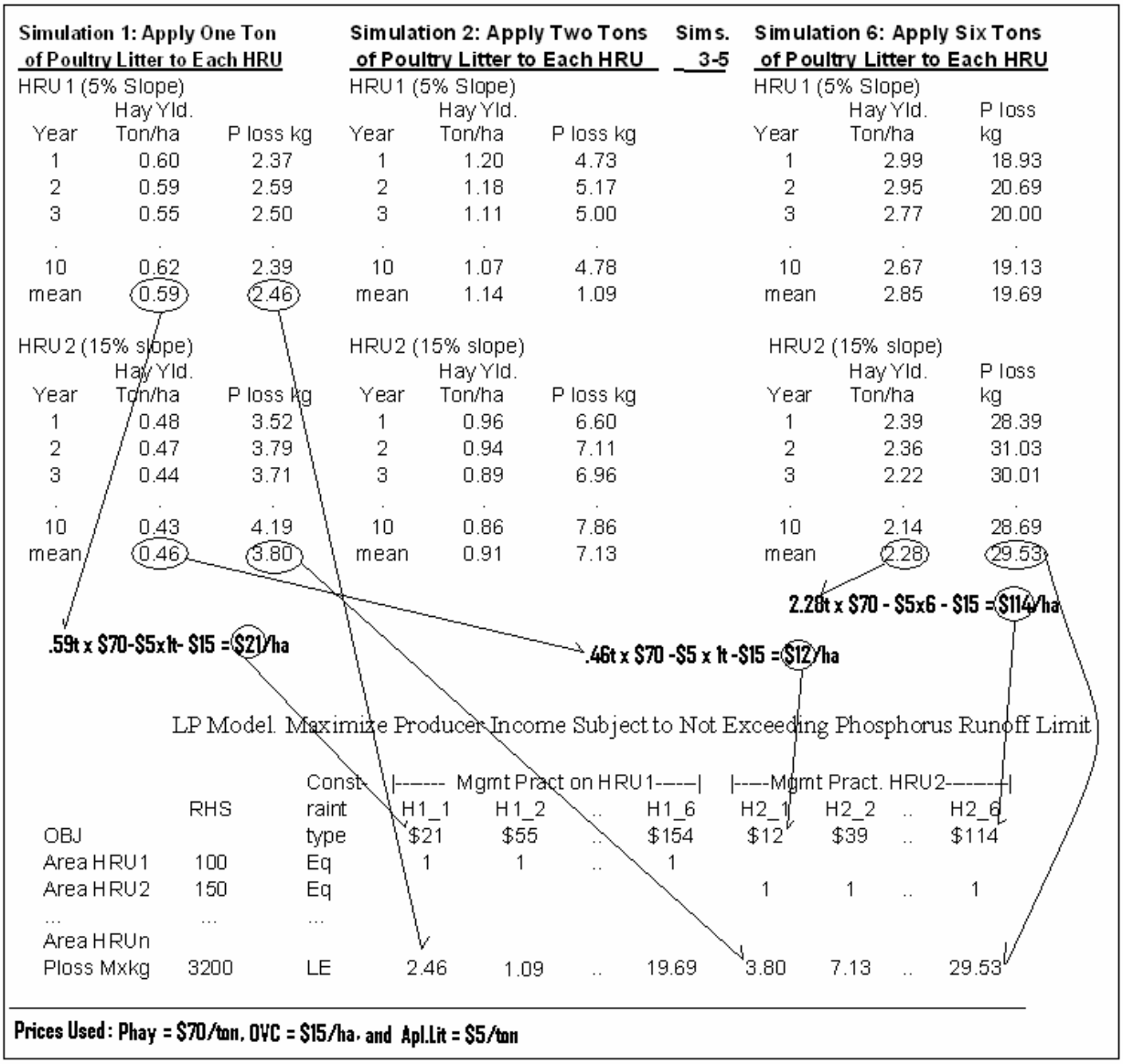


Figure 2. Example Showing Construction of a Watershed Level Programming Model from a Series of Simulation Runs with Different Level of Poultry Litter Applied.

revenue and P loss values are calculated and added to the programming model. The simulation process is repeated with 3, 4, 5, and 6 tons of litter applied to each HRU. After each simulation the net revenue and P loss values for each HRU are calculated and entered in the programming model. After the programming model has been completed, the Maximum Allowable P loss can be varied parametrically to determine the maximum farm income, amount of poultry litter that can be applied in each HRU so the watershed target is met. The treatment cost is obtained by

subtracting the value of the objective function from the maximum farm income that could be obtained in the absence of any limitation of P runoff.

With only the data shown in Figure 2, maximum income ($32,570) is obtained with all producers applying 6 tons of litter. Phosphorus runoff is nearly 6,400 kg. The maximum income that could be obtained with a 3,200 limit on phosphorus loading is $21,770 where producers in HRU1 apply 6 tons of litter but producers in HRU2 reduce litter applications to 2.2 tons per hectare. The minimum treatment cost to meet the 3,200 phosphorus limit is $10,800. In a large watershed with several thousand HRUs, it will be more efficient to determine the least cost pattern of abatement by using a combination of simulation and mathematical programming than by searching with simulation alone. The programming model has the added advantage that treatment costs from municipal and industrial sites can be included along with the damage cost from pollution and the model used to determine optimal level of abatement from point and nonpoint sources of pollution.

Applications of the Methodology

Examples with two types of biosimulation models are provided. The first is the Soil Water Assessment (SWAT) Model. SWAT is a comprehensive model developed by the U.S. Agricultural Research Service at the Blacklands Research Center in Temple, Texas. (Arnold *et al*. 2000). SWAT divides a watershed first into subbasins. Then each subbasin is subdivided into hydrologic response units (HRU). An HRU is an area with a common soil type and land use. The model uses daily rainfall and solar energy to simulate biomass growth, filtration and runoff, nutrient flux, and groundwater drainage. The model must be calibrated for use in a specific subbasin by selecting soil types, land cover, and measuring water and nutrient outflows against recorded stream flow data. (Storm *et al*. 2002).

Use of SWAT and Optimal Phosphorus Abatement in a Watershed.

The Eucha-Spavinaw watershed (Figure 3) that crosses the Western Arkansas- Eastern Oklahoma border has been troubled for a number of years and has been a source of considerable controversy between the two states. The watershed is a primary source of drinking water for the Tulsa metropolitan area (population 1 million). Eutrophication of Lakes Eucha and Spavinaw is blamed on high phosphorus loading in the watershed attributed to excessive land application of litter produced by intensive poultry industry in the area, and discharges of municipal wastewater from the City of Decatur, AR, emitted from a combined treatment plant for the municipality and a poultry processing facility (Storm *et al*., 2002). Water from eutrophic lakes is not suitable for drinking due to bad taste caused by chemicals resulting from algae presence (OWRB, 2002). Drinking water treatment facilities are able to treat the water to achieve established drinking water standards, but find it difficult and extremely expensive to treat the water to remove the bad taste (TMUA, 2003). There are concerns regarding the recreational values of the area lakes, as well as concerns about the overall ecological impacts of phosphorus pollution in the watershed. The estimation of abatement and damage costs is described below.

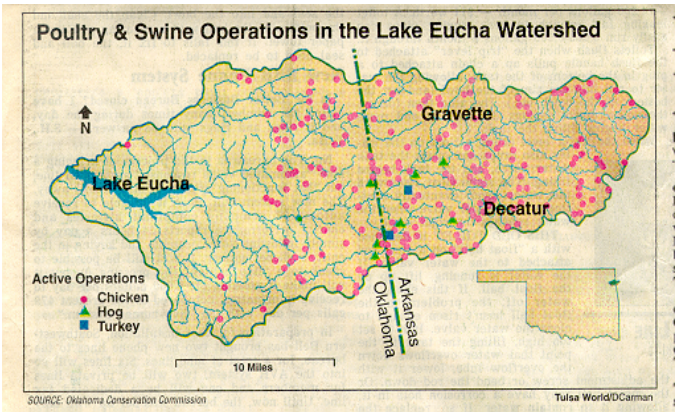


Figure 3. Eucha-Spavinaw Watershed

Abatement costs

Total abatement costs are the sum of point and non-point source abatement costs. Abatement costs for a municipal wastewater treatment represent the costs of employing a particular abatement technology for phosphorus reduction. Abatement costs for non-point

Environmental damage costs

The two main types of environmental damages caused by phosphorus pollution in the watershed were identified as the impairment of the quality of drinking water for the city of Tulsa (OWRB, 2002) and the losses of recreational values from the lakes, Eucha and Spavinaw. The latter was reflected in a drastic reduction in the number of annual visits (OCC, 1997, OTRD, 2003)).

Methods and Procedure

First, the Soil Water Assessment Tool (SWAT) is used as a Geographical Information Systems (GIS) data biophysical simulation model for the Eucha-Spavinaw watershed (Storm *et al*., 2002). The SWAT output data on crop yield, grazed biomass and phosphorus runoff were used in a spatial mathematical programming model to determine optimal allocation of management practices to the point and non-point sources of phosphorus loading within the watershed and to derive the marginal phosphorus abatement costs. Environmental damage costs were calculated as a sum of cost for additional drinking water treatment for the City of Tulsa and the costs of recreational losses of the area lakes.

Step 1: Management Practices, Abatement and Damage Costs

The calibrated SWAT model for the Eucha-Spavinaw watershed (Storm et. al., 2001) was used to conduct biophysical simulation for the alternative BMPs. The BMPs were implemented on 2,416 agricultural hydraulic response units (HRU) from 90 sub-basins in the Eucha-Spavinaw watershed. An HRU represents a combination of a major soil type and land use within a sub-basin. The watershed with broiler houses is shown in Figure 3.

SWAT Delineation of Eucha Watershed

The SWAT simulation model was developed and Calibrated by Storm and White (2005). The 93,115 hectares in the watershed was delineated by SWAT into 2413 HRUs and 27 major

soil types. There are more actual soil types in the basin but SWAT combines similar soils together to reduce the total number of HRUs. The current study concentrates on the `1395 HRUs and the 35,916 hectares classified as pasture The land uses by major soil type are shown in Table 1.

Table 1. Land Use delination of Eucha Watershed by Major Soil Type and Land Use

	Land Use						
Major Soil Type	Crop	Pasture	Range	Forest	Urban	Water	Total
	Hectares						
BRITWATER	5	1111	145	593	27	91	1974
CAPTINA	316	5150	201	404	383	2	6456
CARYTOWN	0	127	16	0	8	1	152
CHEROKEE	0	19	0	0	1	0	20
CLARKSVILLE	11	5932	2327	32810	152	156	41388
DONIPHAN	73	4353	398	1161	172	3	6160
ELDORADO	0	26	0	0	0	0	26
ELSAH	0	85	33	313	4	9	444
HEALING	17	175	7	15	2	0	216
HECTOR	0	6	0	0	0	0	6
JAY	89	985	32	0	27	1	1134
LINKER	0	44	0	0	0	0	44
MACEDONIA	168	1460	111	291	86	0	2116
NEWTONIA	566	2224	84	128	61	0	3063
NIXA	1	5752	994	5659	377	2	12785
NOARK	0	394	220	1793	9	2	2417
PERIDGE	122	1339	2	0	80	0	1544
RAZORT	2	1118	306	3716	22	2	5165
SECESH	2	210	30	258	33	4	537
SHIDLER	0	0	0	0	1	0	1
STIGLER	36	368	0	0	23	1	427
TAFT	1	0	0	0	0	0	1
TALOKA	271	1948	60	0	44	2	2324
TONTI	70	3039	145	237	230	3	3724
WABEN	1	44	0	0	2	0	47
WATER	0	5	3	19	2	912	942
Grand Total	1751	35916	5113	47396	1747	1191	93115
No of HRUs	118	1395	282	241	262	115	2413

The 90 subbasins delineated by SWAT for the Eucha Spavinaw basin are shown in Figure 4. below. Lake Eucha is located in Subbasins 48 and 55. Lake Spavinaw is located to the west of Subbasin 48.

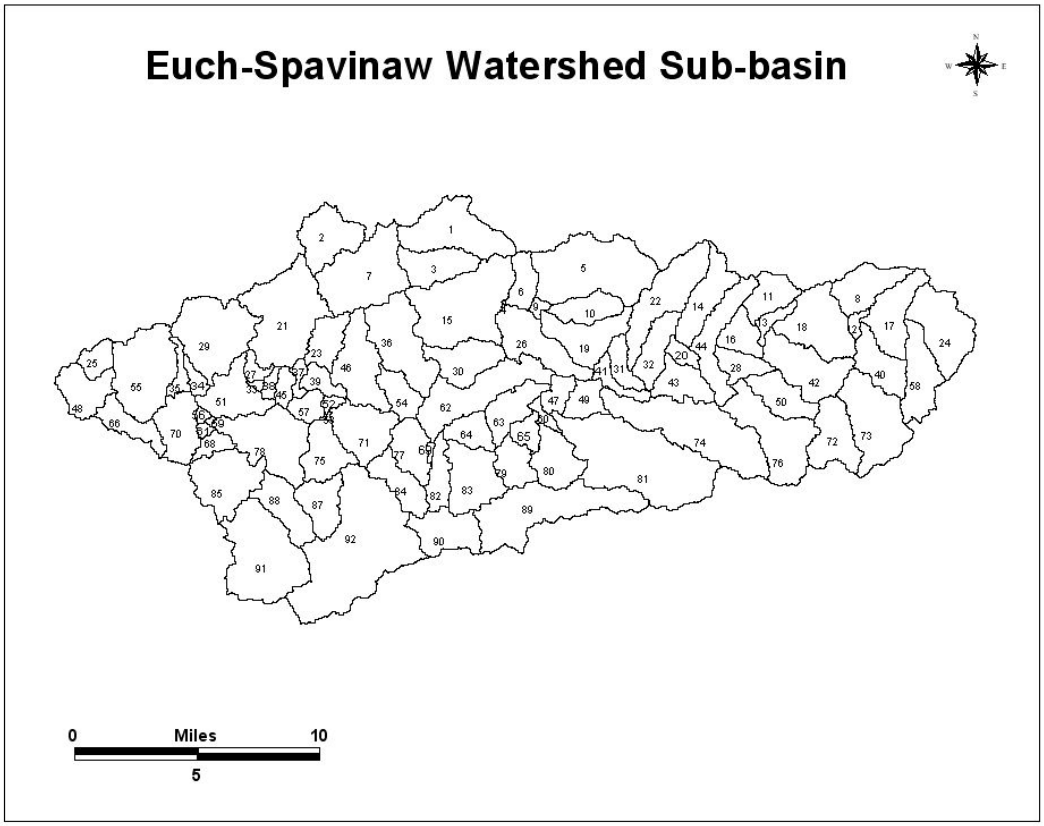


Figure 4. Subbasins Defined for the Eucha-Spavinaw Subbasin.

Modeling Grazing Management Practices in the Eucha Watershed

A previous study by Ancev (2003) indicated that improved pasture management had the potential to become a cost effective BMP for reducing phosphorus runoff. In this study combinations of litter application, commercial nitrogen, minimum biomass maintained during grazing, and stocking densities were simulated. The pasture in the Eucha basin was modeled as tall fescue. Some 48 alternative pasture management combinations were simulated on each of

the 1395 pasture HRUs in the water shed. Alternative pasture management practices were not simulated for HRUs in range and forest. Combinations of litter applied and commercial nitrogen were used to provide a range of fertilizer levels from zero to 240 kg of N per hectare. Each Mg of Litter was assumed to contain 30 kg of N and 14 kg of P. The combinations of Litter and commercial nitrogen used are shown in Figure 5 below and in Table 1.

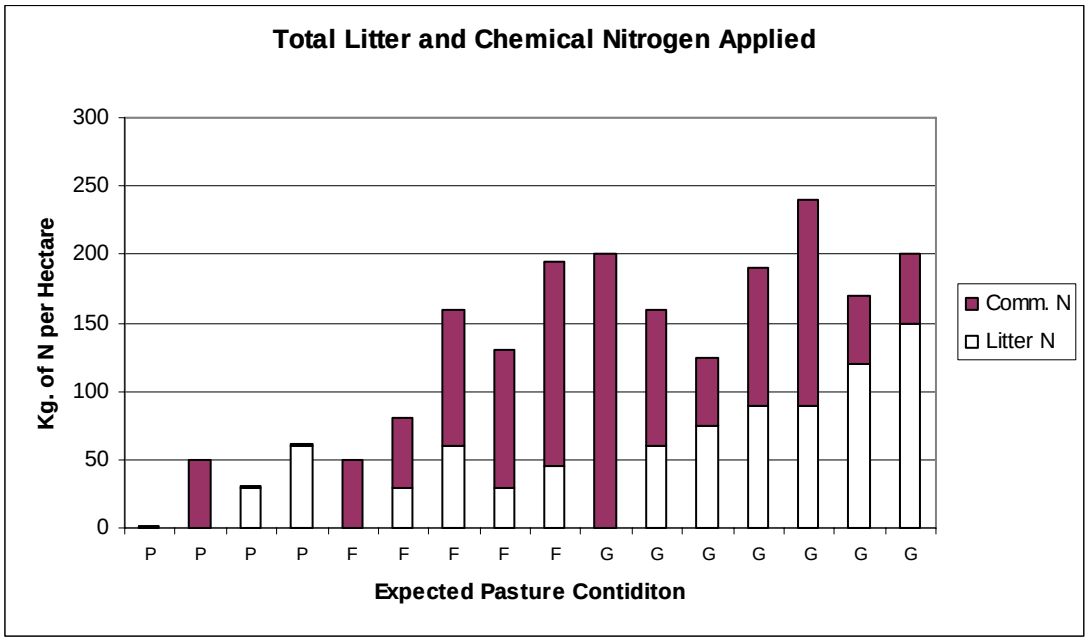


Figure 5 Nitrogen from the Poultry Litter and Commercial Nitrogen Applications Simulated in SWAT to Analyze Grazing Management Practices.

The pasture condition from each of the sixteen litter-fertilizer combinations described above when assigned minimum biomass to be maintained during grazing were assumed to result in ratings of poor, fair, and good. The three levels of minimum biomass specified were

Poor Pasture, 1100 kg.
Fair Pasture, 1600 kg.
Good Pasture, 2000 kg. (was considered Good/Fair with high grazing intensity).

The determination of pasture condition is in part a judgment call but is important because the NRCS curve numbers are assigned or adjusted according to the pasture condition. This adjustment is made exogenous to the SWAT model. In general poor pastures are more

susceptible to runoff because of less land cover and are assigned a higher curve number. The range of curve numbers also depend on the hydrologic code (A, B, C, or D) assigned to each soil type.

The three grazing intensities in terms of consumption per day and animal units/acre were

7.4 Low, 0.67 AU/acre
11.8 Medium, 1.00 AU/acre
14.9 High, 1.26 AU/acre.

The 16 fertilizer combinations used with each grazing intensity are, given in Table 2.

Table 2 . Alternative Litter Application Rates, Commercial Nitrogen, Minimum Biomass Retained During Grazing, Stocking Rates Simulated for each of the 16 Management Practices.

Pasture Condition	Lit kg/ha	qCNit kg/ha	Tot.Nitrogen kg/ha	P Applied	Minimum Biomass	Curve No
			Low Stocking (.67 AU/ha) and Medium Stocking Rates (1 AU/ha			
P	1	1	1.0	0.0	1100	86
P	1	50	50.0	0.0	1100	86
P	1000	1	1.0	14.0	1100	86
P	2000	1	1.0	28.0	1100	86
F	1	50	50.0	0.0	1600	79
F	1000	50	50.0	14.0	1600	79
F	2000	50	50.0	28.0	1600	79
F	1000	100	100.0	14.0	1600	79
F	1500	100	100.0	21.0	1600	79
G	1	200	200.0	0.0	2000	74
G	2000	100	100.0	28.0	2000	74
G	2500	50	50.0	35.0	2000	74
G	3000	100	100.0	42.0	2000	74
G	3000	150	150.0	42.0	2000	74
G	4000	50	50.0	56.0	2000	74
G	5000	50	50	70	2000	74
			High Stocking Rate (1.26 AU/ha.)			
P	1	1	1	0.014	1100	86
P	1	50	50	0.014	1100	86
P	1000	1	1	14	1100	86
P	2000	1	1	28	1100	86
F	1	50	50	0.014	1600	79
F	1000	50	50	14	1600	79
F	2000	50	50	28	1600	79
F	1000	100	100	14	1600	79
F	1500	100	100	21	1600	79
F/G	1	200	200	0.014	2000	76
F/G	2000	100	100	28	2000	76
F/G	2500	50	50	35	2000	76
F/G	3000	100	100	42	2000	76
F/G	3000	150	150	42	2000	76
F/G	4000	50	50	56	2000	76
F/G	5000	50	50	70	2000	76

The SWAT simulation results with respect to biomass eaten, phosphorus runoff for each of the 48 treatments are summarized in the Appendix for the major soils in the watershed. The simulation results for each soil are a weighted average of the results from each individual hru of that soil type. The weights were the area of each hru.

The results show the following.

1. There is much less phosphorus loss under all treatments for the Class B soils than for the class C and D soils.
2. The amount of phosphorus loss for fair and good pastures is much less than for the poor pastures.

However, a simple budget analysis (not shown) found that the higher stocking rate 14.9 kg/day was more profitable than the medium stocking rate of 11.8 kg/day which in turn was more profitable than the low stocking rate of 7.4 kg/day. That is pastures in the upper poor and lower fair range give higher economic returns than good pastures. This assessment was based on the assumption that commercial nitrogen cost $.64 per kg and that the delivered and applied cost of litter would vary from $15 to $20 per metric ton. The net value of consumed grass was valued at $27.88 per metric ton. This value was derived from a modified OSU cow calf budget shown below.

Modifications to OSU Cow Calf Budget.

The census of agriculture provides estimates of the number of cattle sold and the value of these cattle. The value of cattle sold was adjusted to remove an estimate of the number of cull cattle that would likely be sold. Dividing the remaining value by the average price of cattle for each census year then indicated the average weight of all non-cull cattle sold was approximately 623 pounds. This indicated many of the calves were likely kept beyond weaning and sold later.

The OSU budget (2005) was modified by removing the costs for pasture and hay and by assuming that part of the calf crop were kept and sold later as stockers. (Census of Agriculture, 1992, 1997, and 2002).

Table 5. Modified Cow Calf Budget Used to Derive the Value of Biomass Consumed by Grazing.

100 Cow Herd with Stockers Kept

	Weight	Unit	Price	Qt	Revenue
Steer Calves	470	Lbs./hd	$ 93.77	18.91	$ 8,333
Heifer Calves	470	Lbs./hd	$ 86.72	7.49	$ 3,054
Cull Cows	1150	Lbs./hd	$ 38.63	12.0	$ 5,331
Cull Replacement	825	Lbs./hd	$ 76.97	12.0	$ 7,620
Cull Bulls	1750	Lbs./hd	$ 52.24	1.0	$ 914
Stockers	623	Lbs./hd	$ 88.82	40.0	$ 22,134
					$ 47,386
Protein Supp.	1	hd.	$ 41.91	1.1	$ 4,610
Salt	1	hd.	$ 2.33	1.1	$ 256
Minerals	1	hd.	$ 11.50	1.1	$ 1,265
				1.1	
Vet Services	1	hd.	$ 7.14	1.1	$ 785
Vet Supplies	1	hd.	$ 1.16	1.1	$ 128
Marketing	1	hd.	$ 6.14	1	$ 614
Mach. Fuel,Oil, Repairs	1	hd.	$ 20.02	1.1	$ 2,202
Machinery labor	1	hrs.	$ 7.75	2.65	$ 2,054
Other labor	1	hrs.	$ 7.75	3	$ 2,325
Other expense	1	hd.	-	1.1	
Annual Oper. Capital		Dollars	0.0725	179.72	$ 1,303
					$ 15,542
Other Fixed Costs	(OSU budget)				$ 12,577
Net Return to Hay and Pasture					$ 19,266

Hay and Pasture Required per Cow Unit

	Wt	No	lbs/day	days/yr	lbs/yr	kg/yr
Cow	1150	1	25	365	9125	4139
Bull	1200	0.04	25	365	365	166
repHeif	800	0.12	18	365	788	358
Stocker	600	0.4	14	100	560	254
						4916

Net Revenue per Mg Biomass Consumed ($19,266/100hd/4.92) = $ 39.19

The forage calculations at the lower part of the budget in Table 4 indicated that approximately 4.9 Mg of hay and pasture would be required to for the number of cattle associated with each cow unit. Thus the budget provided a potential return of $39.19 for each cow unit if no hay baling were required.

Establishment of Broiler and Subbasin Centroids for Litter Shipment Points

Storm and White (2001) located approximately 1053 broiler houses in the watershed. It was estimated these operations produced 89,460 tons of litter annually. The locations of the poultry houses or clusters of broiler houses are show in Figure 6 below. Most of the poultry operations are located in the eastern part of the watershed in Arkansas.

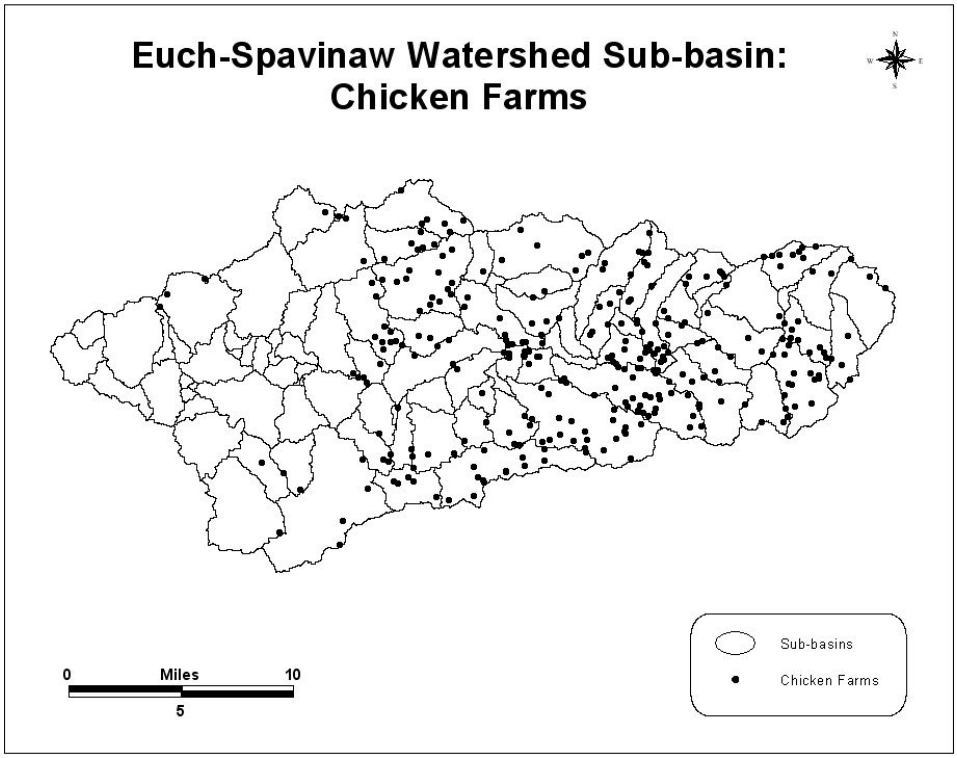


Figure 6. Location of Individual or Clusters of Poultry Operations in the Eucha-Spavinaw Watershed

Methodology for the Transportation Matrix:

Four distance calculations were necessary to develop the transportation matrix. First, the 300 chicken farms were divided into 24 groups such that no chicken farm was more than two miles from a group centroid. This was necessary to limit the number of transportation activities in the linear programming. To obtain the first distance, a script was run in ArcView 3.3® which

determined the average distance from each chicken farm to the centroid of that group. This average distance was needed, since not all the farms were exactly two miles away from the chicken farm centroid.

Second, a nearest feature algorithm was run to determine the distance from each chicken farm centroid to the nearest road. The next distance was determined by utilizing a multi-path script, which started from the point on the road nearest each chicken farm centroid and went to the point on the road nearest each sub-basin centroid. The final distance required was the distance from the road to the sub-basin centroid, in which the nearest feature algorithm was again utilized. By placing each distance in a matrix we were able to obtain the distance from each of the 24 chicken farm centroids to each of the 92 sub-basin centroids, which resulted in 2160 combinations. This same process was utilized to create the transportation matrix from each chicken farm centroid to Jay, Oklahoma for location of a possible litter-to-energy plant.

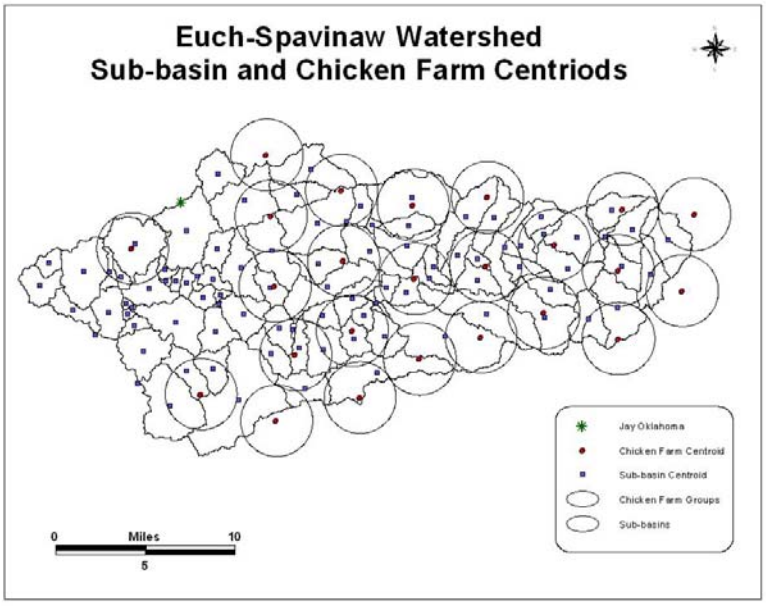


Figure 7. Eucha-Spavinaw Watershed and Sub-basins and Chicken Farm Centroids.

"Currently BMPs, Inc. is coordinating all transactions between the buyers, sellers, and haulers" (Oklahoma Litter Market). The cost for loading, hauling, and spreading through

correspondence with Sheri Herron at BMPs, Inc.,(2006). The cost for loading and coordinating a haul ranges from $7.50 to $8.00 per ton. The cost of hauling ranges from $3.25 to $3.50 per loading mile per truckload, with each truck averaging 23 tons per load and the loaded mileage a one-way distance. There was no direct cost for spreading; however, BMPs, Inc. would coordinate spreading at an average of $6 per short ton.

Step 2: The Basin Level Linear Programming Model.

The purpose of the programming model is to select the best management from the 48 choices available in each of the 1394 pasture HRUs along with the pattern of litter shipments that provides the maximum returns from grazing such that the total phosphorus runoff from the watershed does not exceed a finite amount. The annual phosphorus runoff targets used in the analysis were 30, 25, 20, and 15 Mg.

More formally the model can be stated as,

Maximize $Z = \sum_{i=1,nhru} \sum_{j=1,48} C_{ij} X_{ij} - \sum_{c=1,28} \sum_{b=1,92} s_{cb} T_{cb}$

Subject to:

$\sum_{j=1,48} X_{ij} = Ha_i, \; i = 1, 1395,$ (Land available in each Hru)

$\sum_{s=1, 24} T_{cb} = LS_c, \quad c=1,24$ (All litter must be shipped from each chicken centroid)

$\sum_{h(b)} \sum_{j=1,48} q_{jb} X_{jb} - \sum_{c=1,34} T_{cb} = 0$ (inshipments of litter to each subbasin must equal the quantity of litter applied in each subbasin.)

$\sum_{i=1,nhru} \sum_{j=1,48} p_{ij} X_{ij} \leq P_{max}$ (total runoff from all hrus in the watershed must be less than or equal the maximum allowable phosphorus (30 Mg, 25 Mg, 20 Mg or 15 Mg)

Where

X_{ij} is the area for the j management practice in the i'th hru,
Ha_i is the total number ofhectares in hru I,
T_{cb} is the quantity of litter shipped from chicken centroid c to subbasin b

q_{ij} is the quantity of litter required by management practice j in hru i. The summation is over the hrus in subbasins s
LS_c is the supply of litter in chicken centroid c
p_{ij} is the amount of phosphorus runoff from hru I if management practice j is used.

Results

Scenario 1. Subsidized Hauling to Enid without a Litter-to-Energy Plant.

Enid, Oklahoma was chosen as the repository site for litter hauled from the Basin. This site was chosen because it has a sufficient area of land available that could beneficially receive large quantities of litter from the Eucha basin. The price of litter at Enid was set at $24.82 per Mg ($22.50 per short ton). The cost of hauling from the chicken centroids to Enid (with the subsidy limited to $5.00 per ton) varied from $41 to $46 per Mg ($37 to $42 per short ton). Hauling litter that distance would not be undertaken unless forced by limits on total phosphorus that could leave the watershed. Large additional subsidies would be required to implement the results. If sufficient land that could accept large applications of phosphorus could be located closer the amount of subsidies would be reduced.

Table 6 below provides an aggregate summary of the effects of limiting total phosphorus runoff to 30, 25, 20, and 15 Mg per year when the only method of litter allocation is hauling within the subbasin and exporting westward to Enid. The amount of litter transported to Enid increased from 11.4 Mg to 63,573 Mg as the phosphorus limit was reduced from 30 Mg to 15 Mg per year. At the same time the cost of removing one additional kilogram of phosphorus increased from $55 to $166. The “abatement cost” is the cost in reduced income to from crops and pasture and the increased cost of litter transport.

Table 6. Effect of Meeting Annual Phosphorus Runoff Limits from 30 to 15 Mg On Income, Cost, and Litter Use when the Only Option is Reduced Application and Export to Enid.

Max. Phos. Runoff	Mg	15	20	25	30
Total Poultry Litter	Mg	89460	89460	89460	89460
To Enid	Mg	63573	41261	25474	11378
To Crop-Past. In Basin	Mg	25887	48199	63986	78082
Red. In Crp/Pst Returns	thoudol	1469565	782032	315587	0
Marg.Abat.Cost $/kg P	$/kg P	166.42	113	74.	55.34
Pasture P. Loss Mg	Mg	10.7	14.9	18.8	23.3
Qt. Litter / ha.	Mg/ha	0.61	1.2	1.67	2
Commercial N Apl.	kg/ha	46.6	36.2	33.3	31.2
Total N. Apl.	kg/ha	64.9	73	83.1	92.6
Biomass Min Graz.	Mg/ha	1.59	1.6	1.55	1.5
Biomass Cons.	Mg/ha	1.75	2	2.14	2.3
Nitrogen Runoff		24063	26812	28294	29376

The average litter application rate declined from 2 tons per hectare to only .6 tons per hectare as the annual allowable phosphorus limit was decreased to 15 mg. The minimum biomass retained for cover (to reduce runoff) increased while the actual biomass consumed decreased as the allowable phosphorus runoff was reduced. The amount of commercial nitrogen used steadily increased as the allowable phosphorus runoff was reduced. The actual nitrogen runoff in this series of solutions declined with the level of phosphorus. This is because total nitrogen (commercial nitrogen plus litter N) declined with allowable phosphorus runoff. (The opposite effect was observed in the scenarios described below).

Variation of Litter Applied By Soil Type.

There is considerable variation between the amounts of litter that can be applied to different soil types within each level of phosphorus runoff. Table 7 below shows the overall

quantity of litter applied declines rapidly as the phosphorus limit is reduced from 30 Mg to 15 Mg. However this occurred more rapidly in some soils and not at all in a few soils. In the case of the 15 Mg limit over 15,000 of the nearly 36,000 hectares have rates of one Mg or more. There are some 1600 hectares with application rates of 1.5 Mg or more and over 800 hectares with an application rate of over 2 Mg per hectare. In many cases, the simulated runoff values for soils receiving higher rates of litter application (in many cases the soils receiving the higher litter application rates (Elash, Healing, Linker, Secesh) actually have lower P losses than soils receiving little or no litter (Capitina, Jay, Tonti). The increase in litter application rate on a few soils as the annual P load limit declines occurs because the imputed value of litter in some cases is negative. These results are expected as the least cost phosphorus abatement solution required that marginal abatement costs be equated across soil types which implies the litter application will vary from one soil type to another.

Table 7. Comparison of Optimal Litter Application Rates and Phosphorus Runoff by Soil Type in the Eucha Basin.

			Annual Phosphorus Limit				Annual Phosphorus Limit			
Soil Name	Hyd. Code	Hectares	15 Mg	20 Mg	25 Mg	30 Mg	15 Mg	20 Mg	25 Mg	30 Mg
			Tons of Litter Applied/ha				Phosphorous Runoff kg/ha			
BRITWATER	B	1111.3	0	0	0.3	0.8	0.34	0.34	0.37	0.44
CLARKSVILLE	B	5932.4	0	0.9	1.6	2	0.24	0.41	0.64	0.75
DONIPHAN	B	4352.8	1.4	2.5	2.3	2.1	0.20	0.39	0.49	0.56
ELDORADO	B	26.1	1.3	2.2	2.3	2.4	0.21	0.66	0.74	0.98
ELSAH	B	85.2	2.2	3	2	2	0.24	0.53	0.83	0.83
HEALING	B	174.9	2.9	3.9	4.5	4.6	0.33	0.38	0.49	0.57
LINKER	B	44	5	5	4.9	4.9	0.16	0.22	0.26	0.26
MACEDONIA	B	1460.1	0	0	0	0.6	0.26	0.26	0.26	0.33
NEWTONIA	B	2223.8	1.2	2.8	4.1	4.5	0.21	0.30	0.39	0.42
NOARK	B	393.6	0	0.2	1.1	1.6	0.20	0.27	0.62	0.76
PERIDGE	B	1338.6	1.2	1.7	3	4.3	0.34	0.39	0.53	0.68
RAZORT	B	1118.4	1.9	3.2	3.9	4	0.22	0.29	0.45	0.52
SECESH	B	209.8	4.8	4.9	5	5	0.31	0.42	0.44	0.47
WABEN	B	44	1.8	3.4	2	2	0.24	0.47	0.88	0.88
CAPTINA	C	5150.5	1.1	1.9	2.8	3	0.51	0.76	0.90	1.00
JAY	C	984.8	0.8	2.4	2.6	3.5	0.48	0.65	0.77	0.92
NIXA	C	5752.4	0	0	0	0.5	0.27	0.35	0.40	0.70
TONTI	C	3039.2	0	0	0.1	1.3	0.26	0.26	0.28	0.43
CARYTOWN	D	127.3	0	2.2	3.8	2.6	0.42	0.64	0.82	1.14
CHEROKEE	D	19.5	1.4	2	2.5	2.5	0.13	0.69	1.12	1.31
HECTOR	D	6.2	1.5	0	0	0	0.00	0.66	0.66	0.66
STIGLER	D	367.5	2.1	2.2	3.5	3.7	0.44	0.47	0.55	0.57
TALOKA	D	1948.2	0	0	0	0.1	0.28	0.29	0.31	0.35

The shipment pattern is illustrated in the diagrams below. The general westward movement of litter within the subbasin is observed in all scenarios.

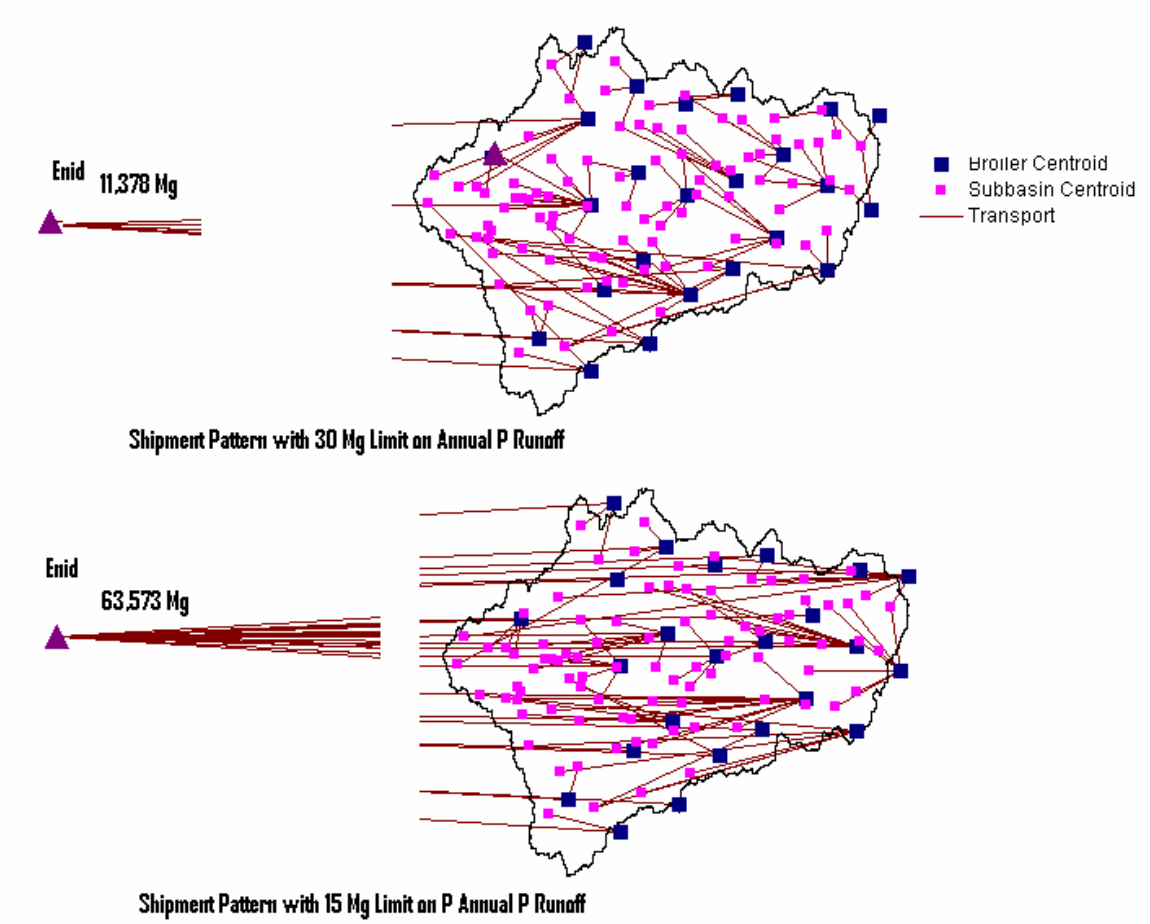


Figure 7, Litter Shipments From Broiler Centroids to Subbasins and Export to Enid when Maximum Phosphorus Runoff is limited to 30 and to 15 Mg per Year.

Scenario 2. A Litter to Energy Plant With Subsidized Hauling to the Plant

In this scenario, the hauling of litter from the broiler house centroids to a Litter to Energy Plant at Jay, Oklahoma was subsidized at the rate of $.05 per ton/mile up to a maximum of $5 per short ton. This is the rate currently used to subsidize hauling of litter outside the basin. The producers were expected to receive a payment of $6.81 for each short ton delivered to the plant. The $6.81 per ton is the midrange of a set of values reported by Chala (2005) for the expected profitability of the proposed litter to energy plant which has been proposed for Jay, Oklahoma (Adam, 2005). The cost of loading and delivery to the plant varied from $9.50 to $13.50 per Mg.

In this scenario, producers would not haul litter to the plant unless the net cost of delivery to the plant exceeded $6.81 – the actual cost of delivery represented the least cost method of disposal.

Table 9 below summarizes the effect of varying phosphorus runoff limits from 15 Mg to 30 Mg per year (12.3 to 27.6 short tons) on the allocation of litter between a litter-to-energy plant and the use on pasture land within the subbasin. (It was also possible to deliver litter to Enid at the same rate as above but this was never a viable option). If only 30 metric tons of phosphorus runoff were allowed each year, then about half of the 89,460 tons of litter would be hauled to the electric plant. The amount of litter hauled to the energy plant steadily increased as the allowable level of phosphorus runoff declined.

Table 9. Summary of Effect of Changing the Annual Phosphorus Runoff Limit from 30 Mg to 15 Mg when there is a Litter-to-Energy Plant and Jay, Oklahoma. Hauling to the Plant is Subsizided at .$05 per Mile.

Max. Phos. Runoff	Mg	15	20	25	30
Total Poultry Litter	Mg	89460	89460	89460	89460
To Electric Plant	Mg	71232	55247	46783	44575
To Crop-Past. In Basin	Mg	18228	34213	42677	44885
Red. In Crp/Pst Returns	thoudol	1306738	667039	256434	0
Marg.Abat.Cost $/kg P	$/kg P	158.244	103.614	61.294	43.32
Pasture P. Loss Mg	Mg	10.6	14.7	18.5	23.2
Qt. Litter / ha.	Mg/ha	0.4	0.83	1.1	1.12
Commercial N Applied.	kg/ha	44	35	32	28.4
Total N. Applied.	kg/ha	50	60.1	63.9	62.2
Biomass Min Graz.	Mg/ha	1.6	1.52	1.5	1.4
Biomass Cons.	Mg/ha	1.7	1.93	2.1	2.2
Nitrogen Runoff		23517	24960	25602	24898

The opportunity to haul litter to the plant at Jay, Oklahoma reduced the cost to remove an additional kilogram of phosphorus at all load levels. The cost to remove one additional ton of phosphorus decreased from over $50 per kg with a 30 ton total limit to $43.32. The cost to remove one additional kilogram of P at the 15 Mg limit declined from $166 to $158. The

marginal cost of removing an additional kilogram rose rapidly in both scenarios when the proposed load limit was reduced below 25 tons. Results by Ancev (2003) using a previous SWAT model with a slightly larger subbasin area, though with less refined land detail, concluded that the optimal level of abatement was around 25 tons per year.

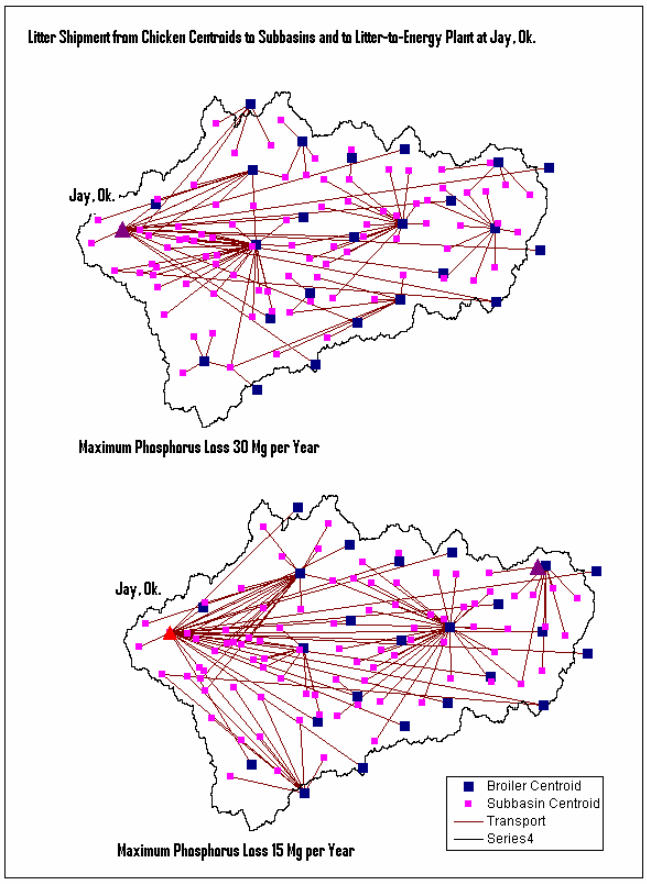


Figure 9. Transportation of Litter Within the Eucha Basin and with Partly Subsidized Transportation to a Possible Litter-to-Energy Plant at Jay, Oklahoma when the Maximum Annual Phosphorus Loss is 15 Mg and 30 Mg.

The transportation patterns of poultry litter from the broiler centroids to the 90 subbasins and to the proposed litter-to-energy plant at Jay, Oklahoma are shown in Figure 9. When the maximum annual phosphorus runoff was limited to 15 Mg, over 71 thousand of the total 89 thousand metric tons are transported to the plant. Hauling to the plant was subsidized at $.05 per mile.

Scenario 3; A Litter to Energy Plant with Complete Subsidization of Hauling to Plant.

Table 10 below contains an overview of the solutions where it was assumed that owners of broiler houses could receive $6.81 per short ton above the cost of delivering the litter to a litter to energy plant at Jay, Oklahoma. This scenario assumed the plant operates as a cooperative with the plant responsible for picking up litter in the basin. This is consistent with the limited cost analysis done so far for the litter-to-energy plant.

As described above, at current prices there is small economic incentive to apply poultry litter to pastures in the subbasin because of high transportation costs and because of the value of litter is limited to its value as a nitrogen fertilizer. The pastures were modeled as grazing units so because of manure deposition there is very little phosphorus removal. There was also little commercial nitrogen used (Price of nitrogen at $063 per kg ($ 0.29/lb plus $2.50 per acre application) because of the relatively high price. The problem with low fertilizer application rates is the lack of biomass to prevent runoff of phosphorus (mainly in the sediment form).

The results indicate at even at the 30 Mg per year limit on phosphorus runoff, that 83.8 mg of the 89.4 Mg of poultry litter would be hauled to the litter-to-energy plant. That is, the value of most litter if applied to land within the basin would yield the broiler owners less than $6.81 per short ton. Currently the Litter Link web site (2006) indicates that producers are averaging $4.50 per ton of litter. As the restriction on the amount of allowable phosphorus runoff is decreased from 30 Mg per year to 25, 20, and 15 Mg, the phosphorus abatement cost in terms of reduction in producer income from crops, pasture, and range increased at an increasing rate. The marginal cost of preventing one additional kilogram of phosphorus loss when 30 tons per year are allowed was estimated to be $23.00. This is considerable less than for scenarios one and two above. However, when only 15 Mg of P per year was allowed, the cost to prevent an

additional kilogram increases to $158. Again, the marginal cost of phosphorus abatement increased rapidly as the phosphorus load was reduced from 20 to 25 tons. 2 The marginal abatement cost is in addition to any subsidies in transportation and in plant construction.

Table 10. Effect of Changing the Annual Allowable Phosphorus Runoff Limit from 30 Mg to 15 Mg when Broiler Owners Receive $7.50 per Mg ($6.81 per short ton) above any Transportation Cost.

	Unit				
Max. Phos. Runoff	Mg	15	20	25	30
Total Poultry Litter	Mg	89460	89460	89460	89460
To Electric Plant	Mg	85642	86214	85055	83805
To Crop-Past. In Basin	Mg	3818	3246	4405	5655
Red. In Crp/Pst Returns	Thou.$	939599	402052	147566	0
Marginal.Abatat. Cost	$/kg P	158.624	73.32	36.68	$23.08
Pasture P. Loss Mg	Mg	10.7	14.3	18.6	23.1
Qt. Litter / ha.	Mg/ha	0.02	0.01	0.04	0.06
Commercial N Apl.	kg/ha	42.5	37.9	34.1	30
Total N. Apl.	kg/ha	43.1	38.4	35.3	31.9
Biomass Min Graz.	Mg/ha	1.5	1.4	1.3	1.23
Biomass Cons.	Mg/ha	1.67	1.89	1.94	1.97
Nitrogen Runoff		21890	22943	22326	21355

The results above indicate that maintaining additional biomass on pastures is required as the allowable phosphorus load is decreased. This is reflected in the value for minimum biomass before grazing is allowed and in the increase in total nitrogen applied to pasture. Conversely, the amount of biomass consumed by grazing declines with the increase in biomass retained for cover. However, as indicated previously, this is not a practice that can be profitably adopted by producers without additional subsidy. That is, producers would have to be compensated to adopt the higher biomass pastures. The increase in pasture biomass from increased nitrogen

fertilization is accompanied by increases in nitrogen runoff. Thus, nitrogen runoff increases as phosphorus runoff decreases.

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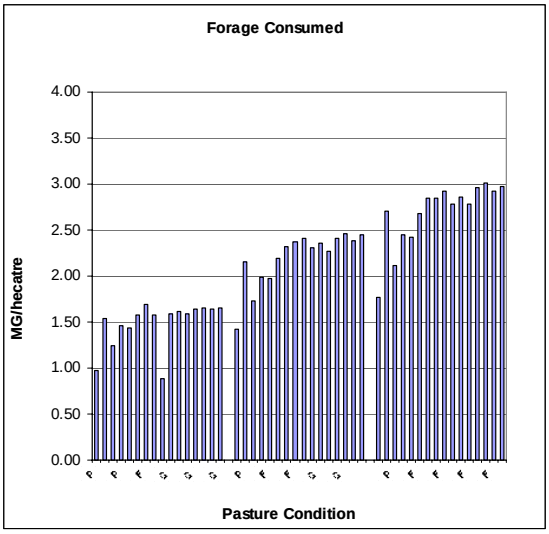
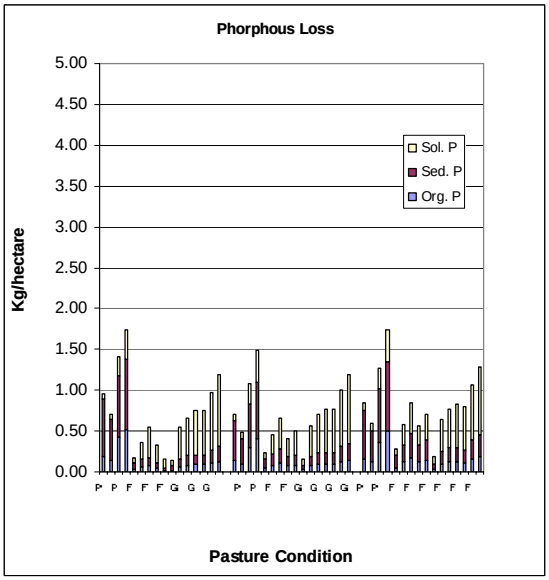
APPENDIX

SWAT Simulation Results for Yield, Phosphorus Runoff for the 48 Alternative Pasture Management Practices Averaged by Major Soil Type

TALOKA

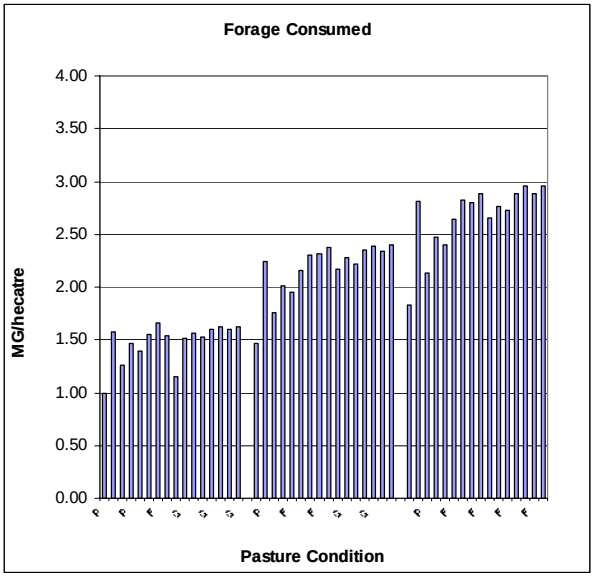
Past Cond	1948 Lit kg/ha	Ha qCNit kg/ha	Hyd Class Tn kg/ha	D P	MnBm	Curve No	OrgP	Sed P	Sol P	Bmeat	NgDys	T Ploss	Nloss
				Low Stocking Rate									
P	1	1	1.0	0.0	1100	86	0.18	0.71	0.06	0.97	41.80	0.95	30.03
P	1	50	50.0	0.0	1100	86	0.14	0.49	0.06	1.54	66.41	0.70	57.89
P	1000	1	1.0	14.0	1100	86	0.42	0.76	0.22	1.24	53.30	1.40	38.77
P	2000	1	1.0	28.0	1100	86	0.52	0.86	0.36	1.46	62.71	1.74	48.12
F	1	50	50.0	0.0	1600	79	0.03	0.08	0.06	1.44	61.86	0.17	58.78
F	1000	50	50.0	14.0	1600	79	0.06	0.09	0.21	1.58	67.97	0.36	69.67
F	2000	100	100.0	28.0	1600	79	0.08	0.10	0.37	1.69	72.60	0.54	129.55
F	1000	100	100.0	14.0	1600	79	0.05	0.07	0.22	1.57	67.81	0.34	119.27
F	1500	150	150.0	21.0	1600	79	0.02	0.03	0.11	0.88	37.99	0.16	75.83
G	1	200	200.0	0.0	2000	74	0.02	0.05	0.08	1.60	68.71	0.15	198.41
G	2000	100	100.0	28.0	2000	74	0.07	0.09	0.38	1.61	69.47	0.54	135.88
G	2500	50	50.0	35.0	2000	74	0.08	0.12	0.46	1.59	68.45	0.66	97.86
G	3000	100	100.0	42.0	2000	74	0.09	0.12	0.55	1.64	70.59	0.76	153.64
G	3000	150	150.0	42.0	2000	74	0.09	0.12	0.55	1.66	71.26	0.76	202.19
G	4000	50	50.0	56.0	2000	74	0.11	0.16	0.70	1.64	70.41	0.97	124.61
G	5000	50	50.0	70.0	2000	74	0.13	0.18	0.87	1.66	71.28	1.19	143.88
				Medium Stocking Rate									
P	1	1	1.0	0.0	1100	86	0.14	0.49	0.08	1.42	61.22	0.71	31.25
P	1	50	50.0	0.0	1100	86	0.09	0.31	0.08	2.15	92.77	0.49	59.03
P	1000	1	1.0	14.0	1100	86	0.30	0.54	0.24	1.73	74.42	1.07	40.09
P	2000	1	1.0	28.0	1100	86	0.41	0.69	0.38	1.99	85.65	1.48	49.62
F	1	50	50.0	0.0	1600	79	0.04	0.12	0.08	1.97	84.74	0.24	60.66
F	1000	50	50.0	14.0	1600	79	0.09	0.14	0.23	2.19	94.12	0.46	70.52
F	2000	50	50.0	28.0	1600	79	0.12	0.17	0.37	2.33	100.14	0.66	81.75
F	1000	100	100.0	14.0	1600	79	0.07	0.11	0.23	2.37	101.94	0.41	110.19
F	1500	100	100.0	21.0	1600	79	0.08	0.12	0.30	2.41	103.80	0.51	116.12
G	1	200	200.0	0.0	2000	74	0.03	0.05	0.08	2.31	99.40	0.16	191.26
G	2000	100	100.0	28.0	2000	74	0.08	0.10	0.38	2.36	101.54	0.56	126.29
G	2500	50	50.0	35.0	2000	74	0.10	0.14	0.46	2.27	97.58	0.70	91.03
G	3000	100	100.0	42.0	2000	74	0.10	0.14	0.53	2.41	103.66	0.77	140.32
G	3000	150	150.0	42.0	2000	74	0.10	0.13	0.53	2.46	105.78	0.76	187.10
G	4000	50	50.0	56.0	2000	74	0.13	0.18	0.68	2.39	102.90	1.00	111.35
G	5000	50	50	70	2000	74	0.15	0.20	0.83	2.45	105.38	1.19	125.75
				High Stocking Rate									
P	1	1	1	0.014	1100	86	0.16	0.59	0.09	1.77	76.20	0.84	32.60
P	1	50	50	0.014	1100	86	0.12	0.39	0.10	2.70	116.41	0.60	60.29
P	1000	1	1	14	1100	86	0.36	0.67	0.25	2.11	90.94	1.27	41.30
P	2000	1	1	28	1100	86	0.50	0.85	0.40	2.44	105.25	1.75	50.81
F	1	50	50	0.014	1600	79	0.05	0.15	0.09	2.43	104.45	0.29	61.38
F	1000	50	50	14	1600	79	0.12	0.21	0.25	2.68	115.39	0.57	70.88
F	2000	50	50	28	1600	79	0.18	0.29	0.39	2.84	122.45	0.85	81.23
F	1000	100	100	14	1600	79	0.12	0.21	0.24	2.84	122.39	0.57	110.23
F	1500	100	100	21	1600	79	0.15	0.24	0.31	2.92	125.61	0.70	115.40
F	1	200	200	0.014	2000	76	0.03	0.07	0.09	2.79	120.04	0.19	188.60
F	2000	100	100	28	2000	76	0.10	0.15	0.39	2.86	123.17	0.64	123.93
F	2500	50	50	35	2000	76	0.12	0.18	0.46	2.78	119.69	0.76	89.92
F	3000	100	100	42	2000	76	0.12	0.18	0.53	2.97	127.76	0.84	136.20
F	3000	150	150	42	2000	76	0.11	0.15	0.53	3.01	129.68	0.79	181.24
F	4000	50	50	56	2000	76	0.16	0.23	0.68	2.93	125.99	1.07	108.40
F	5000	50	50	70	2000	76	0.19	0.27	0.82	2.98	128.10	1.28	121.34

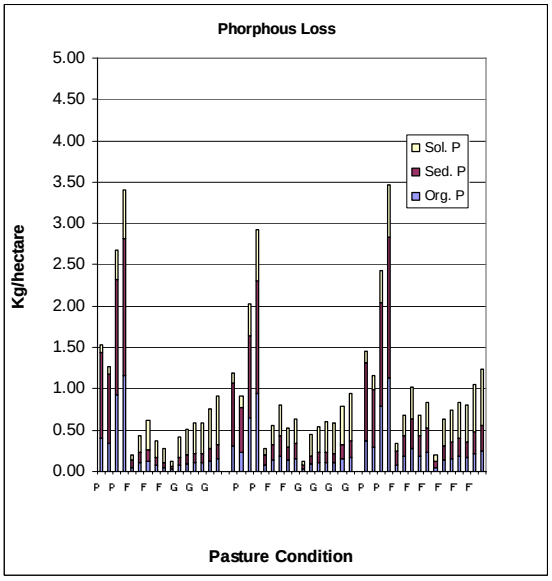

Forage Consumed
MG/hecatre
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pasture Condition


Phorphous Loss
Kg/hectare
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Sol. P
Sed. P
Org. P
Pasture Condition

BRITWATER

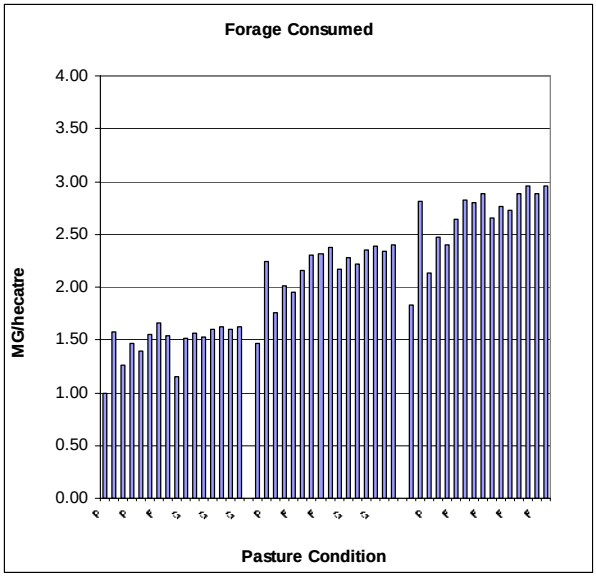
Past Cond	1111 Lit kg/ha	Ha qCNit kg/ha	Hyd Class Tn kg/ha	B P	MnBm	Curve No	OrgP	Sed P	Sol P	Bmeat	NgDys	T Ploss	Nloss
Low Stocking Rate													
P	1	1	1.0	0.0	1100	68	0.40	1.04	0.09	1.00	42.85	1.53	23.55
P	1	50	50.0	0.0	1100	68	0.35	0.83	0.10	1.58	68.03	1.28	46.72
P	1000	1	1.0	14.0	1100	68	0.93	1.40	0.35	1.26	54.07	2.67	30.67
P	2000	1	1.0	28.0	1100	68	1.16	1.66	0.59	1.47	63.12	3.40	37.73
F	1	50	50.0	0.0	1600	49	0.05	0.09	0.06	1.40	60.10	0.21	35.95
F	1000	50	50.0	14.0	1600	49	0.11	0.13	0.20	1.55	66.75	0.44	42.48
F	2000	100	100.0	28.0	1600	49	0.13	0.14	0.36	1.67	71.75	0.63	78.49
F	1000	100	100.0	14.0	1600	49	0.08	0.08	0.21	1.54	66.21	0.37	72.51
F	1500	150	150.0	21.0	1600	49	0.05	0.06	0.18	1.15	49.69	0.28	86.67
G	1	200	200.0	0.0	2000	39	0.03	0.04	0.05	1.52	65.41	0.12	111.03
G	2000	100	100.0	28.0	2000	39	0.08	0.09	0.26	1.56	67.30	0.42	74.35
G	2500	50	50.0	35.0	2000	39	0.10	0.11	0.31	1.53	66.02	0.51	52.45
G	3000	100	100.0	42.0	2000	39	0.10	0.11	0.37	1.60	68.96	0.58	83.80
G	3000	150	150.0	42.0	2000	39	0.10	0.11	0.37	1.62	69.71	0.58	111.14
G	4000	50	50.0	56.0	2000	39	0.13	0.15	0.48	1.59	68.67	0.75	66.51
G	5000	50	50.0	70.0	2000	39	0.15	0.17	0.60	1.62	69.81	0.92	76.82
Medium Stocking Rate													
P	1	1	1.0	0.0	1100	68	0.31	0.76	0.12	1.47	63.18	1.19	24.17
P	1	50	50.0	0.0	1100	68	0.24	0.54	0.13	2.24	96.50	0.91	47.18
P	1000	1	1.0	14.0	1100	68	0.66	0.99	0.38	1.76	75.76	2.03	30.99
P	2000	1	1.0	28.0	1100	68	0.94	1.36	0.62	2.01	86.71	2.93	38.65
F	1	50	50.0	0.0	1600	49	0.07	0.14	0.08	1.95	83.95	0.28	37.25
F	1000	50	50.0	14.0	1600	49	0.14	0.18	0.23	2.16	93.04	0.55	43.27
F	2000	50	50.0	28.0	1600	49	0.19	0.24	0.37	2.30	99.23	0.80	50.17
F	1000	100	100.0	14.0	1600	49	0.13	0.16	0.22	2.31	99.61	0.52	69.84
F	1500	100	100.0	21.0	1600	49	0.15	0.19	0.30	2.38	102.52	0.64	73.08
G	1	200	200.0	0.0	2000	39	0.03	0.04	0.06	2.17	93.53	0.13	107.59
G	2000	100	100.0	28.0	2000	39	0.09	0.10	0.26	2.27	97.94	0.45	70.47
G	2500	50	50.0	35.0	2000	39	0.11	0.13	0.31	2.22	95.50	0.55	49.81
G	3000	100	100.0	42.0	2000	39	0.11	0.12	0.37	2.35	101.31	0.60	78.04
G	3000	150	150.0	42.0	2000	39	0.11	0.12	0.37	2.39	103.01	0.59	105.00
G	4000	50	50.0	56.0	2000	39	0.15	0.17	0.47	2.34	100.75	0.79	60.85
G	5000	50	50	70	2000	39	0.17	0.20	0.57	2.40	103.13	0.94	68.90
High Stocking Rate													
P	1	1	1	0.014	1100	68	0.38	0.94	0.14	1.83	78.85	1.46	25.54
P	1	50	50	0.014	1100	68	0.30	0.70	0.16	2.82	121.32	1.16	48.39
P	1000	1	1	14	1100	68	0.79	1.24	0.40	2.13	91.78	2.44	32.34
P	2000	1	1	28	1100	68	1.12	1.70	0.65	2.47	106.51	3.47	40.00
F	1	50	50	0.014	1600	49	0.08	0.17	0.09	2.40	103.54	0.34	37.92
F	1000	50	50	14	1600	49	0.19	0.25	0.24	2.65	114.03	0.68	44.03
F	2000	50	50	28	1600	49	0.27	0.36	0.39	2.83	121.65	1.02	50.61
F	1000	100	100	14	1600	49	0.19	0.25	0.24	2.80	120.66	0.67	70.11
F	1500	100	100	21	1600	49	0.23	0.30	0.31	2.88	124.04	0.84	73.49
F	1	200	200	0.014	2000	44	0.05	0.08	0.07	2.66	114.48	0.20	108.96
F	2000	100	100	28	2000	44	0.14	0.17	0.32	2.77	119.20	0.63	71.68
F	2500	50	50	35	2000	44	0.16	0.20	0.39	2.72	117.21	0.74	50.81
F	3000	100	100	42	2000	44	0.18	0.22	0.44	2.88	124.17	0.84	78.66
F	3000	150	150	42	2000	44	0.16	0.20	0.44	2.96	127.44	0.80	104.13
F	4000	50	50	56	2000	44	0.22	0.26	0.57	2.88	124.18	1.05	61.29
F	5000	50	50	70	2000	44	0.25	0.30	0.69	2.96	127.25	1.24	68.35

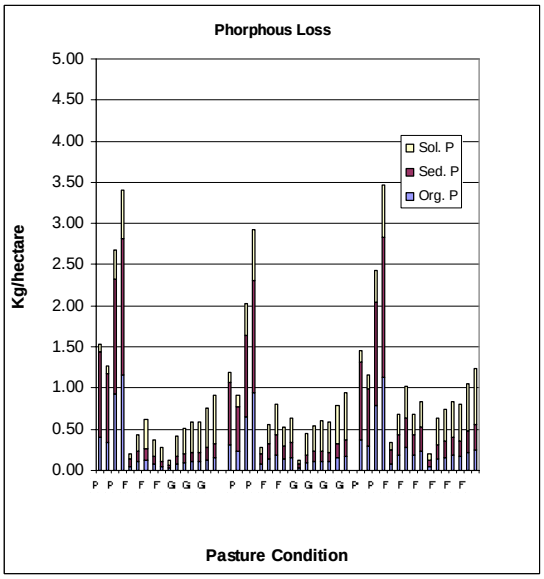

Forage Consumed
MG/hecatre
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pasture Condition


Phorphous Loss
Kg/hectare
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Sol. P
Sed. P
Org. P
Pasture Condition

CLARKSVILLE

Past Cond	5932 Lit kg/ha	Ha qCNit kg/ha	Hyd Class Tn kg/ha	B P	MnBm	Curve No	OrgP	Sed P	Sol P	Bmeat	NgDys	T Ploss	Nloss
				Low Stocking Rate									
P	1	1	1.0	0.0	1100	68	0.40	1.04	0.09	1.00	42.85	1.53	23.55
P	1	50	50.0	0.0	1100	68	0.35	0.83	0.10	1.58	68.03	1.28	46.72
P	1000	1	1.0	14.0	1100	68	0.93	1.40	0.35	1.26	54.07	2.67	30.67
P	2000	1	1.0	28.0	1100	68	1.16	1.66	0.59	1.47	63.12	3.40	37.73
F	1	50	50.0	0.0	1600	49	0.05	0.09	0.06	1.40	60.10	0.21	35.95
F	1000	50	50.0	14.0	1600	49	0.11	0.13	0.20	1.55	66.75	0.44	42.48
F	2000	100	100.0	28.0	1600	49	0.13	0.14	0.36	1.67	71.75	0.63	78.49
F	1000	100	100.0	14.0	1600	49	0.08	0.08	0.21	1.54	66.21	0.37	72.51
F	1500	150	150.0	21.0	1600	49	0.05	0.06	0.18	1.15	49.69	0.28	86.67
G	1	200	200.0	0.0	2000	39	0.03	0.04	0.05	1.52	65.41	0.12	111.03
G	2000	100	100.0	28.0	2000	39	0.08	0.09	0.26	1.56	67.30	0.42	74.35
G	2500	50	50.0	35.0	2000	39	0.10	0.11	0.31	1.53	66.02	0.51	52.45
G	3000	100	100.0	42.0	2000	39	0.10	0.11	0.37	1.60	68.96	0.58	83.80
G	3000	150	150.0	42.0	2000	39	0.10	0.11	0.37	1.62	69.71	0.58	111.14
G	4000	50	50.0	56.0	2000	39	0.13	0.15	0.48	1.59	68.67	0.75	66.51
G	5000	50	50.0	70.0	2000	39	0.15	0.17	0.60	1.62	69.81	0.92	76.82
				Medium Stocking Rate									
P	1	1	1.0	0.0	1100	68	0.31	0.76	0.12	1.47	63.18	1.19	24.17
P	1	50	50.0	0.0	1100	68	0.24	0.54	0.13	2.24	96.50	0.91	47.18
P	1000	1	1.0	14.0	1100	68	0.66	0.99	0.38	1.76	75.76	2.03	30.99
P	2000	1	1.0	28.0	1100	68	0.94	1.36	0.62	2.01	86.71	2.93	38.65
F	1	50	50.0	0.0	1600	49	0.07	0.14	0.08	1.95	83.95	0.28	37.25
F	1000	50	50.0	14.0	1600	49	0.14	0.18	0.23	2.16	93.04	0.55	43.27
F	2000	50	50.0	28.0	1600	49	0.19	0.24	0.37	2.30	99.23	0.80	50.17
F	1000	100	100.0	14.0	1600	49	0.13	0.16	0.22	2.31	99.61	0.52	69.84
F	1500	100	100.0	21.0	1600	49	0.15	0.19	0.30	2.38	102.52	0.64	73.08
G	1	200	200.0	0.0	2000	39	0.03	0.04	0.06	2.17	93.53	0.13	107.59
G	2000	100	100.0	28.0	2000	39	0.09	0.10	0.26	2.27	97.94	0.45	70.47
G	2500	50	50.0	35.0	2000	39	0.11	0.13	0.31	2.22	95.50	0.55	49.81
G	3000	100	100.0	42.0	2000	39	0.11	0.12	0.37	2.35	101.31	0.60	78.04
G	3000	150	150.0	42.0	2000	39	0.11	0.12	0.37	2.39	103.01	0.59	105.00
G	4000	50	50.0	56.0	2000	39	0.15	0.17	0.47	2.34	100.75	0.79	60.85
G	5000	50	50	70	2000	39	0.17	0.20	0.57	2.40	103.13	0.94	68.90
				High Stocking Rate									
P	1	1	1	0.014	1100	68	0.38	0.94	0.14	1.83	78.85	1.46	25.54
P	1	50	50	0.014	1100	68	0.30	0.70	0.16	2.82	121.32	1.16	48.39
P	1000	1	1	14	1100	68	0.79	1.24	0.40	2.13	91.78	2.44	32.34
P	2000	1	1	28	1100	68	1.12	1.70	0.65	2.47	106.51	3.47	40.00
F	1	50	50	0.014	1600	49	0.08	0.17	0.09	2.40	103.54	0.34	37.92
F	1000	50	50	14	1600	49	0.19	0.25	0.24	2.65	114.03	0.68	44.03
F	2000	50	50	28	1600	49	0.27	0.36	0.39	2.83	121.65	1.02	50.61
F	1000	100	100	14	1600	49	0.19	0.25	0.24	2.80	120.66	0.67	70.11
F	1500	100	100	21	1600	49	0.23	0.30	0.31	2.88	124.04	0.84	73.49
F	1	200	200	0.014	2000	44	0.05	0.08	0.07	2.66	114.48	0.20	108.96
F	2000	100	100	28	2000	44	0.14	0.17	0.32	2.77	119.20	0.63	71.68
F	2500	50	50	35	2000	44	0.16	0.20	0.39	2.72	117.21	0.74	50.81
F	3000	100	100	42	2000	44	0.18	0.22	0.44	2.88	124.17	0.84	78.66
F	3000	150	150	42	2000	44	0.16	0.20	0.44	2.96	127.44	0.80	104.13
F	4000	50	50	56	2000	44	0.22	0.26	0.57	2.88	124.18	1.05	61.29
F	5000	50	50	70	2000	44	0.25	0.30	0.69	2.96	127.25	1.24	68.35

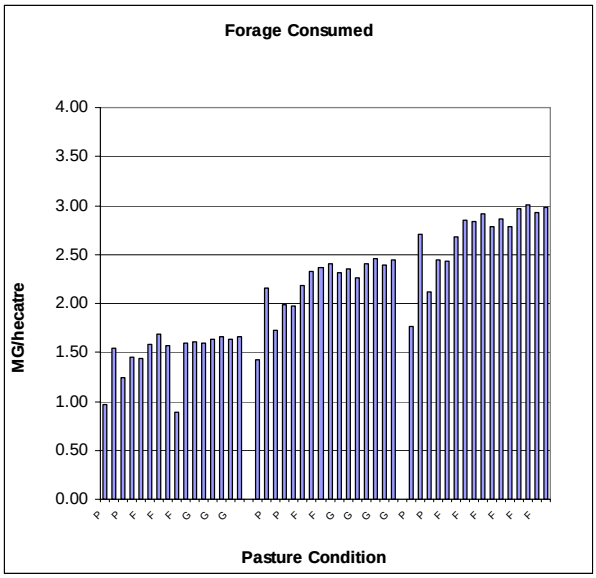

Forage Consumed
MG/hecatre
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pasture Condition

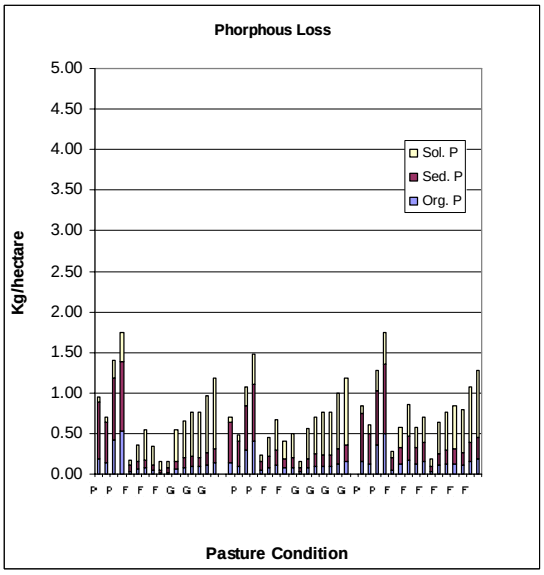

Phorphous Loss
Kg/hectare
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Sol. P
Sed. P
Org. P
Pasture Condition

DONIPHAN

Past Cond	4353 Lit kg/ha	Ha qCNit kg/ha	Hyd Class Tn kg/ha	B P	MnBm	Curve No	Org P	Sed P	Sol P	Bme at	NgDy s	T Ploss	Nlos s
				Low Stocking Rate									
P	1	1	1.0	0.0	1100	86	0.18	0.71	0.06	0.97	41.80	0.95	30.03
P	1	50	50.0	0.0	1100	86	0.14	0.49	0.06	1.54	66.41	0.70	57.89
P	1000	1	1.0	14.0	1100	86	0.42	0.76	0.22	1.24	53.30	1.40	38.77
P	2000	1	1.0	28.0	1100	86	0.52	0.86	0.36	1.46	62.71	1.74	48.12
F	1	50	50.0	0.0	1600	79	0.03	0.08	0.06	1.44	61.86	0.17	58.78
F	1000	50	50.0	14.0	1600	79	0.06	0.09	0.21	1.58	67.97	0.36	69.67
F	2000	100	100.0	28.0	1600	79	0.08	0.10	0.37	1.69	72.60	0.54	129.5 5
F	1000	100	100.0	14.0	1600	79	0.05	0.07	0.22	1.57	67.81	0.34	119.2 7
F	1500	150	150.0	21.0	1600	79	0.02	0.03	0.11	0.88	37.99	0.16	75.83
G	1	200	200.0	0.0	2000	74	0.02	0.05	0.08	1.60	68.71	0.15	198.4 1
G	2000	100	100.0	28.0	2000	74	0.07	0.09	0.38	1.61	69.47	0.54	135.8 8
G	2500	50	50.0	35.0	2000	74	0.08	0.12	0.46	1.59	68.45	0.66	97.86
G	3000	100	100.0	42.0	2000	74	0.09	0.12	0.55	1.64	70.59	0.76	153.6 4
G	3000	150	150.0	42.0	2000	74	0.09	0.12	0.55	1.66	71.26	0.76	202.1 9
G	4000	50	50.0	56.0	2000	74	0.11	0.16	0.70	1.64	70.41	0.97	124.6 1
G	5000	50	50.0	70.0	2000	74	0.13	0.18	0.87	1.66	71.28	1.19	143.8 8
				Medium Stocking Rate									
P	1	1	1.0	0.0	1100	86	0.14	0.49	0.08	1.42	61.22	0.71	31.25
P	1	50	50.0	0.0	1100	86	0.09	0.31	0.08	2.15	92.77	0.49	59.03
P	1000	1	1.0	14.0	1100	86	0.30	0.54	0.24	1.73	74.42	1.07	40.09
P	2000	1	1.0	28.0	1100	86	0.41	0.69	0.38	1.99	85.65	1.48	49.62
F	1	50	50.0	0.0	1600	79	0.04	0.12	0.08	1.97	84.74	0.24	60.66
F	1000	50	50.0	14.0	1600	79	0.09	0.14	0.23	2.19	94.12	0.46	70.52
F	2000	50	50.0	28.0	1600	79	0.12	0.17	0.37	2.33	100.1 4	0.66	81.75
F	1000	100	100.0	14.0	1600	79	0.07	0.11	0.23	2.37	101.9 4	0.41	110.1 9
F	1500	100	100.0	21.0	1600	79	0.08	0.12	0.30	2.41	103.8 0	0.51	116.1 2
G	1	200	200.0	0.0	2000	74	0.03	0.05	0.08	2.31	99.40	0.16	191.2 6
G	2000	100	100.0	28.0	2000	74	0.08	0.10	0.38	2.36	101.5 4	0.56	126.2 9
G	2500	50	50.0	35.0	2000	74	0.10	0.14	0.46	2.27	97.58	0.70	91.03
G	3000	100	100.0	42.0	2000	74	0.10	0.14	0.53	2.41	103.6 6	0.77	140.3 2
G	3000	150	150.0	42.0	2000	74	0.10	0.13	0.53	2.46	105.7 8	0.76	187.1 0
G	4000	50	50.0	56.0	2000	74	0.13	0.18	0.68	2.39	102.9 0	1.00	111.3 5
G	5000	50	50	70	2000	74	0.15	0.20	0.83	2.45	105.3 8	1.19	125.7 5
				High Stocking Rate									
P	1	1	1	0.01 4	1100	86	0.16	0.59	0.09	1.77	76.20	0.84	32.60
P	1	50	50	0.01 4	1100	86	0.12	0.39	0.10	2.70	116.4 1	0.60	60.29
P	1000	1	1	14	1100	86	0.36	0.67	0.25	2.11	90.94	1.27	41.30
P	2000	1	1	28	1100	86	0.50	0.85	0.40	2.44	105.2 5	1.75	50.81
F	1	50	50	0.01 4	1600	79	0.05	0.15	0.09	2.43	104.4 5	0.29	61.38
F	1000	50	50	14	1600	79	0.12	0.21	0.25	2.68	115.3 9	0.57	70.88
F	2000	50	50	28	1600	79	0.18	0.29	0.39	2.84	122.4 5	0.85	81.23
F	1000	100	100	14	1600	79	0.12	0.21	0.24	2.84	122.3 9	0.57	110.2 3
F	1500	100	100	21	1600	79	0.15	0.24	0.31	2.92	125.6 1	0.70	115.4 0
F	1	200	200	0.01 4	2000	76	0.03	0.07	0.09	2.79	120.0 4	0.19	188.6 0
F	2000	100	100	28	2000	76	0.10	0.15	0.39	2.86	123.1	0.64	123.9

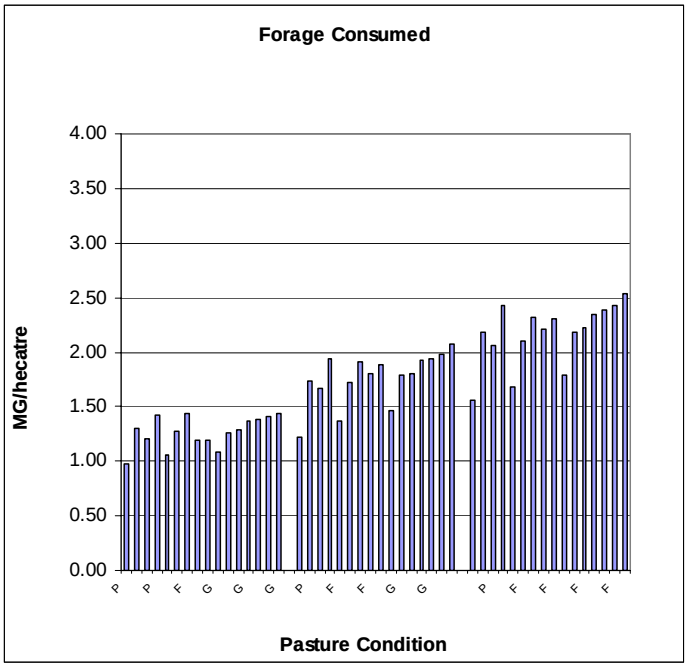
											7		3
F	2500	50	50	35	2000	76	0.12	0.18	0.46	2.78	119.69	0.76	89.92
F	3000	100	100	42	2000	76	0.12	0.18	0.53	2.97	127.76	0.84	136.20
F	3000	150	150	42	2000	76	0.11	0.15	0.53	3.01	129.68	0.79	181.24
F	4000	50	50	56	2000	76	0.16	0.23	0.68	2.93	125.99	1.07	108.40
F	5000	50	50	70	2000	76	0.19	0.27	0.82	2.98	128.10	1.28	121.34

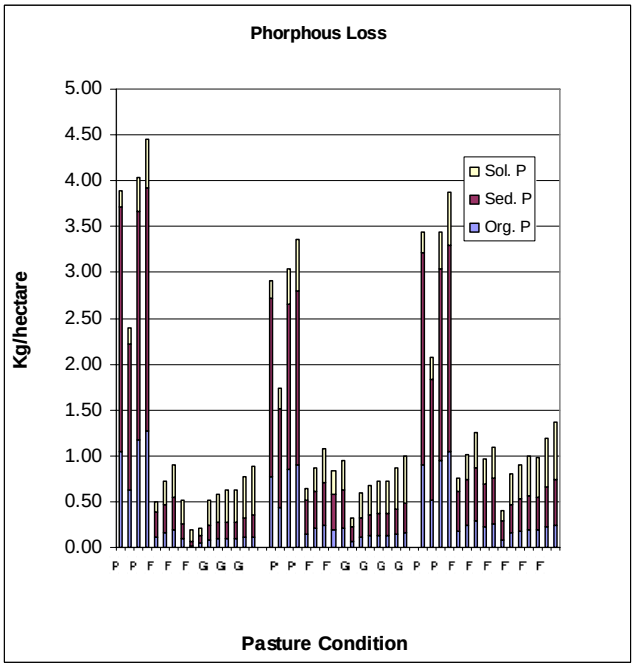




ELDORADO

Past Cond	26 Lit kg/ha	Ha qCNit kg/ha	Tn kg/ha	Hyd Class P	B MnBm	Curve No	Org P	Sed P	Sol P	Bmea t	NgDy s	T Ploss	Nlos s
				Low Stocking Rate									
P	1	1	1.0	0.0	1100	68	1.04	2.68	0.18	0.97	41.94	3.89	30.05
P	1	50	50.0	0.0	1100	68	0.63	1.59	0.19	1.31	56.20	2.40	48.75
P	1000	1	1.0	14.0	1100	68	1.17	2.50	0.37	1.21	52.06	4.04	36.08
P	2000	1	1.0	28.0	1100	68	1.28	2.65	0.53	1.43	61.45	4.45	43.47
F	1	50	50.0	0.0	1600	49	0.12	0.27	0.11	1.06	45.46	0.50	36.26
F	1000	50	50.0	14.0	1600	49	0.16	0.31	0.25	1.27	54.68	0.72	43.63
F	2000	100	100.0	28.0	1600	49	0.19	0.36	0.36	1.44	62.02	0.90	69.24
F	1000	100	100.0	14.0	1600	49	0.09	0.17	0.25	1.19	51.40	0.51	63.85
F	1500	150	150.0	21.0	1600	49	0.02	0.04	0.13	1.19	51.43	0.19	151.8 4
G	1	200	200.0	0.0	2000	39	0.04	0.09	0.08	1.09	46.73	0.21	88.73
G	2000	100	100.0	28.0	2000	39	0.09	0.16	0.27	1.26	54.39	0.51	68.87
G	2500	50	50.0	35.0	2000	39	0.09	0.18	0.31	1.29	55.52	0.58	54.82
G	3000	100	100.0	42.0	2000	39	0.10	0.18	0.35	1.37	59.07	0.63	75.96
G	3000	150	150.0	42.0	2000	39	0.10	0.18	0.35	1.39	59.73	0.63	93.72
G	4000	50	50.0	56.0	2000	39	0.11	0.21	0.44	1.41	60.49	0.76	65.90
G	5000	50	50.0	70.0	2000	39	0.12	0.24	0.53	1.44	62.13	0.89	74.18
				Medium Stocking Rate									
P	1	1	1.0	0.0	1100	68	0.77	1.95	0.20	1.22	52.52	2.92	29.35
P	1	50	50.0	0.0	1100	68	0.43	1.09	0.21	1.74	74.87	1.73	48.97
P	1000	1	1.0	14.0	1100	68	0.85	1.81	0.39	1.67	71.90	3.04	36.07
P	2000	1	1.0	28.0	1100	68	0.90	1.90	0.56	1.94	83.72	3.37	44.25
F	1	50	50.0	0.0	1600	49	0.15	0.36	0.13	1.36	58.68	0.64	37.07
F	1000	50	50.0	14.0	1600	49	0.20	0.41	0.26	1.72	74.12	0.87	44.61
F	2000	50	50.0	28.0	1600	49	0.24	0.47	0.37	1.91	82.08	1.08	51.98
F	1000	100	100.0	14.0	1600	49	0.19	0.39	0.26	1.80	77.50	0.84	63.01
F	1500	100	100.0	21.0	1600	49	0.21	0.41	0.32	1.88	80.91	0.94	66.67
G	1	200	200.0	0.0	2000	39	0.07	0.16	0.09	1.46	62.98	0.32	87.94
G	2000	100	100.0	28.0	2000	39	0.11	0.21	0.28	1.80	77.35	0.60	68.32
G	2500	50	50.0	35.0	2000	39	0.12	0.23	0.32	1.81	77.93	0.68	54.41
G	3000	100	100.0	42.0	2000	39	0.13	0.24	0.36	1.92	82.85	0.73	75.82
G	3000	150	150.0	42.0	2000	39	0.13	0.24	0.36	1.94	83.69	0.73	93.77
G	4000	50	50.0	56.0	2000	39	0.14	0.28	0.44	1.98	85.21	0.87	65.90
G	5000	50	50	70	2000	39	0.16	0.32	0.53	2.07	89.13	1.00	73.24
				High Stocking Rate									
P	1	1	1	0.01 4	1100	68	0.90	2.32	0.22	1.56	67.00	3.43	31.23
P	1	50	50	0.01 4	1100	68	0.51	1.32	0.24	2.19	94.14	2.08	51.27
P	1000	1	1	14	1100	68	0.95	2.08	0.41	2.06	88.69	3.44	38.06
P	2000	1	1	28	1100	68	1.04	2.25	0.58	2.43	104.45	3.87	47.22
F	1	50	50	0.01 4	1600	49	0.18	0.43	0.14	1.68	72.33	0.75	37.98
F	1000	50	50	14	1600	49	0.24	0.50	0.27	2.11	90.76	1.01	45.63
F	2000	50	50	28	1600	49	0.29	0.58	0.38	2.32	99.74	1.25	52.95
F	1000	100	100	14	1600	49	0.23	0.47	0.27	2.21	95.26	0.97	63.94
F	1500	100	100	21	1600	49	0.25	0.51	0.33	2.31	99.31	1.09	67.56
F	1	200	200	0.01 4	2000	44	0.09	0.21	0.11	1.80	77.35	0.41	90.60
F	2000	100	100	28	2000	44	0.16	0.31	0.33	2.18	94.02	0.80	70.36
F	2500	50	50	35	2000	44	0.18	0.35	0.38	2.22	95.59	0.90	56.21
F	3000	100	100	42	2000	44	0.19	0.37	0.43	2.35	101.25	0.99	78.11
F	3000	150	150	42	2000	44	0.19	0.37	0.43	2.38	102.52	0.98	95.97
F	4000	50	50	56	2000	44	0.22	0.44	0.53	2.43	104.45	1.19	67.43
F	5000	50	50	70	2000	44	0.24	0.50	0.62	2.54	109.16	1.36	74.79

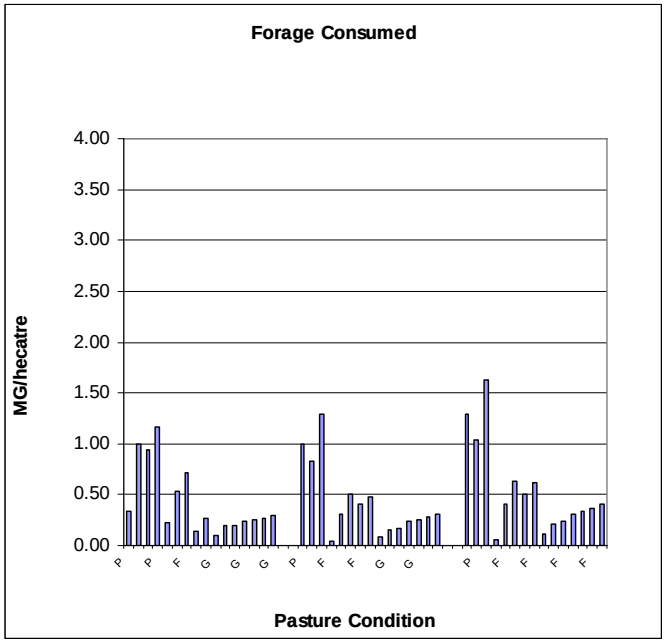

Forage Consumed
MG/hecatre
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pasture Condition

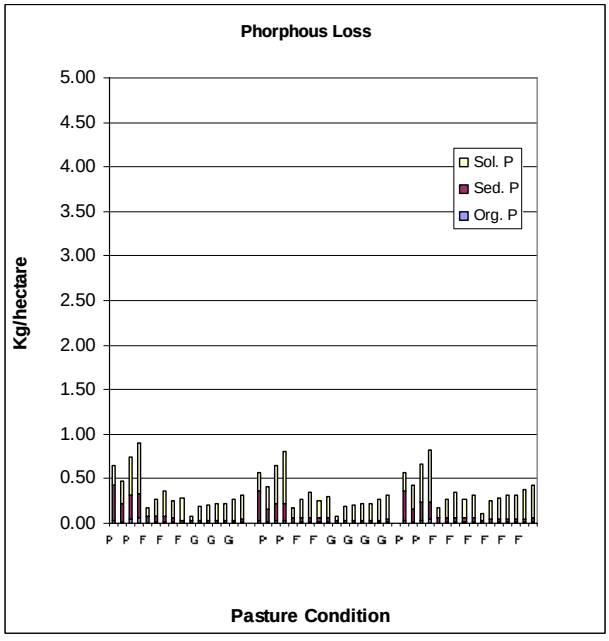

Phorphous Loss
Kg/hectare
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Sol. P
Sed. P
Org. P
Pasture Condition

ELSAH

Past Cond	85 Lit kg/ha	Ha qCNit kg/ha	Hyd Class Tn kg/ha	B P	MnBm	Curve No	Org P	Sed P	Sol P	Bmea t	NgDy s	T Ploss	Nlos s
				Low Stocking Rate									
P	1	1	1.0	0.0	1100	68	0.03	0.39	0.22	0.34	14.70	0.64	27.69
P	1	50	50.0	0.0	1100	68	0.02	0.21	0.24	0.99	42.65	0.47	75.10
P	1000	1	1.0	14.0	1100	68	0.05	0.28	0.42	0.94	40.50	0.74	47.63
P	2000	1	1.0	28.0	1100	68	0.06	0.27	0.57	1.16	50.00	0.90	64.87
F	1	50	50.0	0.0	1600	49	0.01	0.07	0.10	0.22	9.55	0.18	74.88
F	1000	50	50.0	14.0	1600	49	0.01	0.06	0.20	0.53	22.73	0.27	95.77
F	2000	100	100.0	28.0	1600	49	0.01	0.06	0.29	0.72	30.99	0.36	165.0 0
F	1000	100	100.0	14.0	1600	49	0.01	0.05	0.20	0.14	5.85	0.25	144.4 3
F	1500	150	150.0	21.0	1600	49	0.01	0.03	0.26	0.27	11.52	0.29	209.5 0
G	1	200	200.0	0.0	2000	39	0.00	0.02	0.05	0.10	4.30	0.08	222.7 5
G	2000	100	100.0	28.0	2000	39	0.01	0.02	0.15	0.19	8.31	0.18	166.0 3
G	2500	50	50.0	35.0	2000	39	0.01	0.03	0.17	0.20	8.61	0.21	127.2 2
G	3000	100	100.0	42.0	2000	39	0.01	0.03	0.19	0.24	10.54	0.23	187.1 0
G	3000	150	150.0	42.0	2000	39	0.01	0.03	0.19	0.25	10.95	0.23	236.5 9
G	4000	50	50.0	56.0	2000	39	0.01	0.03	0.23	0.27	11.45	0.27	158.7 5
G	5000	50	50.0	70.0	2000	39	0.01	0.03	0.27	0.29	12.63	0.32	179.8 0
				Medium Stocking Rate									
P	1	1	1.0	0.0	1100	68	0.03	0.33	0.21	0.00	0.00	0.57	26.43
P	1	50	50.0	0.0	1100	68	0.01	0.15	0.25	1.00	43.04	0.41	78.05
P	1000	1	1.0	14.0	1100	68	0.03	0.19	0.42	0.83	35.55	0.65	50.03
P	2000	1	1.0	28.0	1100	68	0.04	0.19	0.58	1.28	55.29	0.81	70.60
F	1	50	50.0	0.0	1600	49	0.01	0.06	0.10	0.04	1.91	0.17	74.37
F	1000	50	50.0	14.0	1600	49	0.01	0.05	0.20	0.31	13.55	0.26	96.44
F	2000	50	50.0	28.0	1600	49	0.01	0.05	0.29	0.50	21.72	0.35	117.8 3
F	1000	100	100.0	14.0	1600	49	0.01	0.05	0.20	0.40	17.38	0.26	145.9 7
F	1500	100	100.0	21.0	1600	49	0.01	0.05	0.25	0.48	20.84	0.31	156.6 6
G	1	200	200.0	0.0	2000	39	0.00	0.02	0.05	0.08	3.61	0.08	222.9 6
G	2000	100	100.0	28.0	2000	39	0.01	0.02	0.15	0.16	6.79	0.18	166.3 9
G	2500	50	50.0	35.0	2000	39	0.01	0.03	0.17	0.17	7.16	0.20	127.6 0
G	3000	100	100.0	42.0	2000	39	0.01	0.03	0.19	0.24	10.35	0.23	187.8 7
G	3000	150	150.0	42.0	2000	39	0.01	0.03	0.19	0.25	10.87	0.23	237.3 9
G	4000	50	50.0	56.0	2000	39	0.01	0.03	0.23	0.27	11.79	0.27	159.6 9
G	5000	50	50	70	2000	39	0.01	0.03	0.28	0.31	13.55	0.32	180.9 4
				High Stocking Rate									
P	1	1	1	0.01 4	1100	68	0.03	0.33	0.21	0.00	0.00	0.57	26.43
P	1	50	50	0.01 4	1100	68	0.01	0.15	0.26	1.29	55.62	0.42	80.07
P	1000	1	1	14	1100	68	0.03	0.20	0.43	1.04	44.95	0.66	51.70
P	2000	1	1	28	1100	68	0.04	0.19	0.59	1.63	70.14	0.83	73.13
F	1	50	50	0.01 4	1600	49	0.01	0.06	0.10	0.06	2.46	0.17	74.48
F	1000	50	50	14	1600	49	0.01	0.05	0.20	0.40	17.34	0.27	97.19
F	2000	50	50	28	1600	49	0.01	0.05	0.29	0.64	27.38	0.36	118.9 8
F	1000	100	100	14	1600	49	0.01	0.05	0.20	0.51	21.85	0.26	146.9 2
F	1500	100	100	21	1600	49	0.01	0.05	0.25	0.62	26.72	0.31	157.9 0
F	1	200	200	0.01 4	2000	44	0.00	0.03	0.07	0.12	5.11	0.11	223.0 5

F	2000	100	100	28	2000	44	0.01	0.03	0.21	0.21	8.98	0.25	166.57
F	2500	50	50	35	2000	44	0.01	0.04	0.24	0.24	10.20	0.28	127.90
F	3000	100	100	42	2000	44	0.01	0.04	0.27	0.31	13.49	0.31	188.21
F	3000	150	150	42	2000	44	0.01	0.04	0.27	0.34	14.64	0.31	237.77
F	4000	50	50	56	2000	44	0.01	0.04	0.32	0.37	15.88	0.38	160.07
F	5000	50	50	70	2000	44	0.01	0.04	0.38	0.40	17.41	0.43	181.24

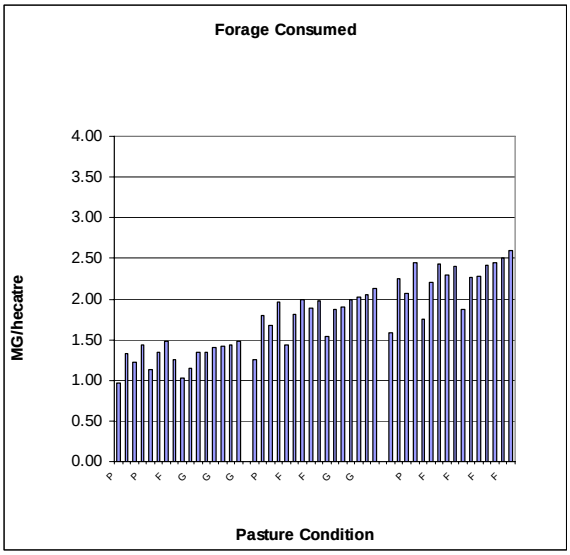


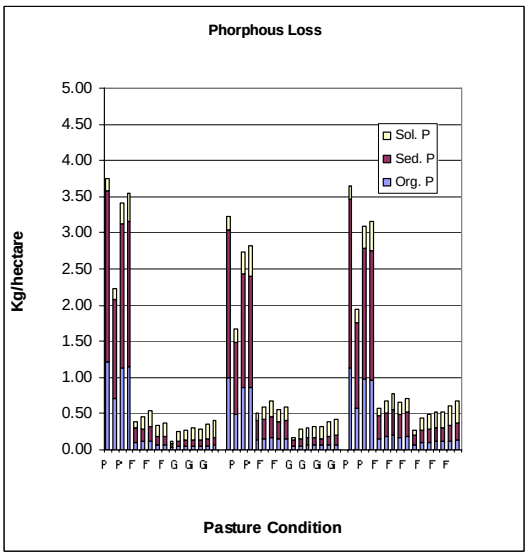


HEALING

Past Cond	175 Lit kg/ha	Ha qCNit kg/ha	Hyd Class Tn kg/ha	B P	MnBm	Curve No	Org P	Sed P	Sol P	Bmea t	NgDy s	T Ploss	Nloss
				Low Stocking Rate									
P	1	1	1.0	0.0	1100	68	1.21	2.38	0.16	0.97	41.65	3.75	72.54
P	1	50	50.0	0.0	1100	68	0.70	1.37	0.16	1.33	57.30	2.24	105.92
P	1000	1	1.0	14.0	1100	68	1.13	1.99	0.29	1.22	52.39	3.41	81.32
P	2000	1	1.0	28.0	1100	68	1.16	2.01	0.38	1.44	61.90	3.55	91.80
F	1	50	50.0	0.0	1600	49	0.10	0.20	0.08	1.13	48.45	0.39	106.18
F	1000	50	50.0	14.0	1600	49	0.11	0.18	0.16	1.34	57.71	0.45	122.75
F	2000	100	100.0	28.0	1600	49	0.12	0.20	0.21	1.48	63.68	0.53	187.16
F	1000	100	100.0	14.0	1600	49	0.07	0.11	0.16	1.25	53.89	0.34	174.33
F	1500	150	150.0	21.0	1600	49	0.07	0.12	0.18	1.03	44.21	0.37	237.71
G	1	200	200.0	0.0	2000	39	0.03	0.05	0.05	1.14	49.09	0.13	251.96
G	2000	100	100.0	28.0	2000	39	0.04	0.07	0.13	1.34	57.78	0.25	193.32
G	2500	50	50.0	35.0	2000	39	0.05	0.08	0.15	1.35	58.10	0.27	154.61
G	3000	100	100.0	42.0	2000	39	0.05	0.08	0.16	1.41	60.54	0.30	212.21
G	3000	150	150.0	42.0	2000	39	0.05	0.08	0.16	1.42	61.17	0.30	261.20
G	4000	50	50.0	56.0	2000	39	0.06	0.09	0.20	1.44	61.96	0.35	182.89
G	5000	50	50.0	70.0	2000	39	0.06	0.11	0.24	1.48	63.68	0.41	203.53
				Medium Stocking Rate									
P	1	1	1.0	0.0	1100	68	1.00	2.05	0.17	1.25	53.81	3.22	73.08
P	1	50	50.0	0.0	1100	68	0.49	1.00	0.18	1.79	77.17	1.67	107.58
P	1000	1	1.0	14.0	1100	68	0.86	1.57	0.31	1.68	72.34	2.73	82.84
P	2000	1	1.0	28.0	1100	68	0.86	1.55	0.41	1.96	84.38	2.81	95.01
F	1	50	50.0	0.0	1600	49	0.14	0.27	0.09	1.43	61.70	0.50	107.97
F	1000	50	50.0	14.0	1600	49	0.15	0.27	0.16	1.81	77.81	0.58	123.45
F	2000	50	50.0	28.0	1600	49	0.17	0.29	0.22	1.99	85.76	0.67	139.77
F	1000	100	100.0	14.0	1600	49	0.14	0.25	0.16	1.88	81.09	0.56	170.42
F	1500	100	100.0	21.0	1600	49	0.15	0.26	0.19	1.97	85.03	0.60	178.73
G	1	200	200.0	0.0	2000	39	0.04	0.08	0.05	1.53	65.98	0.18	251.04
G	2000	100	100.0	28.0	2000	39	0.06	0.10	0.13	1.87	80.63	0.28	191.89
G	2500	50	50.0	35.0	2000	39	0.06	0.10	0.15	1.90	81.68	0.31	153.34
G	3000	100	100.0	42.0	2000	39	0.06	0.10	0.16	1.99	85.63	0.32	209.93
G	3000	150	150.0	42.0	2000	39	0.06	0.10	0.16	2.02	87.01	0.32	258.11
G	4000	50	50.0	56.0	2000	39	0.07	0.12	0.20	2.05	88.28	0.38	180.02
G	5000	50	50	70	2000	39	0.07	0.13	0.23	2.13	91.82	0.43	197.84
				High Stocking Rate									
P	1	1	1	0.01 4	1100	68	1.13	2.33	0.19	1.58	68.07	3.64	75.67
P	1	50	50	0.01 4	1100	68	0.57	1.18	0.20	2.25	96.83	1.95	110.36
P	1000	1	1	14	1100	68	0.98	1.81	0.31	2.07	89.26	3.10	85.79
P	2000	1	1	28	1100	68	0.97	1.78	0.42	2.45	105.48	3.16	99.16
F	1	50	50	0.01 4	1600	49	0.16	0.32	0.10	1.75	75.54	0.57	109.81
F	1000	50	50	14	1600	49	0.18	0.32	0.17	2.20	94.76	0.68	125.50

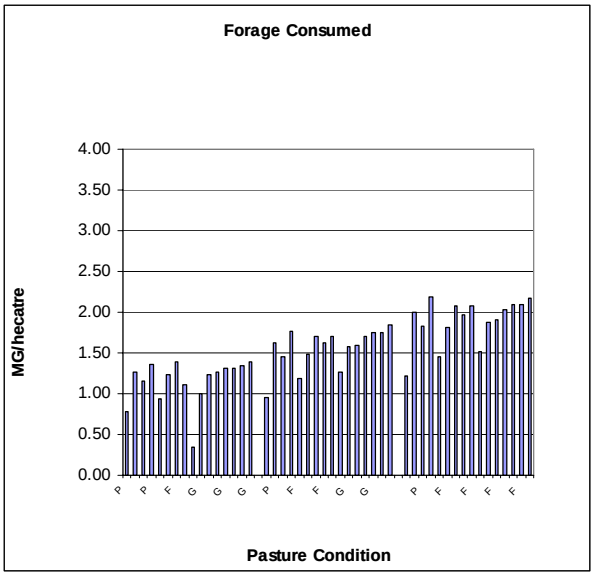
F	2000	50	50	28	1600	49	0.20	0.35	0.22	2.43	104.43	0.78	141.68
F	1000	100	100	14	1600	49	0.18	0.31	0.17	2.29	98.80	0.66	172.56
F	1500	100	100	21	1600	49	0.19	0.33	0.20	2.41	103.65	0.72	180.75
F	1	200	200	0.014	2000	44	0.07	0.13	0.07	1.87	80.71	0.28	251.73
F	2000	100	100	28	2000	44	0.10	0.17	0.17	2.27	97.69	0.44	191.49
F	2500	50	50	35	2000	44	0.11	0.18	0.20	2.28	98.19	0.48	152.61
F	3000	100	100	42	2000	44	0.11	0.19	0.22	2.41	103.71	0.52	208.83
F	3000	150	150	42	2000	44	0.11	0.19	0.22	2.44	105.23	0.52	256.66
F	4000	50	50	56	2000	44	0.12	0.22	0.26	2.50	107.75	0.60	178.42
F	5000	50	50	70	2000	44	0.13	0.24	0.31	2.60	111.96	0.68	195.51

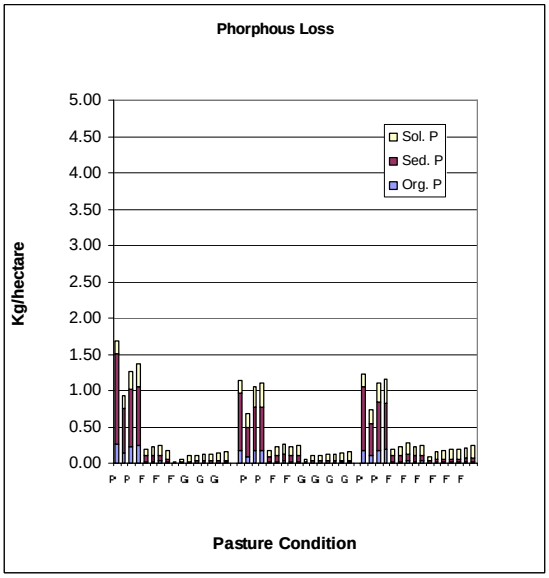




LINKER

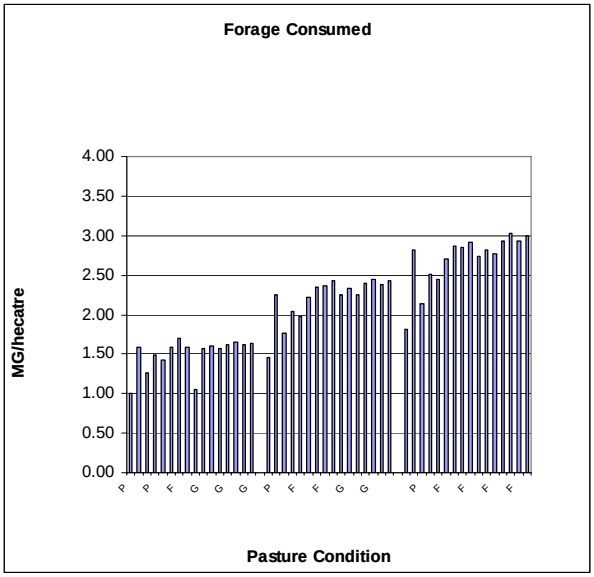
Past Cond	Lit kg/ha	44 Ha qCNit kg/ha	Hyd Class Tn kg/ha	B P	MnBm	Curve No	OrgP	Sed P	Sol P	Bmeat	NgDys	T Ploss	Nloss
					Low Stocking Rate								
P	1	1	1.0	0.0	1100	68	0.26	1.25	0.17	0.78	33.78	1.68	29.49
P	1	50	50.0	0.0	1100	68	0.13	0.62	0.17	1.27	54.62	0.92	66.52
P	1000	1	1.0	14.0	1100	68	0.22	0.79	0.26	1.16	49.90	1.27	42.57
P	2000	1	1.0	28.0	1100	68	0.25	0.80	0.32	1.36	58.63	1.37	56.94
F	1	50	50.0	0.0	1600	49	0.02	0.09	0.07	0.94	40.48	0.19	69.81
F	1000	50	50.0	14.0	1600	49	0.03	0.09	0.12	1.23	53.02	0.23	86.86
F	2000	100	100.0	28.0	1600	49	0.03	0.08	0.15	1.39	59.69	0.25	150.81
F	1000	100	100.0	14.0	1600	49	0.01	0.04	0.12	1.12	48.04	0.17	136.76
F	1500	150	150.0	21.0	1600	49	0.00	0.00	0.02	0.34	14.54	0.02	55.32
G	1	200	200.0	0.0	2000	39	0.00	0.01	0.03	0.99	42.80	0.05	214.82
G	2000	100	100.0	28.0	2000	39	0.01	0.02	0.07	1.23	53.12	0.10	156.99
G	2500	50	50.0	35.0	2000	39	0.01	0.02	0.08	1.26	54.17	0.11	118.81
G	3000	100	100.0	42.0	2000	39	0.01	0.02	0.09	1.31	56.32	0.12	176.10
G	3000	150	150.0	42.0	2000	39	0.01	0.02	0.09	1.32	56.72	0.12	224.20
G	4000	50	50.0	56.0	2000	39	0.01	0.02	0.10	1.34	57.52	0.13	147.43
G	5000	50	50.0	70.0	2000	39	0.01	0.02	0.12	1.39	59.86	0.15	167.50
					Medium Stocking Rate								
P	1	1	1.0	0.0	1100	68	0.17	0.80	0.17	0.95	40.83	1.15	30.66
P	1	50	50.0	0.0	1100	68	0.09	0.40	0.18	1.62	69.71	0.68	70.16
P	1000	1	1.0	14.0	1100	68	0.17	0.61	0.27	1.46	62.89	1.05	46.41
P	2000	1	1.0	28.0	1100	68	0.18	0.58	0.33	1.77	76.26	1.10	62.08
F	1	50	50.0	0.0	1600	49	0.02	0.08	0.08	1.19	51.43	0.17	72.13
F	1000	50	50.0	14.0	1600	49	0.02	0.08	0.12	1.48	63.85	0.23	89.27
F	2000	50	50.0	28.0	1600	49	0.03	0.09	0.15	1.71	73.52	0.27	106.63
F	1000	100	100.0	14.0	1600	49	0.02	0.08	0.12	1.62	69.78	0.22	135.65
F	1500	100	100.0	21.0	1600	49	0.03	0.08	0.14	1.70	73.35	0.24	144.34
G	1	200	200.0	0.0	2000	39	0.00	0.02	0.04	1.26	54.19	0.06	215.81
G	2000	100	100.0	28.0	2000	39	0.01	0.02	0.07	1.57	67.76	0.10	157.53
G	2500	50	50.0	35.0	2000	39	0.01	0.02	0.08	1.60	68.85	0.11	119.12
G	3000	100	100.0	42.0	2000	39	0.01	0.02	0.09	1.71	73.60	0.12	175.88
G	3000	150	150.0	42.0	2000	39	0.01	0.02	0.09	1.74	75.07	0.12	223.81
G	4000	50	50.0	56.0	2000	39	0.01	0.03	0.10	1.75	75.21	0.14	146.31
G	5000	50	50	70	2000	39	0.01	0.03	0.12	1.85	79.57	0.16	165.06
					High Stocking Rate								
P	1	1	1	0.014	1100	68	0.18	0.87	0.18	1.21	52.24	1.24	32.26
P	1	50	50	0.014	1100	68	0.10	0.45	0.19	2.00	86.17	0.74	73.16
P	1000	1	1	14	1100	68	0.18	0.65	0.27	1.83	78.88	1.11	48.84
P	2000	1	1	28	1100	68	0.19	0.63	0.34	2.19	94.08	1.16	65.22
F	1	50	50	0.014	1600	49	0.02	0.08	0.08	1.46	62.73	0.18	74.00
F	1000	50	50	14	1600	49	0.03	0.09	0.12	1.82	78.20	0.24	91.48
F	2000	50	50	28	1600	49	0.03	0.09	0.15	2.07	89.25	0.28	108.84
F	1000	100	100	14	1600	49	0.02	0.08	0.12	1.97	84.86	0.23	137.85
F	1500	100	100	21	1600	49	0.03	0.09	0.14	2.07	89.27	0.25	146.56
F	1	200	200	0.014	2000	44	0.01	0.03	0.06	1.52	65.56	0.10	216.43
F	2000	100	100	28	2000	44	0.01	0.04	0.11	1.88	80.80	0.16	157.94
F	2500	50	50	35	2000	44	0.01	0.04	0.12	1.90	81.99	0.18	119.80
F	3000	100	100	42	2000	44	0.02	0.04	0.13	2.03	87.34	0.19	175.89
F	3000	150	150	42	2000	44	0.01	0.04	0.13	2.10	90.38	0.19	223.64
F	4000	50	50	56	2000	44	0.02	0.05	0.15	2.09	90.06	0.22	146.82
F	5000	50	50	70	2000	44	0.02	0.05	0.17	2.18	93.83	0.24	165.12

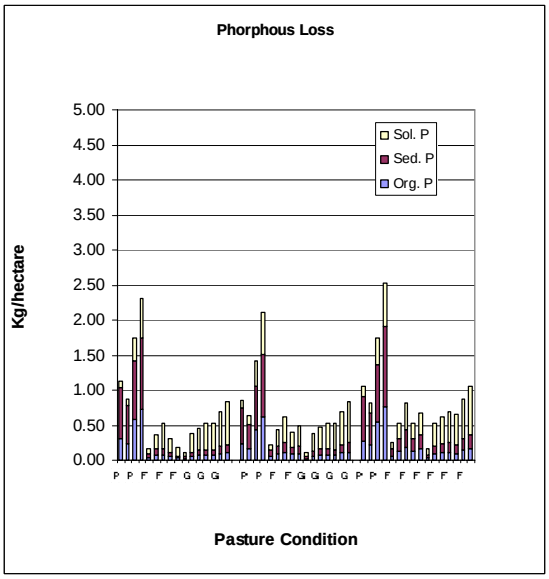

Forage Consumed
MG/hecatre
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pasture Condition


Phorphous Loss
Kg/hectare
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Sol. P
Sed. P
Org. P
Pasture Condition

MACADONIA

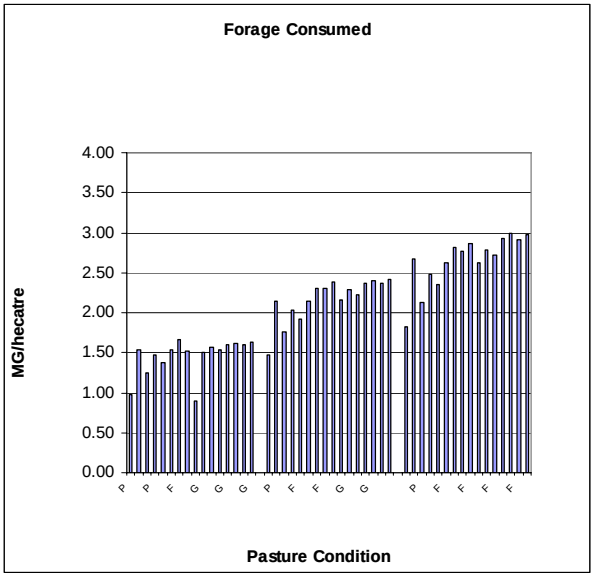
Past Cond	1460 Lit kg/ha	Ha qCNit kg/ha	Hyd Class Tn kg/ha	B P	MnBm	Curve No	Org P	Sed P	Sol P	Bmea t	NgDy s	T Ploss	Nlos s
				Low Stocking Rate									
P	1	1	1.0	0.0	1100	68	0.31	0.74	0.08	1.00	42.89	1.13	21.25
P	1	50	50.0	0.0	1100	68	0.24	0.53	0.10	1.59	68.40	0.88	43.84
P	1000	1	1.0	14.0	1100	68	0.58	0.83	0.34	1.27	54.63	1.75	27.99
P	2000	1	1.0	28.0	1100	68	0.73	1.01	0.57	1.49	64.13	2.31	35.60
F	1	50	50.0	0.0	1600	49	0.04	0.06	0.06	1.43	61.46	0.16	40.00
F	1000	50	50.0	14.0	1600	49	0.07	0.08	0.20	1.58	68.07	0.36	48.26
F	2000	100	100.0	28.0	1600	49	0.08	0.09	0.35	1.70	73.04	0.52	94.76
F	1000	100	100.0	14.0	1600	49	0.05	0.06	0.21	1.58	68.04	0.32	86.34
F	1500	150	150.0	21.0	1600	49	0.03	0.03	0.13	1.05	45.18	0.18	66.26
G	1	200	200.0	0.0	2000	39	0.02	0.03	0.06	1.57	67.73	0.11	137.16
G	2000	100	100.0	28.0	2000	39	0.06	0.06	0.26	1.60	68.98	0.38	92.92
G	2500	50	50.0	35.0	2000	39	0.07	0.07	0.32	1.57	67.55	0.46	66.04
G	3000	100	100.0	42.0	2000	39	0.07	0.08	0.38	1.63	69.97	0.53	105.98
G	3000	150	150.0	42.0	2000	39	0.07	0.07	0.39	1.65	70.97	0.53	140.50
G	4000	50	50.0	56.0	2000	39	0.09	0.10	0.49	1.62	69.80	0.69	85.34
G	5000	50	50.0	70.0	2000	39	0.10	0.12	0.62	1.64	70.76	0.84	98.96
				Medium Stocking Rate									
P	1	1	1.0	0.0	1100	68	0.23	0.52	0.11	1.46	62.79	0.86	21.94
P	1	50	50.0	0.0	1100	68	0.16	0.35	0.12	2.24	96.58	0.64	44.27
P	1000	1	1.0	14.0	1100	68	0.43	0.62	0.37	1.77	76.31	1.42	28.59
P	2000	1	1.0	28.0	1100	68	0.62	0.89	0.60	2.04	87.72	2.11	36.34
F	1	50	50.0	0.0	1600	49	0.05	0.09	0.08	1.98	85.29	0.22	41.28
F	1000	50	50.0	14.0	1600	49	0.09	0.11	0.22	2.22	95.38	0.43	48.89
F	2000	50	50.0	28.0	1600	49	0.12	0.14	0.37	2.35	101.15	0.63	57.45
F	1000	100	100.0	14.0	1600	49	0.08	0.10	0.22	2.36	101.65	0.40	79.59
F	1500	100	100.0	21.0	1600	49	0.09	0.11	0.29	2.43	104.51	0.50	83.87
G	1	200	200.0	0.0	2000	39	0.02	0.03	0.06	2.25	96.96	0.11	130.57
G	2000	100	100.0	28.0	2000	39	0.06	0.07	0.26	2.32	100.10	0.39	85.76
G	2500	50	50.0	35.0	2000	39	0.08	0.09	0.32	2.26	97.11	0.48	61.10
G	3000	100	100.0	42.0	2000	39	0.07	0.08	0.37	2.40	103.38	0.53	96.42
G	3000	150	150.0	42.0	2000	39	0.07	0.08	0.37	2.44	105.12	0.53	130.25
G	4000	50	50.0	56.0	2000	39	0.10	0.12	0.48	2.37	102.22	0.70	76.24
G	5000	50	50	70	2000	39	0.11	0.13	0.58	2.44	104.88	0.83	87.24
				High Stocking Rate									
P	1	1	1	0.01 4	1100	68	0.28	0.64	0.14	1.82	78.33	1.05	23.20
P	1	50	50	0.01 4	1100	68	0.21	0.46	0.15	2.81	121.16	0.83	45.46
P	1000	1	1	14	1100	68	0.54	0.82	0.38	2.14	92.01	1.75	29.79
P	2000	1	1	28	1100	68	0.76	1.14	0.63	2.51	107.87	2.53	37.72
F	1	50	50	0.01 4	1600	49	0.06	0.11	0.09	2.44	105.07	0.26	41.81
F	1000	50	50	14	1600	49	0.13	0.17	0.24	2.70	116.26	0.54	49.36
F	2000	50	50	28	1600	49	0.19	0.25	0.38	2.87	123.65	0.81	57.30
F	1000	100	100	14	1600	49	0.13	0.17	0.23	2.85	122.53	0.53	79.40
F	1500	100	100	21	1600	49	0.16	0.21	0.30	2.92	125.64	0.67	83.61
F	1	200	200	0.01 4	2000	44	0.03	0.05	0.08	2.74	118.08	0.16	131.94
F	2000	100	100	28	2000	44	0.09	0.11	0.32	2.82	121.34	0.53	87.07
F	2500	50	50	35	2000	44	0.10	0.13	0.39	2.78	119.55	0.62	61.75
F	3000	100	100	42	2000	44	0.12	0.14	0.44	2.94	126.47	0.70	95.67
F	3000	150	150	42	2000	44	0.10	0.12	0.44	3.03	130.27	0.66	127.90
F	4000	50	50	56	2000	44	0.14	0.17	0.57	2.93	126.36	0.88	75.64
F	5000	50	50	70	2000	44	0.16	0.20	0.69	2.99	128.81	1.05	85.52

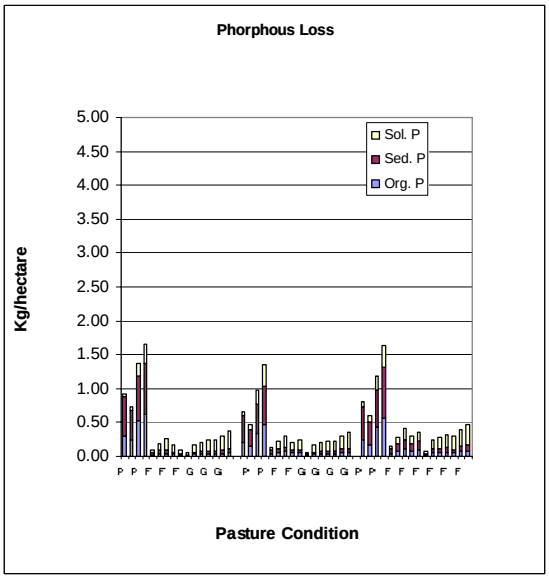

Forage Consumed
MG/hecatre
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
P P F G G G P F F G G P F F F F
Pasture Condition


Phorphous Loss
Kg/hectare
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Sol. P
Sed. P
Org. P
P P F F F G G G P P F F G G G P P F F F F F F
Pasture Condition

NEWTONIA

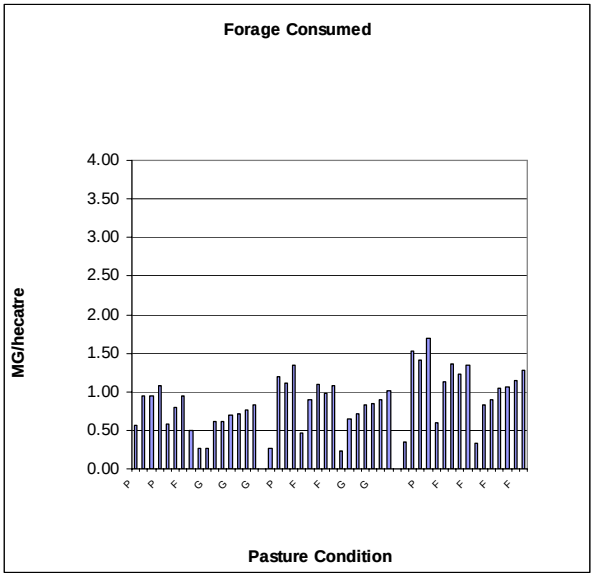
Past Cond	2224 Lit kg/ha	Ha qCNit kg/ha	Hyd Class Tn kg/ha	B P	MnBm	Curve No	OrgP	Sed P	Sol P	Bmeat	NgDys	T Ploss	Nloss
Low Stocking Rate													
P	1	1	1.0	0.0	1100	68	0.29	0.59	0.05	0.98	42.33	0.93	27.72
P	1	50	50.0	0.0	1100	68	0.24	0.45	0.05	1.54	66.20	0.74	56.78
P	1000	1	1.0	14.0	1100	68	0.53	0.67	0.18	1.24	53.53	1.37	35.94
P	2000	1	1.0	28.0	1100	68	0.61	0.76	0.29	1.47	63.45	1.66	44.73
F	1	50	50.0	0.0	1600	49	0.02	0.04	0.03	1.38	59.23	0.09	57.51
F	1000	50	50.0	14.0	1600	49	0.04	0.04	0.10	1.54	66.38	0.18	68.62
F	2000	100	100.0	28.0	1600	49	0.04	0.04	0.17	1.66	71.46	0.26	128.51
F	1000	100	100.0	14.0	1600	49	0.03	0.03	0.10	1.53	65.68	0.16	118.54
F	1500	150	150.0	21.0	1600	49	0.02	0.02	0.07	0.90	38.84	0.10	121.06
G	1	200	200.0	0.0	2000	39	0.01	0.02	0.03	1.50	64.74	0.06	197.64
G	2000	100	100.0	28.0	2000	39	0.03	0.03	0.12	1.57	67.62	0.17	135.70
G	2500	50	50.0	35.0	2000	39	0.03	0.04	0.14	1.54	66.47	0.21	98.25
G	3000	100	100.0	42.0	2000	39	0.04	0.04	0.17	1.60	69.07	0.24	153.72
G	3000	150	150.0	42.0	2000	39	0.04	0.04	0.17	1.62	69.71	0.24	201.94
G	4000	50	50.0	56.0	2000	39	0.05	0.05	0.21	1.60	68.83	0.31	125.37
G	5000	50	50.0	70.0	2000	39	0.05	0.06	0.27	1.62	69.94	0.38	144.78
Medium Stocking Rate													
P	1	1	1.0	0.0	1100	68	0.21	0.40	0.06	1.47	63.25	0.67	28.50
P	1	50	50.0	0.0	1100	68	0.14	0.26	0.07	2.14	92.20	0.47	57.29
P	1000	1	1.0	14.0	1100	68	0.34	0.43	0.19	1.76	75.59	0.97	36.53
P	2000	1	1.0	28.0	1100	68	0.46	0.58	0.31	2.03	87.30	1.35	45.93
F	1	50	50.0	0.0	1600	49	0.04	0.06	0.04	1.92	82.73	0.13	59.71
F	1000	50	50.0	14.0	1600	49	0.06	0.06	0.10	2.15	92.49	0.22	69.75
F	2000	50	50.0	28.0	1600	49	0.07	0.07	0.17	2.31	99.26	0.31	81.18
F	1000	100	100.0	14.0	1600	49	0.05	0.05	0.10	2.31	99.49	0.20	109.76
F	1500	100	100.0	21.0	1600	49	0.05	0.05	0.13	2.39	102.74	0.24	115.74
G	1	200	200.0	0.0	2000	39	0.01	0.02	0.03	2.16	92.82	0.06	190.87
G	2000	100	100.0	28.0	2000	39	0.03	0.03	0.11	2.28	98.20	0.17	127.41
G	2500	50	50.0	35.0	2000	39	0.04	0.04	0.13	2.22	95.55	0.21	92.12
G	3000	100	100.0	42.0	2000	39	0.04	0.04	0.16	2.37	101.89	0.23	141.61
G	3000	150	150.0	42.0	2000	39	0.04	0.04	0.16	2.40	103.38	0.23	188.37
G	4000	50	50.0	56.0	2000	39	0.05	0.05	0.20	2.37	101.88	0.30	112.88
G	5000	50	50	70	2000	39	0.06	0.06	0.24	2.42	104.17	0.36	128.09
High Stocking Rate													
P	1	1	1	0.014	1100	68	0.25	0.49	0.08	1.82	78.46	0.82	29.99
P	1	50	50	0.014	1100	68	0.18	0.33	0.08	2.67	115.05	0.59	58.66
P	1000	1	1	14	1100	68	0.42	0.56	0.20	2.13	91.63	1.19	37.90
P	2000	1	1	28	1100	68	0.57	0.74	0.32	2.49	107.05	1.63	47.20
F	1	50	50	0.014	1600	49	0.04	0.07	0.04	2.36	101.52	0.16	60.46
F	1000	50	50	14	1600	49	0.08	0.10	0.11	2.62	112.73	0.29	70.05
F	2000	50	50	28	1600	49	0.12	0.14	0.17	2.81	121.09	0.42	80.73
F	1000	100	100	14	1600	49	0.08	0.10	0.11	2.77	119.43	0.29	109.82
F	1500	100	100	21	1600	49	0.10	0.12	0.14	2.86	123.00	0.36	115.08
F	1	200	200	0.014	2000	44	0.02	0.03	0.04	2.63	113.13	0.08	187.69
F	2000	100	100	28	2000	44	0.05	0.06	0.14	2.78	119.82	0.25	125.28
F	2500	50	50	35	2000	44	0.06	0.06	0.17	2.73	117.37	0.29	90.56
F	3000	100	100	42	2000	44	0.06	0.07	0.19	2.92	125.80	0.32	137.29
F	3000	150	150	42	2000	44	0.05	0.05	0.19	2.99	128.57	0.29	182.07
F	4000	50	50	56	2000	44	0.07	0.08	0.24	2.92	125.56	0.40	109.26
F	5000	50	50	70	2000	44	0.08	0.09	0.30	2.98	128.17	0.47	122.52

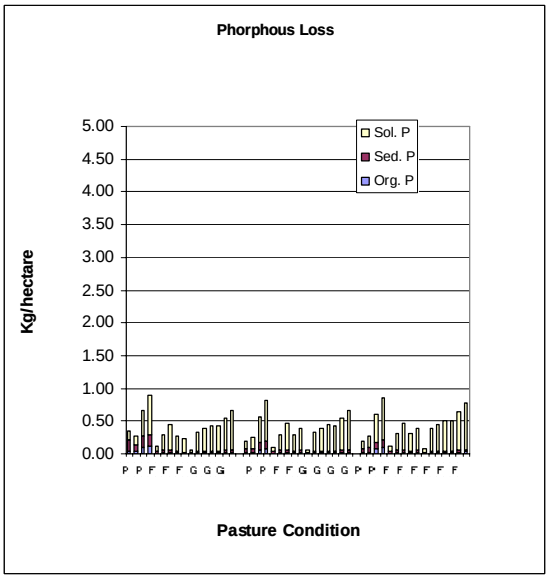

Forage Consumed
MG/hecatre
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pasture Condition


Phorphous Loss
Kg/hectare
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Sol. P
Sed. P
Org. P
Pasture Condition

NOARK

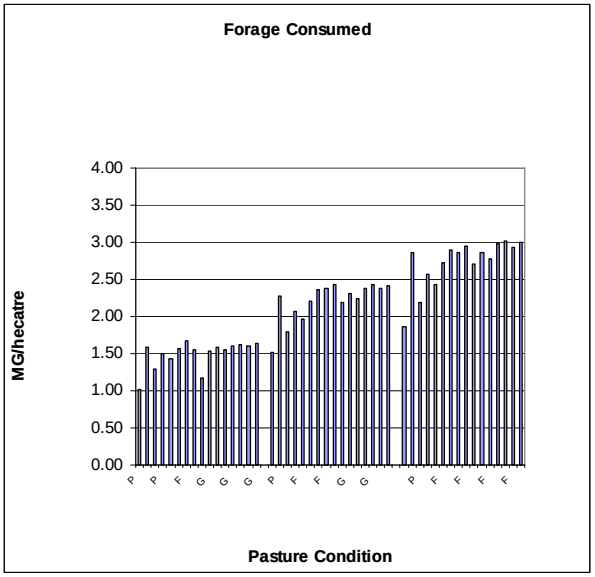
Past Cond	394 Lit kg/ha	Ha qCNit kg/ha	Hyd Class Tn kg/ha	B P	MnBm	Curve No	OrgP	Sed P	Sol P	Bmeat	NgDys	T Ploss	Nloss
				Low Stocking Rate									
P	1	1	1.0	0.0	1100	68	0.05	0.17	0.13	0.57	24.61	0.36	9.73
P	1	50	50.0	0.0	1100	68	0.03	0.11	0.15	0.95	40.98	0.28	20.40
P	1000	1	1.0	14.0	1100	68	0.10	0.17	0.39	0.95	40.77	0.65	12.59
P	2000	1	1.0	28.0	1100	68	0.13	0.17	0.60	1.08	46.55	0.90	15.94
F	1	50	50.0	0.0	1600	49	0.01	0.03	0.08	0.58	24.83	0.12	13.38
F	1000	50	50.0	14.0	1600	49	0.02	0.03	0.24	0.79	33.97	0.29	15.84
F	2000	100	100.0	28.0	1600	49	0.03	0.03	0.39	0.95	40.80	0.45	25.66
F	1000	100	100.0	14.0	1600	49	0.01	0.02	0.23	0.49	21.12	0.27	22.72
F	1500	150	150.0	21.0	1600	49	0.01	0.02	0.21	0.27	11.69	0.24	22.60
G	1	200	200.0	0.0	2000	39	0.00	0.01	0.05	0.26	11.20	0.07	31.34
G	2000	100	100.0	28.0	2000	39	0.02	0.02	0.29	0.61	26.33	0.32	23.06
G	2500	50	50.0	35.0	2000	39	0.02	0.02	0.34	0.62	26.71	0.38	18.00
G	3000	100	100.0	42.0	2000	39	0.02	0.02	0.39	0.70	30.32	0.43	25.34
G	3000	150	150.0	42.0	2000	39	0.02	0.02	0.39	0.71	30.76	0.43	31.61
G	4000	50	50.0	56.0	2000	39	0.02	0.03	0.50	0.77	33.06	0.55	21.51
G	5000	50	50.0	70.0	2000	39	0.03	0.03	0.60	0.83	35.53	0.65	24.02
				Medium Stocking Rate									
P	1	1	1.0	0.0	1100	68	0.02	0.06	0.11	0.26	11.25	0.19	9.34
P	1	50	50.0	0.0	1100	68	0.02	0.06	0.16	1.20	51.61	0.25	20.93
P	1000	1	1.0	14.0	1100	68	0.06	0.11	0.40	1.10	47.55	0.57	13.26
P	2000	1	1.0	28.0	1100	68	0.08	0.11	0.62	1.34	57.75	0.82	16.54
F	1	50	50.0	0.0	1600	49	0.01	0.02	0.07	0.47	20.23	0.11	13.54
F	1000	50	50.0	14.0	1600	49	0.02	0.03	0.25	0.90	38.94	0.30	16.08
F	2000	50	50.0	28.0	1600	49	0.03	0.03	0.40	1.09	47.02	0.46	18.71
F	1000	100	100.0	14.0	1600	49	0.02	0.03	0.25	0.98	42.38	0.30	23.35
F	1500	100	100.0	21.0	1600	49	0.02	0.03	0.33	1.08	46.51	0.38	24.65
G	1	200	200.0	0.0	2000	39	0.00	0.01	0.05	0.22	9.66	0.07	31.46
G	2000	100	100.0	28.0	2000	39	0.02	0.02	0.29	0.64	27.76	0.32	23.36
G	2500	50	50.0	35.0	2000	39	0.02	0.02	0.34	0.71	30.44	0.38	18.30
G	3000	100	100.0	42.0	2000	39	0.02	0.02	0.40	0.82	35.45	0.44	25.80
G	3000	150	150.0	42.0	2000	39	0.02	0.02	0.40	0.84	36.27	0.44	32.13
G	4000	50	50.0	56.0	2000	39	0.02	0.03	0.50	0.90	38.80	0.55	21.91
G	5000	50	50	70	2000	39	0.03	0.03	0.60	1.01	43.33	0.66	24.36
				High Stocking Rate									
P	1	1	1	0.014	1100	68	0.02	0.07	0.12	0.34	14.76	0.20	9.40
P	1	50	50	0.014	1100	68	0.02	0.07	0.18	1.52	65.55	0.28	21.38
P	1000	1	1	14	1100	68	0.07	0.12	0.42	1.40	60.47	0.60	13.73
P	2000	1	1	28	1100	68	0.09	0.12	0.64	1.69	72.57	0.85	17.09
F	1	50	50	0.014	1600	49	0.01	0.02	0.08	0.59	25.61	0.11	13.65
F	1000	50	50	14	1600	49	0.02	0.03	0.26	1.13	48.47	0.31	16.33
F	2000	50	50	28	1600	49	0.03	0.03	0.41	1.37	58.81	0.47	19.07
F	1000	100	100	14	1600	49	0.02	0.03	0.26	1.23	52.82	0.31	23.59
F	1500	100	100	21	1600	49	0.02	0.03	0.34	1.35	58.20	0.39	25.00
F	1	200	200	0.014	2000	44	0.00	0.01	0.06	0.33	14.19	0.08	33.48
F	2000	100	100	28	2000	44	0.02	0.02	0.34	0.84	35.99	0.38	24.99
F	2500	50	50	35	2000	44	0.02	0.03	0.40	0.89	38.48	0.45	19.46
F	3000	100	100	42	2000	44	0.02	0.03	0.47	1.04	44.79	0.52	27.38
F	3000	150	150	42	2000	44	0.02	0.03	0.47	1.06	45.60	0.51	34.11
F	4000	50	50	56	2000	44	0.03	0.03	0.59	1.14	49.05	0.65	23.01
F	5000	50	50	70	2000	44	0.03	0.03	0.71	1.28	54.98	0.77	25.57

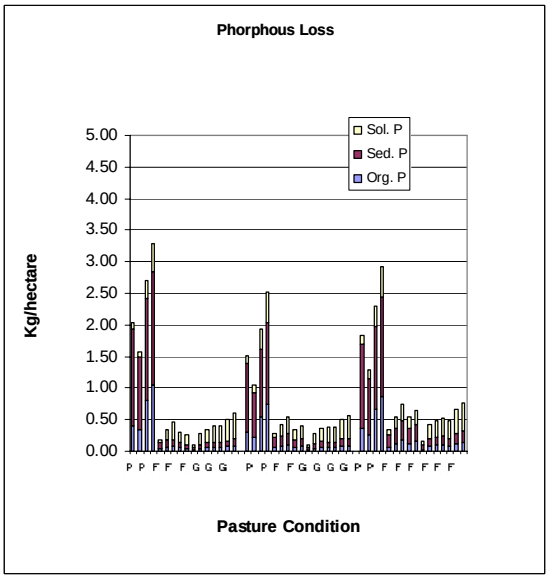

Forage Consumed
MG/hecatre
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pasture Condition


Phorphous Loss
Kg/hectare
Sol. P
Sed. P
Org. P
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pasture Condition

PERIDGE

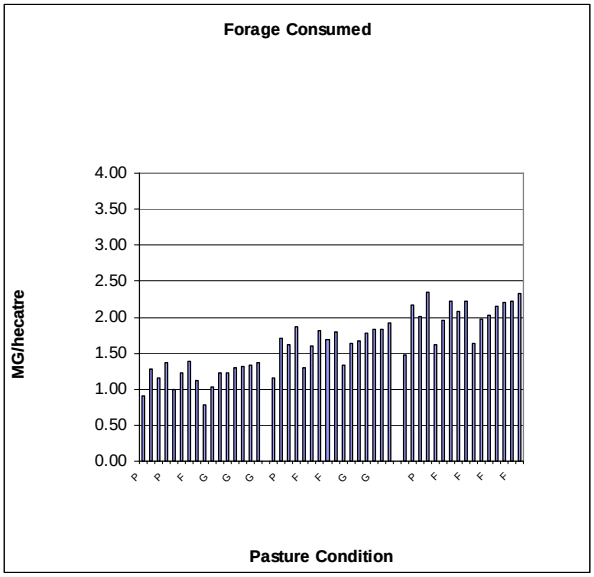
Past Cond	1339 Lit kg/ha	Ha qCNit kg/ha	Hyd Class Tn kg/ha	B P	MnBm	Curve No	Org P	Sed P	Sol P	Bmea t	NgDy s	T Ploss	Nlos s
				Low Stocking Rate									
P	1	1	1.0	0.0	1100	68	0.41	1.53	0.10	1.02	43.78	2.04	28.70
P	1	50	50.0	0.0	1100	68	0.33	1.15	0.09	1.58	68.16	1.58	49.55
P	1000	1	1.0	14.0	1100	68	0.81	1.60	0.29	1.29	55.45	2.70	34.23
P	2000	1	1.0	28.0	1100	68	1.04	1.80	0.45	1.49	64.31	3.29	40.19
F	1	50	50.0	0.0	1600	49	0.04	0.11	0.05	1.43	61.46	0.19	45.52
F	1000	50	50.0	14.0	1600	49	0.07	0.11	0.16	1.57	67.57	0.33	54.44
F	2000	100	100.0	28.0	1600	49	0.08	0.11	0.27	1.68	72.15	0.46	97.79
F	1000	100	100.0	14.0	1600	49	0.05	0.08	0.17	1.54	66.52	0.30	91.17
F	1500	150	150.0	21.0	1600	49	0.04	0.06	0.16	1.18	50.70	0.25	122.70
G	1	200	200.0	0.0	2000	39	0.02	0.04	0.04	1.53	65.86	0.10	142.90
G	2000	100	100.0	28.0	2000	39	0.05	0.06	0.18	1.58	67.93	0.29	96.88
G	2500	50	50.0	35.0	2000	39	0.06	0.08	0.21	1.55	66.88	0.35	69.78
G	3000	100	100.0	42.0	2000	39	0.06	0.08	0.26	1.61	69.19	0.39	109.26
G	3000	150	150.0	42.0	2000	39	0.06	0.08	0.26	1.62	69.74	0.40	143.86
G	4000	50	50.0	56.0	2000	39	0.07	0.10	0.32	1.60	68.97	0.50	88.09
G	5000	50	50.0	70.0	2000	39	0.09	0.11	0.40	1.63	70.19	0.60	101.52
				Medium Stocking Rate									
P	1	1	1.0	0.0	1100	68	0.30	1.08	0.12	1.53	65.69	1.51	28.77
P	1	50	50.0	0.0	1100	68	0.21	0.71	0.12	2.28	98.34	1.04	49.12
P	1000	1	1.0	14.0	1100	68	0.55	1.07	0.32	1.80	77.36	1.94	34.11
P	2000	1	1.0	28.0	1100	68	0.75	1.30	0.48	2.08	89.38	2.53	40.00
F	1	50	50.0	0.0	1600	49	0.06	0.17	0.06	1.97	84.71	0.28	46.32
F	1000	50	50.0	14.0	1600	49	0.09	0.16	0.17	2.21	94.98	0.42	52.61
F	2000	50	50.0	28.0	1600	49	0.11	0.16	0.27	2.35	101.36	0.54	60.08
F	1000	100	100.0	14.0	1600	49	0.07	0.11	0.17	2.39	102.76	0.35	82.91
F	1500	100	100.0	21.0	1600	49	0.08	0.12	0.22	2.42	104.35	0.41	86.78
G	1	200	200.0	0.0	2000	39	0.02	0.04	0.04	2.19	94.09	0.10	137.86
G	2000	100	100.0	28.0	2000	39	0.05	0.07	0.18	2.31	99.50	0.29	91.84
G	2500	50	50.0	35.0	2000	39	0.06	0.09	0.21	2.24	96.55	0.36	65.66
G	3000	100	100.0	42.0	2000	39	0.06	0.08	0.24	2.38	102.33	0.38	101.39
G	3000	150	150.0	42.0	2000	39	0.06	0.08	0.25	2.42	104.37	0.39	134.37
G	4000	50	50.0	56.0	2000	39	0.08	0.11	0.30	2.38	102.58	0.50	79.78
G	5000	50	50	70	2000	39	0.09	0.11	0.37	2.42	104.23	0.57	90.41
				High Stocking Rate									
P	1	1	1	0.01 4	1100	68	0.37	1.33	0.13	1.86	79.92	1.84	30.13
P	1	50	50	0.01 4	1100	68	0.26	0.88	0.14	2.86	123.23	1.29	50.21
P	1000	1	1	14	1100	68	0.66	1.32	0.33	2.19	94.42	2.30	35.27
P	2000	1	1	28	1100	68	0.87	1.56	0.50	2.56	110.25	2.93	40.99
F	1	50	50	0.01 4	1600	49	0.06	0.20	0.07	2.42	104.35	0.34	46.65
F	1000	50	50	14	1600	49	0.13	0.24	0.18	2.72	117.01	0.55	52.70
F	2000	50	50	28	1600	49	0.18	0.29	0.28	2.89	124.52	0.75	59.53
F	1000	100	100	14	1600	49	0.13	0.24	0.18	2.86	123.16	0.54	81.67
F	1500	100	100	21	1600	49	0.16	0.26	0.23	2.95	127.19	0.65	85.11
F	1	200	200	0.01 4	2000	44	0.03	0.07	0.05	2.71	116.76	0.15	140.67
F	2000	100	100	28	2000	44	0.08	0.12	0.22	2.86	123.31	0.43	92.40
F	2500	50	50	35	2000	44	0.09	0.14	0.26	2.78	119.57	0.49	66.15
F	3000	100	100	42	2000	44	0.10	0.14	0.30	2.98	128.24	0.53	100.71
F	3000	150	150	42	2000	44	0.08	0.11	0.30	3.01	129.69	0.49	134.94
F	4000	50	50	56	2000	44	0.12	0.17	0.37	2.94	126.46	0.66	79.14
F	5000	50	50	70	2000	44	0.14	0.19	0.44	2.99	128.84	0.77	87.88

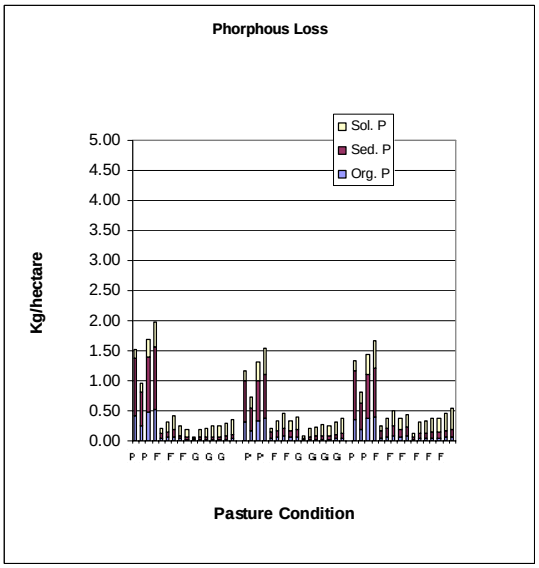

Forage Consumed
MG/hecatre
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pasture Condition


Phorphous Loss
Sol. P
Sed. P
Org. P
Kg/hectare
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pasture Condition

RAZORT

Past Cond	1118 Lit kg/ha	Ha qCNit kg/ha	Hyd Class Tn kg/ha	B P	MnBm	Curve No	OrgP	Sed P	Sol P	Bmeat	NgDys	T Ploss	Nloss
				Low Stocking Rate									
P	1	1	1.0	0.0	1100	68	0.42	0.96	0.15	0.90	38.74	1.52	56.59
P	1	50	50.0	0.0	1100	68	0.25	0.56	0.15	1.28	54.99	0.97	94.48
P	1000	1	1.0	14.0	1100	68	0.47	0.92	0.30	1.15	49.68	1.69	68.67
P	2000	1	1.0	28.0	1100	68	0.53	1.02	0.43	1.38	59.27	1.98	82.27
F	1	50	50.0	0.0	1600	49	0.04	0.09	0.07	1.00	43.00	0.21	95.75
F	1000	50	50.0	14.0	1600	49	0.05	0.10	0.16	1.23	52.94	0.31	113.48
F	2000	100	100.0	28.0	1600	49	0.07	0.12	0.23	1.39	59.70	0.42	178.26
F	1000	100	100.0	14.0	1600	49	0.03	0.06	0.16	1.12	48.18	0.25	163.93
F	1500	150	150.0	21.0	1600	49	0.02	0.04	0.12	0.78	33.42	0.18	162.07
G	1	200	200.0	0.0	2000	39	0.01	0.02	0.04	1.03	44.16	0.07	241.56
G	2000	100	100.0	28.0	2000	39	0.02	0.04	0.13	1.22	52.51	0.19	183.91
G	2500	50	50.0	35.0	2000	39	0.02	0.04	0.15	1.23	53.13	0.22	145.56
G	3000	100	100.0	42.0	2000	39	0.03	0.05	0.17	1.30	55.81	0.24	203.08
G	3000	150	150.0	42.0	2000	39	0.03	0.05	0.17	1.31	56.47	0.24	251.59
G	4000	50	50.0	56.0	2000	39	0.03	0.05	0.21	1.33	57.44	0.30	174.01
G	5000	50	50.0	70.0	2000	39	0.03	0.06	0.26	1.38	59.28	0.35	193.31
				Medium Stocking Rate									
P	1	1	1.0	0.0	1100	68	0.31	0.70	0.16	1.15	49.61	1.17	57.93
P	1	50	50.0	0.0	1100	68	0.17	0.38	0.18	1.71	73.63	0.72	97.82
P	1000	1	1.0	14.0	1100	68	0.33	0.66	0.32	1.62	69.69	1.31	71.59
P	2000	1	1.0	28.0	1100	68	0.37	0.73	0.45	1.86	80.07	1.55	86.91
F	1	50	50.0	0.0	1600	49	0.04	0.09	0.08	1.30	55.95	0.22	98.14
F	1000	50	50.0	14.0	1600	49	0.06	0.11	0.17	1.60	68.88	0.34	115.47
F	2000	50	50.0	28.0	1600	49	0.08	0.14	0.24	1.81	77.93	0.46	133.50
F	1000	100	100.0	14.0	1600	49	0.06	0.11	0.17	1.69	72.72	0.34	163.01
F	1500	100	100.0	21.0	1600	49	0.07	0.12	0.20	1.80	77.69	0.39	172.03
G	1	200	200.0	0.0	2000	39	0.01	0.03	0.04	1.33	57.33	0.09	242.44
G	2000	100	100.0	28.0	2000	39	0.03	0.05	0.13	1.63	70.29	0.20	184.28
G	2500	50	50.0	35.0	2000	39	0.03	0.05	0.15	1.67	71.82	0.23	146.11
G	3000	100	100.0	42.0	2000	39	0.03	0.06	0.17	1.78	76.69	0.26	203.52
G	3000	150	150.0	42.0	2000	39	0.03	0.06	0.17	1.83	78.67	0.26	251.79
G	4000	50	50.0	56.0	2000	39	0.04	0.07	0.21	1.83	78.84	0.31	174.31
G	5000	50	50	70	2000	39	0.04	0.08	0.25	1.93	82.91	0.37	193.03
				High Stocking Rate									
P	1	1	1	0.014	1100	68	0.35	0.81	0.18	1.47	63.31	1.34	59.93
P	1	50	50	0.014	1100	68	0.19	0.44	0.20	2.16	93.05	0.82	100.53
P	1000	1	1	14	1100	68	0.37	0.74	0.33	2.01	86.70	1.44	74.31
P	2000	1	1	28	1100	68	0.40	0.80	0.47	2.34	100.71	1.67	90.75
F	1	50	50	0.014	1600	49	0.05	0.11	0.09	1.62	69.80	0.25	100.30
F	1000	50	50	14	1600	49	0.07	0.13	0.17	1.95	84.07	0.38	118.16
F	2000	50	50	28	1600	49	0.09	0.16	0.25	2.22	95.71	0.50	136.36
F	1000	100	100	14	1600	49	0.07	0.13	0.17	2.08	89.51	0.37	165.87
F	1500	100	100	21	1600	49	0.08	0.15	0.21	2.23	95.85	0.43	175.02
F	1	200	200	0.014	2000	44	0.02	0.05	0.06	1.64	70.51	0.13	243.67
F	2000	100	100	28	2000	44	0.04	0.08	0.18	1.98	85.19	0.30	185.62
F	2500	50	50	35	2000	44	0.05	0.08	0.21	2.02	86.96	0.34	147.15
F	3000	100	100	42	2000	44	0.05	0.09	0.24	2.16	92.80	0.38	204.57
F	3000	150	150	42	2000	44	0.05	0.09	0.24	2.21	95.11	0.38	252.53
F	4000	50	50	56	2000	44	0.06	0.11	0.29	2.22	95.54	0.46	174.95
F	5000	50	50	70	2000	44	0.06	0.13	0.35	2.33	100.39	0.54	193.29

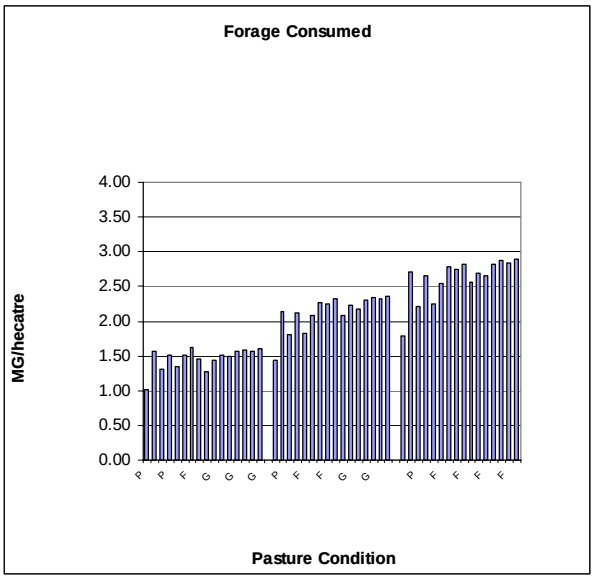

Forage Consumed
MG/hecatre
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pasture Condition

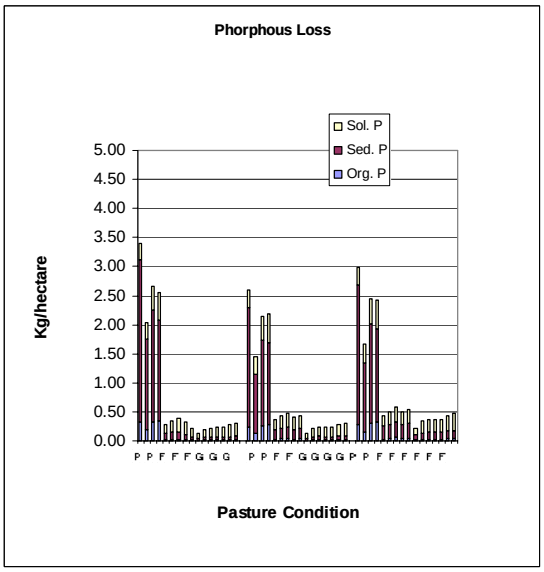

Phorphous Loss
Sol. P
Sed. P
Org. P
Kg/hectare
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pasture Condition

SECESH

Past Cond	210 Lit kg/ha	Ha qCNit kg/ha	Tn kg/ha	Hyd Class P	B MnBm	Curve No	Org P	Sed P	Sol P	Bmea t	NgDy s	T Ploss	Nlos s
				Low Stocking Rate									
P	1	1	1.0	0.0	1100	68	0.32	2.79	0.29	1.02	43.78	3.40	23.44
P	1	50	50.0	0.0	1100	68	0.19	1.56	0.28	1.57	67.53	2.03	53.02
P	1000	1	1.0	14.0	1100	68	0.33	1.93	0.40	1.31	56.20	2.66	32.53
P	2000	1	1.0	28.0	1100	68	0.35	1.72	0.47	1.52	65.46	2.55	42.41
F	1	50	50.0	0.0	1600	49	0.02	0.12	0.14	1.34	57.78	0.28	57.37
F	1000	50	50.0	14.0	1600	49	0.02	0.12	0.21	1.52	65.41	0.35	71.75
F	2000	100	100.0	28.0	1600	49	0.03	0.12	0.25	1.62	69.90	0.40	135.6 0
F	1000	100	100.0	14.0	1600	49	0.02	0.09	0.21	1.47	63.08	0.32	123.9 6
F	1500	150	150.0	21.0	1600	49	0.01	0.06	0.15	1.27	54.64	0.22	153.1 1
G	1	200	200.0	0.0	2000	39	0.01	0.04	0.08	1.43	61.64	0.12	200.6 7
G	2000	100	100.0	28.0	2000	39	0.01	0.05	0.14	1.52	65.38	0.20	142.2 2
G	2500	50	50.0	35.0	2000	39	0.01	0.05	0.16	1.50	64.60	0.22	104.7 3
G	3000	100	100.0	42.0	2000	39	0.01	0.05	0.17	1.56	67.29	0.24	162.7 9
G	3000	150	150.0	42.0	2000	39	0.01	0.05	0.17	1.58	68.06	0.24	211.6 0
G	4000	50	50.0	56.0	2000	39	0.02	0.06	0.20	1.57	67.65	0.27	135.1 9
G	5000	50	50.0	70.0	2000	39	0.02	0.06	0.22	1.60	68.87	0.31	156.1 3
				Medium Stocking Rate									
P	1	1	1.0	0.0	1100	68	0.24	2.05	0.30	1.44	61.86	2.60	24.86
P	1	50	50.0	0.0	1100	68	0.13	1.03	0.30	2.15	92.43	1.46	55.64
P	1000	1	1.0	14.0	1100	68	0.25	1.48	0.41	1.80	77.49	2.14	34.75
P	2000	1	1.0	28.0	1100	68	0.28	1.41	0.49	2.13	91.53	2.18	44.92
F	1	50	50.0	0.0	1600	49	0.02	0.18	0.15	1.82	78.53	0.36	58.75
F	1000	50	50.0	14.0	1600	49	0.03	0.18	0.22	2.08	89.59	0.43	72.34
F	2000	50	50.0	28.0	1600	49	0.04	0.19	0.26	2.26	97.31	0.49	85.84
F	1000	100	100.0	14.0	1600	49	0.03	0.17	0.21	2.25	96.67	0.41	115.5 3
F	1500	100	100.0	21.0	1600	49	0.03	0.17	0.24	2.31	99.63	0.44	122.6 5
G	1	200	200.0	0.0	2000	39	0.01	0.04	0.08	2.08	89.59	0.13	195.8 8
G	2000	100	100.0	28.0	2000	39	0.01	0.06	0.14	2.22	95.73	0.21	136.4 7
G	2500	50	50.0	35.0	2000	39	0.01	0.06	0.16	2.17	93.64	0.23	99.89
G	3000	100	100.0	42.0	2000	39	0.01	0.06	0.17	2.31	99.52	0.24	153.3 2
G	3000	150	150.0	42.0	2000	39	0.01	0.06	0.17	2.34	100.57	0.24	200.1 8
G	4000	50	50.0	56.0	2000	39	0.02	0.07	0.19	2.33	100.13	0.28	124.3 9
G	5000	50	50	70	2000	39	0.02	0.07	0.22	2.36	101.57	0.30	142.4 9
				High Stocking Rate									
P	1	1	1	0.01 4	1100	68	0.28	2.39	0.31	1.80	77.37	2.99	26.87
P	1	50	50	0.01 4	1100	68	0.15	1.19	0.32	2.70	116.34	1.66	58.06
P	1000	1	1	14	1100	68	0.29	1.72	0.42	2.22	95.58	2.44	36.96
P	2000	1	1	28	1100	68	0.32	1.60	0.50	2.66	114.43	2.42	48.65
F	1	50	50	0.01 4	1600	49	0.03	0.24	0.16	2.25	96.83	0.42	60.67
F	1000	50	50	14	1600	49	0.04	0.24	0.22	2.55	109.65	0.51	73.42
F	2000	50	50	28	1600	49	0.06	0.27	0.26	2.78	119.52	0.58	86.66
F	1000	100	100	14	1600	49	0.04	0.23	0.22	2.75	118.46	0.49	116.3 3
F	1500	100	100	21	1600	49	0.05	0.25	0.24	2.83	121.77	0.53	123.2 6
F	1	200	200	0.01 4	2000	44	0.01	0.09	0.12	2.56	110.30	0.21	195.1 2
F	2000	100	100	28	2000	44	0.02	0.12	0.20	2.69	115.95	0.34	134.5 4
F	2500	50	50	35	2000	44	0.03	0.12	0.21	2.65	114.22	0.36	98.09

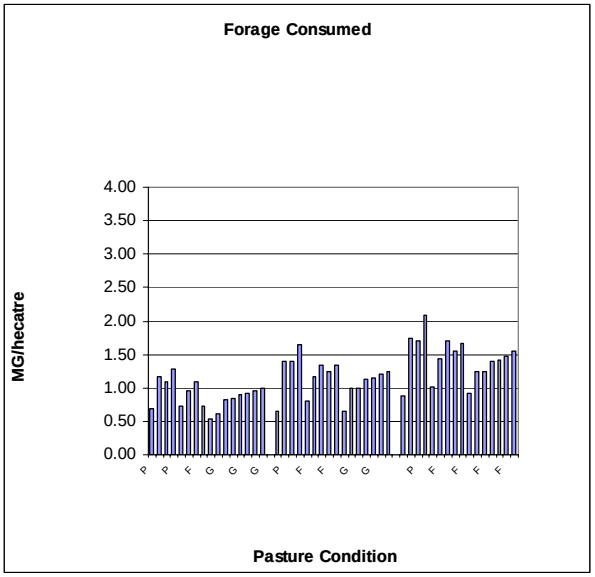
F	3000	100	100	42	2000	44	0.03	0.12	0.23	2.82	121.35	0.38	149.39
F	3000	150	150	42	2000	44	0.03	0.12	0.23	2.88	124.06	0.37	195.15
F	4000	50	50	56	2000	44	0.03	0.14	0.26	2.83	121.93	0.44	120.05
F	5000	50	50	70	2000	44	0.04	0.14	0.29	2.89	124.55	0.47	135.53

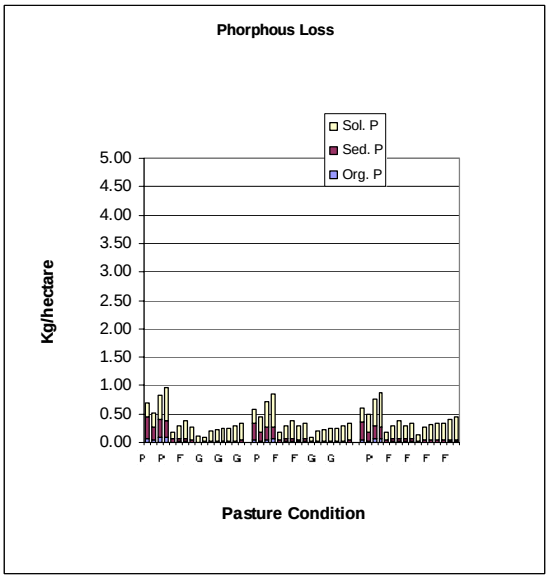




WABEN

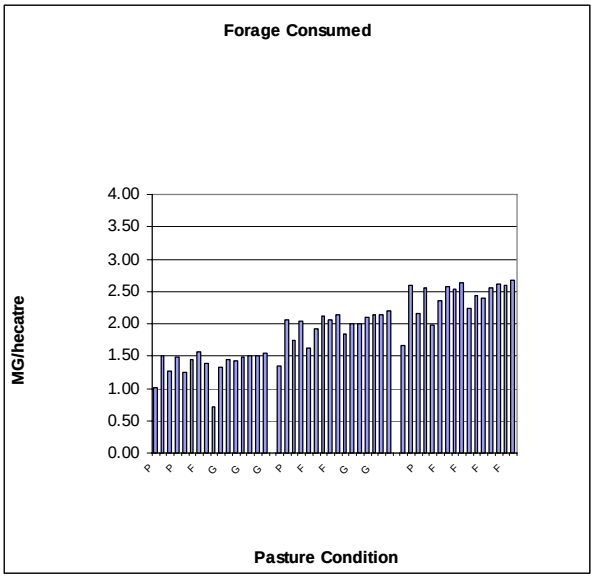
Past Cond	44 Lit kg/ha	Ha qCNit kg/ha	Hyd Class Tn kg/ha	B P	MnBm	Curve No	OrgP	Sed P	Sol P	Bmeat	NgDys	T Ploss	Nloss
				Low Stocking Rate									
P	1	1	1.0	0.0	1100	68	0.07	0.38	0.25	0.69	29.87	0.70	35.83
P	1	50	50.0	0.0	1100	68	0.04	0.22	0.26	1.17	50.24	0.53	80.34
P	1000	1	1.0	14.0	1100	68	0.08	0.31	0.44	1.10	47.30	0.84	50.88
P	2000	1	1.0	28.0	1100	68	0.09	0.30	0.57	1.28	55.32	0.96	65.69
F	1	50	50.0	0.0	1600	49	0.01	0.05	0.13	0.73	31.51	0.19	76.76
F	1000	50	50.0	14.0	1600	49	0.01	0.05	0.23	0.97	41.58	0.29	92.93
F	2000	100	100.0	28.0	1600	49	0.02	0.05	0.30	1.09	46.73	0.37	156.20
F	1000	100	100.0	14.0	1600	49	0.01	0.03	0.22	0.73	31.42	0.27	140.53
F	1500	150	150.0	21.0	1600	49	0.00	0.01	0.10	0.53	22.81	0.11	75.63
G	1	200	200.0	0.0	2000	39	0.00	0.02	0.07	0.61	26.06	0.09	193.94
G	2000	100	100.0	28.0	2000	39	0.01	0.02	0.17	0.82	35.20	0.20	144.17
G	2500	50	50.0	35.0	2000	39	0.01	0.02	0.19	0.83	35.87	0.22	111.47
G	3000	100	100.0	42.0	2000	39	0.01	0.02	0.21	0.90	38.61	0.24	161.31
G	3000	150	150.0	42.0	2000	39	0.01	0.02	0.21	0.91	39.39	0.24	202.94
G	4000	50	50.0	56.0	2000	39	0.01	0.02	0.26	0.95	41.01	0.29	137.18
G	5000	50	50.0	70.0	2000	39	0.01	0.03	0.30	0.99	42.48	0.33	154.60
				Medium Stocking Rate									
P	1	1	1.0	0.0	1100	68	0.05	0.28	0.25	0.65	27.80	0.58	37.16
P	1	50	50.0	0.0	1100	68	0.03	0.15	0.28	1.40	60.39	0.45	83.57
P	1000	1	1.0	14.0	1100	68	0.06	0.21	0.46	1.41	60.54	0.72	54.96
P	2000	1	1.0	28.0	1100	68	0.06	0.20	0.59	1.64	70.66	0.85	71.75
F	1	50	50.0	0.0	1600	49	0.01	0.04	0.13	0.80	34.41	0.18	78.33
F	1000	50	50.0	14.0	1600	49	0.01	0.04	0.23	1.16	50.13	0.29	96.99
F	2000	50	50.0	28.0	1600	49	0.02	0.05	0.31	1.35	57.93	0.38	114.98
F	1000	100	100.0	14.0	1600	49	0.01	0.04	0.23	1.24	53.23	0.29	143.26
F	1500	100	100.0	21.0	1600	49	0.01	0.05	0.28	1.34	57.50	0.33	152.18
G	1	200	200.0	0.0	2000	39	0.00	0.02	0.07	0.66	28.29	0.09	195.16
G	2000	100	100.0	28.0	2000	39	0.01	0.02	0.17	0.99	42.59	0.20	146.55
G	2500	50	50.0	35.0	2000	39	0.01	0.02	0.20	1.00	43.14	0.22	113.47
G	3000	100	100.0	42.0	2000	39	0.01	0.02	0.22	1.13	48.57	0.25	163.41
G	3000	150	150.0	42.0	2000	39	0.01	0.02	0.22	1.15	49.72	0.25	204.89
G	4000	50	50.0	56.0	2000	39	0.01	0.02	0.26	1.20	51.63	0.29	138.59
G	5000	50	50	70	2000	39	0.01	0.03	0.30	1.24	53.55	0.33	155.53
				High Stocking Rate									
P	1	1	1	0.014	1100	68	0.05	0.30	0.26	0.87	37.55	0.61	38.44
P	1	50	50	0.014	1100	68	0.03	0.16	0.30	1.74	74.82	0.48	85.91
P	1000	1	1	14	1100	68	0.06	0.22	0.47	1.71	73.71	0.75	57.43
P	2000	1	1	28	1100	68	0.06	0.21	0.60	2.08	89.44	0.88	74.85
F	1	50	50	0.014	1600	49	0.01	0.04	0.13	1.01	43.58	0.19	79.87
F	1000	50	50	14	1600	49	0.01	0.05	0.24	1.44	61.91	0.30	98.98
F	2000	50	50	28	1600	49	0.02	0.05	0.32	1.70	73.23	0.38	117.19
F	1000	100	100	14	1600	49	0.01	0.04	0.24	1.55	66.53	0.30	145.26
F	1500	100	100	21	1600	49	0.01	0.05	0.28	1.67	71.96	0.34	154.40
F	1	200	200	0.014	2000	44	0.00	0.02	0.10	0.92	39.70	0.12	206.54
F	2000	100	100	28	2000	44	0.01	0.03	0.24	1.24	53.44	0.27	155.23
F	2500	50	50	35	2000	44	0.01	0.03	0.27	1.25	53.96	0.30	120.38
F	3000	100	100	42	2000	44	0.01	0.03	0.30	1.39	59.94	0.34	173.39
F	3000	150	150	42	2000	44	0.01	0.03	0.30	1.42	60.99	0.34	217.37
F	4000	50	50	56	2000	44	0.01	0.03	0.35	1.47	63.43	0.40	146.96
F	5000	50	50	70	2000	44	0.01	0.04	0.41	1.54	66.52	0.46	164.77

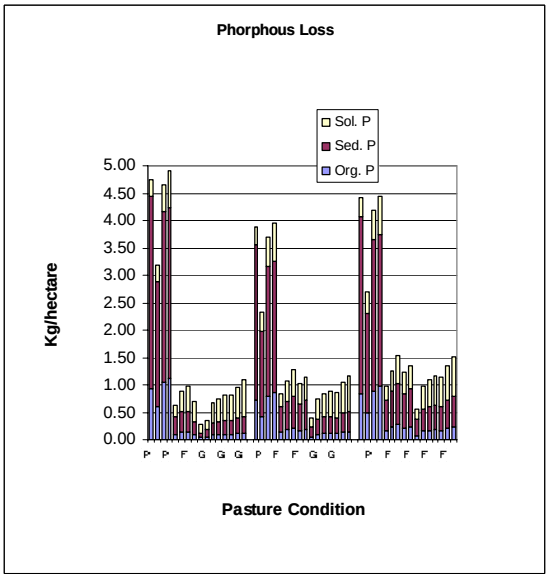

Forage Consumed
MG/hecatre
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pasture Condition


Phorphous Loss
Sol. P
Sed. P
Org. P
Kg/hectare
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pasture Condition

CAPTINA

Past Cond	5150 Lit kg/ha	Ha qCNit kg/ha	Hyd Class Tn kg/ha	C P	MnBm	Curve No	Org P	Sed P	Sol P	Bmea t	NgDy s	T Ploss	Nlos s
				Low Stocking Rate									
P	1	1	1.0	0.0	1100	79	0.92	3.52	0.31	1.00	43.23	4.75	33.94
P	1	50	50.0	0.0	1100	79	0.61	2.27	0.31	1.51	65.10	3.19	49.05
P	1000	1	1.0	14.0	1100	79	1.04	3.11	0.50	1.27	54.78	4.65	37.64
P	2000	1	1.0	28.0	1100	79	1.13	3.11	0.66	1.49	63.96	4.90	42.53
F	1	50	50.0	0.0	1600	69	0.09	0.32	0.21	1.26	54.11	0.63	52.79
F	1000	50	50.0	14.0	1600	69	0.14	0.38	0.36	1.44	62.07	0.88	62.38
F	2000	100	100.0	28.0	1600	69	0.14	0.37	0.47	1.57	67.50	0.98	104.9 8
F	1000	100	100.0	14.0	1600	69	0.09	0.24	0.36	1.38	59.50	0.69	99.13
F	1500	150	150.0	21.0	1600	69	0.03	0.09	0.16	0.71	30.65	0.28	59.84
G	1	200	200.0	0.0	2000	61	0.04	0.15	0.16	1.33	57.37	0.35	149.3 9
G	2000	100	100.0	28.0	2000	61	0.09	0.22	0.37	1.44	61.84	0.68	108.3 0
G	2500	50	50.0	35.0	2000	61	0.09	0.23	0.42	1.43	61.36	0.75	82.17
G	3000	100	100.0	42.0	2000	61	0.10	0.25	0.47	1.49	64.07	0.82	121.6 7
G	3000	150	150.0	42.0	2000	61	0.10	0.24	0.47	1.51	64.90	0.81	155.1 6
G	4000	50	50.0	56.0	2000	61	0.11	0.28	0.57	1.50	64.79	0.96	102.0 6
G	5000	50	50.0	70.0	2000	61	0.12	0.30	0.66	1.55	66.55	1.09	115.9 9
				Medium Stocking Rate									
P	1	1	1.0	0.0	1100	79	0.73	2.82	0.33	1.34	57.66	3.88	34.02
P	1	50	50.0	0.0	1100	79	0.42	1.55	0.36	2.05	88.28	2.33	49.95
P	1000	1	1.0	14.0	1100	79	0.79	2.38	0.53	1.74	74.80	3.70	38.32
P	2000	1	1.0	28.0	1100	79	0.86	2.41	0.69	2.04	87.87	3.96	44.03
F	1	50	50.0	0.0	1600	69	0.13	0.48	0.23	1.63	69.98	0.84	54.27
F	1000	50	50.0	14.0	1600	69	0.18	0.51	0.37	1.93	83.11	1.06	63.66
F	2000	50	50.0	28.0	1600	69	0.22	0.58	0.48	2.11	90.88	1.28	73.49
F	1000	100	100.0	14.0	1600	69	0.17	0.49	0.37	2.07	89.05	1.03	94.42
F	1500	100	100.0	21.0	1600	69	0.19	0.52	0.42	2.14	92.27	1.14	99.35
G	1	200	200.0	0.0	2000	61	0.05	0.18	0.17	1.83	78.99	0.41	147.9 8
G	2000	100	100.0	28.0	2000	61	0.10	0.27	0.38	2.01	86.52	0.75	106.5 8
G	2500	50	50.0	35.0	2000	61	0.11	0.29	0.42	1.99	85.69	0.83	80.84
G	3000	100	100.0	42.0	2000	61	0.12	0.29	0.47	2.11	90.77	0.88	117.8 9
G	3000	150	150.0	42.0	2000	61	0.11	0.28	0.47	2.15	92.46	0.86	150.0 4
G	4000	50	50.0	56.0	2000	61	0.14	0.34	0.56	2.13	91.79	1.04	97.53
G	5000	50	50	70	2000	61	0.15	0.36	0.65	2.20	94.63	1.16	109.1 6
				High Stocking Rate									
P	1	1	1	0.01 4	1100	79	0.84	3.23	0.35	1.66	71.53	4.41	35.64
P	1	50	50	0.01 4	1100	79	0.48	1.82	0.39	2.58	111.28	2.69	51.82
P	1000	1	1	14	1100	79	0.89	2.76	0.54	2.16	92.83	4.19	40.20
P	2000	1	1	28	1100	79	0.97	2.77	0.71	2.56	110.11	4.45	46.64
F	1	50	50	0.01 4	1600	69	0.16	0.57	0.24	1.98	85.12	0.97	55.78
F	1000	50	50	14	1600	69	0.22	0.65	0.38	2.35	101.12	1.26	65.10
F	2000	50	50	28	1600	69	0.28	0.76	0.49	2.58	111.03	1.52	74.63
F	1000	100	100	14	1600	69	0.22	0.63	0.38	2.53	108.76	1.22	95.60
F	1500	100	100	21	1600	69	0.24	0.68	0.43	2.63	113.15	1.36	100.4 1
F	1	200	200	0.01 4	2000	65	0.08	0.28	0.20	2.23	96.08	0.56	149.2 3
F	2000	100	100	28	2000	65	0.15	0.40	0.42	2.43	104.82	0.98	107.0 3
F	2500	50	50	35	2000	65	0.17	0.44	0.47	2.39	103.06	1.09	81.19
F	3000	100	100	42	2000	65	0.18	0.46	0.52	2.56	110.20	1.15	117.6 4
F	3000	150	150	42	2000	65	0.17	0.44	0.52	2.61	112.25	1.13	149.4 4
F	4000	50	50	56	2000	65	0.20	0.52	0.62	2.60	111.79	1.34	96.99
F	5000	50	50	70	2000	65	0.22	0.57	0.72	2.67	115.10	1.51	107.7 5

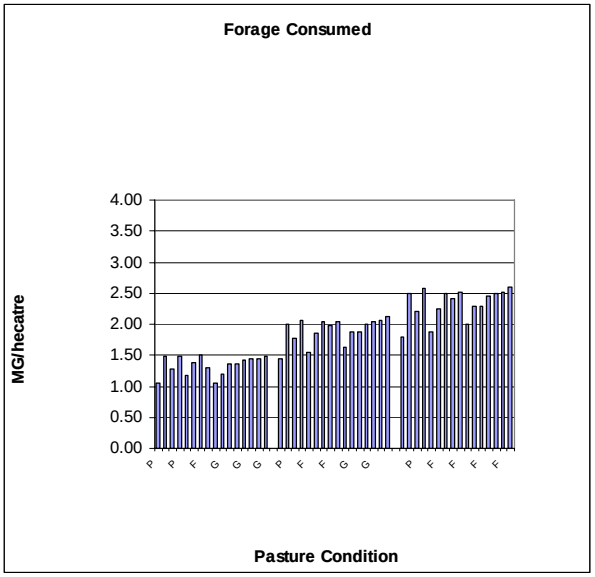

Forage Consumed
MG/hecatre
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pasture Condition

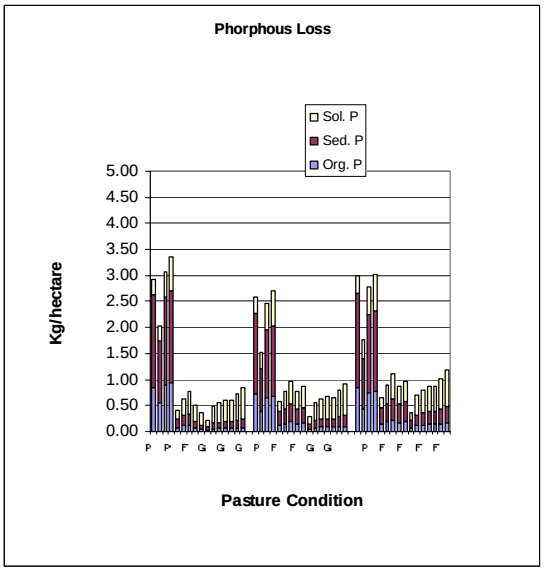

Phorphous Loss
Sol. P
Sed. P
Org. P
Kg/hectare
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pasture Condition

JAY

985Ha Hyd ClassC

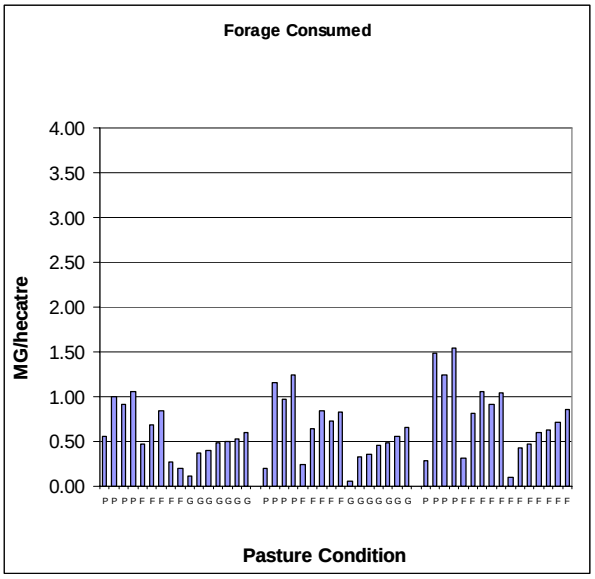
Past	Cond	Lit kg/ha	qCNit kg/ha	Tn kg/ha	P	MnBm	Curve No	OrgP	Sed P	Sol P	Bmeat	NgDys	T Ploss	Nloss
				Low Stocking Rate										
P		1	1	1.0	0.0	1100	79	0.85	1.79	0.28	1.04	44.85	2.91	44.83
P		1	50	50.0	0.0	1100	79	0.56	1.18	0.29	1.48	63.86	2.02	67.90
P		1000	1	1.0	14.0	1100	79	0.88	1.70	0.48	1.29	55.44	3.07	50.30
P		2000	1	1.0	28.0	1100	79	0.94	1.78	0.65	1.49	64.13	3.36	57.38
F		1	50	50.0	0.0	1600	69	0.08	0.16	0.17	1.18	50.69	0.42	90.86
F		1000	50	50.0	14.0	1600	69	0.11	0.20	0.32	1.37	59.18	0.63	106.05
F		2000	100	100.0	28.0	1600	69	0.13	0.22	0.43	1.51	65.08	0.78	167.67
F		1000	100	100.0	14.0	1600	69	0.07	0.12	0.33	1.30	55.87	0.51	156.65
F		1500	150	150.0	21.0	1600	69	0.05	0.08	0.23	1.05	45.16	0.36	124.24
G		1	200	200.0	0.0	2000	61	0.03	0.07	0.12	1.20	51.81	0.22	238.28
G		2000	100	100.0	28.0	2000	61	0.06	0.10	0.33	1.35	58.31	0.49	177.55
G		2500	50	50.0	35.0	2000	61	0.06	0.11	0.38	1.35	58.30	0.56	138.53
G		3000	100	100.0	42.0	2000	61	0.07	0.12	0.42	1.42	61.09	0.61	195.84
G		3000	150	150.0	42.0	2000	61	0.07	0.12	0.42	1.43	61.78	0.61	244.69
G		4000	50	50.0	56.0	2000	61	0.08	0.14	0.51	1.45	62.30	0.73	166.47
G		5000	50	50.0	70.0	2000	61	0.08	0.16	0.61	1.49	64.13	0.85	186.65
				Medium Stocking Rate										
P		1	1	1.0	0.0	1100	79	0.72	1.55	0.31	1.44	61.81	2.59	45.76
P		1	50	50.0	0.0	1100	79	0.38	0.81	0.32	1.99	85.69	1.52	69.71
P		1000	1	1.0	14.0	1100	79	0.66	1.29	0.51	1.78	76.55	2.46	51.74
P		2000	1	1.0	28.0	1100	79	0.69	1.33	0.68	2.07	89.00	2.71	59.90
F		1	50	50.0	0.0	1600	69	0.12	0.25	0.19	1.54	66.34	0.57	92.59
F		1000	50	50.0	14.0	1600	69	0.15	0.28	0.34	1.85	79.72	0.78	107.28
F		2000	50	50.0	28.0	1600	69	0.18	0.34	0.45	2.03	87.48	0.97	122.37
F		1000	100	100.0	14.0	1600	69	0.15	0.28	0.33	1.97	84.87	0.76	153.08
F		1500	100	100.0	21.0	1600	69	0.17	0.30	0.39	2.05	88.16	0.86	160.54
G		1	200	200.0	0.0	2000	61	0.05	0.10	0.13	1.64	70.54	0.28	237.99
G		2000	100	100.0	28.0	2000	61	0.08	0.14	0.33	1.88	81.03	0.55	176.91
G		2500	50	50.0	35.0	2000	61	0.09	0.16	0.38	1.89	81.20	0.63	138.00
G		3000	100	100.0	42.0	2000	61	0.09	0.16	0.42	2.01	86.47	0.67	194.26
G		3000	150	150.0	42.0	2000	61	0.09	0.16	0.42	2.04	87.85	0.66	242.26
G		4000	50	50.0	56.0	2000	61	0.10	0.19	0.51	2.06	88.80	0.80	163.83
G		5000	50	50	70	2000	61	0.11	0.20	0.60	2.13	91.64	0.91	181.07
				High Stocking Rate										
P		1	1	1	0.014	1100	79	0.84	1.82	0.33	1.79	77.11	2.99	47.92
P		1	50	50	0.014	1100	79	0.44	0.96	0.36	2.50	107.84	1.76	72.36
P		1000	1	1	14	1100	79	0.75	1.50	0.53	2.21	95.34	2.78	54.24
P		2000	1	1	28	1100	79	0.78	1.54	0.71	2.59	111.30	3.03	63.33
F		1	50	50	0.014	1600	69	0.15	0.30	0.21	1.87	80.49	0.66	94.47
F		1000	50	50	14	1600	69	0.19	0.35	0.35	2.24	96.40	0.89	109.48
F		2000	50	50	28	1600	69	0.22	0.42	0.46	2.50	107.57	1.10	124.50
F		1000	100	100	14	1600	69	0.18	0.34	0.35	2.41	103.63	0.87	155.13
F		1500	100	100	21	1600	69	0.20	0.37	0.40	2.52	108.45	0.98	162.59
F		1	200	200	0.014	2000	65	0.07	0.14	0.17	2.01	86.50	0.37	237.66
F		2000	100	100	28	2000	65	0.11	0.21	0.39	2.29	98.39	0.71	175.93
F		2500	50	50	35	2000	65	0.13	0.23	0.44	2.29	98.45	0.79	137.03
F		3000	100	100	42	2000	65	0.13	0.25	0.49	2.45	105.67	0.86	192.77
F		3000	150	150	42	2000	65	0.13	0.25	0.48	2.50	107.77	0.86	240.06
F		4000	50	50	56	2000	65	0.15	0.29	0.59	2.52	108.67	1.02	161.76
F		5000	50	50	70	2000	65	0.17	0.32	0.69	2.61	112.19	1.17	178.13

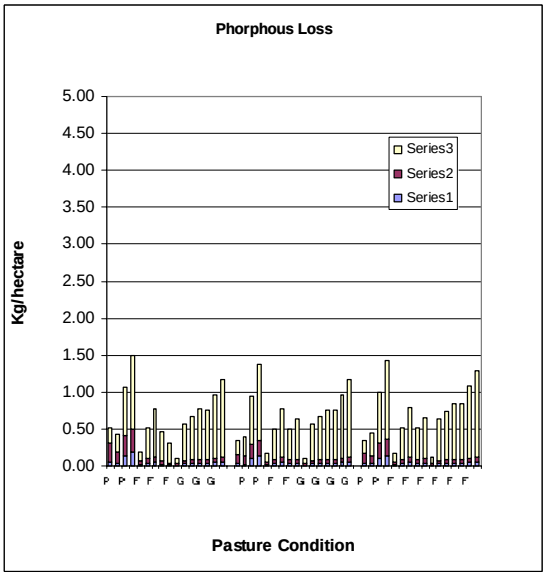

Forage Consumed
MG/hecatre
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pasture Condition


Phorphous Loss
Sol. P
Sed. P
Org. P
Kg/hectare
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pasture Condition

NIXA

Past Cond	Lit kg/ha	5752 qCNit kg/ha	Ha Tn kg/ha	Hyd Class P	C MnBm	Curve No	OrgP	Cost Nit Sed P	0.63 Sol P	Valu BM Bmeat	39.19 NgDys	Cost Litter T Ploss	Nloss
		Low Stocking Rate										Low Stocking Rate	
P	1.0	1	1	0	1100	79	0.05	0.26	0.21	0.55	23.77	$ 0.52	$ 23.05
P	1.0	50	50	0	1100	79	0.04	0.16	0.24	1.00	42.91	$ 0.44	$ 59.83
P	1000.0	1	1	14	1100	79	0.14	0.27	0.64	0.91	39.07	$ 1.06	$ 33.04
P	2000.0	1	1	28	1100	79	0.20	0.30	0.99	1.05	45.30	$ 1.49	$ 43.94
F	1.0	50	50	0	1600	69	0.01	0.05	0.12	0.47	20.38	$ 0.19	$ 42.07
F	1000.0	50	50	14	1600	69	0.04	0.07	0.41	0.69	29.72	$ 0.51	$ 50.62
F	2000.0	100	100	28	1600	69	0.05	0.07	0.65	0.85	36.52	$ 0.78	$ 87.90
F	1000.0	100	100	14	1600	69	0.03	0.05	0.39	0.28	11.87	$ 0.46	$ 78.32
F	1500.0	150	150	21	1600	69	0.01	0.02	0.27	0.20	8.67	$ 0.31	$ 68.80
G	1.0	200	200	0	2000	61	0.01	0.03	0.08	0.12	5.08	$ 0.11	$ 104.60
G	2000.0	100	100	28	2000	61	0.03	0.04	0.49	0.38	16.21	$ 0.57	$ 73.18
G	2500.0	50	50	35	2000	61	0.04	0.05	0.58	0.39	17.00	$ 0.67	$ 53.91
G	3000.0	100	100	42	2000	61	0.04	0.05	0.68	0.48	20.67	$ 0.76	$ 80.99
G	3000.0	150	150	42	2000	61	0.04	0.05	0.68	0.49	21.24	$ 0.76	$ 104.25
G	4000.0	50	50	56	2000	61	0.05	0.06	0.86	0.53	22.84	$ 0.96	$ 65.39
G	5000.0	50	50	70	2000	61	0.06	0.06	1.04	0.60	26.02	$ 1.16	$ 73.02
		Medium Stocking Rate										#REF!	#REF!
P	1.0	1	1	0	1100	79	0.03	0.13	0.18	0.20	8.61	$ 0.34	$ 22.35
P	1.0	50	50	0	1100	79	0.03	0.11	0.26	1.15	49.62	$ 0.39	$ 61.86
P	1000.0	1	1	14	1100	79	0.10	0.19	0.66	0.98	42.10	$ 0.95	$ 35.34
P	2000.0	1	1	28	1100	79	0.14	0.21	1.02	1.24	53.38	$ 1.37	$ 46.85
F	1.0	50	50	0	1600	69	0.01	0.04	0.11	0.24	10.40	$ 0.17	$ 42.33
F	1000.0	50	50	14	1600	69	0.03	0.06	0.41	0.64	27.60	$ 0.50	$ 51.49
F	2000.0	50	50	28	1600	69	0.05	0.07	0.66	0.84	36.11	$ 0.78	$ 60.58
F	1000.0	100	100	14	1600	69	0.03	0.05	0.41	0.73	31.26	$ 0.50	$ 79.71
F	1500.0	100	100	21	1600	69	0.04	0.06	0.54	0.82	35.49	$ 0.64	$ 84.16
G	1.0	200	200	0	2000	61	0.01	0.03	0.07	0.06	2.58	$ 0.11	$ 104.57
G	2000.0	100	100	28	2000	61	0.03	0.04	0.49	0.32	13.90	$ 0.56	$ 73.62
G	2500.0	50	50	35	2000	61	0.04	0.05	0.58	0.36	15.47	$ 0.67	$ 54.50
G	3000.0	100	100	42	2000	61	0.04	0.05	0.68	0.46	19.92	$ 0.76	$ 81.59
G	3000.0	150	150	42	2000	61	0.04	0.05	0.68	0.48	20.67	$ 0.76	$ 104.77
G	4000.0	50	50	56	2000	61	0.05	0.05	0.86	0.56	23.95	$ 0.96	$ 66.22
G	5000	50	50	70	2000	61	0.05	0.06	1.05	0.66	28.40	1.16	74.07
		High Stocking Rate											
P	1	1	1	0	1100	79	0.03	0.14	0.18	0.28	12.07	0.35	22.52
P	1	50	50	0	1100	79	0.03	0.12	0.30	1.49	64.16	0.45	63.46
P	1000	1	1	14	1100	79	0.10	0.20	0.69	1.25	53.66	0.99	36.67
P	2000	1	1	28	1100	79	0.14	0.22	1.05	1.55	66.65	1.42	48.50
F	1	50	50	0	1600	69	0.01	0.04	0.12	0.31	13.44	0.17	42.61
F	1000	50	50	14	1600	69	0.03	0.06	0.42	0.81	34.85	0.51	52.20
F	2000	50	50	28	1600	69	0.05	0.07	0.68	1.06	45.77	0.79	61.53
F	1000	100	100	14	1600	69	0.03	0.05	0.43	0.91	39.38	0.51	80.46
F	1500	100	100	21	1600	69	0.04	0.06	0.56	1.04	44.84	0.65	85.12
F	1	200	200	0	2000	65	0.01	0.03	0.08	0.11	4.54	0.12	111.29
F	2000	100	100	28	2000	65	0.03	0.04	0.55	0.42	18.29	0.63	78.03
F	2500	50	50	35	2000	65	0.04	0.05	0.65	0.47	20.31	0.74	57.71
F	3000	100	100	42	2000	65	0.04	0.05	0.76	0.60	26.02	0.85	86.41
F	3000	150	150	42	2000	65	0.04	0.05	0.76	0.63	26.95	0.85	110.96
F	4000	50	50	56	2000	65	0.05	0.06	0.96	0.72	30.85	1.07	70.21
F	5000	50	50	70	2000	65	0.06	0.07	1.17	0.85	36.81	1.29	78.68

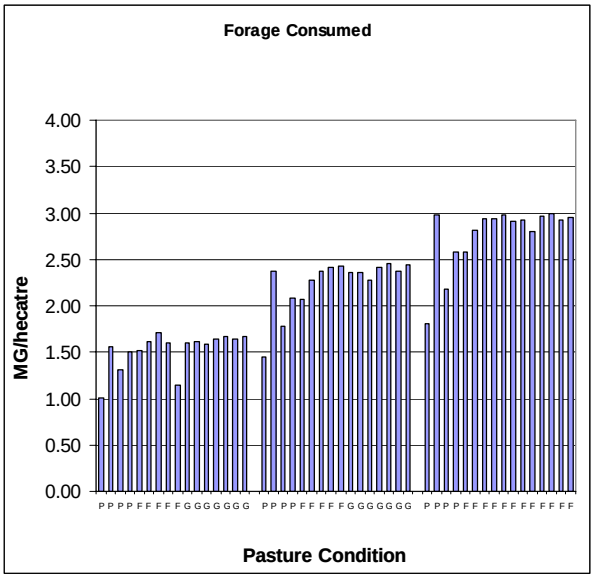

Forage Consumed
MG/hecatre
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pasture Condition

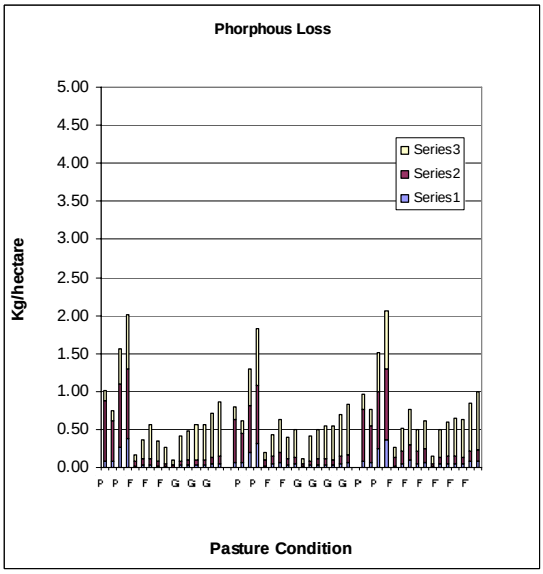

Phorphous Loss
Kg/hectare
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Series3
Series2
Series1
Pasture Condition

TONTI

Past Cond	Lit kg/ha	3039 qCNit kg/ha	Ha Tn kg/ha	P	Hyd Class	C MnBm	Curve No	Org P	Cost Nit Sed P	0.63 Sol P	Valu BM Bmeat	39.19 NgDys	Cost Litter T Ploss	Nloss
		Low Stocking Rate											Low Stocking Rate	
P	1.0	1	1		0	1100	79	0.09	0.79	0.14	1.01	43.49	$ 1.02	$ 11.26
P	1.0	50	50		0	1100	79	0.08	0.54	0.12	1.56	67.27	$ 0.74	$ 25.91
P	1000.0	1	1		14	1100	79	0.27	0.83	0.45	1.30	56.12	$ 1.56	$ 16.29
P	2000.0	1	1		28	1100	79	0.39	0.92	0.70	1.51	65.01	$ 2.01	$ 21.52
F	1.0	50	50		0	1600	69	0.01	0.07	0.08	1.51	65.16	$ 0.16	$ 37.78
F	1000.0	50	50		14	1600	69	0.03	0.08	0.26	1.61	69.49	$ 0.37	$ 47.66
F	2000.0	100	100		28	1600	69	0.04	0.07	0.44	1.71	73.56	$ 0.56	$ 107.33
F	1000.0	100	100		14	1600	69	0.02	0.05	0.27	1.60	68.91	$ 0.35	$ 95.66
F	1500.0	150	150		21	1600	69	0.02	0.03	0.21	1.15	49.48	$ 0.26	$ 97.99
G	1.0	200	200		0	2000	61	0.01	0.03	0.07	1.60	68.88	$ 0.11	$ 172.24
G	2000.0	100	100		28	2000	61	0.03	0.05	0.33	1.61	69.43	$ 0.41	$ 112.49
G	2500.0	50	50		35	2000	61	0.03	0.06	0.39	1.59	68.46	$ 0.48	$ 75.88
G	3000.0	100	100		42	2000	61	0.04	0.06	0.46	1.65	70.88	$ 0.56	$ 130.44
G	3000.0	150	150		42	2000	61	0.04	0.06	0.47	1.66	71.63	$ 0.56	$ 177.88
G	4000.0	50	50		56	2000	61	0.05	0.08	0.58	1.64	70.52	$ 0.71	$ 101.82
G	5000.0	50	50		70	2000	61	0.06	0.09	0.72	1.66	71.59	$ 0.86	$ 120.13
		Medium Stocking Rate												
P	1.0	1	1		0	1100	79	0.07	0.56	0.17	1.45	62.58	$ 0.80	$ 11.97
P	1.0	50	50		0	1100	79	0.06	0.39	0.17	2.37	102.2 1	$ 0.62	$ 26.17
P	1000.0	1	1		14	1100	79	0.20	0.61	0.48	1.78	76.52	$ 1.30	$ 16.94
P	2000.0	1	1		28	1100	79	0.31	0.77	0.74	2.08	89.47	$ 1.82	$ 22.14
F	1.0	50	50		0	1600	69	0.02	0.09	0.10	2.08	89.37	$ 0.20	$ 38.98
F	1000.0	50	50		14	1600	69	0.04	0.10	0.29	2.28	98.07	$ 0.43	$ 47.88
F	2000.0	50	50		28	1600	69	0.06	0.13	0.44	2.37	102.1 1	$ 0.64	$ 58.32
F	1000.0	100	100		14	1600	69	0.04	0.08	0.27	2.41	103.6 8	$ 0.39	$ 83.44
F	1500.0	100	100		21	1600	69	0.04	0.09	0.35	2.43	104.4 9	$ 0.49	$ 89.39
G	1.0	200	200		0	2000	61	0.01	0.03	0.07	2.35	101.3 8	$ 0.12	$ 161.37
G	2000.0	100	100		28	2000	61	0.03	0.06	0.32	2.36	101.7 4	$ 0.41	$ 101.25
G	2500.0	50	50		35	2000	61	0.04	0.07	0.38	2.28	98.04	$ 0.49	$ 68.81
G	3000.0	100	100		42	2000	61	0.04	0.07	0.44	2.42	104.0 1	$ 0.55	$ 114.87
G	3000.0	150	150		42	2000	61	0.04	0.07	0.44	2.46	105.7 3	$ 0.55	$ 159.29
G	4000.0	50	50		56	2000	61	0.05	0.09	0.56	2.38	102.3 0	$ 0.70	$ 87.71
G	5000	50	50		70	2000	61	0.06	0.10	0.67	2.44	105.0 2	0.84	$ 101.37
		High Stocking Rate												
P	1	1	1		0	1100	79	0.08	0.69	0.20	1.80	77.68	0.97	12.77
P	1	50	50		0	1100	79	0.07	0.48	0.21	2.98	128.3 9	0.77	27.11
P	1000	1	1		14	1100	79	0.24	0.76	0.51	2.19	94.11	1.51	17.81
P	2000	1	1		28	1100	79	0.37	0.93	0.77	2.58	110.9 5	2.07	23.11
F	1	50	50		0	1600	69	0.02	0.12	0.12	2.58	111.0	0.26	39.41

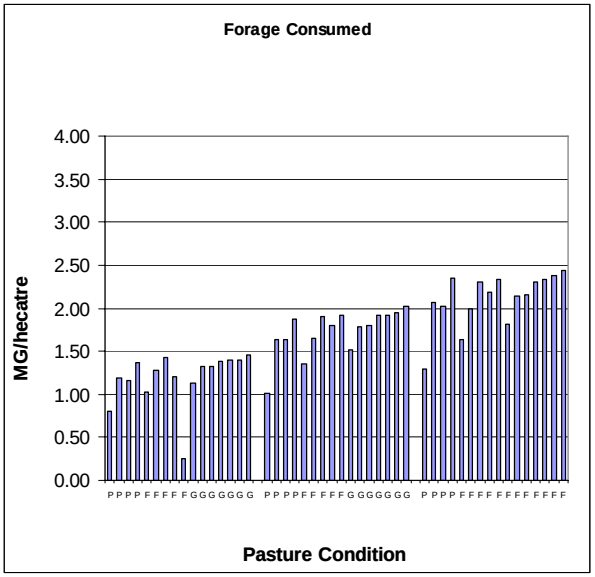
F	1000	50	50	14	1600	69	0.06	0.16	0.31	2.81	120.91	0.52	47.98
F	2000	50	50	28	1600	69	0.09	0.20	0.46	2.93	126.24	0.76	57.34
F	1000	100	100	14	1600	69	0.06	0.15	0.29	2.94	126.67	0.50	83.66
F	1500	100	100	21	1600	69	0.07	0.17	0.37	2.98	128.11	0.62	88.71
F	1	200	200	0	2000	65	0.01	0.05	0.09	2.91	125.35	0.14	157.43
F	2000	100	100	28	2000	65	0.04	0.09	0.37	2.93	126.05	0.50	97.74
F	2500	50	50	35	2000	65	0.05	0.10	0.44	2.81	120.82	0.60	66.88
F	3000	100	100	42	2000	65	0.06	0.10	0.50	2.97	127.88	0.66	110.03
F	3000	150	150	42	2000	65	0.05	0.09	0.50	2.99	128.83	0.64	152.35
F	4000	50	50	56	2000	65	0.08	0.13	0.64	2.92	125.65	0.84	84.55
F	5000	50	50	70	2000	65	0.09	0.15	0.76	2.96	127.24	1.00	97.28

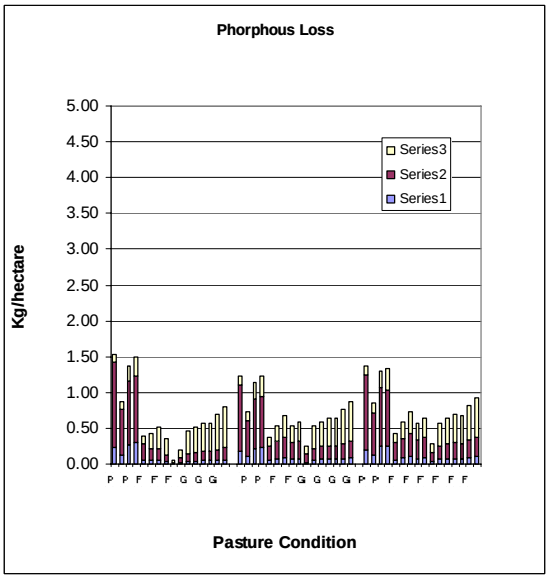




CARYTOWN

Past Cond	Lit kg/ha	127.3 qCNit kg/ha	Ha Tn kg/ha	Hyd Class P	D MnBm	Curve No	OrgP	Cost Nit Sed P	0.63 Sol P	Valu BM Bmeat	39.19 NgDys	Cost Litter T Ploss	Nloss
		Low Stocking Rate									Low Stocking Rate		
P	1.0	1	1	0	1100	86	0.23	1.20	0.11	0.80	34.35	$ 1.54	$ 31.22
P	1.0	50	50	0	1100	86	0.13	0.63	0.11	1.20	51.53	$ 0.87	$ 71.89
P	1000.0	1	1	14	1100	86	0.27	0.90	0.21	1.16	49.99	$ 1.37	$ 43.96
P	2000.0	1	1	28	1100	86	0.30	0.92	0.28	1.38	59.22	$ 1.50	$ 57.68
F	1.0	50	50	0	1600	79	0.05	0.23	0.11	1.03	44.26	$ 0.39	$ 73.10
F	1000.0	50	50	14	1600	79	0.05	0.16	0.22	1.28	55.19	$ 0.43	$ 89.33
F	2000.0	100	100	28	1600	79	0.06	0.16	0.29	1.43	61.61	$ 0.51	$ 153.84
F	1000.0	100	100	14	1600	79	0.03	0.09	0.23	1.20	51.73	$ 0.35	$ 140.02
F	1500.0	150	150	21	1600	79	0.00	0.01	0.03	0.25	10.90	$ 0.04	$ 19.87
G	1.0	200	200	0	2000	74	0.02	0.07	0.11	1.13	48.64	$ 0.20	$ 218.36
G	2000.0	100	100	28	2000	74	0.04	0.11	0.31	1.33	57.06	$ 0.46	$ 158.75
G	2500.0	50	50	35	2000	74	0.04	0.12	0.35	1.32	57.01	$ 0.52	$ 119.56
G	3000.0	100	100	42	2000	74	0.05	0.13	0.40	1.39	59.81	$ 0.57	$ 177.11
G	3000.0	150	150	42	2000	74	0.05	0.13	0.40	1.40	60.23	$ 0.57	$ 226.00
G	4000.0	50	50	56	2000	74	0.05	0.15	0.48	1.40	60.40	$ 0.69	$ 147.55
G	5000.0	50	50	70	2000	74	0.06	0.16	0.57	1.46	62.83	$ 0.80	$ 167.89
		Medium Stocking Rate											
P	1.0	1	1	0	1100	86	0.18	0.92	0.12	1.02	43.82	$ 1.22	$ 33.03
P	1.0	50	50	0	1100	86	0.10	0.51	0.13	1.63	70.16	$ 0.73	$ 75.63
P	1000.0	1	1	14	1100	86	0.21	0.70	0.23	1.63	70.23	$ 1.14	$ 47.08
P	2000.0	1	1	28	1100	86	0.23	0.71	0.30	1.87	80.67	$ 1.23	$ 62.78
F	1.0	50	50	0	1600	79	0.04	0.21	0.12	1.35	58.03	$ 0.38	$ 74.78
F	1000.0	50	50	14	1600	79	0.07	0.24	0.23	1.64	70.77	$ 0.54	$ 91.37
F	2000.0	50	50	28	1600	79	0.09	0.28	0.30	1.90	81.70	$ 0.67	$ 109.01
F	1000.0	100	100	14	1600	79	0.07	0.23	0.23	1.81	77.76	$ 0.53	$ 139.15
F	1500.0	100	100	21	1600	79	0.08	0.25	0.26	1.92	82.78	$ 0.59	$ 147.76
G	1.0	200	200	0	2000	74	0.02	0.11	0.12	1.52	65.55	$ 0.26	$ 218.79
G	2000.0	100	100	28	2000	74	0.06	0.16	0.31	1.78	76.82	$ 0.53	$ 158.82
G	2500.0	50	50	35	2000	74	0.06	0.18	0.35	1.80	77.37	$ 0.59	$ 119.98
G	3000.0	100	100	42	2000	74	0.07	0.19	0.39	1.92	82.51	$ 0.64	$ 176.72
G	3000.0	150	150	42	2000	74	0.07	0.19	0.39	1.93	82.91	$ 0.64	$ 224.91
G	4000.0	50	50	56	2000	74	0.08	0.21	0.47	1.95	84.12	$ 0.76	$ 146.31
G	5000	50	50	70	2000	74	0.09	0.24	0.55	2.02	86.82	0.88	164.44
		High Stocking Rate											
P	1	1	1	0	1100	86	0.20	1.04	0.13	1.29	55.52	1.37	34.60
P	1	50	50	0	1100	86	0.12	0.59	0.14	2.07	89.17	0.85	79.01
P	1000	1	1	14	1100	86	0.24	0.83	0.23	2.03	87.30	1.30	50.79
P	2000	1	1	28	1100	86	0.25	0.78	0.30	2.35	101.00	1.34	66.50
F	1	50	50	0	1600	79	0.05	0.24	0.13	1.64	70.64	0.42	76.60
F	1000	50	50	14	1600	79	0.08	0.27	0.23	1.99	85.88	0.59	93.42
F	2000	50	50	28	1600	79	0.10	0.32	0.31	2.31	99.46	0.74	111.75
F	1000	100	100	14	1600	79	0.08	0.27	0.23	2.19	94.40	0.58	141.09
F	1500	100	100	21	1600	79	0.09	0.29	0.27	2.34	100.63	0.65	150.18
F	1	200	200	0	2000	76	0.03	0.13	0.13	1.82	78.40	0.29	219.87
F	2000	100	100	28	2000	76	0.06	0.19	0.31	2.14	91.98	0.57	159.36
F	2500	50	50	35	2000	76	0.07	0.21	0.35	2.15	92.55	0.64	120.61
F	3000	100	100	42	2000	76	0.08	0.22	0.39	2.31	99.42	0.69	177.23
F	3000	150	150	42	2000	76	0.07	0.22	0.39	2.33	100.27	0.68	225.31
F	4000	50	50	56	2000	76	0.09	0.25	0.47	2.38	102.38	0.81	146.63
F	5000	50	50	70	2000	76	0.10	0.28	0.55	2.43	104.75	0.93	164.23

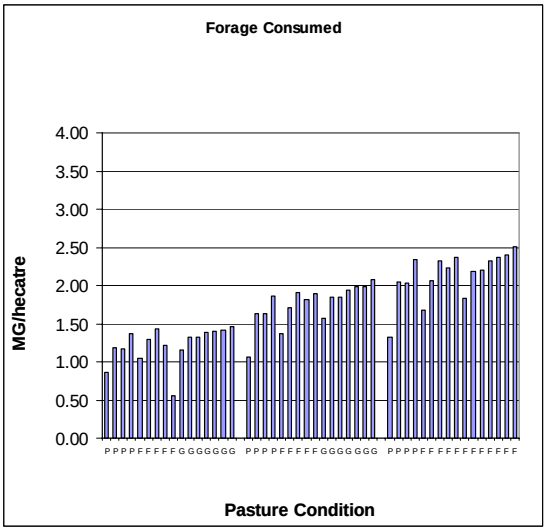

Forage Consumed
MG/hecatre
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Pasture Condition

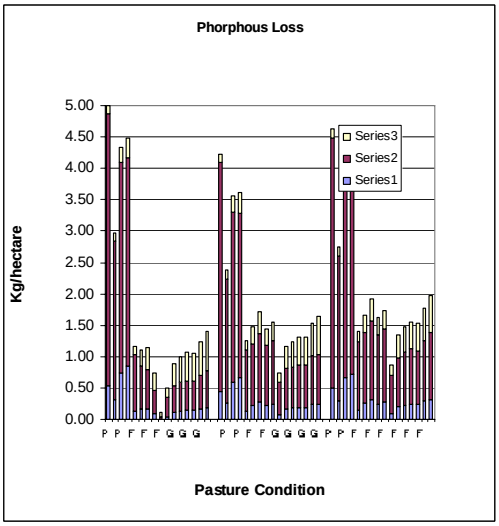

Phorphous Loss
Kg/hectare
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Series3
Series2
Series1
Pasture Condition

CHEROKEE

Past Cond	Lit kg/ha	19.5 qCNit kg/ha	Ha Tn kg/ha	Hyd Class P	D MnBm	Curve No	OrgP	Cost Nit Sed P	0.63 Sol P	Valu BM Bmeat	39.19 NgDys	Cost Litter T Ploss	Nloss
		Low Stocking Rate										Low Stocking Rate	
P	1.0	1	1	0	1100	86	0.53	4.34	0.13	0.86	37.00	$ 5.00	$ 31.73
P	1.0	50	50	0	1100	86	0.32	2.53	0.13	1.19	51.20	$ 2.98	$ 71.70
P	1000.0	1	1	14	1100	86	0.73	3.36	0.24	1.16	50.07	$ 4.33	$ 44.37
P	2000.0	1	1	28	1100	86	0.84	3.33	0.31	1.37	59.02	$ 4.48	$ 58.23
F	1.0	50	50	0	1600	79	0.12	0.91	0.13	1.05	45.17	$ 1.16	$ 2.06
F	1000.0	50	50	14	1600	79	0.16	0.69	0.25	1.29	55.52	$ 1.10	$ 8.57
F	2000.0	100	100	28	1600	79	0.17	0.63	0.34	1.43	61.62	$ 1.13	$ 53.59
F	1000.0	100	100	14	1600	79	0.09	0.38	0.26	1.22	52.36	$ 0.73	$ 39.50
F	1500.0	150	150	21	1600	79	0.01	0.04	0.07	0.55	23.71	$ 0.11	$ 8.97
G	1.0	200	200	0	2000	74	0.05	0.31	0.14	1.16	49.88	$ 0.49	$ 18.41
G	2000.0	100	100	28	2000	74	0.11	0.42	0.36	1.33	57.20	$ 0.89	$ 58.40
G	2500.0	50	50	35	2000	74	0.13	0.46	0.40	1.32	57.02	$ 0.99	$ 19.52
G	3000.0	100	100	42	2000	74	0.14	0.48	0.44	1.38	59.63	$ 1.06	$ 77.49
G	3000.0	150	150	42	2000	74	0.14	0.48	0.44	1.40	60.45	$ 1.06	$ 26.55
G	4000.0	50	50	56	2000	74	0.16	0.54	0.53	1.41	60.69	$ 1.24	$ 47.88
G	5000.0	50	50	70	2000	74	0.19	0.59	0.63	1.46	62.71	$ 1.40	$ 68.83
		Medium Stocking Rate										#REF!	#REF!
P	1.0	1	1	0	1100	86	0.45	3.64	0.14	1.05	45.41	$ 4.23	$ 33.60
P	1.0	50	50	0	1100	86	0.25	1.97	0.15	1.62	69.90	$ 2.37	$ 74.80
P	1000.0	1	1	14	1100	86	0.59	2.71	0.26	1.64	70.50	$ 3.55	$ 7.47
P	2000.0	1	1	28	1100	86	0.67	2.62	0.33	1.87	80.37	$ 3.62	$ 2.64
F	1.0	50	50	0	1600	79	0.13	0.97	0.14	1.37	59.00	$ 1.25	$ 74.07
F	1000.0	50	50	14	1600	79	0.22	0.99	0.26	1.71	73.68	$ 1.47	$ 0.73
F	2000.0	50	50	28	1600	79	0.28	1.09	0.35	1.91	82.22	$ 1.71	$ 07.57
F	1000.0	100	100	14	1600	79	0.21	0.96	0.26	1.82	78.38	$ 1.44	$ 37.80
F	1500.0	100	100	21	1600	79	0.25	1.01	0.30	1.90	81.78	$ 1.56	$ 46.50
G	1.0	200	200	0	2000	74	0.07	0.52	0.14	1.57	67.52	$ 0.73	$ 18.32
G	2000.0	100	100	28	2000	74	0.17	0.64	0.36	1.85	79.55	$ 1.16	$ 57.97
G	2500.0	50	50	35	2000	74	0.18	0.65	0.40	1.85	79.59	$ 1.23	$ 19.09
G	3000.0	100	100	42	2000	74	0.19	0.67	0.44	1.94	83.36	$ 1.30	$ 75.99
G	3000.0	150	150	42	2000	74	0.19	0.67	0.44	1.98	85.16	$ 1.30	$ 24.32
G	4000.0	50	50	56	2000	74	0.23	0.78	0.52	1.99	85.73	$ 1.54	$ 45.82
G	5000	50	50	70	2000	74	0.24	0.78	0.61	2.08	89.50	1.63	163.78
		High Stocking Rate											
P	1	1	1	0	1100	86	0.50	3.98	0.14	1.33	57.27	4.62	35.42
P	1	50	50	0	1100	86	0.30	2.30	0.16	2.05	88.09	2.75	77.83
P	1000	1	1	14	1100	86	0.66	3.08	0.26	2.03	87.23	4.00	50.23
P	2000	1	1	28	1100	86	0.73	2.91	0.34	2.34	100.79	3.98	66.38
F	1	50	50	0	1600	79	0.15	1.10	0.15	1.68	72.18	1.40	75.89
F	1000	50	50	14	1600	79	0.25	1.14	0.27	2.06	88.73	1.65	92.95
F	2000	50	50	28	1600	79	0.32	1.24	0.35	2.32	99.99	1.91	109.96

F	1000	100	100	14	1600	79	0.24	1.11	0.27	2.23	95.98	1.62	140.23
F	1500	100	100	21	1600	79	0.28	1.16	0.31	2.37	102.11	1.74	149.01
F	1	200	200	0	2000	76	0.09	0.62	0.15	1.83	78.66	0.86	219.39
F	2000	100	100	28	2000	76	0.20	0.78	0.36	2.19	94.23	1.34	158.35
F	2500	50	50	35	2000	76	0.23	0.84	0.40	2.21	94.96	1.47	119.43
F	3000	100	100	42	2000	76	0.25	0.87	0.44	2.32	99.91	1.56	176.31
F	3000	150	150	42	2000	76	0.24	0.85	0.44	2.37	102.08	1.53	224.52
F	4000	50	50	56	2000	76	0.29	0.97	0.52	2.39	103.11	1.78	145.73
F	5000	50	50	70	2000	76	0.32	1.06	0.60	2.50	107.81	1.98	163.73

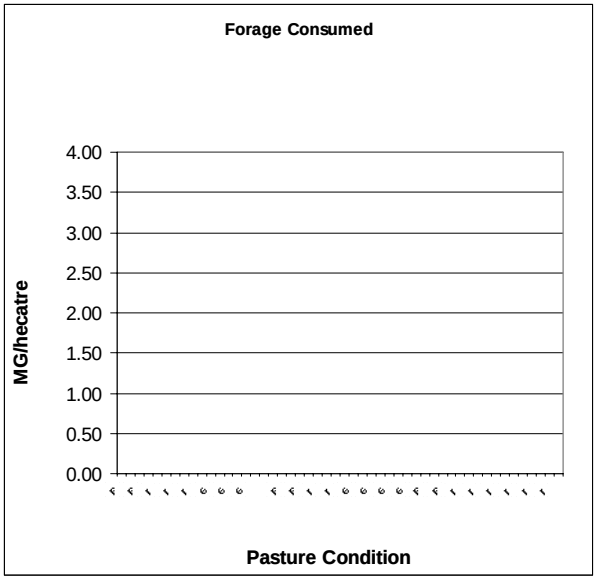




HECTOR

Past Cond	Lit kg/ha	6.201 qCNit kg/ha	Ha Tn kg/ha	P	Hyd Class	D MnBm	Curve No	Org P	Cost Nit Sed P	0.63 Sol P	Valu BM Bmeat	39.19 NgDys	Cost Litter T Ploss	Nloss
		Low Stocking Rate											Low Stocking Rate	
P	1.0	1	1		0	1100	86	0.12	0.52	0.01	0.00	0.00	$ 0.66	$ 21.92
P	1.0	50	50		0	1100	86	0.08	0.33	0.01	0.00	0.00	$ 0.42	$ 69.21
P	1000.0	1	1		14	1100	86	0.38	0.52	0.13	0.00	0.00	$ 1.03	$ 41.11
P	2000.0	1	1		28	1100	86	0.57	0.70	0.24	0.00	0.00	$ 1.51	$ 60.94
F	1.0	50	50		0	1600	79	0.08	0.33	0.01	0.00	0.00	$ 0.42	$ 69.21
F	1000.0	50	50		14	1600	79	0.34	0.46	0.13	0.00	0.00	$ 0.94	$ 89.29
F	2000.0	100	100		28	1600	79	0.54	0.66	0.24	0.00	0.00	$ 1.44	$159.54
F	1000.0	100	100		14	1600	79	0.33	0.45	0.13	0.00	0.00	$ 0.91	$139.13
F	1500.0	150	150		21	1600	79	0.00	0.00	0.00	0.00	0.00	$ -	$ -
G	1.0	200	200		0	2000	74	0.07	0.30	0.01	0.00	0.00	$ 0.38	$218.54
G	2000.0	100	100		28	2000	74	0.54	0.66	0.24	0.00	0.00	$ 1.44	$159.54
G	2500.0	50	50		35	2000	74	0.64	0.77	0.30	0.00	0.00	$ 1.72	$119.89
G	3000.0	100	100		42	2000	74	0.74	0.88	0.36	0.00	0.00	$ 1.98	$180.14
G	3000.0	150	150		42	2000	74	0.74	0.88	0.36	0.00	0.00	$ 1.98	$230.13
G	4000.0	50	50		56	2000	74	0.94	1.09	0.47	0.00	0.00	$ 2.50	$150.82
G	5000.0	50	50		70	2000	74	1.13	1.30	0.58	0.00	0.00	$ 3.02	$171.56
		Medium Stocking Rate												
P	1.0		1		1	0 1100	86	0.12	0.52	0.01	0.00	0.00	$ 0.66	$ 21.92
P	1.0		50		50	0 1100	86	0.08	0.33	0.01	0.00	0.00	$ 0.42	$ 69.21
P	1000.0		1		1	14 1100	86	0.38	0.52	0.13	0.00	0.00	$ 1.03	$ 41.11
P	2000.0		1		1	28 1100	86	0.57	0.70	0.24	0.00	0.00	$ 1.51	$ 60.94
F	1.0		50		50	0 1600	79	0.08	0.33	0.01	0.00	0.00	$ 0.42	$ 69.21
F	1000.0		50		50	14 1600	79	0.34	0.46	0.13	0.00	0.00	$ 0.94	$ 89.29
F	2000.0		50		50	28 1600	79	0.54	0.66	0.24	0.00	0.00	$ 1.44	$109.62
F	1000.0		100		100	14 1600	79	0.33	0.45	0.13	0.00	0.00	$ 0.91	$139.13
F	1500.0		100		100	21 1600	79	0.44	0.56	0.19	0.00	0.00	$ 1.18	$149.32
G	1.0		200		200	0 2000	74	0.07	0.30	0.01	0.00	0.00	$ 0.38	$218.54
G	2000.0		100		100	28 2000	74	0.54	0.66	0.24	0.00	0.00	$ 1.44	$159.54
G	2500.0		50		50	35 2000	74	0.64	0.77	0.30	0.00	0.00	$ 1.72	119.89
G	3000.0		100		100	42 2000	74	0.74	0.88	0.36	0.00	0.00	$ 1.98	$180.1

														4
G	3000.0	150	150	4 2	200 0	74	0.74	0.88	0.36	0.00	0.00	$ 1.98		$230.13
G	4000.0	50	50	5 6	200 0	74	0.94	1.09	0.47	0.00	0.00	$ 2.50		$150.82
G	5000	50	50	7 0	200 0	74	1.13	1.30	0.58	0.00	0.00		3.02	171.56
		High Stocking Rate												
P	1	1	1	0	110 0	86	0.12	0.52	0.01	0.00	0.00		0.66	21.92
P	1	50	50	0	110 0	86	0.08	0.33	0.01	0.00	0.00		0.42	69.21
P	1000	1	1	1 4	110 0	86	0.38	0.52	0.13	0.00	0.00		1.03	41.11
P	2000	1	1	2 8	110 0	86	0.57	0.70	0.24	0.00	0.00		1.51	60.94
F	1	50	50	0	160 0	79	0.08	0.33	0.01	0.00	0.00		0.42	69.21
F	1000	50	50	1 4	160 0	79	0.34	0.46	0.13	0.00	0.00		0.94	89.29
F	2000	50	50	2 8	160 0	79	0.54	0.66	0.24	0.00	0.00		1.44	109.62
F	1000	100	100	1 4	160 0	79	0.33	0.45	0.13	0.00	0.00		0.91	139.13
F	1500	100	100	2 1	160 0	79	0.44	0.56	0.19	0.00	0.00		1.18	149.32
F	1	200	200	0	200 0	76	0.07	0.30	0.01	0.00	0.00		0.38	218.54
F	2000	100	100	2 8	200 0	76	0.54	0.66	0.24	0.00	0.00		1.44	159.54
F	2500	50	50	3 5	200 0	76	0.64	0.77	0.30	0.00	0.00		1.72	119.89
F	3000	100	100	4 2	200 0	76	0.74	0.88	0.36	0.00	0.00		1.98	180.14
F	3000	150	150	4 2	200 0	76	0.74	0.88	0.36	0.00	0.00		1.98	230.13
F	4000	50	50	5 6	200 0	76	0.94	1.09	0.47	0.00	0.00		2.50	150.82
F	5000	50	50	7 0	200 0	76	1.13	1.30	0.58	0.00	0.00		3.02	171.56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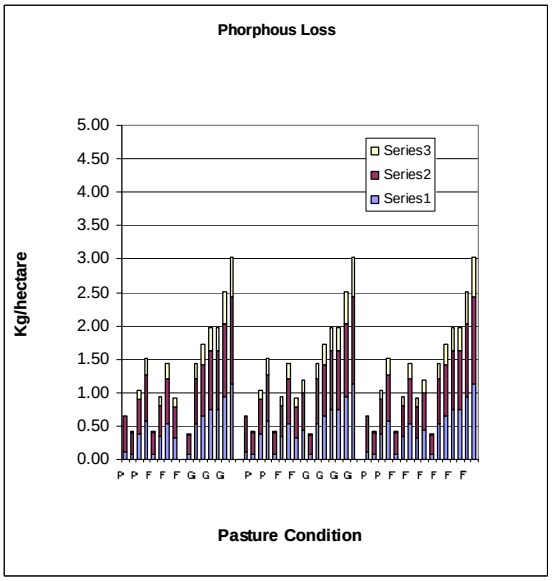
3.00
2.50
2.00
1.50
1.00
0.50
0.00
MG/hecatre
Pasture Condition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4.00
3.50
3.00
2.50
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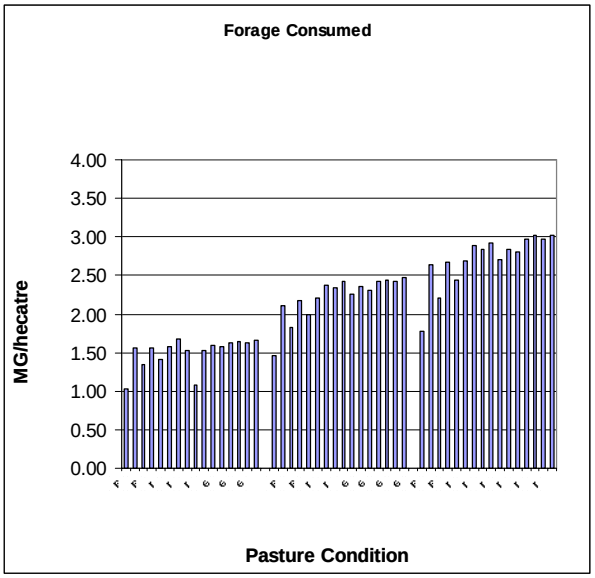
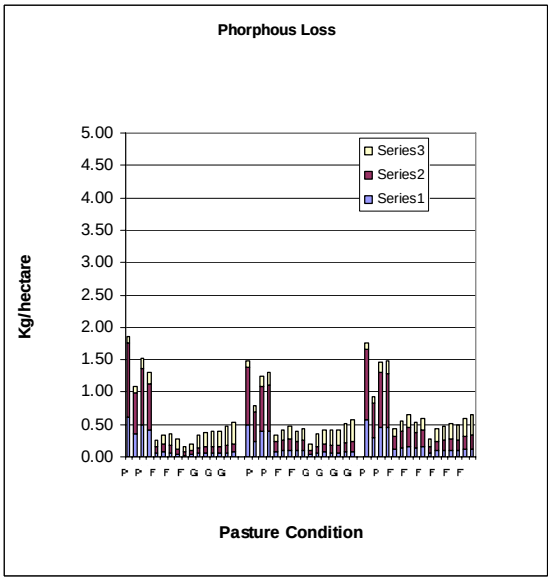
1.50
1.00
0.50
0.00
Series3
Series2
Series1
Kg/hectare
Pasture Condition

STIGLER

Past Cond	Lit kg/ha	367.5 qCNit kg/ha	Ha Tn kg/ha	Hyd Class P	D MnB m	Curve No	Org P	Cost Nit Sed P	0.63 Sol P	Valu BM Bmeat	39.19 NgDys	Cost Litter T Ploss	Nloss
		Low Stocking Rate									Low Stocking Rate		
P	1.0	1	1	0	1100	86	0.62	1.13	0.10	1.03	44.20	$ 1.85	$ 60.36
P	1.0	50	50	0	1100	86	0.35	0.63	0.10	1.56	67.33	$ 1.08	$ 87.46
P	1000.0	1	1	14	1100	86	0.50	0.88	0.15	1.34	57.60	$ 1.52	$ 68.82
P	2000.0	1	1	28	1100	86	0.41	0.71	0.19	1.57	67.42	$ 1.31	$ 78.85
F	1.0	50	50	0	1600	79	0.06	0.10	0.09	1.42	61.05	$ 0.25	$ 89.64
F	1000.0	50	50	14	1600	79	0.07	0.12	0.15	1.58	68.17	$ 0.34	$ 103.74
F	2000.0	100	100	28	1600	79	0.07	0.11	0.19	1.67	71.92	$ 0.37	$ 168.01
F	1000.0	100	100	14	1600	79	0.05	0.08	0.15	1.52	65.60	$ 0.28	$ 155.95
F	1500.0	150	150	21	1600	79	0.03	0.04	0.09	1.08	46.35	$ 0.16	$ 133.09
G	1.0	200	200	0	2000	74	0.04	0.06	0.09	1.53	65.87	$ 0.19	$ 231.33
G	2000.0	100	100	28	2000	74	0.05	0.09	0.19	1.59	68.42	$ 0.34	$ 174.69
G	2500.0	50	50	35	2000	74	0.06	0.10	0.22	1.57	67.77	$ 0.38	$ 137.97
G	3000.0	100	100	42	2000	74	0.06	0.10	0.24	1.63	70.00	$ 0.40	$ 195.32
G	3000.0	150	150	42	2000	74	0.06	0.10	0.24	1.64	70.41	$ 0.40	$ 243.70
G	4000.0	50	50	56	2000	74	0.07	0.12	0.29	1.63	70.13	$ 0.47	$ 169.15
G	5000.0	50	50	70	2000	74	0.07	0.13	0.34	1.65	71.12	$ 0.54	$ 190.92
		Medium Stocking Rate											
P	1.0	1	1	0	1100	86	0.49	0.89	0.11	1.46	62.81	$ 1.49	$ 60.97
P	1.0	50	50	0	1100	86	0.24	0.44	0.11	2.11	90.83	$ 0.79	$ 88.79
P	1000.0	1	1	14	1100	86	0.39	0.70	0.16	1.82	78.44	$ 1.24	$ 70.43
P	2000.0	1	1	28	1100	86	0.40	0.71	0.20	2.17	93.46	$ 1.31	$ 81.44
F	1.0	50	50	0	1600	79	0.08	0.14	0.10	2.00	86.10	$ 0.33	$ 90.29
F	1000.0	50	50	14	1600	79	0.10	0.16	0.15	2.21	95.10	$ 0.41	$ 103.40
F	2000.0	50	50	28	1600	79	0.10	0.18	0.19	2.37	101.99	$ 0.47	$ 116.91
F	1000.0	100	100	14	1600	79	0.09	0.15	0.15	2.34	100.74	$ 0.40	$ 144.43
F	1500.0	100	100	21	1600	79	0.10	0.16	0.17	2.42	104.18	$ 0.43	$ 151.70
G	1.0	200	200	0	2000	74	0.04	0.07	0.10	2.25	96.83	$ 0.21	$ 225.20
G	2000.0	100	100	28	2000	74	0.06	0.10	0.19	2.35	101.17	$ 0.36	$ 166.50
G	2500.0	50	50	35	2000	74	0.07	0.12	0.22	2.31	99.50	$ 0.41	$ 130.14
G	3000.0	100	100	42	2000	74	0.07	0.12	0.24	2.43	104.42	$ 0.42	$ 183.92
G	3000.0	150	150	42	2000	74	0.07	0.11	0.24	2.45	105.31	$ 0.42	$ 230.64
G	4000.0	50	50	56	2000	74	0.08	0.14	0.28	2.43	104.55	$ 0.51	$ 156.08
G	5000	50	50	70	2000	74	0.08	0.15	0.33	2.47	106.37	0.56	174.26
		High Stocking Rate											
P	1	1	1	0	1100	86	0.58	1.07	0.11	1.78	76.76	1.77	63.10
P	1	50	50	0	1100	86	0.29	0.53	0.11	2.63	113.43	0.94	91.10
P	1000	1	1	14	1100	86	0.46	0.84	0.16	2.21	94.95	1.47	72.77
P	2000	1	1	28	1100	86	0.45	0.84	0.20	2.68	115.20	1.49	84.45
F	1	50	50	0	1600	79	0.12	0.21	0.11	2.44	104.92	0.44	91.19
F	1000	50	50	14	1600	79	0.14	0.25	0.16	2.69	115.68	0.55	103.56
F	2000	50	50	28	1600	79	0.16	0.29	0.20	2.88	124.02	0.65	116.27
F	1000	100	100	14	1600	79	0.14	0.24	0.16	2.84	122.24	0.53	144.65

F	1500	100	100	21	1600	79	0.15	0.26	0.18	2.91	125.43	0.59	151.34
F	1	200	200	0	2000	76	0.06	0.10	0.11	2.71	116.65	0.27	221.06
F	2000	100	100	28	2000	76	0.09	0.16	0.20	2.83	121.87	0.44	162.28
F	2500	50	50	35	2000	76	0.10	0.17	0.22	2.81	120.89	0.48	127.72
F	3000	100	100	42	2000	76	0.10	0.17	0.24	2.97	127.79	0.51	176.86
F	3000	150	150	42	2000	76	0.09	0.16	0.24	3.02	129.85	0.49	222.51
F	4000	50	50	56	2000	76	0.11	0.20	0.28	2.97	127.96	0.59	149.38
F	5000	50	50	70	2000	76	0.12	0.22	0.33	3.01	129.71	0.66	165.99





Estimating the orientation and intensity of fractures in sedimentary rocks using multi-component 3-D ground-penetrating radar (GPR)

Basic Information

Title:	Estimating the orientation and intensity of fractures in sedimentary rocks using multi-component 3-D ground-penetrating radar (GPR)
Project Number:	2005OK42B
Start Date:	3/1/2005
End Date:	2/28/2006
Funding Source:	104B
Congressional District:	3rd
Research Category:	Climate and Hydrologic Processes
Focus Category:	Methods, Groundwater, Hydrology
Descriptors:	Ground Penetrating Radar, Fracture Mapping, Hydrology, Gypsum Karst
Principal Investigators:	Surinder Sahai, Todd Halihan, Roger Young

Publication

1. Sahai, Surinder; Roger Young, Todd Halihan, David Ramirez, Sterve Hadaway, and Charles Kaun. 2005, "Application of Geophysical and Hydrological Techiniques to map Fractures in a Karst Region of Western Oklahoma, GSA Abstracts with Programs, v. 37, no.7.
2. Ramirez-Mejia, David; Roger Young, Surinder Sahai, and Todd Halihan. 2006, Fracture Orientation Determination in Sedimentary Rocks Using Multicomponent Ground Penetrating Radar Measurements, submitted for presentation at the SEG Annual Meeting.

Research Report

Submitted to:

The Oklahoma Water Resource Research Institute

Title:

Estimating the orientation and intensity of fractures in sedimentary rocks using multi-component 3-D ground-penetrating radar (GPR)

Submitted by:

Surinder Sahai, Associate Professor and Geophysics Chair
Oklahoma State University
Roger A. Young, Associate Professor
University of Oklahoma
Todd Halihan, Assistant Professor
Oklahoma State University

Starting Date: 3/1/05

End Date: 2/28/06

Problem and Research Objectives

Fractures in sedimentary rocks influence the hydraulic properties of aquifers. Not only are the fractures important for the flow of water through an aquifer but the recharge of the aquifer is greatly influenced by the fracture aperture, orientation, and density. Therefore, an understanding of the orientation and hydraulic parameters of fractures is crucial to ground water flow modelers in research institutions, and government agencies charged with managing the water resources for the state of Oklahoma (Sahai and others, 2005).

Ground Penetrating Radar (GPR) has been shown to have the potential to detect vertical fractures in sedimentary formations (Tsoflias and others, 2004). Figure 1 illustrates the multicomponent GPR method described by Tsoflias and others (2004). The presence of a fracture affects the phase of the GPR signal differently in the H-polarization and E-polarization. In H-polarization, the magnetic field is parallel to the fracture. In E-polarization, the electric field is parallel to the fracture. By acquiring radar data with different antenna orientations at various angles to the fracture, the phase differences in the receiver signal can be used to determine the presence and orientation of the fracture. Therefore, fracture detection should be possible from polarization studies.

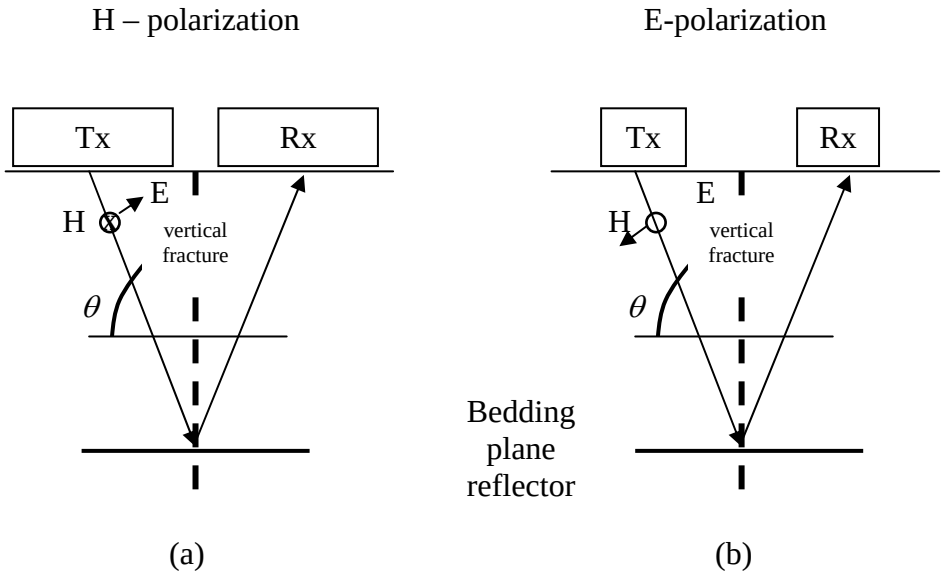


Figure 1. Schematic of the H-polarization and E-polarization response to a fracture at a large angle of incidence. (a) Antennas in endfire orientation. The magnetic field is parallel to the fracture in H-polarization. (b) Antennas in parallel orientation. The electric field is parallel to the fracture in E-polarization. Tx and Rx are the transmitter and receiver antennas respectively and the sizes of the boxes signify the length and width of the antennas. (after Tsoflias et al., 2004).

Tsoflias and others (2001) show that for a horizontal fracture plane, the amplitude of the GPR signal is affected by the fracture aperture and saturation. Therefore, GPR has the potential to not only detect fractures but also provide quantitative information about the hydraulic properties of fractures.

The research work presented in this report was undertaken with the objective of detecting the orientation and intensity of fractures in sedimentary rocks and possibly extend the work of Tsoflias and others (2004). The site selected for our study was a former gypsum quarry in a karstic region of western Oklahoma (Figure 2). The quarry floor is replete with fractures of varying sizes. The dominant fracture trend is NE-SW although there are cross-cutting fractures, as well as some fractures that run predominantly E-W. Many fractures have sub-millimeter apertures while others can be classified as the surface expressions of large sinkholes in the subsurface (Figure 3). In addition to being an ideal place for the investigation of geophysical and hydrological techniques to map fractures in gypsum, the site is also of interest to the Oklahoma Department of Transportation because the karst topography in the area is a potential hazard to the integrity of highways. The geophysical techniques used in our study included Ground Penetrating Radar (GPR) and a Global Positioning System (GPS).

Figure 2. A view of the quarry floor looking east. The fractures in the quarry floor are visible in the foreground.

Figure 3. Some fractures are surface expressions of large sinkholes in gypsum. Buckets of water poured into these fractures disappears quickly.

Methodology

The field work for this project involved GPS mapping, subsurface imaging with ground penetrating radar, and hydraulic testing. The locations of all geophysical observations, geologic mapping, and hydrologic testing were established by differential GPS measurements. A Trimble GPS and base station, Pathfinder software to log waypoints, and ArcView software were used to map important features with a spatial accuracy of 0.1 m. Figure 4 shows the trend of some of the fractures mapped at the survey site, the locations of GPR lines, and polarization and hydraulic tests.

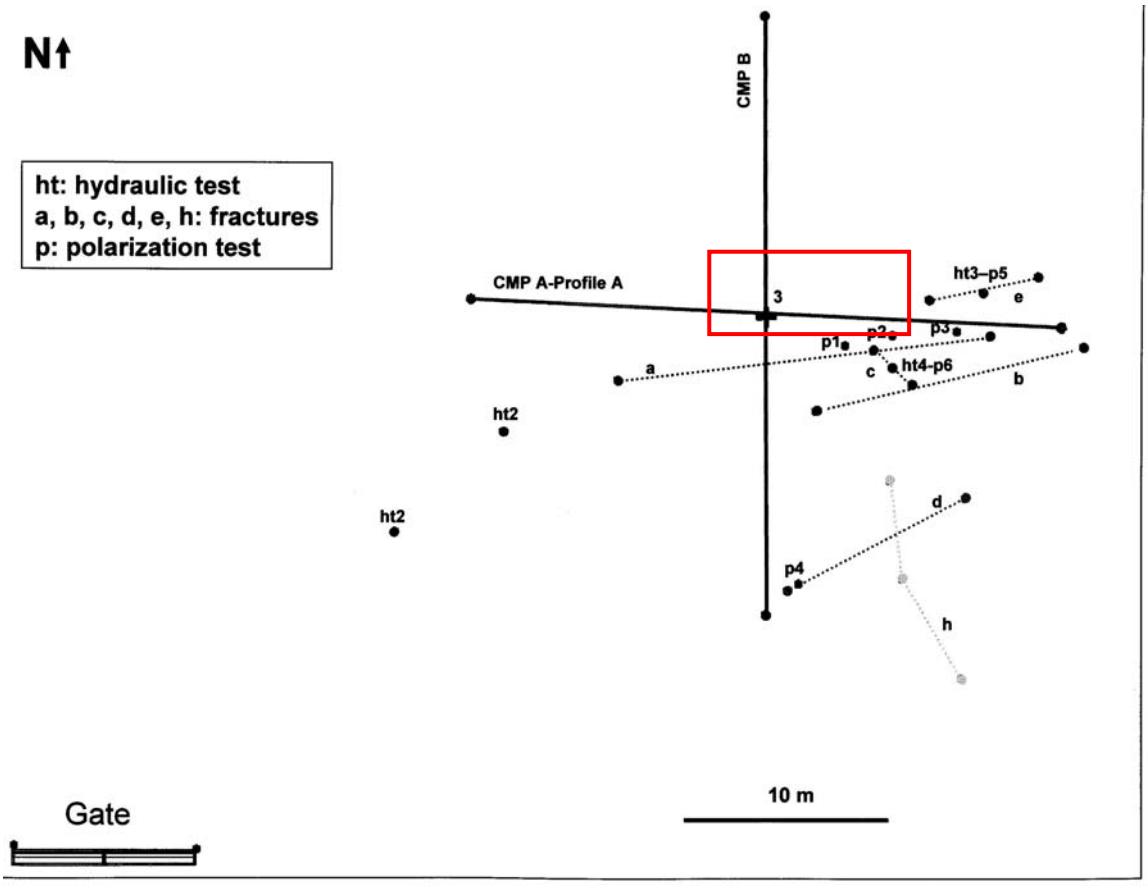


Figure 4. GPS mapped location of some fractures on the quarry floor (dotted lines), the locations of GPR profiles A and B, the locations of common mid-point measurements for radar velocity analyses, and polarization and hydraulic tests. The rectangular box shows the location of 24 common mid-point surveys conducted for mapping the velocity field in the vicinity of the large fracture shown in Figure 3.

The fracture aperture influences the GPR signal polarization. The fracture aperture can be measured directly by mechanical means, i.e., measuring the fracture opening with a fine scaled ruler. This method works well when the fracture aperture is large enough to be measured accurately. Another method is to do infiltration experiments to determine the rate of flow of water through the fracture for a given hydraulic head and then use Darcy's equation to calculate the fracture aperture. In our work, infiltration experiments were conducted by bonding a 6" diameter PVC coupler to the quarry floor using Bondo® body filler (Figure 5). The coupler was filled with water and allowed to infiltrate. The water level change as a function of time was measured with a ruler. In one case, it was difficult to bond the plastic pipe to the quarry floor to produce a water tight seal. In another case, the hydraulic aperture of the fracture was too large to conduct any meaningful measurement of hydraulic conductivity (Figure 3). Therefore, the results for only one fracture (location ht4 in Figure 4) are presented.

Figure 5. Six inch PVC coupler bonded to a NE-SW fracture (left) in preparation for infiltration experiment. The picture on the right shows the polarization experiment Being conducted for dry and saturated fracture conditions.

The GPR data was acquired using standard techniques (Davis and Annan, 1989; Jol and Bristow, 2003). A PulseEkko100 system with 200 MHz antenna was used to image the subsurface with GPR. The antenna spacing of 0.5 m and step size of 0.075 m (3 inches) was used to acquire two N-S and W-E trending GPR lines. These lines were centered on a fracture with a large hydraulic aperture measuring several centimeters (Figure 3). Twenty-four locations were selected for common mid-point data acquisition on a 1m x 1m grid in order to map the velocity field in the vicinity of the fracture seen in Figure 3.

The polarization data was acquired with 100 MHz and 200 MHz transmitter antennas oriented in the parallel and endfire configurations. The experiments with the 100 MHz antenna were conducted in the absence of the infiltration experiments. The 200 MHz data was acquired for both dry and water saturated fracture conditions (Figure 5). Figure 6 shows the antenna configuration used to acquire the polarization data.

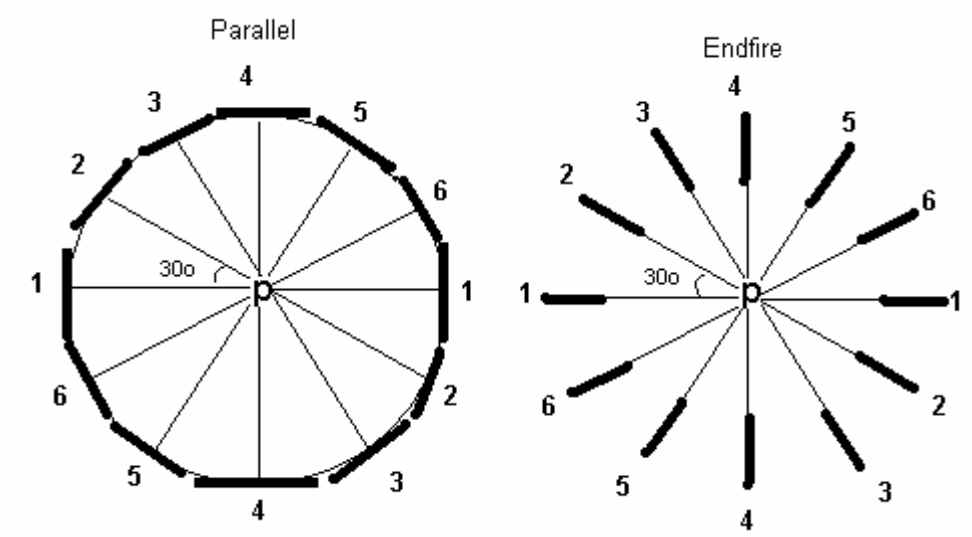


Figure 6. The parallel and endfire antenna configurations. The transmitter-receiver pair was rotated by 30 degree increments to complete a circle around a fracture.

Results and Discussion

Figure 7 shows the water infiltration data obtained from fracture h4 (Figure 4). The fluid flow through the fracture is steady because the rate of change of water level in the PVC coupler is directly related to the elapsed time. Therefore, a straight line relationship can be used to determine the rate of infiltration through the fracture. The fracture aperture "b" can be calculated from the well known Darcy's equation:

$$Q = K I A$$

where Q is the infiltration rate, K is the hydraulic conductivity, I is the hydraulic gradient, and A is the surface area of the fracture. The area is related to the fracture aperture b and width w by:

$$A = b w$$

The width of the fracture in our case is the diameter of the PVC coupler.

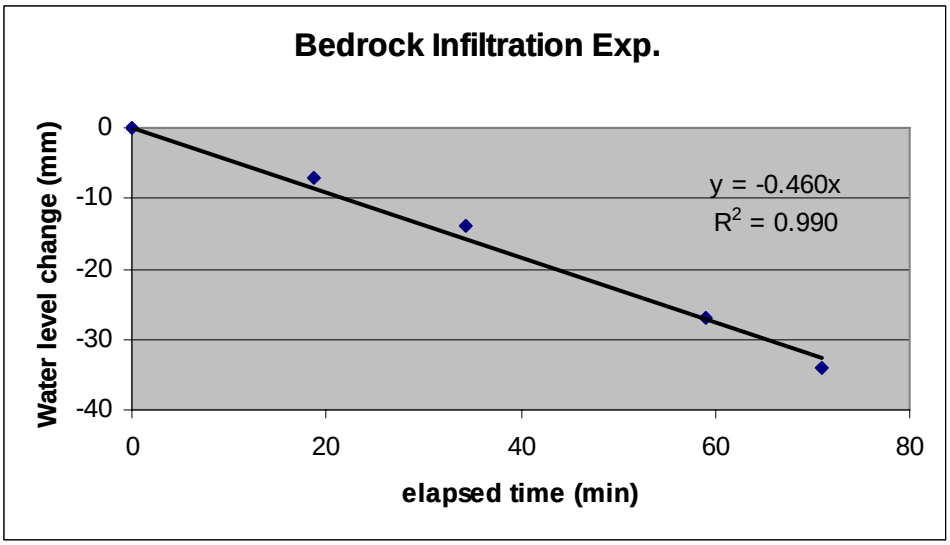


Figure 7. The change in water level in the PVC coupler plotted as a function of elapsed time for fracture h4 (figure 4).

From our data, the bulk infiltration rate for fracture h4 was calculated to be 7.66×10^{-6} m/s. Assuming a hydraulic gradient of 1.0 for the fracture, the hydraulic conductivity of the fracture is 8.49×10^{-3} m/s which corresponds to a hydraulic fracture aperture of 0.108 mm or 108 microns. A large number of fractures visible in the quarry floor seem to have similar apertures. Although an individual fracture of sub-millimeter aperture may have very little influence on the GPR polarization results (Tsoflias, personal communication), a large number of fractures (thus high fracture density) may result in a measurable polarization effect on the GPR signal.

Knowledge of the velocity of radar waves at the gypsum quarry site is important for converting a time section to a depth section. Moreover, the velocity field at the site can aid in the interpretation of the subsurface geology. Figure 8 shows a typical common mid-point gather obtained at the site. The direct waves traveling from the transmitter to the receiver appear as linear events on the time versus offset position plot on the left. The reflection events are hyperbolic in appearance. It is these events that are important for determining the velocity. The right hand side of Figure 8 shows the semblance plot generated from the common mid-point data. The velocity at a point in depth is given by the bull's-eye picks on the semblance plot. Two observations can be made from this plot. First, the velocity of radar waves in gypsum is of the order of 0.1 m/ns. Second, there is a slight increase in velocity with depth. We used a velocity of 0.1 m/ns to convert the time to depth sections.

Figure 9 shows an east-west GPR line (marked A in Figure 4). It is apparent that the data is replete with diffractions that possibly result from sharp discontinuities in the subsurface. Some examples of these discontinuities are faults and fracture, and in our case, possibly sinkholes. The GPR profile shows a lack of horizontal bedding within the gypsum. Instead small relief depocenters and fractures are abundant throughout the section.

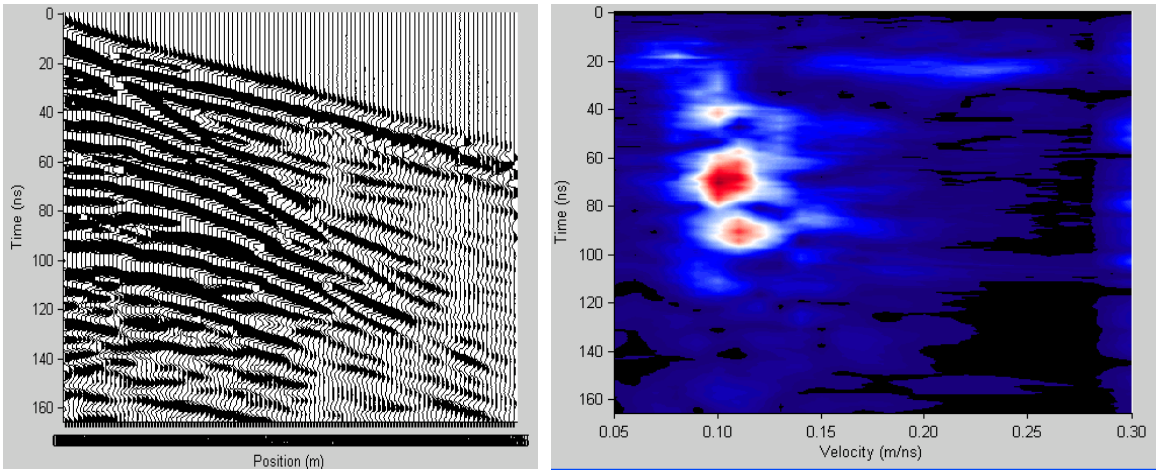


Figure 8. A common mid-point profile (left) and the corresponding semblance plot (right) for velocity picks.

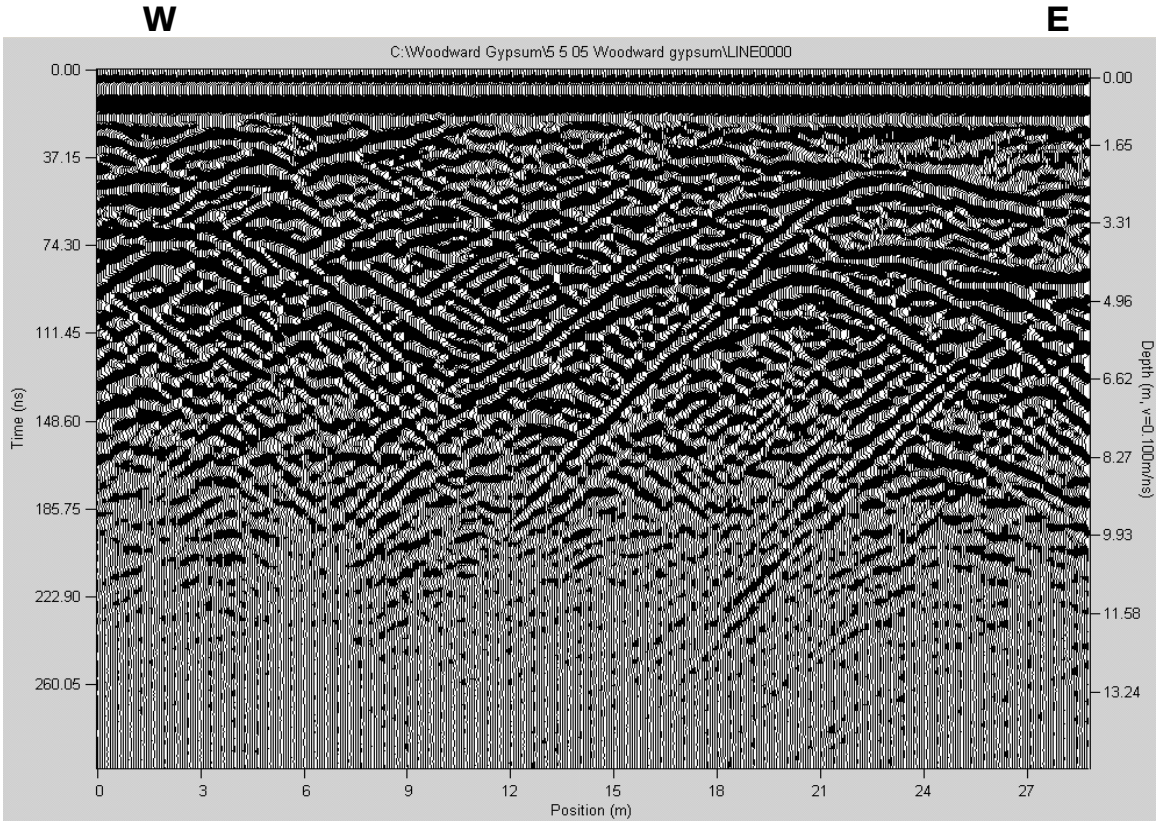


Figure 9. An east-west GPR line (line A in figure 4). The diffractions are the result of faults and fractures, and possibly sinkholes in gypsum. The large fracture shown in Figure 3 is located at the center of the line.

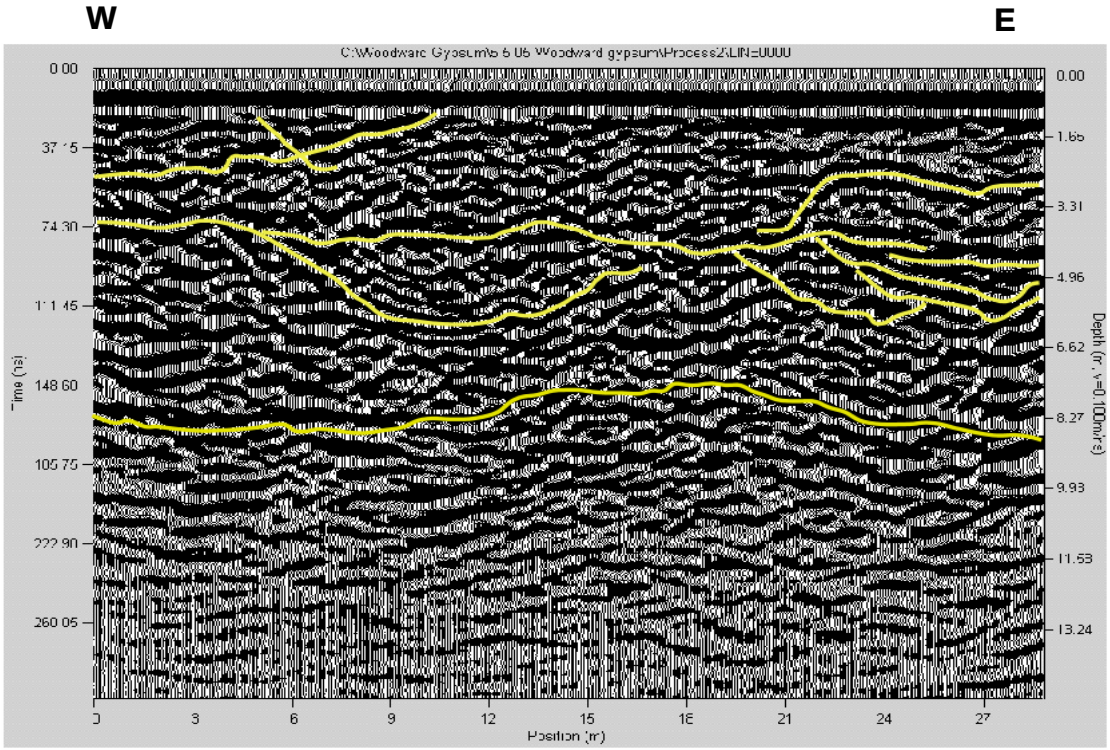


Figure 10. Migration of line A shown in figure 9. The diffraction energy is collapsed, revealing highly irregular subsurface.

Many applications of GPR involve detection of buried objects such as pipelines, rebar, etc. These objects produce diffractions which are used as indicators of their presence or absence. However, diffractions obscure geological information. Migration of GPR data is necessary to collapse the diffraction energy to the point of origin. Figure 10 shows the migrated data for line A. The discontinuous nature of the subsurface is quite evident in this data. There are numerous fractures and faults beneath the quarry floor. A synclinal feature between 75 and 120 nsec (approximately 4 to 6 meter depth) could be a sinkhole or collapsed feature because the large fracture (Figure 3) is at the center of line A. A map of the radar velocity field is shown in Figure 11. A high velocity anomaly is centered in the vicinity of the large fracture which could be further evidence of a sinkhole. As noted previously, the hydraulic conductivity of the large fracture could not be measured. The water disappeared in the fracture as fast it could be poured.

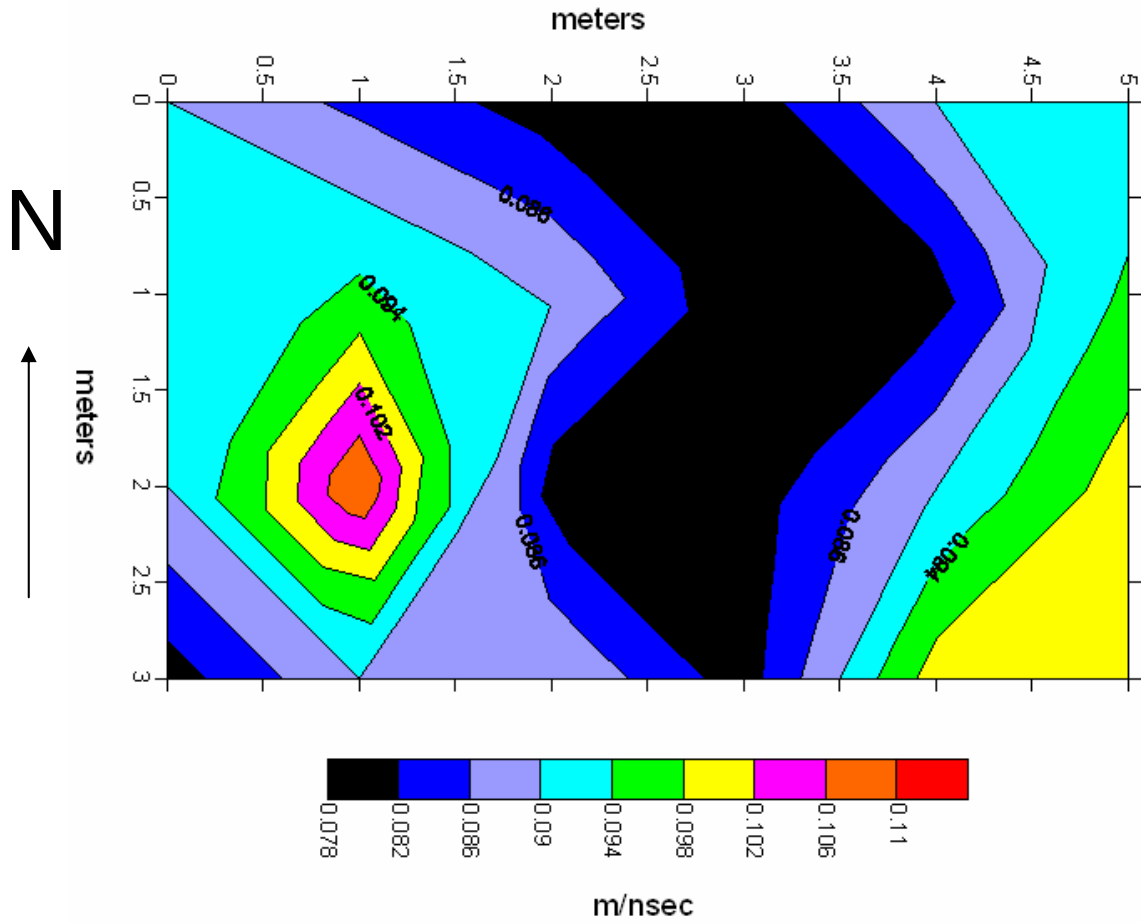


Figure 11. The radar velocity field in the area of the large fracture (figure 3). The high velocity (bull's-eye in the lower left) occurs in the vicinity of the fracture. The velocity variations are predominantly in an east-west direction.

The GPR polarization tests performed across fracture ht4 for both the dry and wet fracture are shown in Figure 12. Five traces in succession were recorded at each location occupied by the transmitter-receiver pair. When the GPR transmitter is fired, the generated E-field of the electromagnetic pulse is parallel to the length of the transmitter antenna. Figures 12 shows that there are definite differences in the arrival time of the GPR signal when the data are acquired in the parallel or endfire configuration, i.e., the E-field is parallel or perpendicular to the fracture. The delay times translate into phase differences between the recorded signals. The data acquired with the endfire configuration appears to have higher frequency content than the parallel configuration data. In the parallel configuration (a and c), there is a slight delay in the reflector time in the shallow section when the electric field is oriented perpendicular to the fracture (orientation c) than parallel to the fracture (orientation a). This is consistent with the work of Tsoflias and others (2004). However, there are large differences in reflector times greater than 60 nsec in panels a and c or b and d that cannot be explained by the presence of a fracture with an aperture of only 0.108 mm. One plausible explanation is

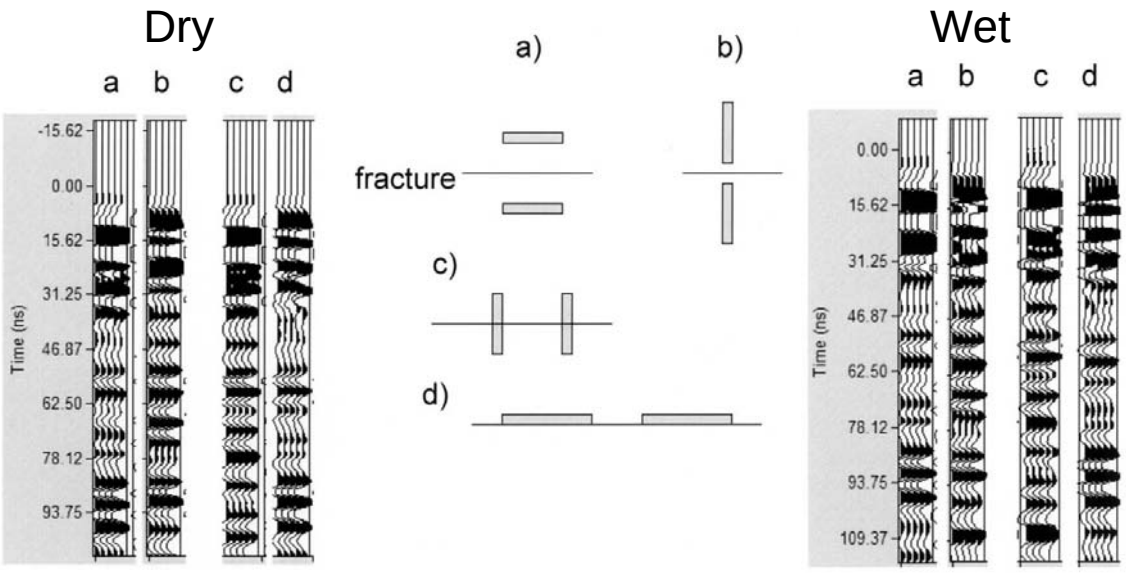


Figure 12. Comparison of parallel and endfire antenna configurations for dry and wet fractures.

the velocity anisotropy due to karst features below the quarry floor (Tsoflias, personal communication).

Figure 13 shows a comparison of the reflection event times for the dry and wet fracture for the endfire antenna configuration. The electric field is perpendicular to the fracture for the zero degree case. The antenna pair was rotated by 30 degree increments around the fracture. In the shallow section, there is a delay in the arrival times of the reflectors when the electric field is perpendicular to the fracture. As the antenna pair is rotated around the fracture, the arrival times get smaller until the electric field is parallel to the fracture. Once again this is consistent with theory. One major difference between the dry and wet case is the highlighted zone where the amplitude and frequency content of the events is greater for the wet fracture than the dry fracture, even though the hydraulic fracture aperture is only 0.108 mm. Therefore, small fracture aperture can lead to measurable change in the amplitude and frequency of the recorded signal. As noted previously, large differences in reflector times below about 60 nsec in various panels are present. Once again, these differences cannot be explained by the presence of a fracture with an aperture of only 0.108 mm.

Another test of the polarization effects on radar waves at fracture h4 (figure 4) was conducted. In this example, the 100 MHz transmitter antenna was used. The antennas were rotated by 45 degrees around the fracture for the parallel and endfire configurations and three traces were acquired at each location. Analyses of reflection delay times were translated into phase differences. Figures 14 and 15 show the cumulative phase for the average trace plotted against time for the four main orientations of the antennas around

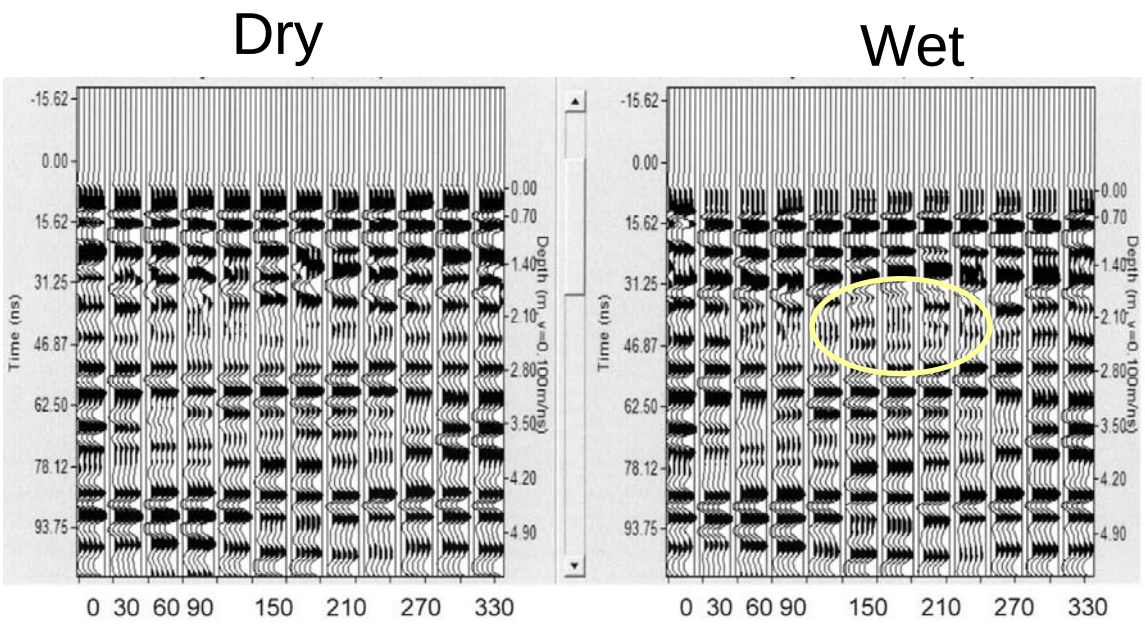


Figure 13. Comparison of dry and wet fracture response at different angles of antenna orientation for the endfire configuration. The electric field is perpendicular to the fracture for the zero degree case.

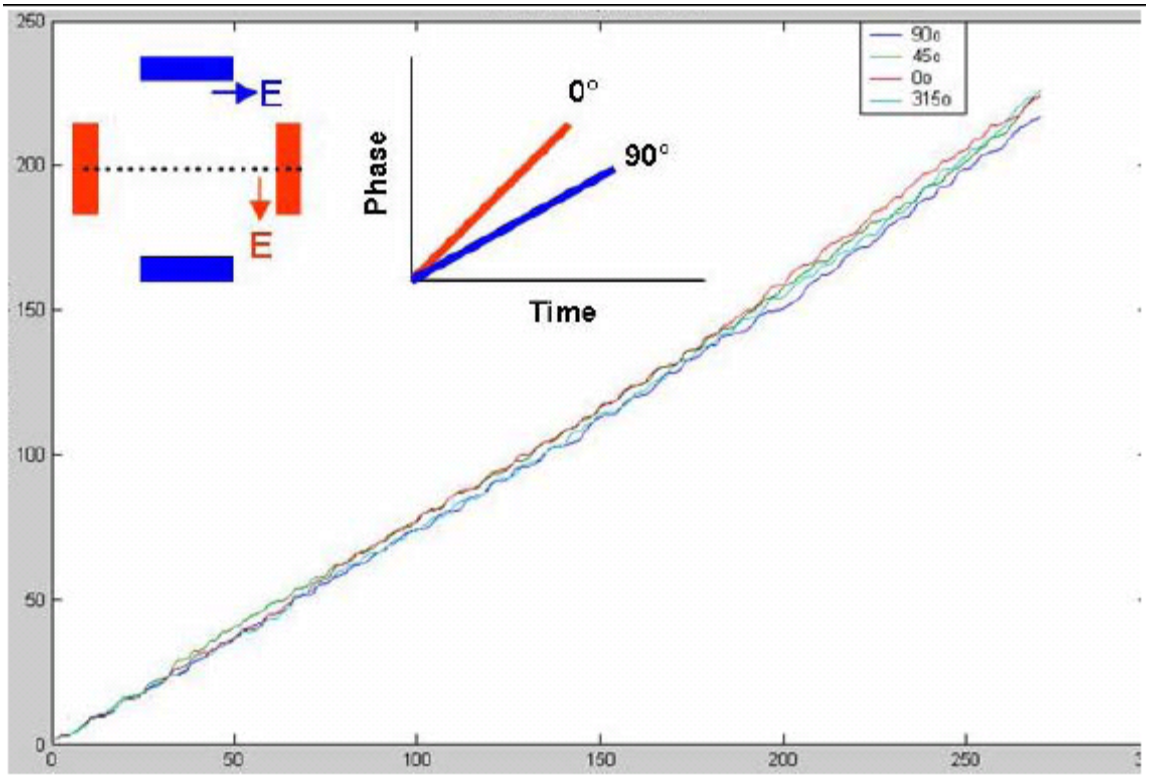


Figure 14. Plot of cumulative phase (vertical axis) and time in nsec (horizontal axis) for the parallel antenna configuration. The inset shows a hypothetical phase plot color-coded to correspond with the colors on the antenna orientations.

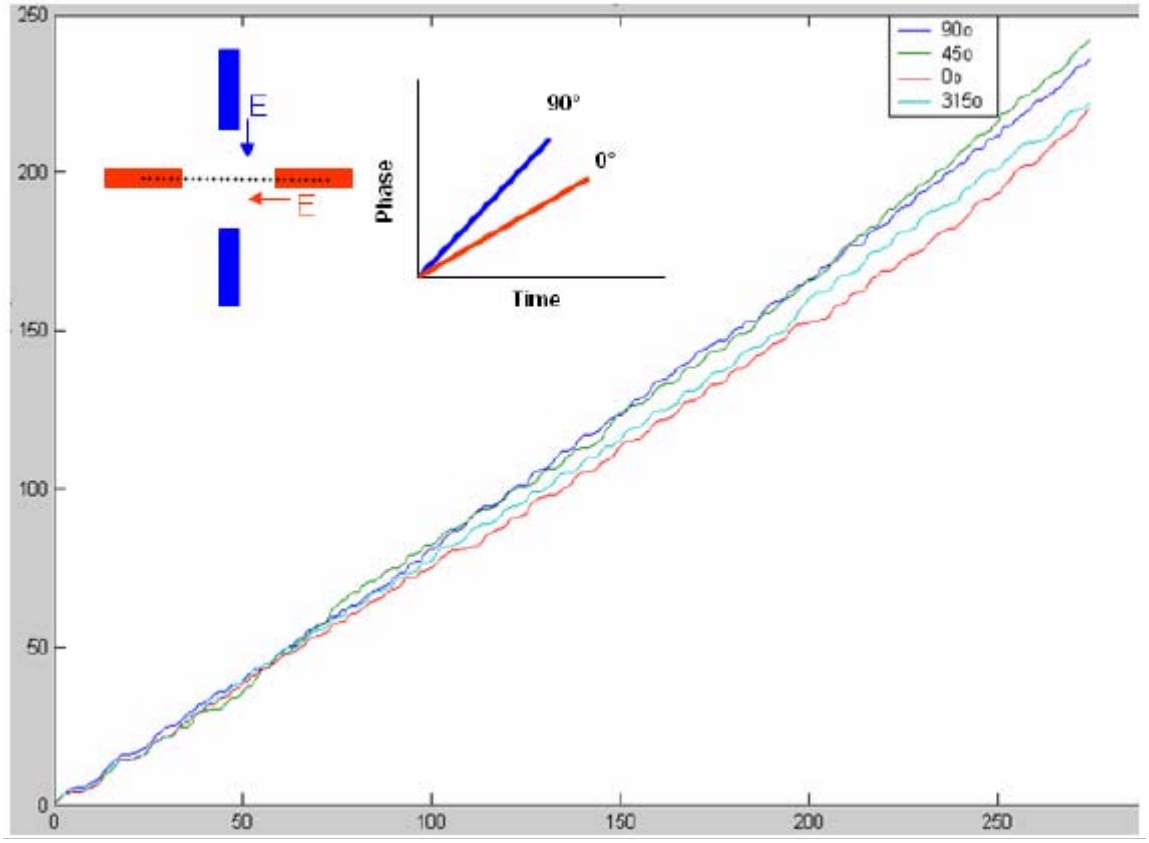


Figure 15. Plot of cumulative phase (vertical axis) and time in nsec (horizontal axis) for the endfire antenna configuration. The inset shows a hypothetical phase plot color-coded to correspond with the colors on the antenna orientations.

the fracture. For the parallel configuration case (Figure 14), the average trace recorded at 0 deg with respect to the fracture plane is expected to experience a greater time delay since the electric field is oriented perpendicular to the fracture plane. A bigger positive phase shift is therefore visible on the plot with respect to the other traces recorded at different orientations. On the other hand, the average trace recorded at 90 degrees with respect to the fracture plane is expected to experience a smaller time delay since the electric field is now oriented parallel to the fracture plane. In this case, the trace is less affected by the presence of the fracture and its cumulative phase response with time is plotted below the other traces. For the endfire case (Figure 15), the resulting phase response is reversed.

Conclusions

The azimuth of fractures can be determined by exploiting the polarization properties of GPR signals because EM waves are affected by the presence of fractures in the subsurface. The orientation of the electric field with respect to the fracture plane affects the reflection time of events on the GPR data. The delay times observed at different antenna orientations and configurations can be quantified into phase information of the recorded traces.

The velocity field at the survey area is laterally heterogeneous. Further careful work on velocities may lead us to a method to predict underground karst features, thus complementing the work reported by other workers (e.g., Tarhule and others, 2003).

The saturated vertical fractures increase the amplitude and frequency of the recorded signal. The increase in amplitude is consistent with the work reported in the literature (Tsoflias and others, 2001). However, the effect on the frequency content of the signal is a new observation and should be confirmed by additional data and analysis.

Bonding a PVC coupler to the quarry floor is an easy and reliable method for determining the fracture aperture. However, further work is needed to understand the relationship between hydraulic conductivity and the attributes of the GPR data.

Recommendations

The gypsum quarry site is probably not an ideal place to conduct GPR multicomponent surveys because the karst features greatly influence the polarization data. At the outset, the gypsum site seemed an ideal place to carry out this work due to easy accessibility and interest by the Oklahoma Department of Transportation (Sahai and others, 2005). Our recommendation is that further studies should be conducted at a different site.

The GPR profiles and common mid-point data should be acquired by using various configurations of the transmitter-receiver antennas. Soon and others (2001) have suggested that fractures with different azimuths can be preferentially imaged depending of on the configuration used when running the GPR profiles.

Acknowledgements

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Science, Development & Public Opinion: The Adjudication of Groundwater Policy for the Arbuckle-Simpson Aquifer

Basic Information

Title:	Science, Development & Public Opinion: The Adjudication of Groundwater Policy for the Arbuckle-Simpson Aquifer
Project Number:	2005OK45B
Start Date:	3/1/2005
End Date:	2/28/2006
Funding Source:	104B
Congressional District:	3rd
Research Category:	Social Sciences
Focus Category:	Groundwater, Law, Institutions, and Policy, Education
Descriptors:	Arbuckle-Simpson Aquifer, Oklahoma Water Resources Board, Science, Stakeholders, Environmental Sociology
Principal Investigators:	Beth Caniglia, Marc Krein

Publication

1. Caniglia, Beth Schaefer; Kris Smith, and Mark Vermillion. 2005, "The Making of a Moratorium: Cultural Context & the Battle over the Arbuckle-Simpson Aquifer," presented at the American Sociological Association. Scheduled for sumission to "Rural Sociology" August 2006.
2. Caniglia, Beth Schaefer; Rodney Clayton. 2005, "The Institutional Context of Policy Science: Lessons from the Arbuckle-Simpson Aquifer," presented at the annual meeting of the Geological Society of America. Scheduled for submission to "Environmental Management" December 2006.

Title: Science, Development & Public Opinion: The Adjudication of Groundwater Policy for the Arbuckle-Simpson Aquifer

Start Date: 03/01/05

End Date: 2/28/06

Principal Investigators:

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Problem and Research Objectives:

Purpose

- To collect benchmark public opinion data from relevant representatives of citizen groups, public agencies and legislators toward: development trajectories of the Arbuckle-Simpson aquifer, the present moratorium on permits for extra-county use of Arbuckle-Simpson groundwater resources (Senate Bill 288); and the Arbuckle-Simpson Aquifer Hydrogeology Study being conducted by the Oklahoma Water Resources Board
- To systematically assess over time the impact of the Arbuckle-Simpson Aquifer Hydrogeology Study on public opinion in the above mentioned areas
- To assess the ultimate impact of the Arbuckle-Simpson Aquifer Hydrogeology Study on groundwater law in the State of Oklahoma

Project Description

In May 2004, the Oklahoma State Legislature passed Senate Bill 288, which places a moratorium on the issuance of temporary permits that would result in the usage of water from a "sensitive sole source" aquifer outside of its home county, until a scientific study is conducted by the Oklahoma Water Resources Board (OWRB). The purpose of the OWRB study is to approve "a maximum annual yield that will ensure that any permit for the removal of water from a sensitive sole source groundwater basin or subbasin will not reduce the natural flow of water from springs or streams emanating from said basin or subbasin" (ENR. S. B. NO. 288). Senate Bill 288 may add a new provision to Oklahoma's water law, and that possibility has motivated unprecedented activist engagement targeted at OWRB. Literally thousands of public comment letters have poured into OWRB offices. One lawsuit, which was filed just hours after passage of the Bill, resulted in a ruling that the Bill is constitutional, and the appeal filed with the Oklahoma Supreme Court reiterated the original ruling. Therefore, the adjudication of cross-county water transfer permits hinges upon science.

Following the impact of this hydrological study is of intellectual import. Environmental policy is frequently based upon natural science. While natural science is often billed as the central determinant in environmental policy decision-making, sociologists argue that the impact of policy science studies varies based on several factors including: the extent to which findings and predictions are certain, the extent to which the scientific processes and findings are clearly communicated to various publics, and the extent to which relevant authorities possess political capacity and will to enact the recommendations of scientists. To date, we have been unable to find extant systematic studies within the sociology of science, technology and environment that empirically measure the impact of policy science from its inception to its policy conclusions. The current study is designed to fill this gap. By systematically examining the impact of information related to the OWRB study on public opinion and legislative decisions, our research will provide an empirically informed model of the role of science in the formation of environmental policy in the Arbuckle-Simpson case.

Methodology:

This longitudinal study will follow the impact of a scientific study being conducted by the Oklahoma Water Resources Board until its completion. Phase I of the project, which was funded by this grant, assembled baseline public opinion data from newspaper articles, public comment letters and in-depth semi-structured interviews. A database of national, regional and local newspaper articles was assembled using a variety of search mechanisms, including google news and lexus-nexus. The time period of the search spanned from May 2001 – July 2005, and the resulting database includes full-text versions of all discovered articles. The articles were uploaded into a qualitative analysis software package, and specific text string searches were performed to facilitate correlation of stakeholder group identities and the corresponding frames used to express concerns and preferences toward the development and distribution of Arbuckle-Simpson Aquifer resources. Because this project focused on the viewpoints of stakeholders prior to the passage of Senate Bill 288, we restricted our analyses to approximately 100 articles which were published during the 12 months preceding the bills passage.

Public comment letters were photocopied by staff at the Oklahoma Water Resources Board. These letters formed the basis for the creation of an SPSS database that includes such information as the county of residence of letter authors, “yes/no” dummy variables indicating whether particular subjects of relevance were mentioned, indicators for whether the letter was hand written, typed or a form letter, and indicators of stakeholder group affiliations.

Finally, a total of twenty-five (25) in-depth interviews were conducted with members of most target publics (or stakeholder groups) indicated in the OWRB public participation plan (see attached questionnaire). The interviews followed a semi-structured format, allowing easy comparison of answers across respondents. While two interviews were conducted face-to-face, the rest were telephone interviews that ranged between fifteen minutes and one hour. We were unable to interview members of the Indian tribes from the region, but every other stakeholder group was included. The interviews were transcribed by the social science research bureau at Oklahoma State University, and the transcripts were uploaded into a qualitative software package for systematic analysis.

These baseline data allow triangulation of public opinion toward the Arbuckle-Simpson aquifer prior to the release of significant scientific findings from the Oklahoma Water Resources Board. These baseline data will be used to compare with subsequent data collected to analyze public opinion change over time. The resulting data will provide important insights into the role of science in the adjudication of groundwater policy in the Arbuckle-Simpson case. In the final analyses, we hope to discern the ultimate impact of science on Oklahoma groundwater law.

Principal Findings and Significance:

The principal findings from this phase of the research are two-fold. First, the analyses of newspaper articles, newsletters and websites indicate that stakeholders frame the debate of the Arbuckle-Simpson aquifer from four primary perspectives: water security, sustainable development, wildlife conservation, and property rights. Wildlife conservation is primarily expressed by state and national agency personnel, while the property rights perspective is predominate among property owners in the Arbuckle region and those who wish to purchase Arbuckle-Simpson water. Water security and sustainable development concerns were expressed by members of all sides of the debate, and these perspectives represent the majority of those cited or expressed in the documents. It is noteworthy that science is not a central theme in the documents.

Several findings emanate from the interview data. First, OWRB target publics are generally supportive of the Arbuckle-Simpson Hydrology Study (76% of respondents). A minority (12%) is either opposed to the study or skeptical regarding the technical skills of OWRB researchers, with the remaining respondents undecided. All respondents who oppose the study expressed desires to buy, sell or broker Arbuckle-Simpson water, while those who expressed support had more diverse stakeholder affiliations (i.e. state agency personnel, regional environmental groups, industry groups, municipalities, etc.). Comments related to expected outcomes of the Arbuckle-Simpson Hydrology Study suggest potential for future conflict, since people express divergent expected outcomes. These two quotes are illustrative:

- "Well, I believe that if the scientific study is done correctly that it will come forward saying that it is permissible for all cities to transfer this water."

- "I expect the study is going to show that there's not adequate recharge for the aquifer to allow it to export very much water."

Another interesting finding from the interviews suggests that those actively following the Arbuckle-Simpson Hydrology Study are primarily residents of the counties overlying the aquifer (corroborated by public comment letter data), those who wish to buy the water, or employees of state agencies. Several potential respondents refused to be interviewed because they did not feel the Arbuckle-Simpson issue was relevant to them and/or their organization. With few exceptions, every respondent viewed the decision over allocation of Arbuckle-Simpson water as a fight that will last until the final decision is made. While many are optimistic that they will be satisfied with the outcome, others doubt that wholly beneficial results will occur.

These findings suggest that the final decision regarding the cross-county transfer of Arbuckle-Simpson Aquifer water will be contentious. It is likely that political and economic interests will compete heavily with the scientific findings. The best opportunity for consensus building seems to lie with shared concerns over water security, while the most important potential barrier to consensus stems from divergent views regarding private property rights.

The Making of a Moratorium: Science, Development & Public Opinion

Phase I Interview Questions

Introduction:

Read Consent form:

Consent to tape record:

General Background

1. Can you share with us your [organization/agency/tribe's] view of the role of science in the creation of environmental policy?

2. To your knowledge, what is your [organization/agency/tribe's] position toward the future development of the Arbuckle-Simpson aquifer groundwater resources?

3. What factors led your [organization/agency/tribe] to develop this position?

4. To your knowledge, what is your [organization/agency/tribe's] position toward Senate Bill 288, which places a moratorium on the issuance of temporary permits for the transfer of Arbuckle-Simpson aquifer groundwater resources outside the counties of origin?

5. What factors led your [organization/agency/tribe] to develop this position?

6. To your knowledge, what is your [organization/agency/tribe's] position toward the Arbuckle-Simpson Hydrology Study, which is being conducted by the Oklahoma Water Resources Board?

7. What factors led your [organization/agency/tribe] to develop this position?

Sources of Information

8. What are the primary sources consulted by you and other members of your [organization/agency/tribe] to acquire information pertaining to the Arbuckle-Simpson aquifer?

9. Are you satisfied that these sources provide you with the quality and quantity of information you need?

10. Are there other types or sources of information that you would like to have available?

Is this a fight?

11. Some of the individuals we have spoken to during the course of this research project have referred to their involvement in the future of the Arbuckle-Simpson aquifer as a fight – whether a legal fight, a political fight, an ethical or moral fight, an environmental fight. Do you think your [organization/agency/tribe] considers itself part of a fight? If so, can you flesh that out for us?

12. [If yes to #11] Are there other organizations, agencies, tribes or individuals that you feel are on your side in this fight?

    a. If so, can you name them and explain why you place them on your side?
    b. Also, are there certain organizations, agencies, tribes or individuals that you feel are on the other side of the fight?
    c. If so, can you name them and explain why you place them on the other side?
    d. Are there certain organizations, agencies, tribes or individuals that you consider important players in this fight who are unaligned or who you are unsure where you would place them, in terms of sides?
    e. If so, can you name them and explain why you might consider them unaligned or are unclear where to place them?

13. [If yes to #11] What kinds of strategies does your [organization/agency/tribe] use to influence the outcome of this fight?

14. Some of the individuals we have spoken to during the course of this research project have said that the Arbuckle-Simpson issue was a fight earlier – whether a legal fight, a political fight, an ethical or moral fight, an environmental fight, but it doesn't appear to be a fight anymore. Do you think your [organization/agency/tribe] would agree that the fight itself seems to have passed? If so, can you flesh that out for us?

Expectations for the future

The following questions refer to your expectations regarding the processes and outcomes that may occur.

15. Can you share with us the various steps you think should be taken as we move toward a more permanent policy decision on cross-county transfer of these water resources?

16. Can you share with us the various steps you expect will be taken?

17. Do you anticipate that your [organization/agency/tribe] will be wholly satisfied with this process? Please explain.

18. Can you share with us the particular outcomes you expect from the scientific study being conducted by the Oklahoma Water Resources Board?

19. Do you anticipate that your [organization/agency/tribe] will be wholly satisfied with the study outcomes? Please explain.

20. Can you share with us the particular outcomes you expect in terms of final policy decisions?

21. Do you anticipate that your [organization/agency/tribe] will be wholly satisfied with the policy outcomes? Please explain.

Final Comments

22. Are there other concerns, preferences, viewpoints or pieces of information you would like to share with us regarding the future of the Arbuckle-Simpson aquifer?

23. Are there other concerns, preferences, viewpoints or pieces of information you would like to share with us regarding Senate Bill 288 or the current moratorium?

24. Are there other concerns, preferences, viewpoints or pieces of information you would like to share with us regarding the Arbuckle-Simpson Hydrology Study being conducted by the Oklahoma Water Resources Board?

25. Would you be willing to speak with us again in the future?

Thank you for taking time to participate in this study!!!

Protocol to determine the optimal placement of riparian/buffer strips in watersheds

Basic Information

Title:	Protocol to determine the optimal placement of riparian/buffer strips in watersheds
Project Number:	2005OK46B
Start Date:	3/1/2005
End Date:	2/28/2006
Funding Source:	104B
Congressional District:	3rd
Research Category:	Water Quality
Focus Category:	Conservation, Hydrology, Water Quality
Descriptors:	Targeting, Riparian, SWAT, REMM, Spavinaw, GIS, Flow, Accumulation, Phosporus, Sediment, Buffer, Model
Principal Investigators:	Daniel E. Storm, Glenn Brown, Chang-Xing Jin, Michael Smolen

Publication

Protocol to Determine the Optimal Placement of Riparian/Buffer Strips in Watersheds

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Congressional District: 2nd and 3rd Oklahoma Congressional Districts

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Descriptors: Targeting, Riparian, SWAT, REMM, Spavinaw, GIS, Flow Accumulation, Phosphorus, Sediment, Buffer, Model

Principal Investigators: Daniel E. Storm[1], Michael J. White[1], Glenn O. Brown[1], Michael D. Smolen[1], and Ranbir S. Kang[2]

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Publications: None Pending

Table of Contents

(1) Problem and Research Objectives

(1.1) Introduction

Riparian buffers are a commonly recommended Best Management Practice (BMP) in Okalahoma and in other parts of the United States. Their use is promoted by federal programs such as the Conservation Reserve Program (CRP), Conservation Reserve Enhancement Program (CREP), Environmental Quality Incentives Program (EQIP), and various cost-share programs funded by USEPA 319(h) and state funds. Buffers are among the primary BMPs recommended by environmental agencies to reduce nonpoint sources pollution (Fields, 1992). Effectiveness of buffers in the removal of sediment and nutrients at a field scale has been extensively explored (Barling and Moose, 1994; Hill, 1996; Fennessy and Cronk, 1997; Lowrance *et al.*, 2002). However, the effect of riparian buffers placement within a watershed has not been well studied. Placement is, nevertheless, likely to have a significant effect on the effectiveness of the BMP. Optimizing overall BMP performance through proper placement is a critical issue (Tomer *et al.*, 2003; Marcelo and Conrad, 2003). Establishment of buffers is expensive and funding is limited; only a small fraction of streams within a watershed generally receive the BMP. By evaluating the effectiveness of riparian buffers at all potential sites within a watershed we can optimally place the buffers in targeted areas to generate the most environmental benefit per dollar spent.

(1.2) Objectives

The primary objective of this project was to develop a methodology to identify the optimal placement of riparian buffer strips in a watershed and to test that method in the Spavinaw Creek watershed in northeast Oklahoma. A secondary objective was to evaluate the use of SWAT and REMM models for the purpose of optimizing riparian BMP placement and to evaluate the effect of riparian buffer widths.

Since the proposal was submitted, we discovered that the secondary objective has been addressed by other research. Work elsewhere linking SWAT and REMM has progressed and it is clear that REMM and SWAT can be linked and will be useful for predicting the effects of riparian BMPs (Cerucci 2002, Amanjot 2003). It was not within the scope of this project to develop a comprehensive linkage between SWAT and REMM, since that work is currently underway by other researchers. Models such as SWAT and REMM are complex; to properly link these models in a useful manor requires a great deal of experience with both models. The linkage will certainly require involvement of the developers of both models, and is beyond both the budget and the duration of this project. For these reasons we have chosen to focus on the development of a methodology to target riparian areas based on currently available tools, and to demonstrate this methodology in the Spavinaw Creek watershed.

(1.3) Previous Work

Even though the effect of buffer placement has not been well studied, it is generally agreed that targeting areas for buffer establishment does improve the total environmental benefit. Several studies have performed targeting to identify areas for riparian buffer establishment (Table 1.1.3). The optimal placement of riparian buffers within a watershed has been based on a number of metrics, models, and or other criteria.

The primary criterion used in previous studies is landcover within the riparian corridor (Wilkinson *et al.,* 2004; Christianson *et al.*, 2005; Zhaoning *et al.*, 2005). Landcover data are readily available or can be developed from remotely sensed imagery, and can be used to asses the status of vegetation within the riparian corridor. Areas with little vegetation or erosive land uses are natural candidates for riparian BMPs.

Hydrologic models have been used by researchers to identify areas with the most potential for improvement (Wanhong and Weersink 2004; Marcelo and Conrad, 2003). In these studies two separate types of models were used to predict BMP benefits, an upland model like the Soil and Water Assessment Tool (SWAT), Erosion Productivity Impact Calculator (EPIC), or AGricultural Non-Point Source (AGNPS), and a riparian zone model such as Riparian Ecosystem Management Model (REMM). The data and computational requirements of these models are tremendous. Researchers have reduced these requirements by limiting spatial detail (resolution) and or spatial extent (area) considered, giving rise to very detailed field scale models and less detailed basin scale models.

Because basin scale models have large spatial extents, many field scale processes are aggregated or not considered. Models such as SWAT cleverly reduce the number of calculations by aggregating input GIS data into subbasins and Hydrologic Response Units (HRU). Both Wanhong and Weersink (2004) and Marcelo and Conrad (2003) used basin scale upland models (AnnAGNPS, SWAT) in conjunction with field scale riparian models (REMM, Vegetated Filter Strip (VFS)). This combination allowed both researchers to estimate pollutant reduction with riparian BMPs. SWAT and AnnAGNPS operate by aggregating individual gridcells from the original landcover, soils and elevation data into subbasins. Targeting is also aggregated to the subbasin level; a limitation of all commonly used basin scale hydrologic and nutrient models. In reality there may be optimal locations within each subbasin. This limitation is reduced by using smaller subbasins, Wanhong and Weersink (2004) and Marcelo and Conrad (2003) had average subbasin sizes of 0.51 km^2 and 1.2 km^2, respectively. To utilize the same subbasin areas for targeting riparian areas in the Lake Eucha basin or the Illinois River basin, two of Oklahoma's priority basins, would require 1,300 and 5,500 subbasins, which is excessive and beyond the limits for most models. With the current generation of models it will be problematic to resolve

both the fine scale at which riparian buffer processes operate and the spatial extent required to perform targeting in large basins.

Tomer *et al.* (2003) used terrain analysis to identify optimal locations for wetlands. They found that 57% of riparian gridcells received runoff from less than 1 acre, making these locations poor choices for riparian BMPs. A riparian area can only filter water which moves through it from adjacent areas. Therefore, when the drainage area of BMP is less than its design capacity, the less effective the practice is to the overall water quality of the basin.

(1.4) Study Area

The area selected for this study was Brush Creek and lower Beaty Creek in northeast Oklahoma, which are portions of the Lake Eucha Basin. The Lake Eucha Basin has been studied extensively by Oklahoma State University, the City of Tulsa, and the Oklahoma Conservation Commission. Data, such as IKONOS imagery, were available in the Lake Eucha Basin and not available elsewhere. These sites were selected because they contained both forested and degraded riparian areas and a variety of landcovers. The study area covered 47,000 acres within the Oklahoma portion of the Lake Eucha Basin (Figure 1.4).

Table 1.3.1 Criteria used for riparian targeting in previous studies.

Study (Reference)	Targeting Criteria
Murrumbidgee Catchment (Wilkinson *et al.*, 2004)	Remotely sensed canopy cover, estimated stream power, and SedNet predicted gulley erosion.
Turkey Creek (Christianson *et al.*, 2005)	Remotely sensed landcover weighted by unit/area estimated erosion.
Rapidan River (Tipett *et al.*, 2001)	Extensive field survey, summarized and ranked in a GIS.
Canagagigue Creek (Wanhong and Weersink, 2004)	Annualized Agricultural Nonpoint Source Pollution (AnnAGNPS) and Vegetation Filter Strip (VFS) model predicted sediment delivery.
Tipton Creek (Tomer *et al.,* 2003)	Wetness and erosion indices based on contributing area and slope.
Beijing GuanTing Watershed (Zhaoning *et al.*, 2005)	Remotely sensed landcover and stream proximity.
Townbrook Watershed (Marcelo and Conrad, 2003)	SWAT upland model and REMM riparian model Subbasin Level.

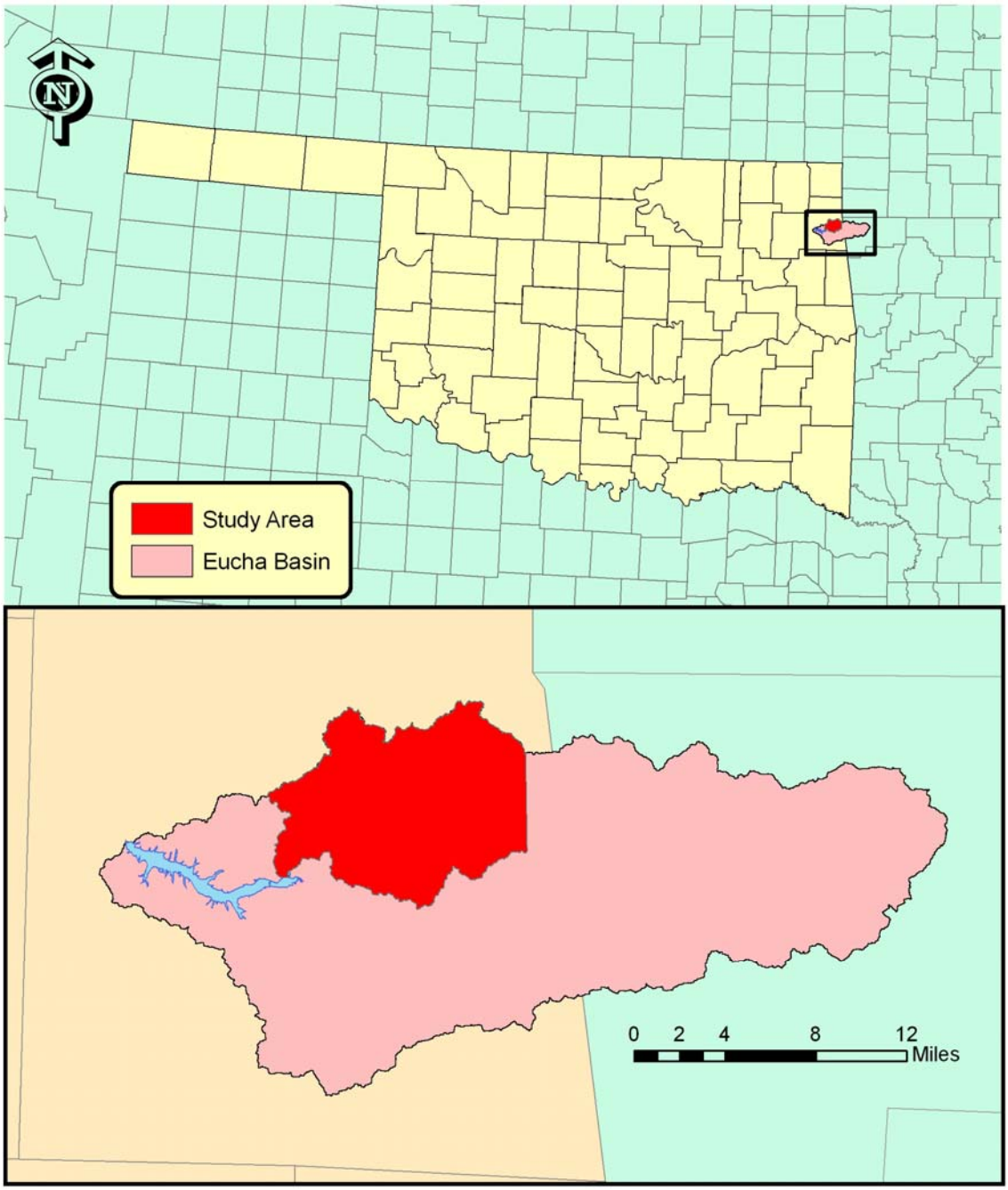


Figure 1.4 Brush Creek and lower Beaty Creek study site within the Lake Eucha Basin.

(2) Methodology

For this project, the use of models to optimally locate riparian BMPs was rejected in favor of using simpler GIS based means. Currently available watershed models, such as SWAT, must be linked with a riparian model to evaluate riparian BMPs, a significant undertaking. This linkage is currently underway by other researches and will likely become available in a few years. While models can quantitatively predict improvement by the establishment of a BMP in any location, current models lack the ability to do so with high resolution and large extents. For these reasons we believe that the best solution, given current technology, is to use simpler methods to target riparian BMPs using primarily qualitative means.

Our approach is to use multiple indicators of riparian BMP suitability and effectiveness obtainable from various GIS data and weight them into a single indicator. This master indicator will then be used to rank all possible riparian BMP sites from most to least effective. GIS derived indicators are listed below and explained in more detail in the following sections.

1) Landcover within the riparian zone
2) RUSLE gridcell predicted erosion
3) Extrapolated SWAT runoff volume and soluble phosphorus yield
4) Flow accumulation from adjacent areas
5) Stream curvature
6) Stream order and gradient

(2.1) RUSLE gridcell predicted erosion

Erosion is highly correlated with the transport of sediment-bound nutrients, including phosphorus. The Universal Soil Loss Equation (USLE) (Wischmeier and Smith, 1978) and the more recent Revised Universal Soil Loss Equation (RUSLE) (Renard *et al.*, 1991) can be applied to readily available GIS data to generate rainfall erosion estimates for large areas. USLE and RUSLE are designed to predict long term average annual soil loss using an extensive database of parameter values developed across the US. Both the USLE and RUSLE are calculated as:

$$A = R\ K\ L\ S\ C\ P$$

where R is the rainfall factor, K is the soil erodibility factor, L is the slope length factor, S is the slope gradient factor, C is the crop management factor and P is the conservation practice factor. RUSLE improves prediction over the USLE by incorporating sub factors to better represent field conditions and management.

C Factor

USLE C factors are based directly on landcover and land use. Landcover/land use is the most important contributor to erosion. The landcover data affects the amount and distribution of pasture, small grains, row crop, and forest in the basin. These landcovers are very different. Forested areas contribute little to the sediment loading, while pastures, small grains and row crops are thought to be the primary source of sediment and nutrients.

It is important that landcover data be based on the most current data available, since landcover changes over time. Therefore, landcover was derived from four meter IKONOS imagery, digital aerial photos, ground truth data points provided by the Oklahoma Conservation Commission, and a stream corridor manually digitized from IKONOS 1 meter panchromatic imagery by Oklahoma State University (OSU) personnel (Storm *et al.*, 2005). Ten IKONOS images captured February 17, 2005 were obtained and classified by Applied Analysis Inc. (AAI). An unsupervised iterative self-organizing data analysis (ISODATA) clustering algorithm was applied by AAI to define spectral categories. After several iterations these categories combined into individual landcovers (Figure 2.1.1). OSU personnel georeferenced the classified images to existing aerial photography.

USLE C factors were derived from a variety of sources and are listed in Table 2.1.1. A final map of USLE C factor for the study area is given in Figure 2.1.2.

LS Factor

LS factor was estimated from topographic data. Moore and Wilson (1992) approximated the LS factor in the RUSLE as:

$$LS = (A_s / 22.13)^{0.6} (\text{Sin}\ \theta / 0.0896)^{1.3}$$

where A_s is the upslope contributing area divided by the width of the pixel and θ is the slope of the pixel. A_s is derived from a flow accumulation (Figure 2.1.3) of the DEM performed in ArcView using Hydrotools 1.0 (Schäuble 2003). Hydrotools includes a multi-path algorithm based on Quinn *et al. (*1991) which produces more realistic flow accumulations than traditional methods. This multi-flow algorithm directs a portion of the flow to all down slope cells, not only to the most down slope cell as done by traditional algorithms. Since RUSLE is intended to predict rill and interrill erosion, we limited the flow accumulation to a maximum of 15 cells, which is equivalent to a maximum flow length of 150 meters.

Pixel slope was also derived from the DEM. A map of the combined LS factor is given in Figure 2.1.4. These data have a resolution equivalent to the original DEM, 1/3 arc second (~10m).

K Factor

The USLE K factor represents soil erodibility. Soil information was given in SSURGO (State Soil Survey Geographic) data provided by the Natural Resources Conservation Service (NRCS). These data are essentially digitized soil survey manuals. The USLE K factor was included in the SSURGO database. These data are natively vector, but were sampled into raster format at the resolution of the DEM (Figure 2.1.5).

R Factor

The rainfall factor was taken as a constant of 120 ft*ton*in/acre*hr*storm for Delaware County, Oklahoma (Haan *et al.*, 1994). Although R factor varies spatially we did not consider it to vary significantly across the study area.

P Factor

The conservation practice factor was assumed to be equal to one, i.e. no conservation practices in effect. Without specific information about what practices were used on which fields within the study area, a uniform conservation practice factor was necessary to prevent biasing the targeting results.

RUSLE Predicted Erosion

RUSLE predicted erosion is given in Figure 2.1.6. Erosion estimated ranged from 0.0 to 275 tons per acre with an average of 0.65 tons/acre for the study area. High rates of erosion were not realistic and were primarily due to errors in the input data in isolated cells. For this reason erosion was limited to 25 tons/acre. For the purpose of targeting, absolute rates are less important than the relative differences between cells.

Table 2.1.1 USLE C factors for landcovers in the study area.

Landcover	USLE Crop Factor	Notes
High Biomass Pasture[2]	0.003	Grass 95% cover
Low Biomass Pasture[2]	0.035	50% tall weeds over 60% grass cover
Rangeland[2]	0.013	25% brush over 80% grass cover
Urban[2]	0.042	Grass 60% cover
Wheat/beans[1]	0.25	Estimated from soybeans and winter wheat.
Forest[1]	0.0001	
Bare[2]	0.20	20% Grass cover
Water	0.0	Not Applicable
Stream[2]	0.003	50% Brush, 95% ground cover

1) C. T. Haan, Barfield, B.J., and J.C. Hayes. 1994. Design hydrology and sedimentology for small catchments. New York: Academic Press.
2) 1977, Procedure for computing rill and interrill erosion on project areas, SCS (NRCS) technical release 51.

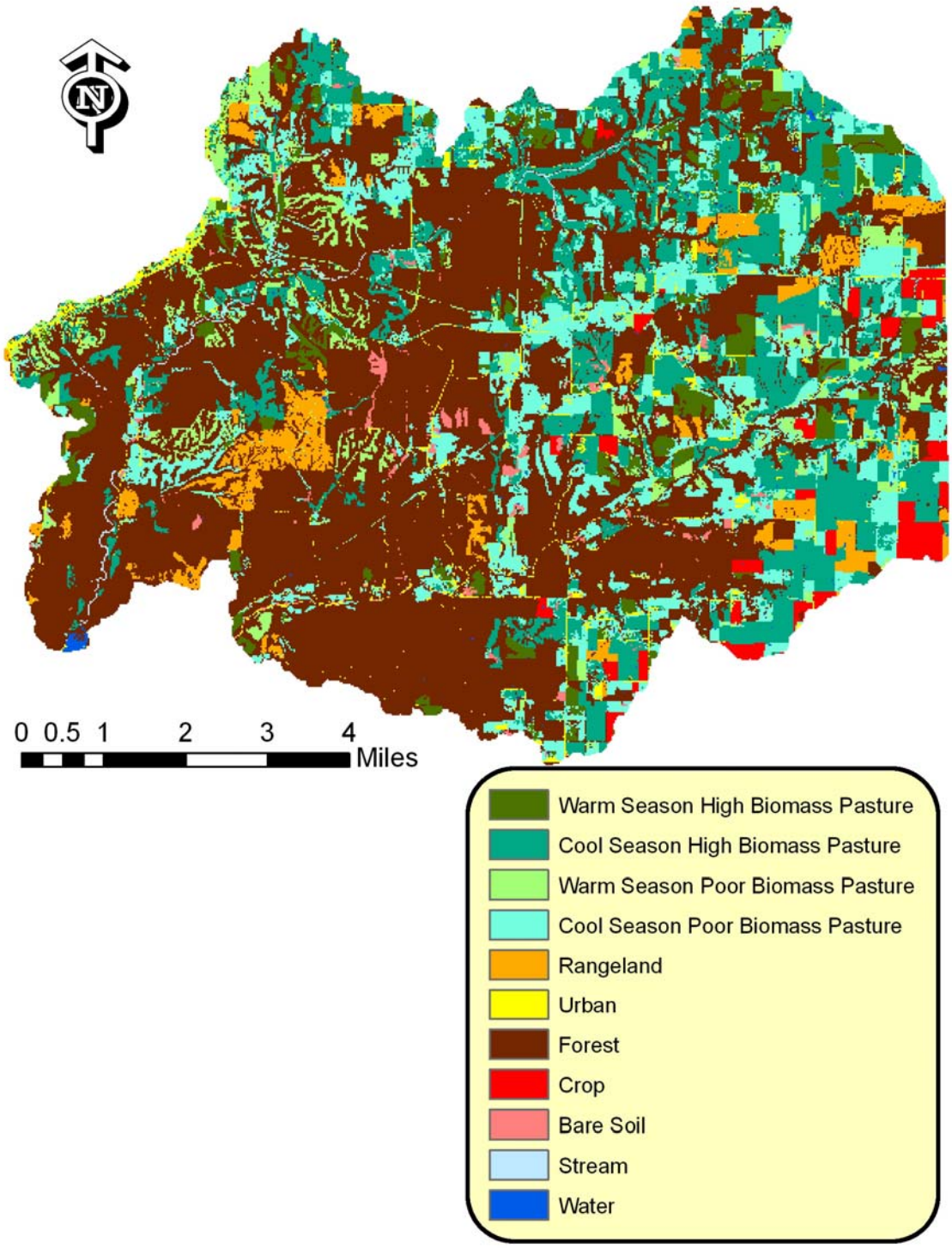


Figure 2.1.1 IKONOS derived landcover.

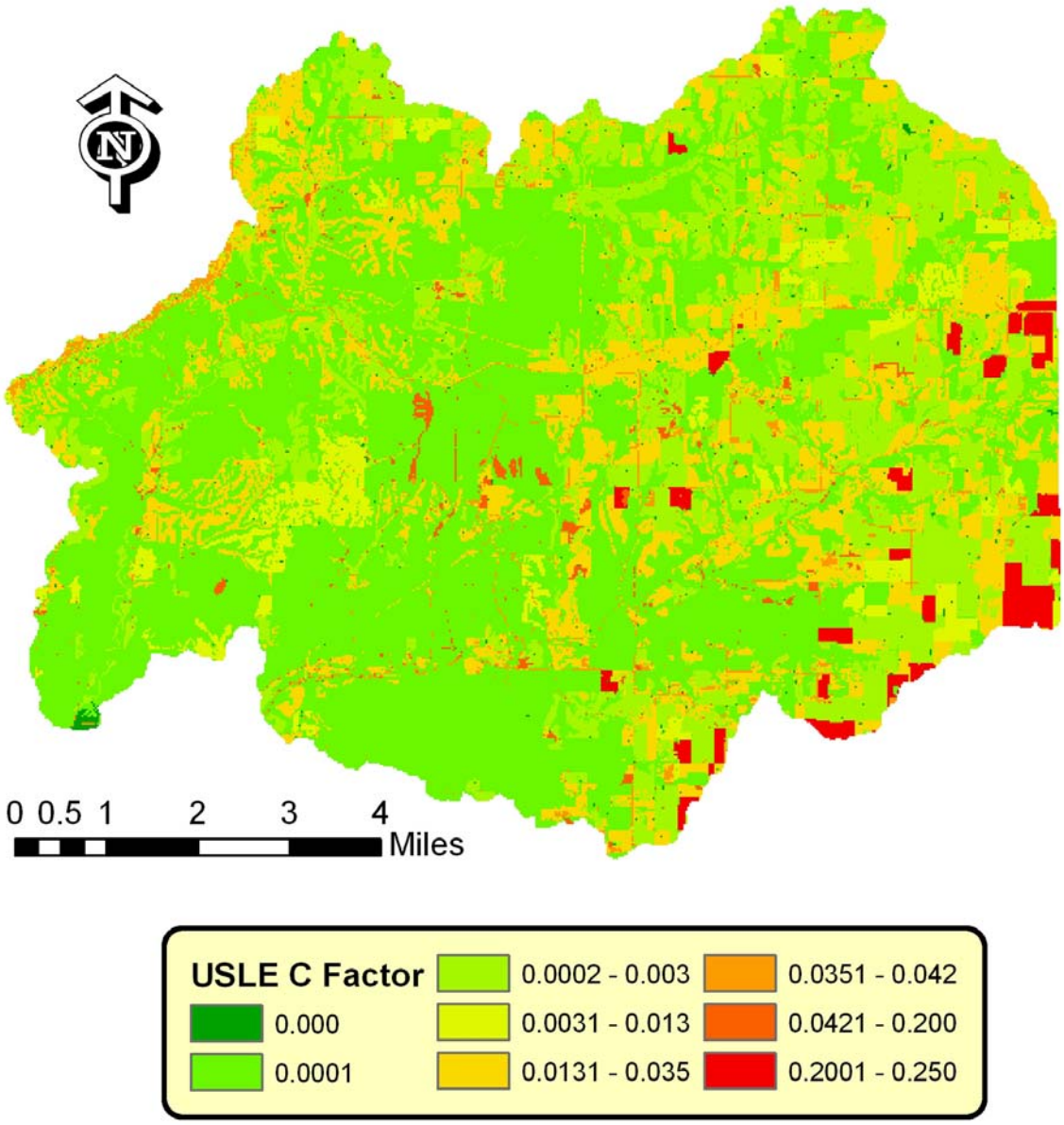


Figure 2.1.2 Universal Soil Loss Equation (USLE) C factors based on Landcover.

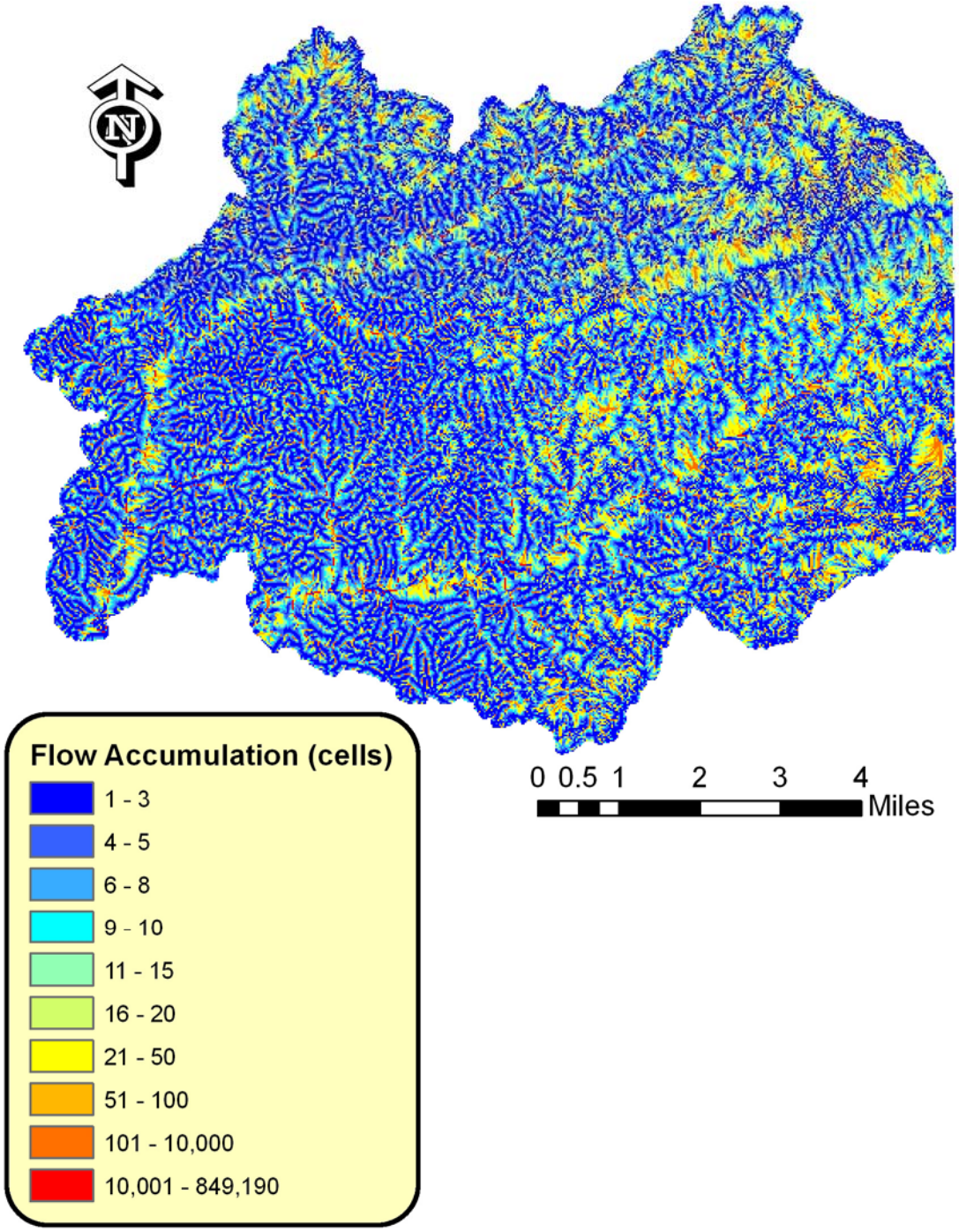


Figure 2.1.3 Flow accumulation used to estimate flow lengths for Revised Universal Soil Loss Equation (RUSLE) LS factor.

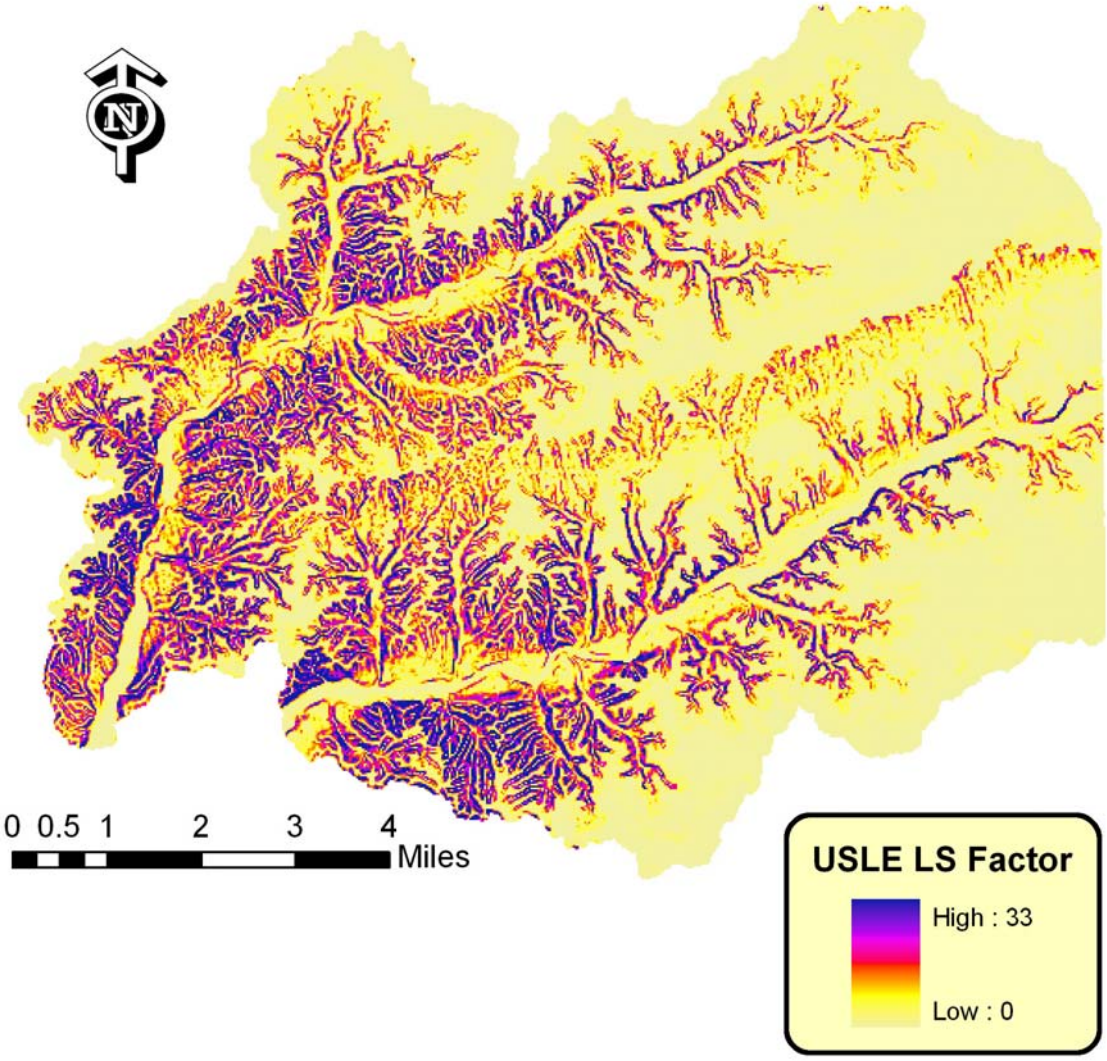


Figure 2.1.4 Revised Universal Soil Loss Equation (RUSLE) LS factor used to predict gridcell erosion.

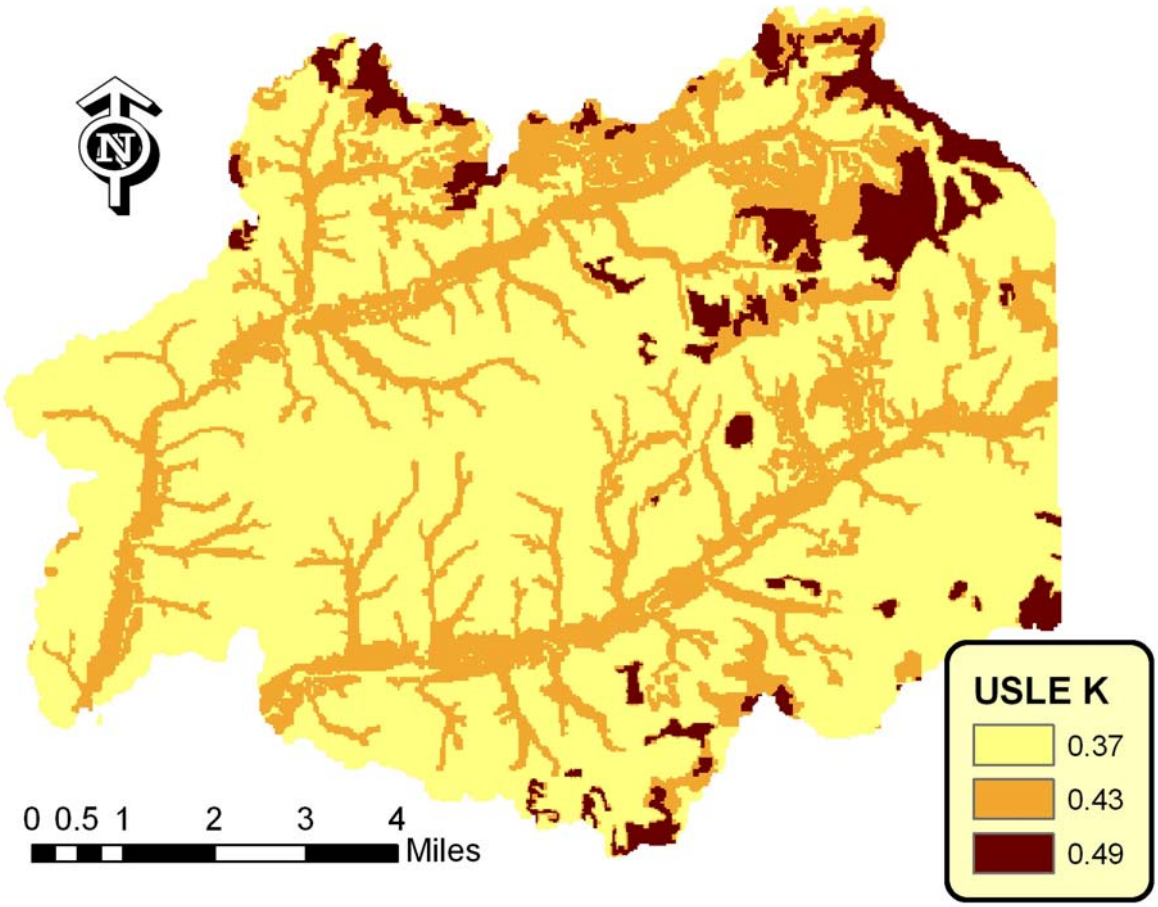


Figure 2.1.5 Universal Soil Loss Equation (USLE) K factor (English units) used to predict gridcell erosion, derived from State Soil Geographic (SSURGO) data.

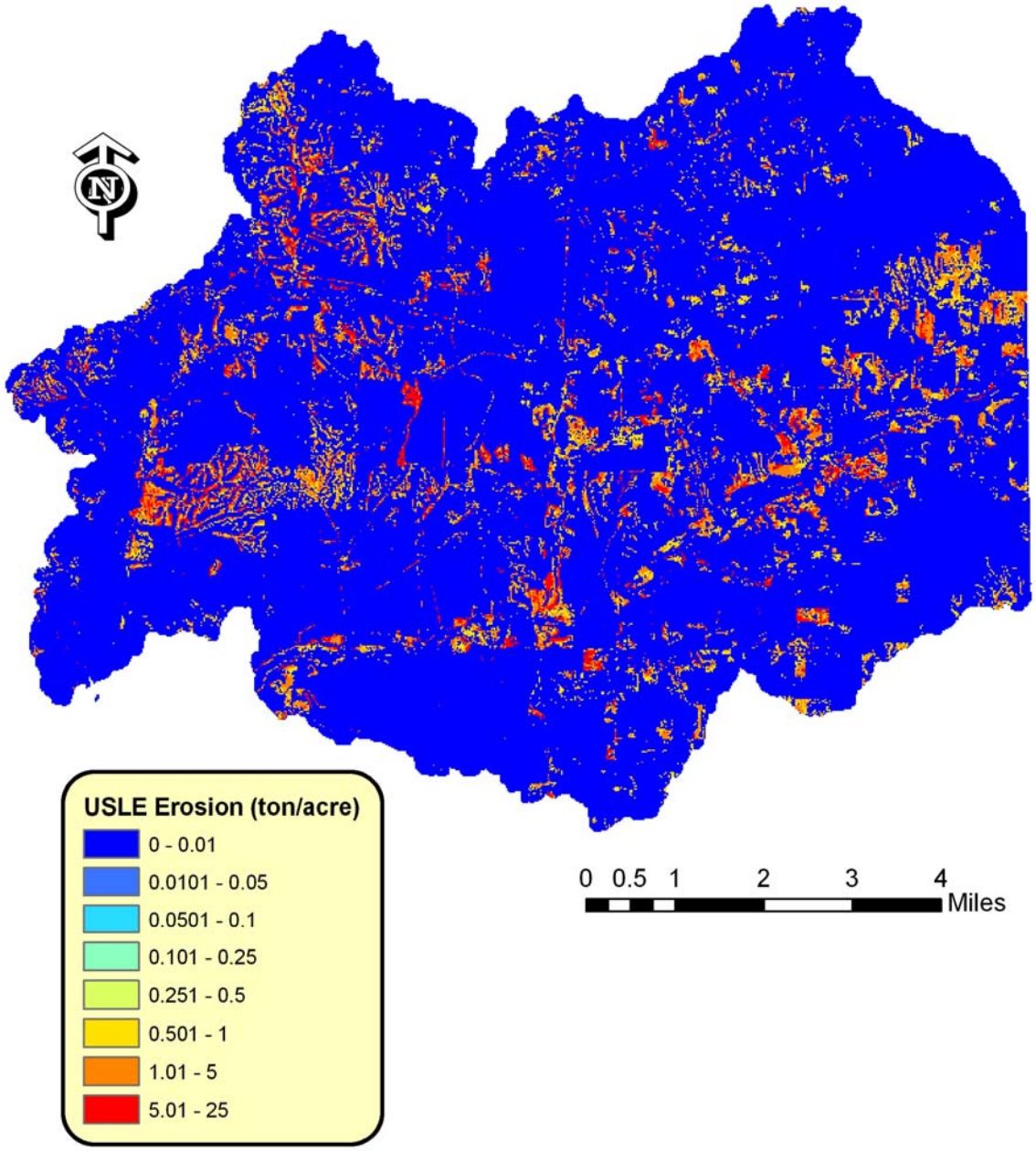


Figure 2.1.6 Universal Soil Loss Equation (USLE) predicted gridcell erosion.

(2.2) Extrapolated SWAT Runoff and Soluble Phosphorus

In pasture systems with little erosion, most phosphorus is transported in soluble forms. The amount of soluble phosphorus lost from a field is primarily a function of weather, land use, management, and soils. It is difficult to estimate the quantity of soluble phosphorus lost from every gridcell in the basin. Models such as SWAT can be used, but it is difficult to run SWAT on a gridcell basis, and out of the scope of this project. As a surrogate for true gridcell model predictions, estimates by landcover and soil were interpolated from SWAT model results by Storm *et al.* (2005) in the neighboring Spavinaw Creek Basin. SWAT model predictions were summarized by hydrologic soil group and landcover (Table 2.2.1). Gridcells within the original GIS data with the same hydrologic soil group and landcover were assigned runoff and soluble phosphorus yields from Table 2.2.1. Because the original landcover data did not specify littered or non-littered pasture, the average of three scenarios was used. The three scenarios were pasture with litter, pasture with commercial nitrogen, and pasture with no fertilization of any kind. If litter pastures locations and their boundaries were known, this information could be included.

Runoff and soluble phosphorus yields vary widely across the study area (Figure 2.2.1 and 2.2.2). Both runoff volume and soluble phosphorus yield are higher in the upland portions of the study areas. The areas have different soils, as illustrated in Figure 2.2.3, with higher runoff potential in the upland areas. Fraction of rock, silt, and clay are given in Figures 2.2.4 to 2.2.6, illustrating the differences in soil properties across the study areas.

Table 2.2.1 SWAT predictions summarized by landcover and hydrologic soil group by Storm *et al.* (2005).

Land Cover	Hydrologic Soil Group								
	B			C			D		
	Surface Runoff (mm)	Soluble P (kg/ha)	Total P (kg/ha) .	Surface Runoff (mm)	Soluble P (kg/ha)	Total P (kg/ha) .	Surface Runoff (mm)	Soluble P (kg/ha)	Total P (kg/ha) .
Cultivated	118	0.193	0.726	218	0.386	2.448	257	0.488	2.822
Bare	146	0.248	0.597	222	0.263	0.912	255	0.380	1.498
Forest	18	0.001	0.003	110	0.004	0.018	142	0.004	0.047
Range	42	0.010	0.012	139	0.026	0.037	196	0.052	0.068
Stream	50	0.002	0.006	147	0.004	0.010	197	0.005	0.010
Urban	118	0.695	0.773	193	1.015	1.172	223	1.230	1.429
Water	0	0.000	0.000	0	0.000	0.000	0	0.000	0.000
Litter-Good Condition Warm Season Pasture	45	0.685	0.708	119	1.880	2.008	152	2.576	2.780
Litter-Good Condition Cool Season Pasture	50	0.752	0.787	128	1.984	2.174	173	2.823	3.090
Urea-Good Condition Warm Season Pasture	43	0.150	0.165	114	0.444	0.519	153	0.599	0.713
Urea-Good Condition Cool Season Pasture	48	0.154	0.164	124	0.394	0.445	169	0.577	0.650
No Fert-Poor Condition Warm Season Pasture	142	0.326	0.757	227	0.556	1.807	251	0.606	2.241
No Fert-Poor Condition Cool Season Pasture	152	0.399	0.665	232	0.610	1.351	268	0.758	1.744
No Fert-Good Condition Warm Season Pasture	41	0.093	0.108	113	0.266	0.349	147	0.349	0.486
No Fert-Good Condition Cool Season Pasture	48	0.070	0.081	125	0.182	0.234	166	0.280	0.356
Good Condition Warm Season (Average)*	43	0.310	0.327	115	0.863	0.958	151	1.175	1.326
Good Condition Cool Season (Average)*	49	0.325	0.344	126	0.854	0.951	169	1.226	1.365

* Average of all good condition pasture

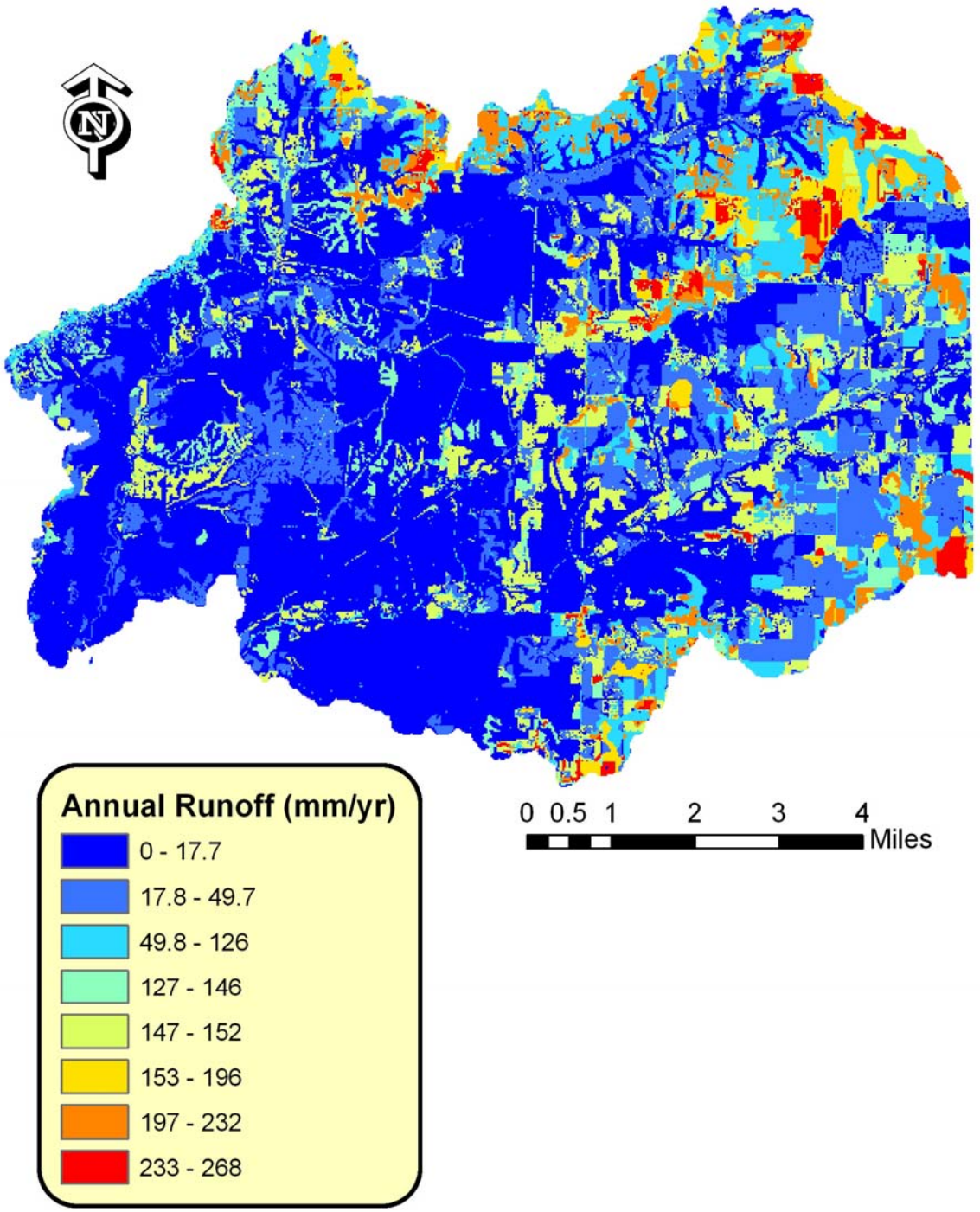


Figure 2.2.1 Gridcell annual runoff volume extrapolated from SWAT model prediction of the Spavinaw Creek Basin (Storm *et al.*, 2005).

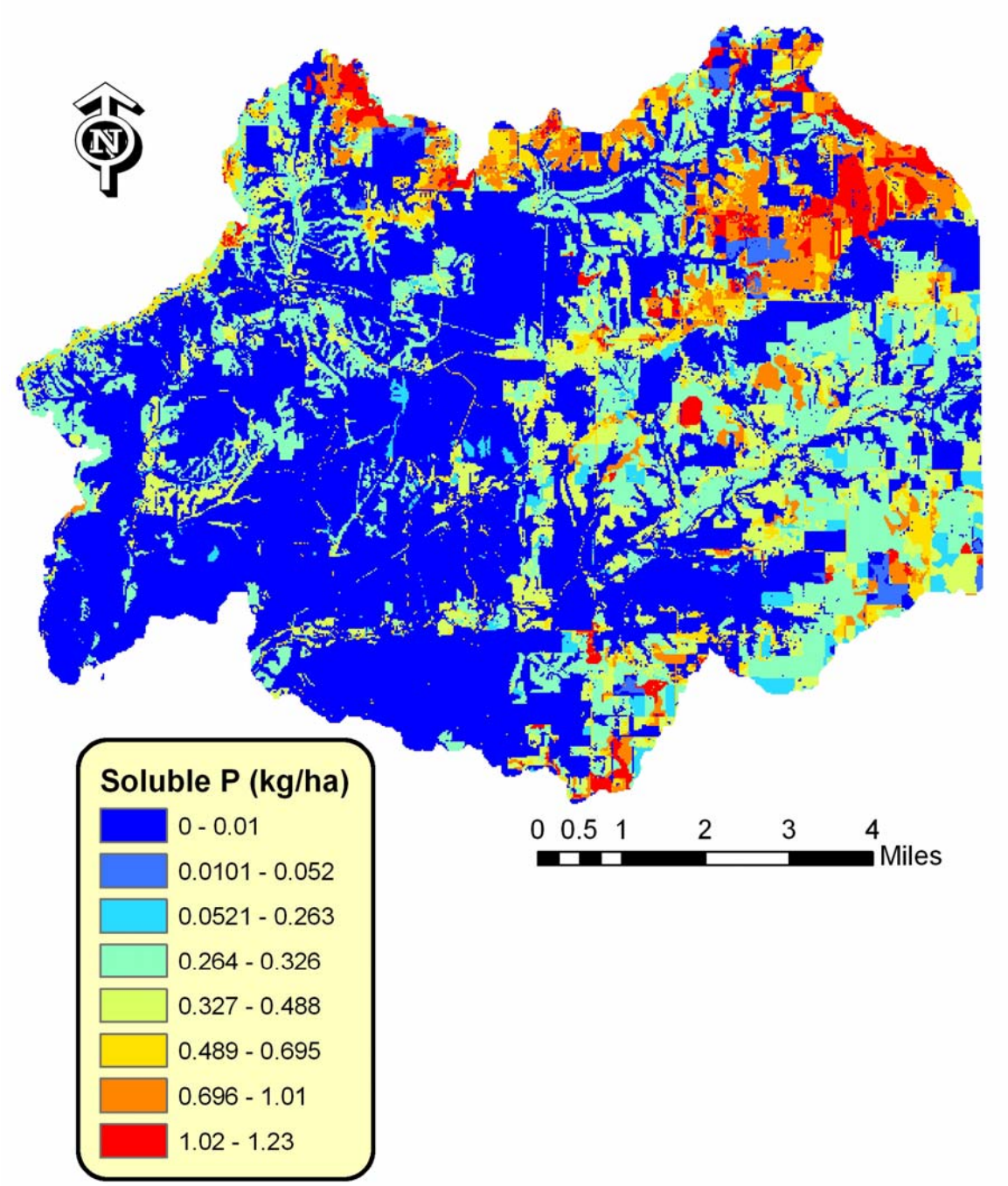


Figure 2.2.2 Gridcell annual soluble phosphorus load extrapolated from SWAT model prediction of the Spavinaw Creek Basin (Storm *et al.,* 2005).

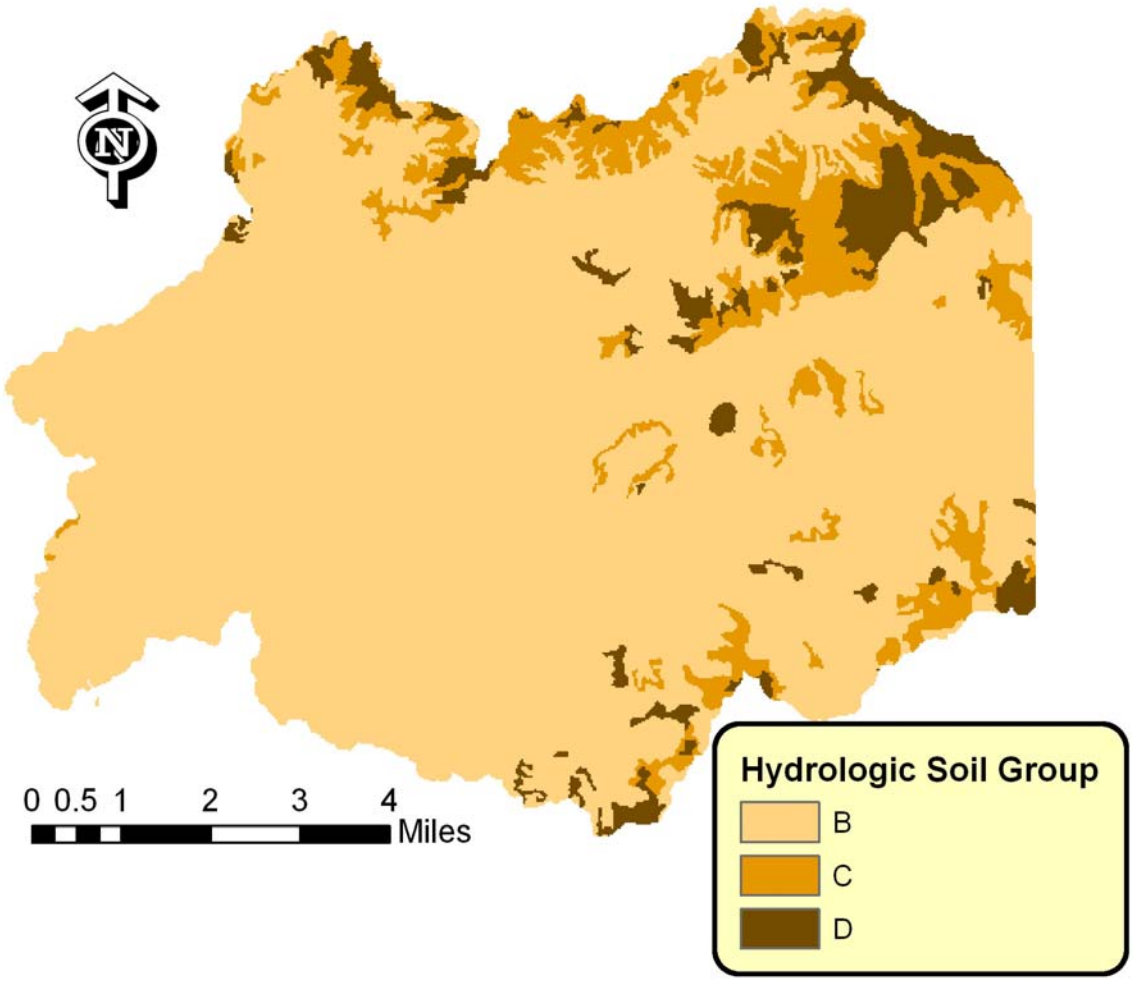


Figure 2.2.3 Hydrologic soil group derived from State Soil Geographic (SSURGO) data.

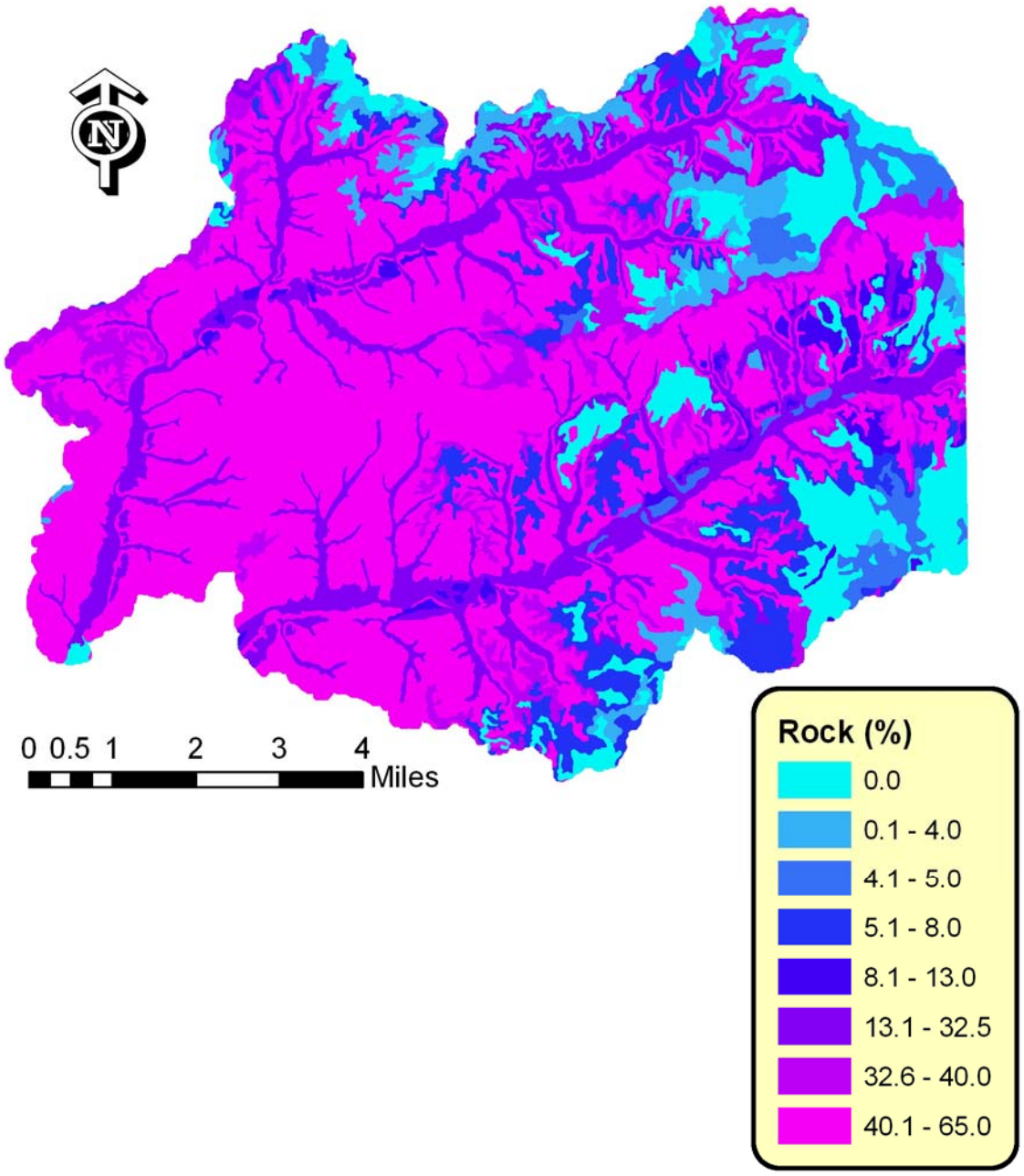


Figure 2.2.4 Fraction of rock in the surface soil layer across the study area. Derived from State Soil Geographic (SSURGO) data.

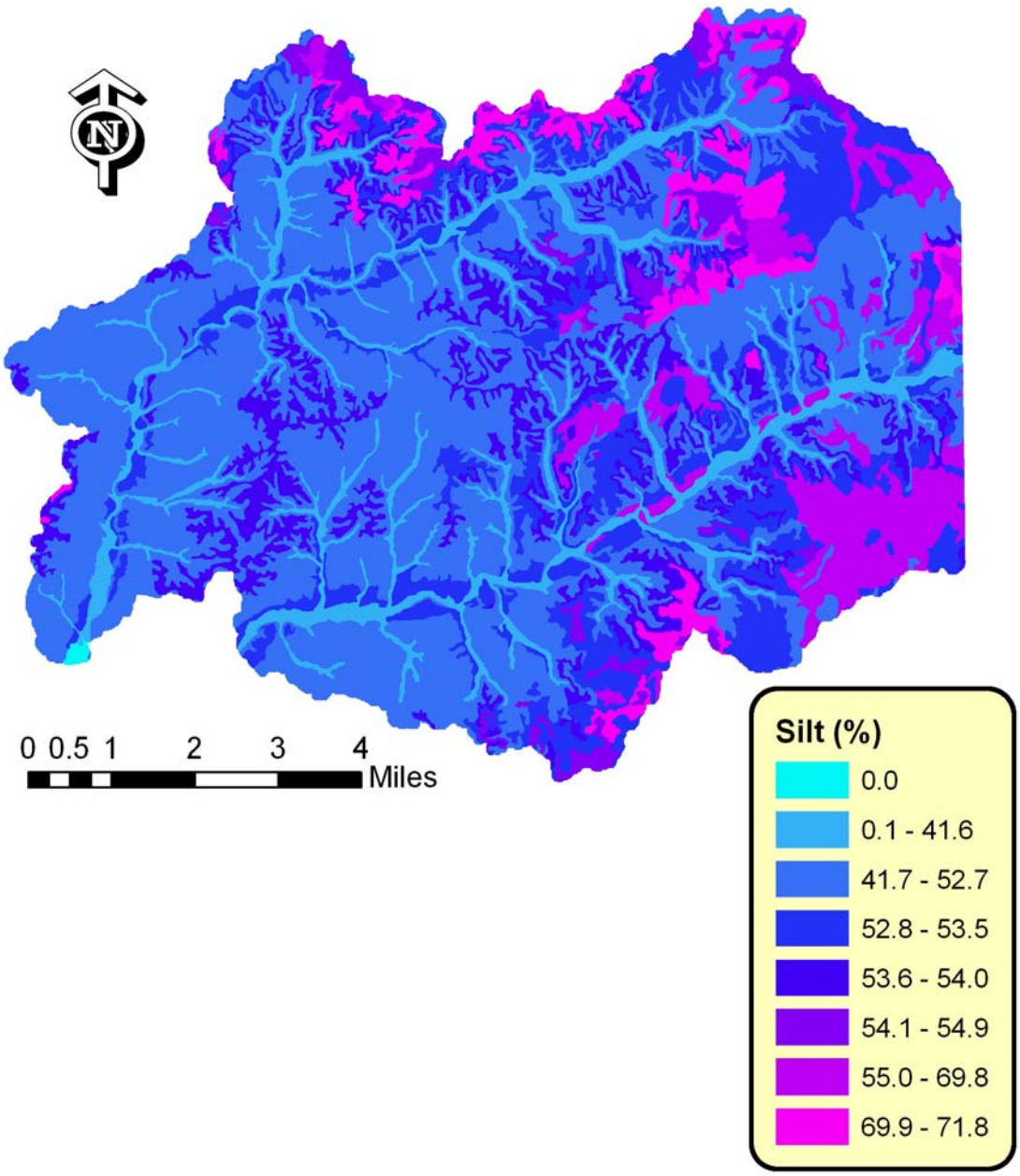


Figure 2.2.5 The fraction of silt in the surface soil layer across the study area. Derived from State Soil Geographic (SSURGO) data.

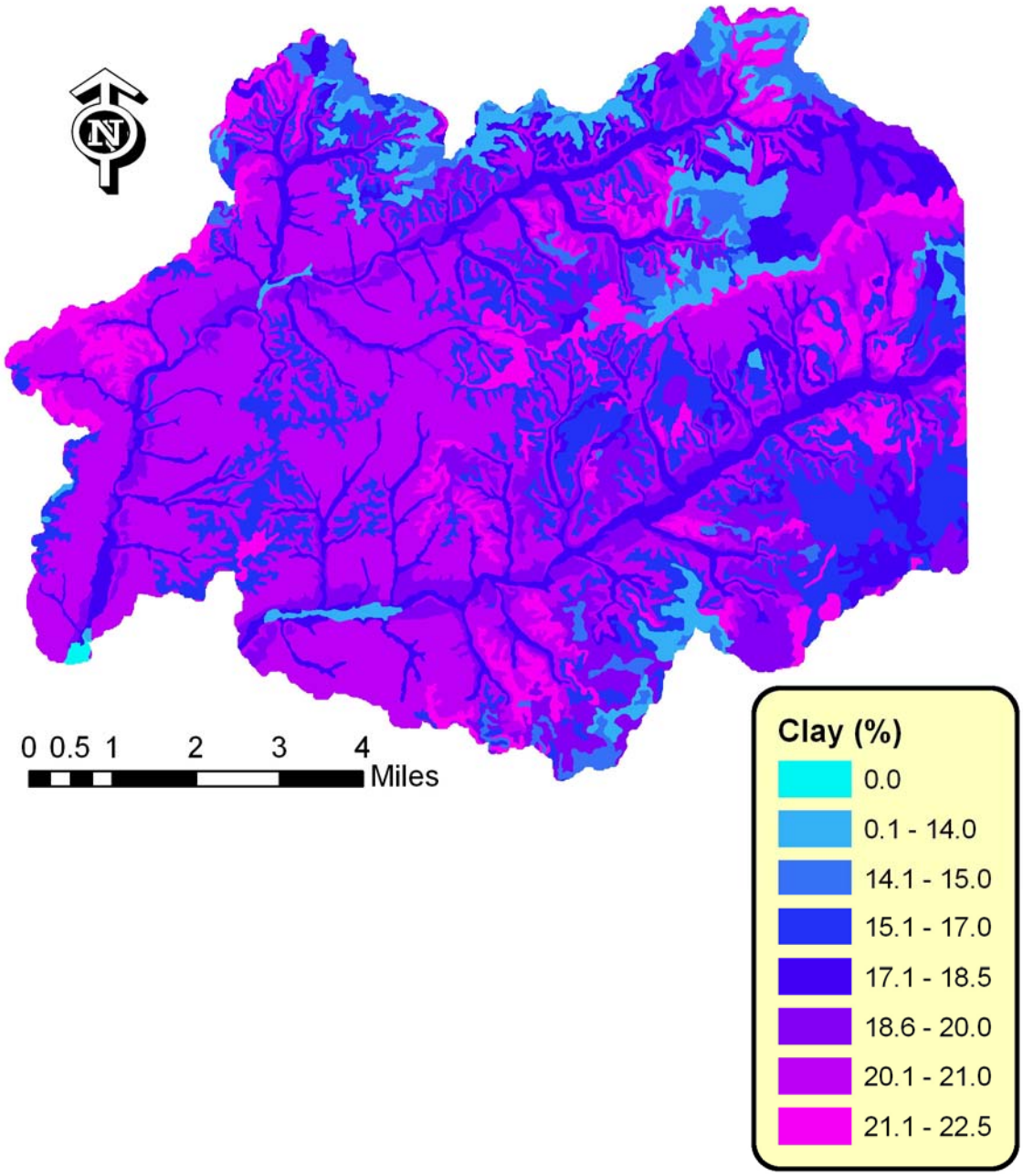


Figure 2.2.6 The fraction of clay in the surface soil layer across the study area. Derived from State Soil Geographic (SSURGO) data.

(2.3) Flow Accumulation

The RUSLE gridcell sediment, SWAT extrapolated runoff volume, and soluble phosphorus yield provided estimates of the production of sediment and nutrients, but not how and where these are transported to the stream. Flow accumulation provided estimates of how water moves across the land surface to streams and rivers. In order to evaluate a site for riparian performance we must know how much water, sediment, and nutrients enter a particular riparian zone. A riparian buffer cannot filter water which does not pass through it. However, if the flow is too concentrated it will channelize, the riparian buffer will be bypassed and will not function properly.

Flow accumulation can be used to determine the amount of runoff flowing through any given cell of a DEM. Traditional flow accumulations assume all gridcells produce one unit of runoff, and are therefore a measure of drainage area, not flow as the name implies. An example of traditional flow accumulation is given in Figure 2.3.1. One weakness of the traditional flow accumulation is that the entire flow accumulated in a cell is transferred to the most down slope adjacent cell, even if other adjacent cells are also down slope. This is not realistic using a DEM based on the average elevation for a 10*10 meter cell. It is likely that parts of each 10 meter cell will pass flow down slope to multiple cells. This weakness is overcome by using the ArcView Extension Hydrotools 1.0 (Schäuble, 2003). Hydrotools has a multi-path algorithm based on Quinn *et al*. (1991), which produces more realistic flow accumulations by routing flow to all down slope cells based on the elevation difference. Once the flow accumulation reaches 500 cells, the traditional method of routing all flow to the most down slope cell is used. The multi-path algorithm was applied to the study area and is shown in Figure 2.3.2.

Estimates of erosion, runoff volume and soluble phosphorus were made in sections 2.1 and 2.2 for each 10 meter gridcell in the study area. How these were transported to the stream determine in part the effectiveness of a riparian buffer. Most flow accumulation algorithms assume one unit of runoff per gridcell; however Hydrotools 1.0 can utilize a weighting grid to allow an estimated runoff for each gridcell to be utilized. This function was used to produce flow accumulation of runoff volume, sediment, and soluble phosphorus load. This procedure ignored losses due to deposition, even though significant. Before accumulation the runoff volume, sediment and soluble phosphorus load grids were normalized such that the average gridcell value was 1.00 to make all accumulation grids relative in magnitude. The results are given in Figures 2.3.3 to 2.3.5. The sediment accumulation grid had higher values in steeper sloping areas and erosive landcovers. Runoff volume accumulation was higher in the B and C hydrologic group soils in the eastern and north eastern portions of the study area. Soluble phosphorus accumulation was similar to that of runoff volume.

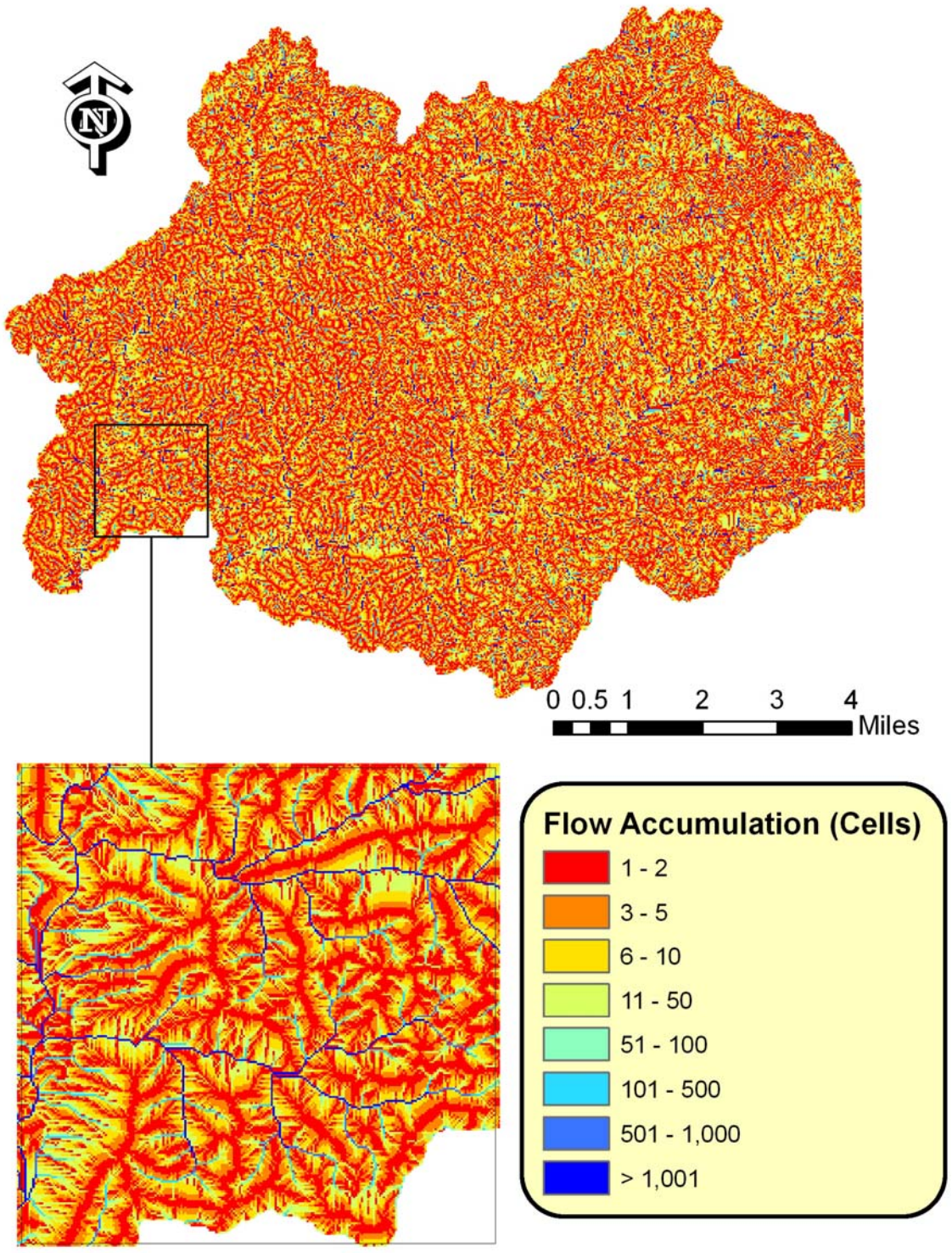


Figure 2.3.1 Traditional flow accumulation in the study area.

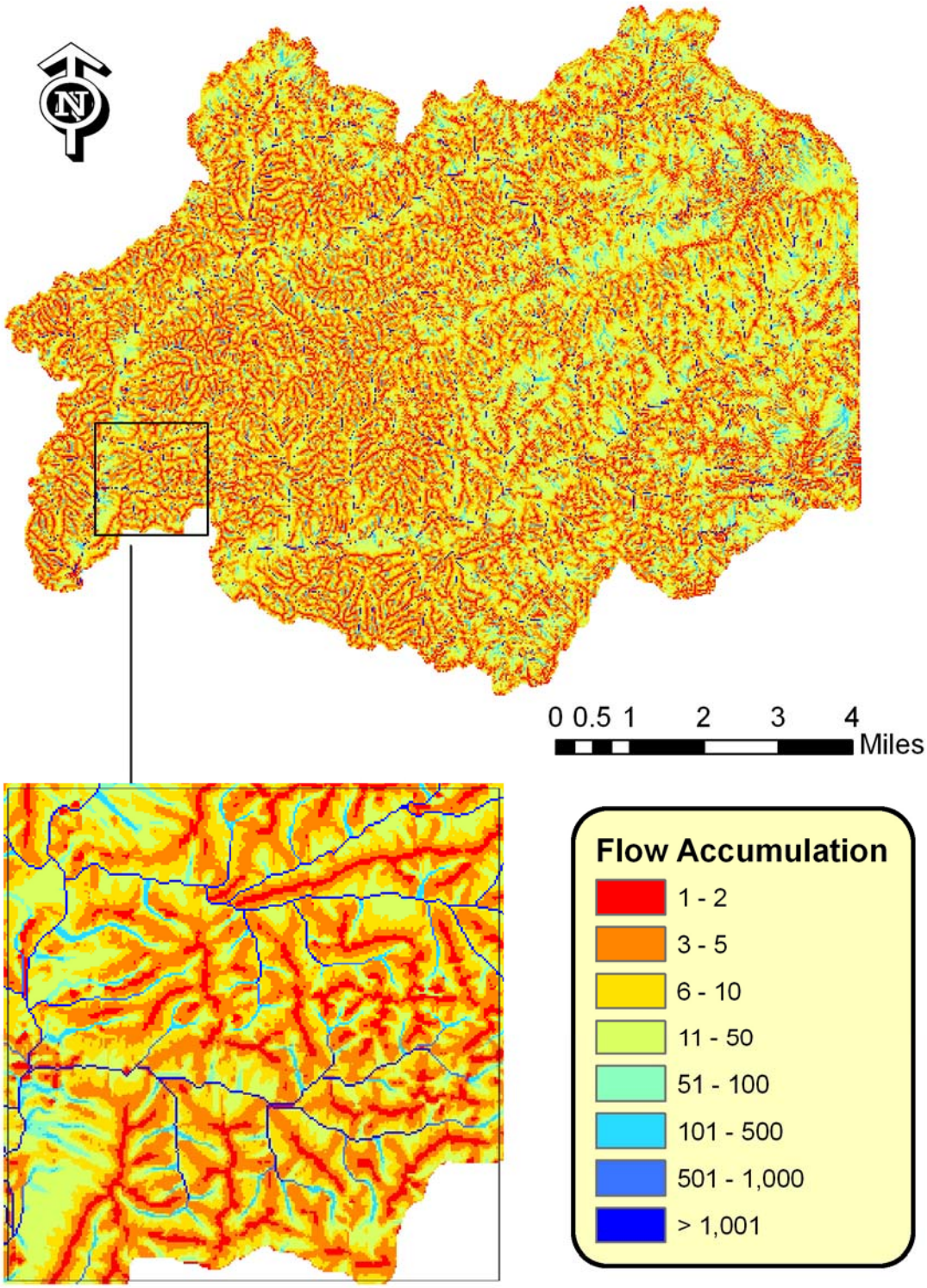


Figure 2.3.2 Flow accumulation in the study area using multi-path algorithm.

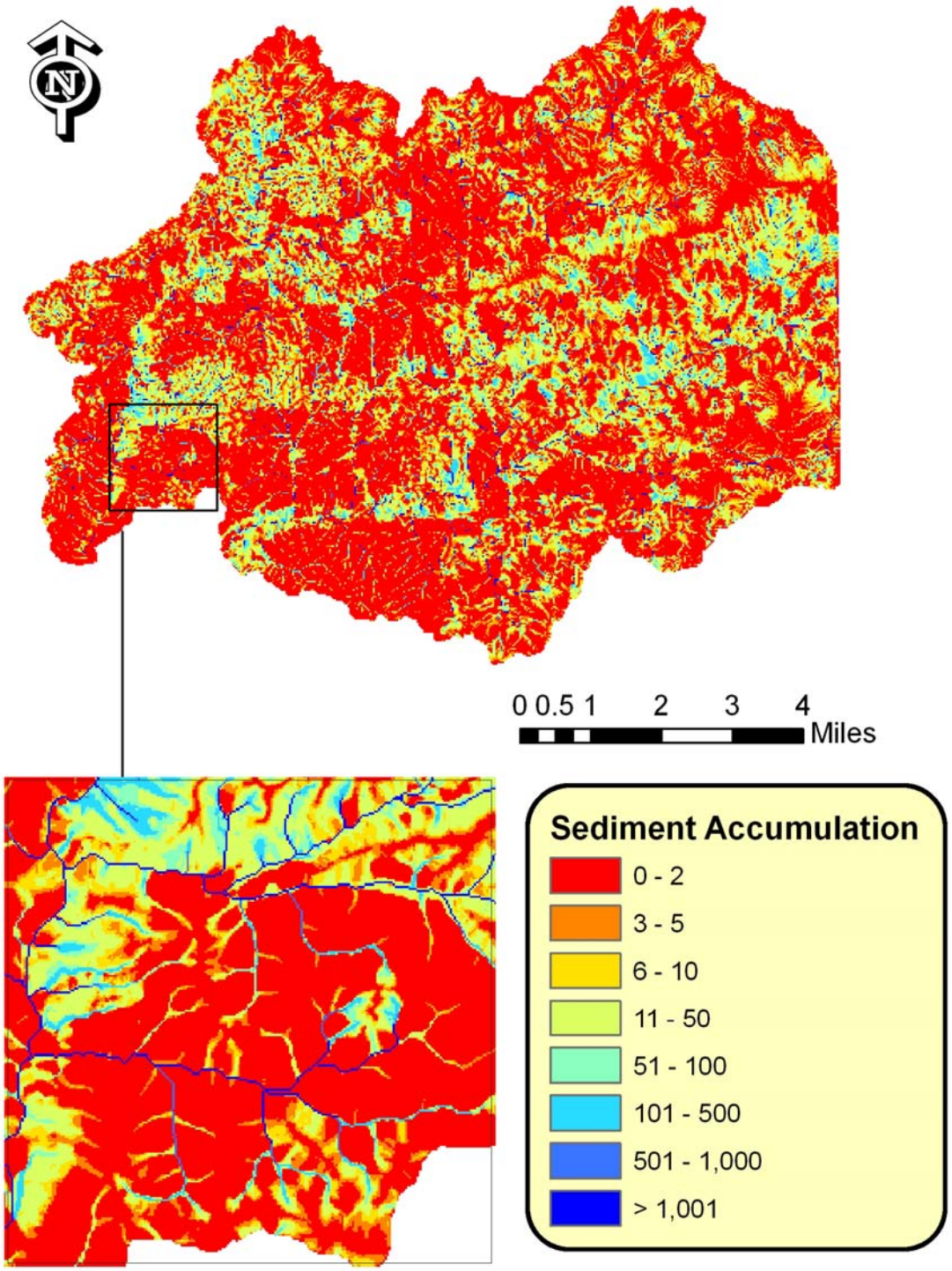


Figure 2.3.3 Sediment accumulation in the study area using multi-path algorithm and relative erosion as predicted by the Revised Universal Soil Loss Equation (RULSE). Does not account for sediment deposition.

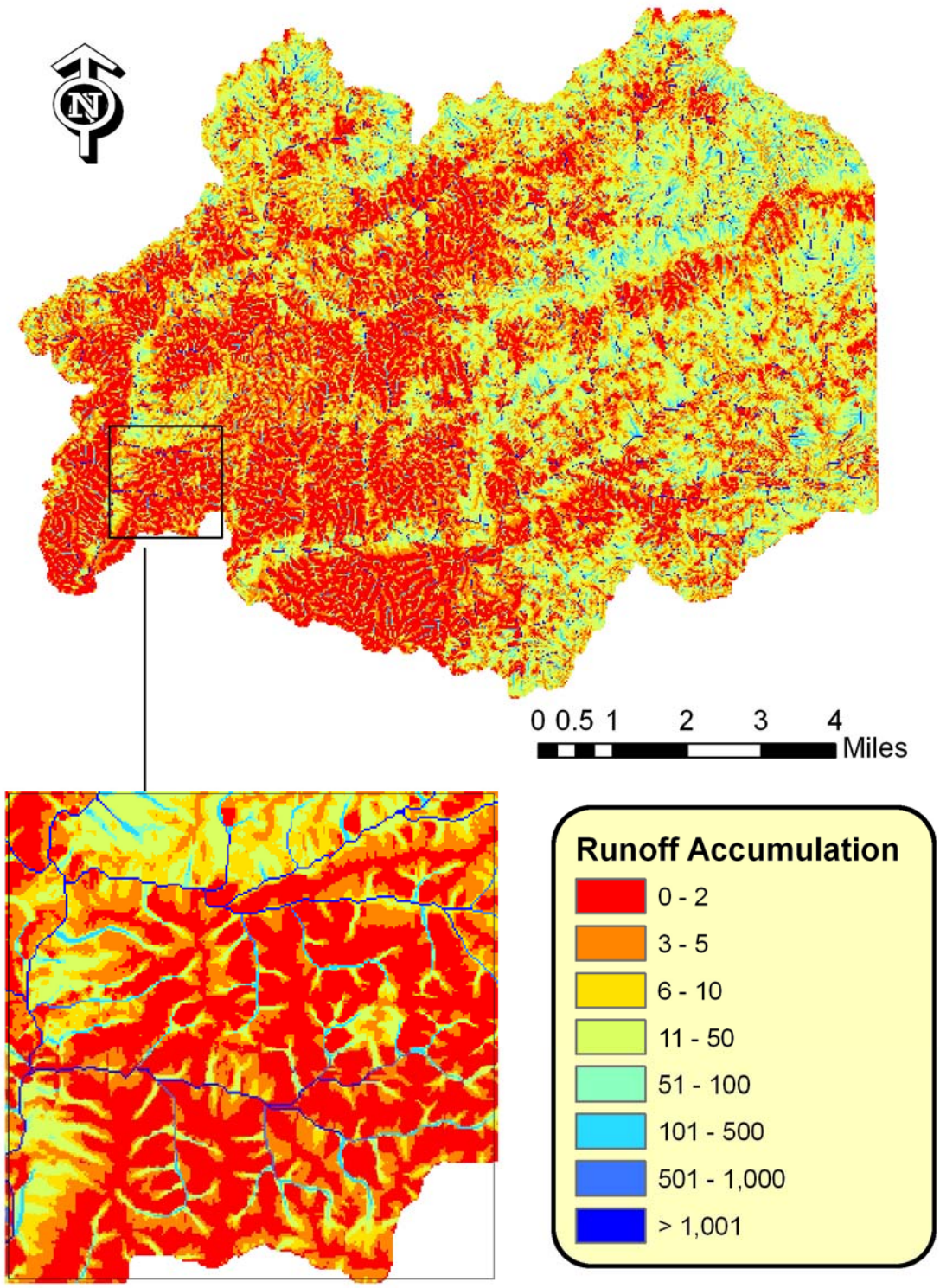


Figure 2.3.4 Runoff accumulation in the study area using multi-path algorithm and relative surface runoff as extrapolated from Soil and Water Assessment Tool (SWAT) predictions in neighboring Spavinaw Creek.

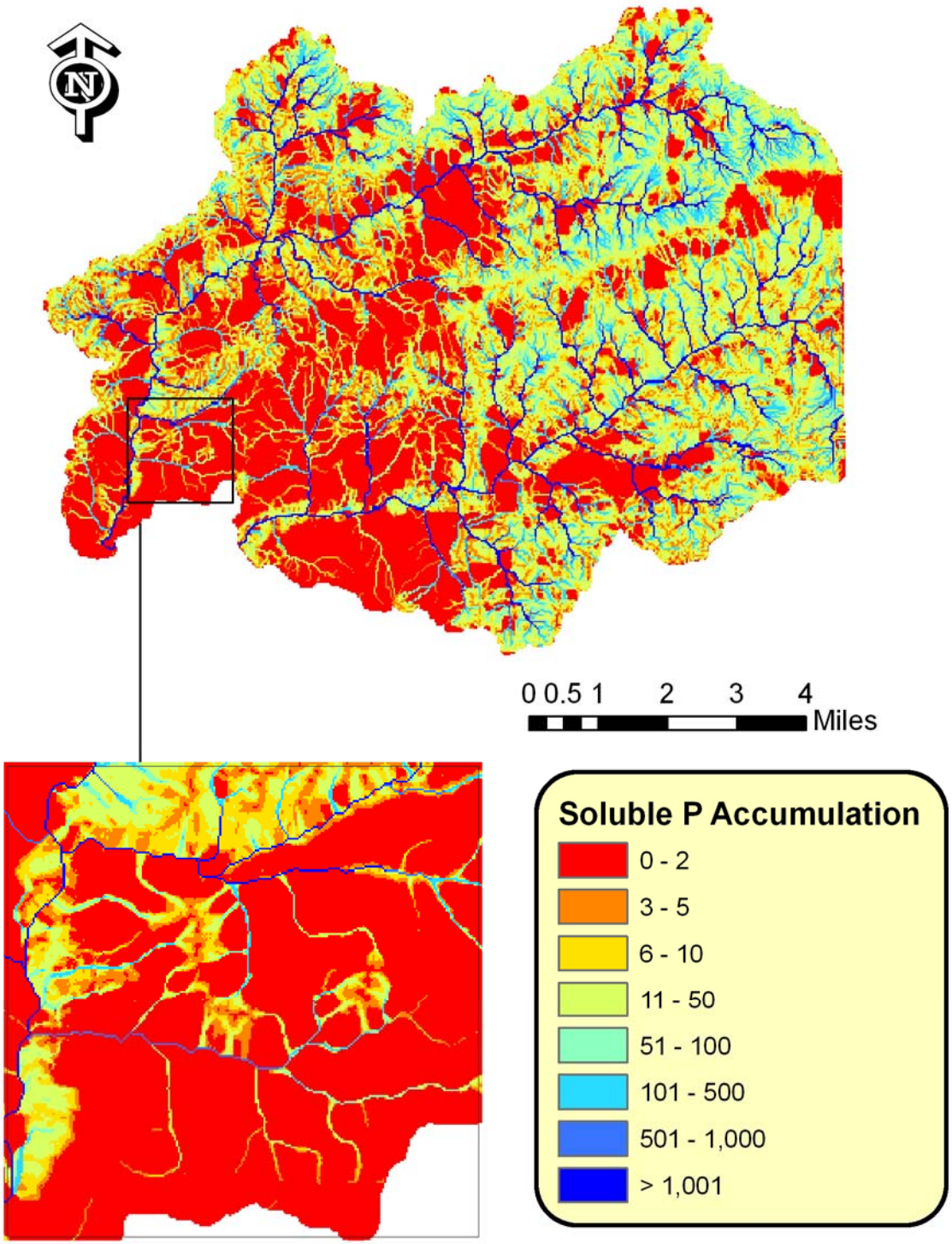


Figure 2.3.5 Soluble P accumulation in the study area using multi-path algorithm and relative soluble phosphorus yield as extrapolated from Soil and Water Assessment Tool (SWAT) predictions in neighboring Spavinaw Creek.

(2.4) Stream Shape and Sinuosity

Stream shape and sinuosity influences the distribution of erosive energy within the stream channel. Energy is concentrated on the outside of each meander resulting in an area of more active stream bank erosion known as a cut bank. A Point bar is an area of deposition occurring on the inside of each meander where flow velocity is reduced. As sediment is eroded from cut banks and deposited in point bars meanders may migrate outward or translocate in a downstream direction, consuming riparian area and reducing the available buffer.

Complex flow models can predict the migration of streams (Furbish 2001). For the purposes of targeting, it is not necessary to quantitatively estimate cut bank migration or stream bank erosion. In lieu of a model, we chose to identify stream segments with tight curvatures as potential sites with increased bank erosion and stream bank instability. In particular the outside of tight curvatures were considered a higher priority, and the inside of the curve was lower, and straight segments were neutral.

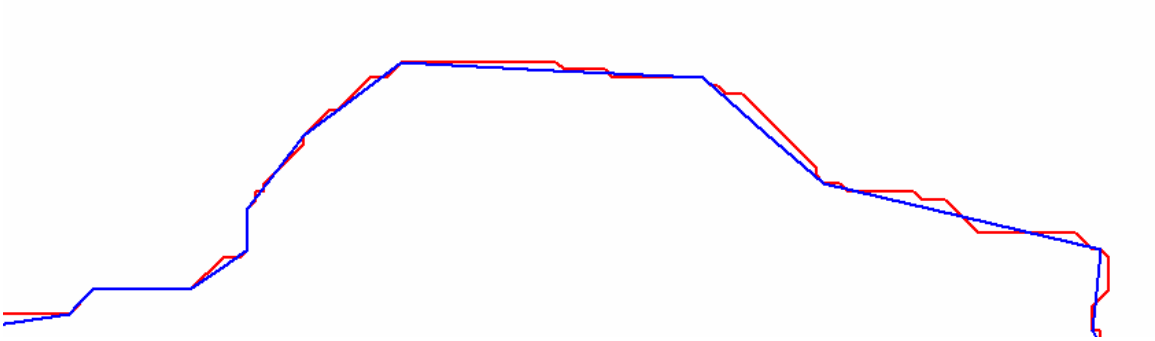
Data

Highly accurate stream GIS data were important. Streams were derived from the Digital Elevation model using flow accumulation. Streams were defined using a minimum contributing area of 50 ha to form a stream. We considered using the National Hydrography Dataset (NHD) (Figure 2.4.1) to define streams within the study area; however there were significant discrepancies between the two stream networks. Due to the extensive use of flow accumulations in this project, we elected to use streams derived from the DEM to ensure proper overlay between the curvature based and the flow accumulation based factors. This issue should be addressed in future projects. Visual inspections of smaller streams using aerial photography indicated errors in both NHD and DEM derived streams, the best dataset was not clear. NHD was created by the U.S. Environmental Protection Agency (EPA) and the U.S. Geological Survey (USGS) by combining USGS digital line graph (DLG) hydrography files and USEPA Reach File (RF3), and is not directly linked to elevation data, hence the discrepancies between datasets.

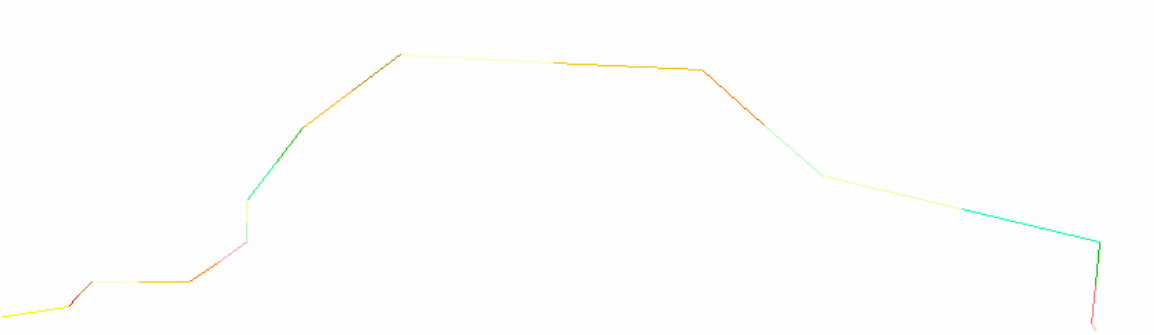
Processing

Suitable tools to identify curvature within GIS stream coverage were not currently available. The methods used to quantify curvature used in this project were rather crude. It was certainly possible to create better programs, but it would have required writing complex Avenue or Visual Basic for Applications software to directly report this information from the GIS. This was beyond the scope of this project, but may be necessary in future implementations of this methodology. The methods presented here were based on simplifying the stream network to straight segments, then estimating the angle between each segment and the two connecting segments based on buffer areas. The steps are listed below:

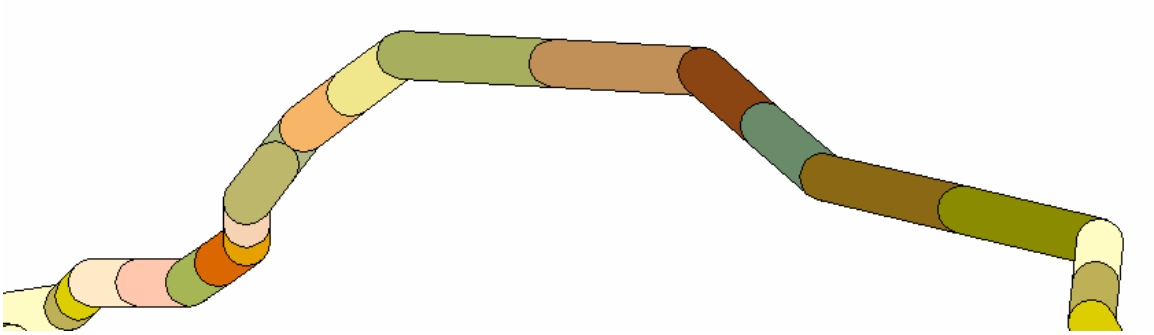
1) The stream network was generalized into straight segments using an Arcview 3.x user extension Point and Polyline tools V1.2 (Alsleben 2001) using a tolerance of 21 meters (~2 DEM grid cells). The complexity of the stream network was reduced as a requirement of this method. A visual comparison between the generalized and original data is given in Figure 2.4.1, and below:



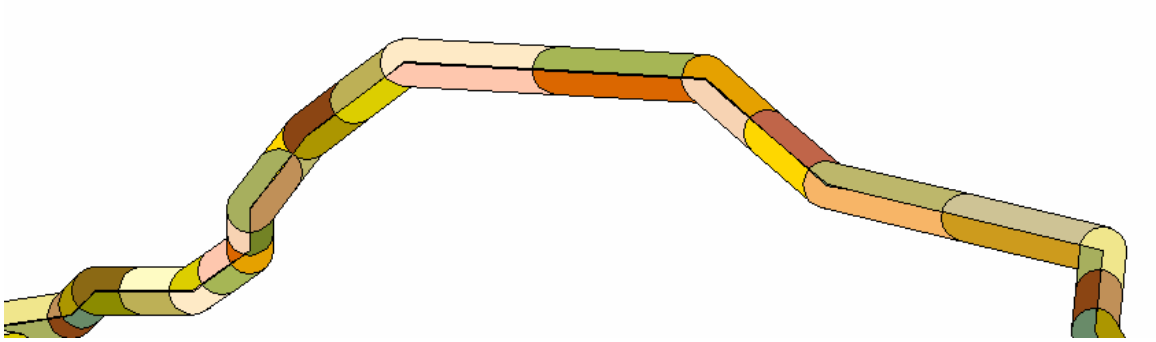
2) The generalized stream data were converted from a connected network to simple unconnected line segments using Point and Polyline tools V1. Each segment was again broken at it midpoint. See the example below, each color represents a separate entity.



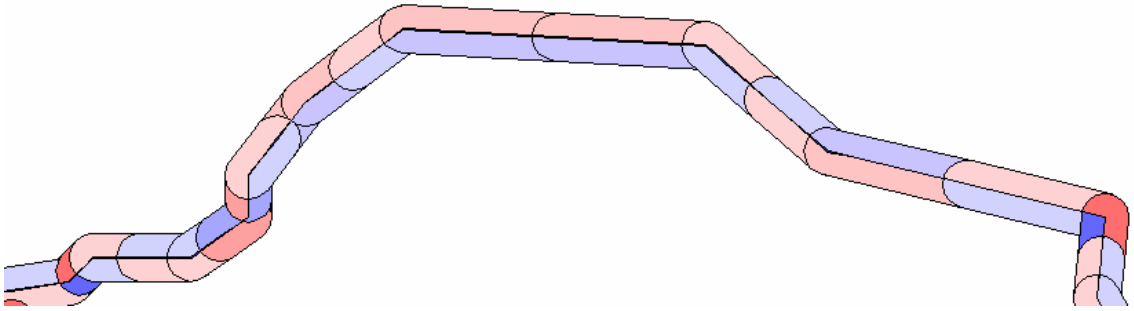
3) The length of each individual segment was added to the table, and each segment was buffered by 50 meters. Each buffer was an independent polygon. The attribute information from the original segment was retained in the buffers attribute table.



4) The buffered segments were split by the stream network to create separate buffers on each side of the stream for each segment. The area of each buffer was calculated.

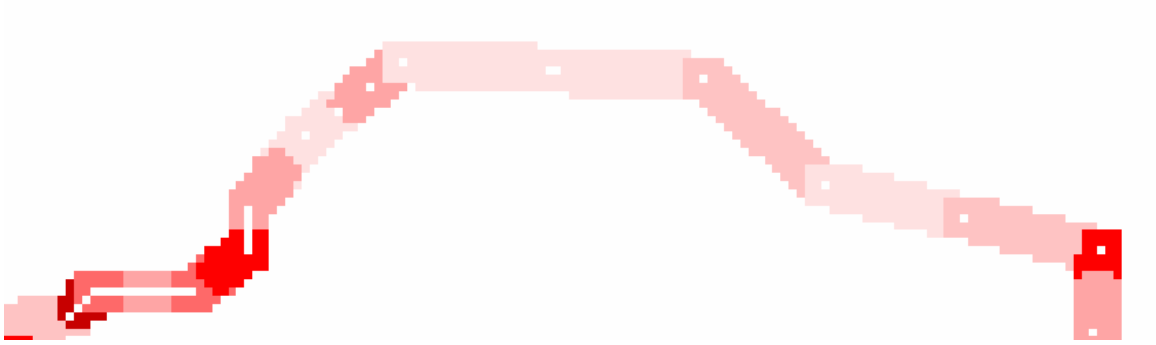


5) Differences in area between buffers on each side of a line segment were used to calculate the amount of bend in degrees in each segment. Angle was normalized by segment length for units of degrees per meter of stream length. Red areas in the image below show areas with greater potential for stream bank instability.



6) Finally the vector data were converted to a grid with the same resolution as the original DEM. The final map is shown in Figure 2.4.2.

7) Because of the generalization, there can be up to a 21 meter displacement between the generalized stream location and the flow accumulation stream. To reduce overlay errors we used only the absolute value of the curvature.



The method presented here was done without software development using freely available ArcView extensions and scripts, but has limitations in both scope and resolution. It required simplifying the original GIS data into straight segments, which resulted in the loss of data detail. Because this method relied on paired buffers, it did not work at stream intersections. The method resulted in many overlapping polygons. There were not suitable tools available in ArcView to properly resolve these overlapping areas into grids. Software packages, such as ArcGIS, can be extended via custom software to properly identify curvature in the original curvilinear GIS data. We recommend this approach for future projects.

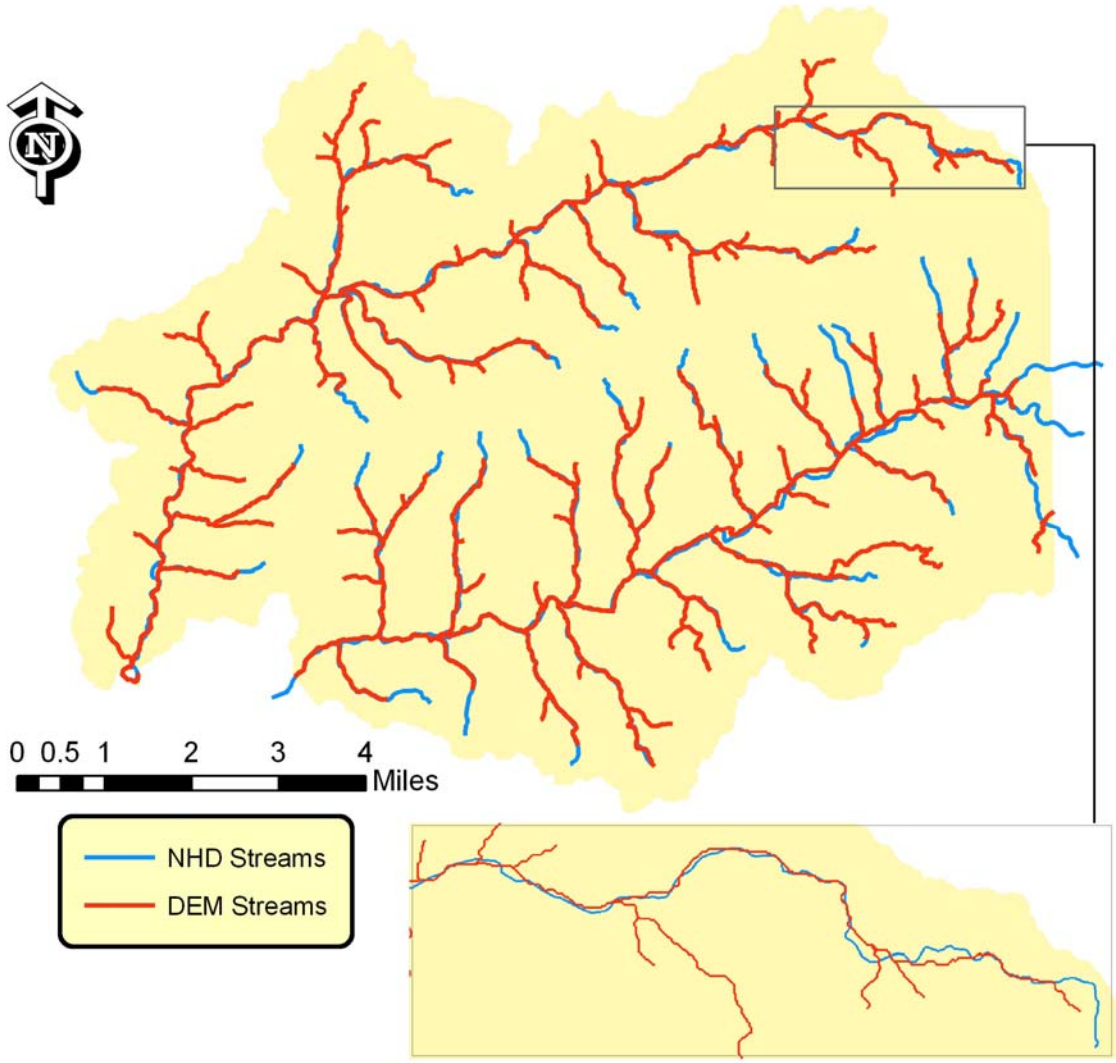


Figure 2.4.1 National Hydrography Dataset (NHD) and Digital Elevation Model (DEM) derived streams.

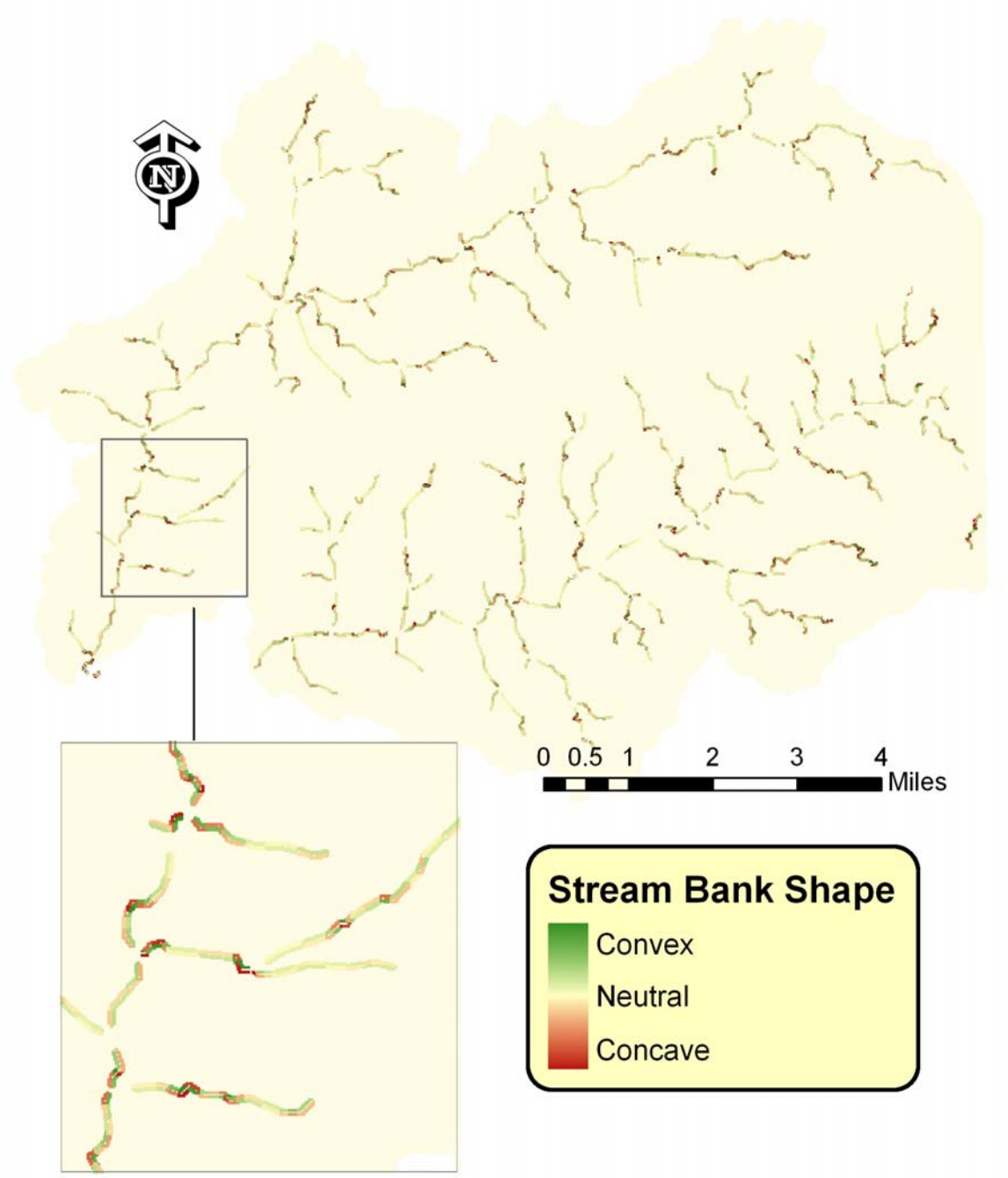


Figure 2.4.2 Curvature by stream segment within the study area. Higher stream bank erosion potential in concave sections (cut banks). Deposition likely in convex point bars.

(2.5) Stream Order and Gradient

Stream order is a simple method of stream classification based on the number of upstream tributaries. There are several methods of used to calculate stream order. We chose to use Strahler (1952). According to this method, a stream with no tributaries is a first order stream. The confluence of two first order streams forms a second order stream. A third order stream is formed by the confluence of two second order streams. Stream order increases in a downstream direction with drainage area. Stream characteristics are generally correlated with stream order in a given basin. Stream orders in the study area are given in Figure 2.5.1.

Stream gradient was estimated using the watershed delineation functions of the SWAT model (Figure 2.5.2). Stream gradient was highly correlated with stream order. Gradient deceased in a down stream direction with a typical concave line (Figure 2.5.3). Other stream characteristics such as drainage area, channel width and depth, flow velocity, bed grain size, stored sediment, and discharge were also correlated with stream order. These other characteristics were not measured in this study, but the general relationships are well documented (Stream Corridor Restoration: Principles, Processes, and Practices, (1998)). The general relationships are given in Figure 2.5.4.

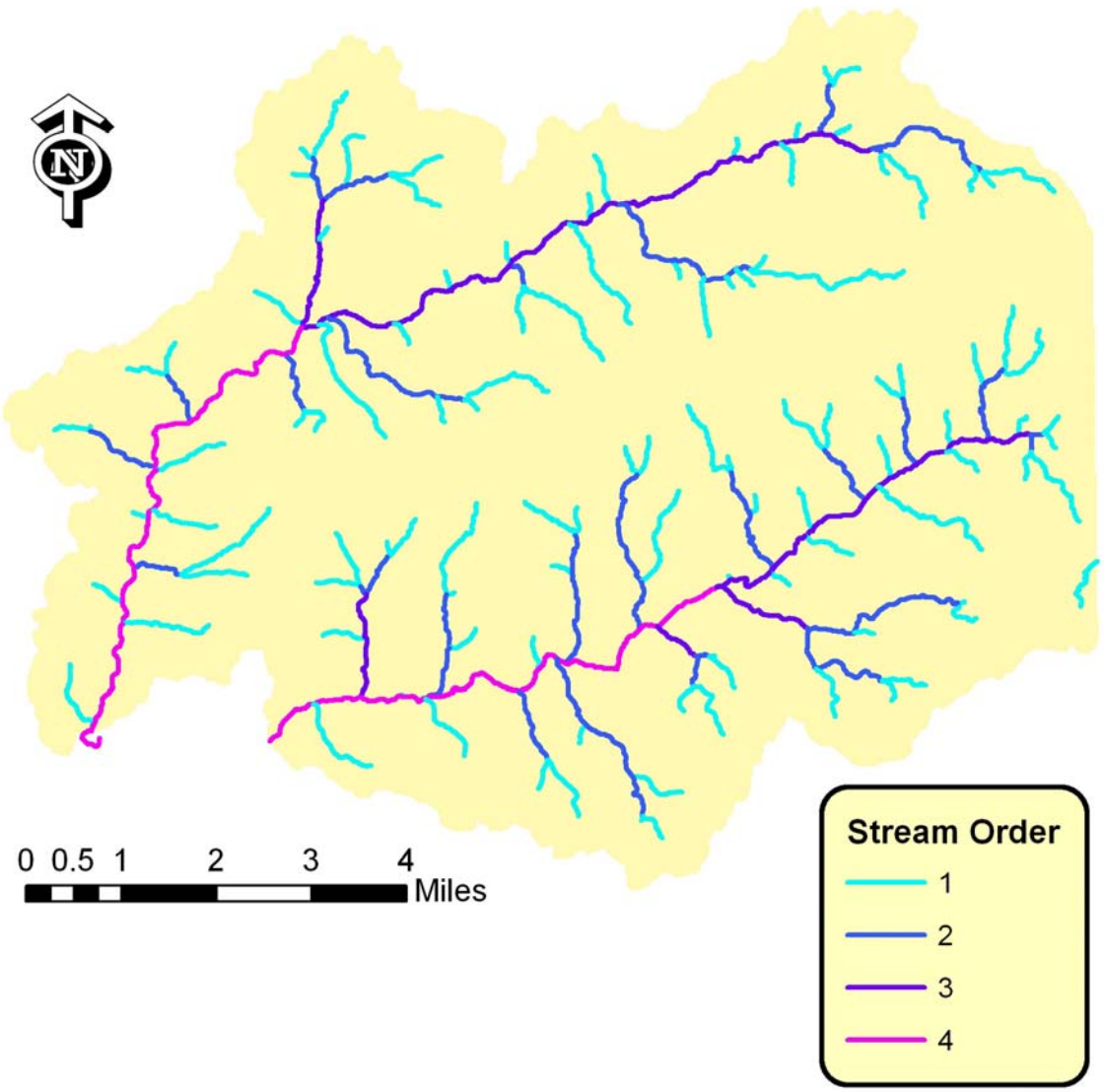


Figure 2.5.1 Straher (1952) stream order in the study area.

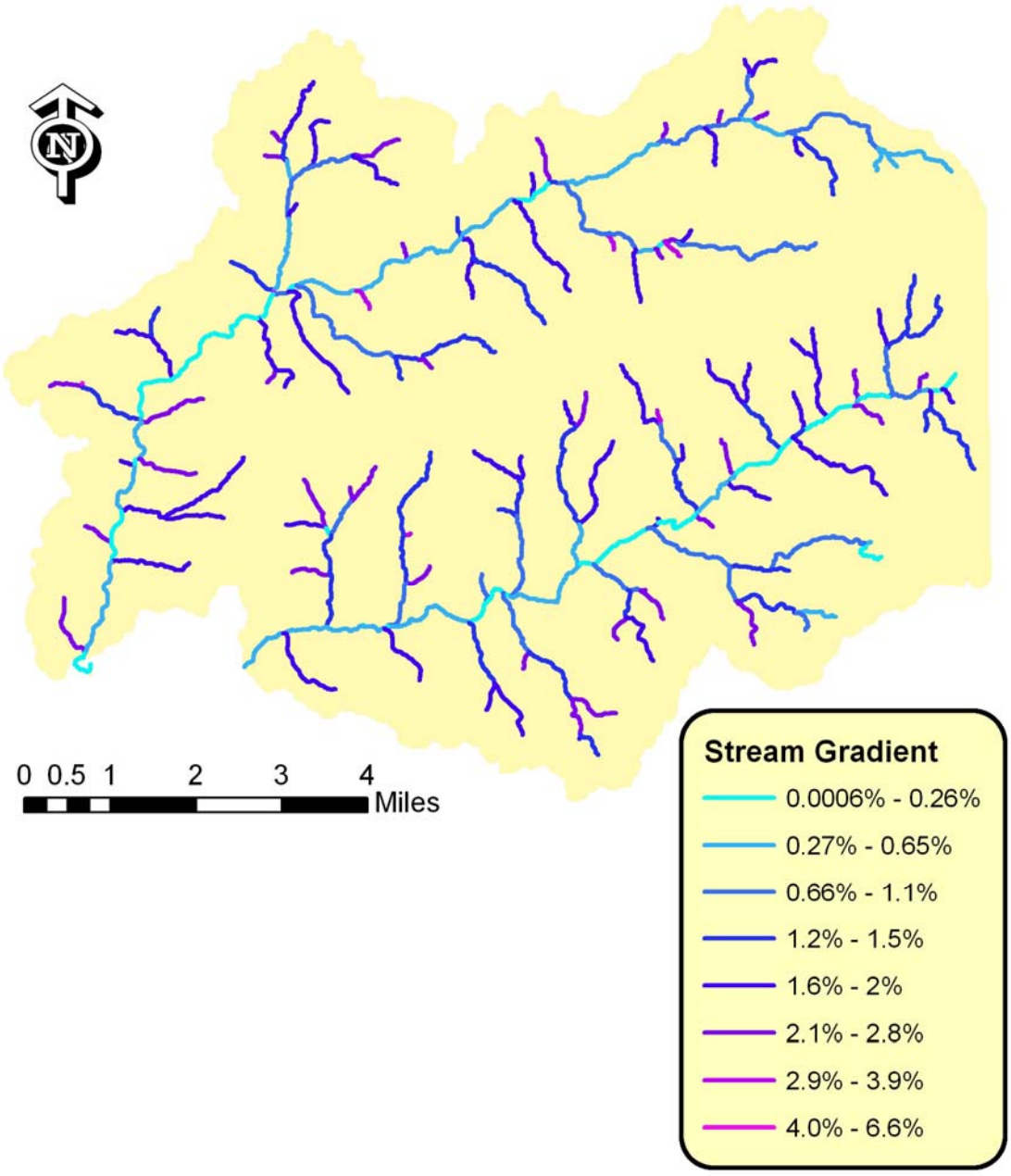


Figure 2.5.2 Soil and Water Assessment Tool (SWAT) estimated stream gradient in the study area.

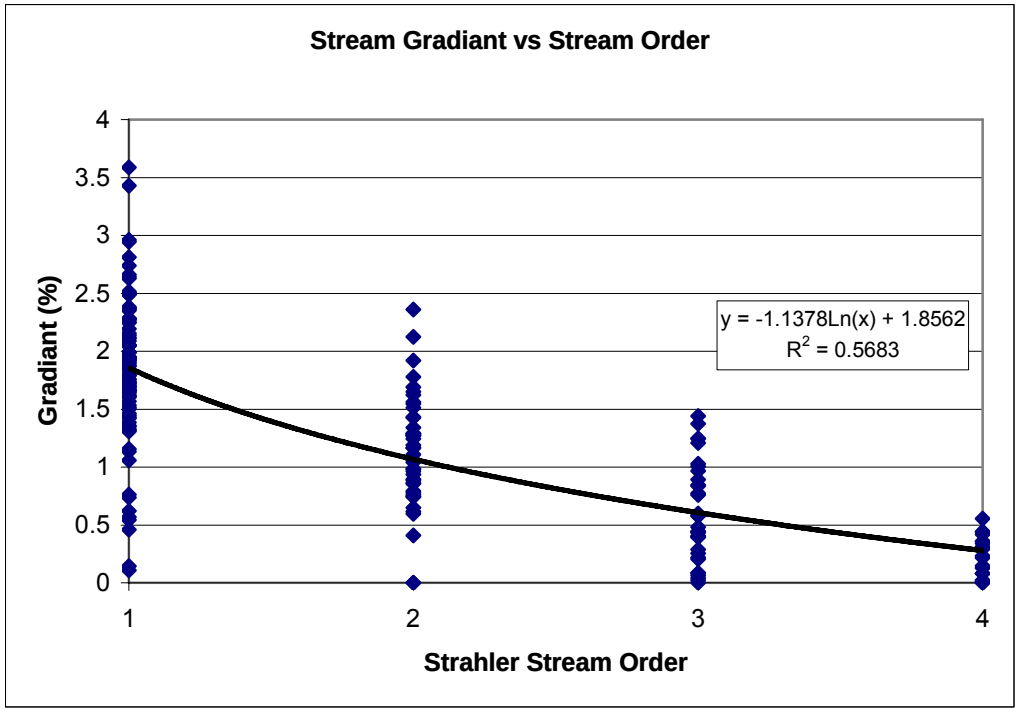


Figure 2.5.3 Relationship between stream order and stream gradient for segments longer than 200 meters within the study area.

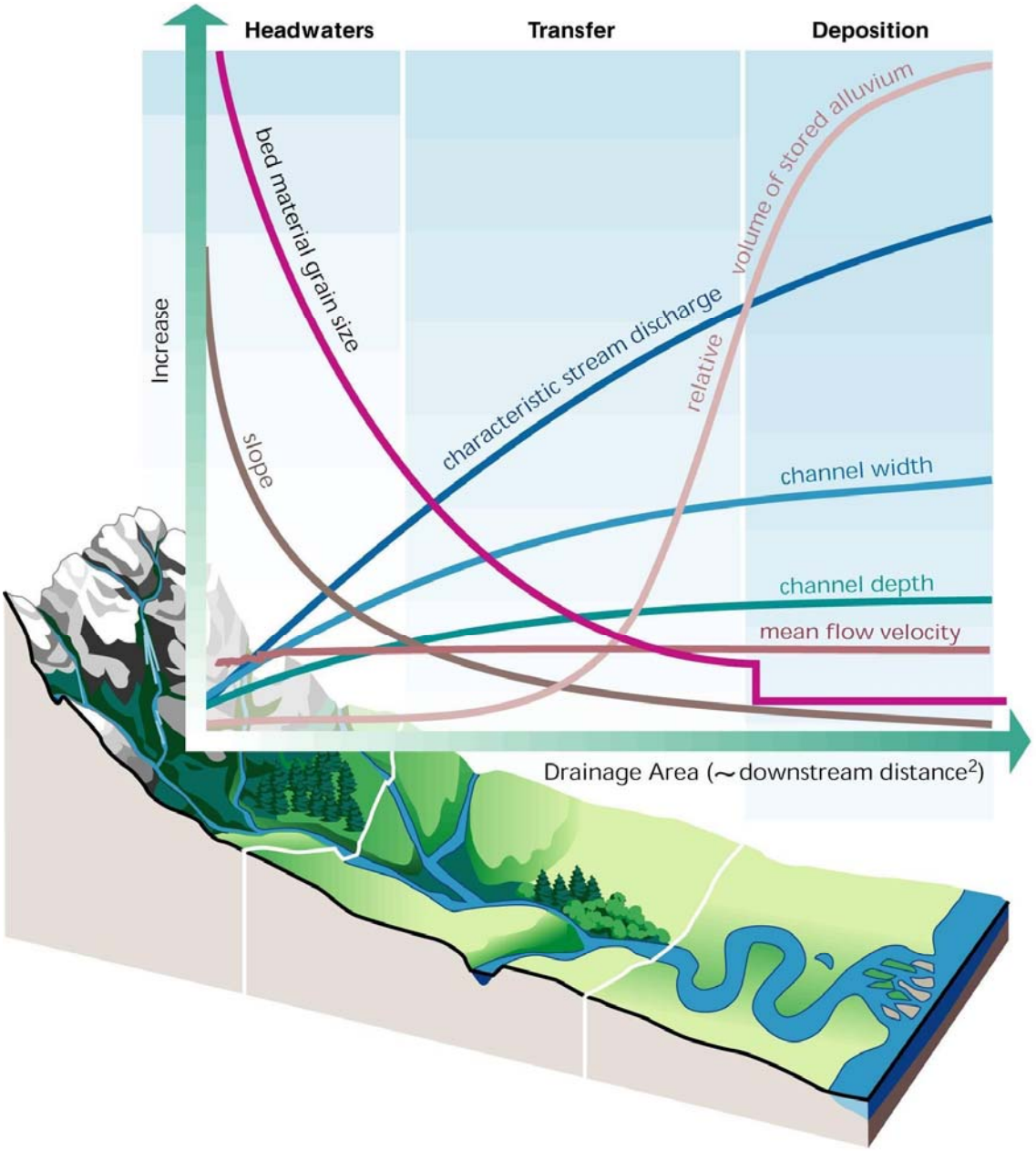


Figure 2.5.4 Generalized relationships by drainage area. Reproduced with permission (Stream Corridor Restoration: Principles, Processes, and Practices, (1998)).

(2.6) Riparian Targeting

Buffer and Mask development

Only a small fraction of the basin was considered for riparian targeting. We applied a buffer of 50 meters to each side of streams with a drainage area of at least 50 ha. It is unlikely that perennial streams would have a smaller drainage area unless spring fed, which is possible given the karst topography. Only areas within this 50 meter buffer were considered.

Additional areas within the buffer were excluded based on the runoff accumulation grid. Accumulations produce exceedingly high values in cells with channelized flow. This includes both the stream channel on which the 50 meter buffer was based and channelized flow from areas with drainage less that the 50 ha required to form a stream. Cells which contain channelized flow were not suitable for riparian buffers, which function with sheet flow only. Channelized flow short circuits the buffer, delivering the flow directly to the stream. The amount of runoff accumulation required to produce flow which was sufficiently channelized to short circuit riparian buffers was not clear. In future projects, this should be determined by field examination of sites with various levels of runoff accumulation. The focus of this project was to explore and define methodology, which can be refined with field data when applied. For this reason a runoff accumulation cutoff of 1000 was selected based on visual inspection of the GIS data and professional judgment. Because the runoff accumulation grid was normalized to an average value of 1 before the accumulation, a value of 1000 is roughly equal to the runoff produced from 1000 average cells or about 10 ha. The actual area will be larger in low runoff producing areas such as forest and smaller in higher runoff producing areas. The final buffer which was used to mask all data layers is given in Figure 2.6.1.

To perform targeting, we must consider whether the goal was restoration of degraded riparian zones or protection of existing forested ones. Both can be done using the same data by examining the current landcover in conjunction with the factors detailed in previous sections. Landcover was reclassified into a boolean grid based on the quality of the existing landcover with respect to riparian buffers, for use as a mask. Landcovers having a positive benefit in riparian zones were reclassified as true, all others were considered false. The reclassification scheme is shown in Table 2.6.1 and mapped in Figure 2.6.2. These data were used to assess the current state of riparian zones within the area of study.

Targeting

The final targeting maps were a compilation many factors. Each of the following factors is an indicator of riparian effectiveness or characteristics:

1. Landcover within riparian zone (Boolean) (LC)
2. Erosion predicted in riparian zone (ER)

3. Erosion accumulation from adjacent areas (EA)
4. Runoff accumulation from adjacent areas (RA)
5. Soluble phosphorus accumulation from adjacent areas (PA)
6. Stream curvature (SC)
7. Stream order (categorical)
8. Drainage area (DA)
9. Stream gradient (SG)
10. Buffer slope (BS)

The final targeting map was calculated as:

$$TI = LC\ (W_{ER}*ER + W_{EA}*EA + W_{RA}*RA + W_{PA}*PA + W_{SC}*SC + W_{DA}*DA + W_{SG}*SG + W_{BS}*BS)$$

where TI is the targeting index, W_X is the respective weighting factor for factor X, and factor abbreviations were defined in the list above.

Because these data had different means and distributions, factors were not directly comparable. Without comparable factors it was very difficult to define appropriate weights. To make these factors more comparable, each was transformed and normalized. No single parametric transformation could be applied to all factors to generate similar distributions. Similar distributions were required to make factors directly comparable. Nonparametric statistical tests avoid assumptions of data distribution by ranking data; a similar approach was applied here. After clipping each parameter grid to the 50 meter buffer mask, these data were broken into 20 quantiles using Arcview. Each quantile had roughly the same number of cells, 5% of the buffer area. The 20 quantiles were reclassified from 0 to 1 in 0.05 increments; the 0.95-1 quantile contained the highest valued original parameter cells. The resulting range was defined from 0-1 and the distribution was uniform with a mean of 0.5. The transformed and normalized data are given in Figures 2.6.3 to 2.6.10.

Weighting factors determined the relative importance of each factor in the final targeting map. Some data such as stream order were categorical and could not be directly used in the targeting. However, stream order is highly corrected with the drainage area factor and thus was indirectly accounted for in the final targeting. How each factor contributes to the effectiveness of a proposed or existing forested riparian buffer cannot be easily quantified. Without additional data the weighting factors can only be subjective estimated, based on professional judgment and a general understanding of how riparian buffers function. Each factor was selected and calculated to be an indicator of riparian functionality, however some factors were likely to be more important that others. Several factors were correlated with other factors, indicating that both contain at least in part, the same information. Correlation between factors must be considered when setting weighting factors. Correlation between factor grids is given in Table 2.6.2. As expected, factors based on flow accumulation are highly correlated (>0.81). The curvature factor was poorly correlated with any other factor, indicating that the information it contains is unique among all factors.

The intended benefit of the riparian buffer must also be considered. There are many pollutants, such as nitrogen, phosphorus, pesticides, metals, and sediment, which are controlled to varying degrees by riparian buffers. Riparian buffers also impact stream temperature, woody debris content, and wildlife populations. Although these were not considered in this research, other factors could be included based on landscape metrics, such as connectivity and diversity, which were correlated with habitat quality.

To better grasp the importance of each factor, field data are needed. Site visits could verify the utility of flow accumulation based factors. Although the removal of nutrients cannot be directly observed without expensive and elaborate field studies, areas of sediment deposition, stream bank instability, and channelization are visible. Although not quantitative, these data are still useful. Other data such as stream gradient and buffer slope can be easily verified. Unfortunately, field data were not collected in the study area to verify factors or to provide guidance determining factor weights. For this reason factor weights were not estimated, and assumed to be uniform.

Targeting maps were developed assuming all weighing factors to be 1.0 with the exception of buffer slope which was assumed to be -1.0. Buffer slope was known to have an inverse impact on trapping efficiency. Degraded riparian areas wee targeted and presented in Figure 2.6.10. Intact riparian buffers which were targeted for preservation are given in Figure 2.6.11.

Classification

Methods developed for remote sensing applications were adapted for use with these data. Image classification is the classification of an image consisting of several bands of correlated information. Each band is a measurement of the reflected radiant energy within a narrow band of frequencies. A natural color image is comprised of three bands: red, green, and blue. Image classifiers seek to identify surface features based on patterns within these bands. This pattern is the spectral signature. Many features, such as a particular landcover, have unique spectral signatures that can be used to identify all pixels of that landcover within an entire image. Signatures are developed by examining pixels at several locations within an image known to be the feature of interest. Our factors can be thought of as bands. If field surveys of the study area could determine several examples of highly effective riparian buffers, we can locate similar areas for restoration or preservation by developing the signature of an efficient buffer in the available factors, and locating that pattern elsewhere in the study area.

Signature development requires knowledge of existing features or in this case riparian effectiveness. Although we did not have ground truth data characterizing riparian buffers, the factor grids were calculated as indicators of riparian effectiveness. There were methods to classify data without predefined signatures called unsupervised classifiers. These methods identify groups of

pixels with similar characteristics, without any knowledge of exactly what features each group represents. One such method called isoclustering, or iterative optimization clustering procedure, was used to define categories within the study area with similar riparian characteristics as defined by our factors. ArcMap had an isoclustering component, but we were unable to include more than three bands using this software. To simplify these data into three bands, principal components analysis was used. Principle components analysis is a procedure to reduce the number of bands or dimensions of a dataset to facilitate further analysis while preserving the information present within the original data. Eight factors were reduced to three bands, shown in Figure 2.6.12. In this figure, bands were shown in primary colors; areas with similar factors had similar colors. These three bands were processed using isodata clustering to generate 30 categories (Figure 2.6.13). The mean factor values for each class are given in Table 2.6.3. Ideally ground truth would be used to identify which categories best identify riparian buffer suitability.

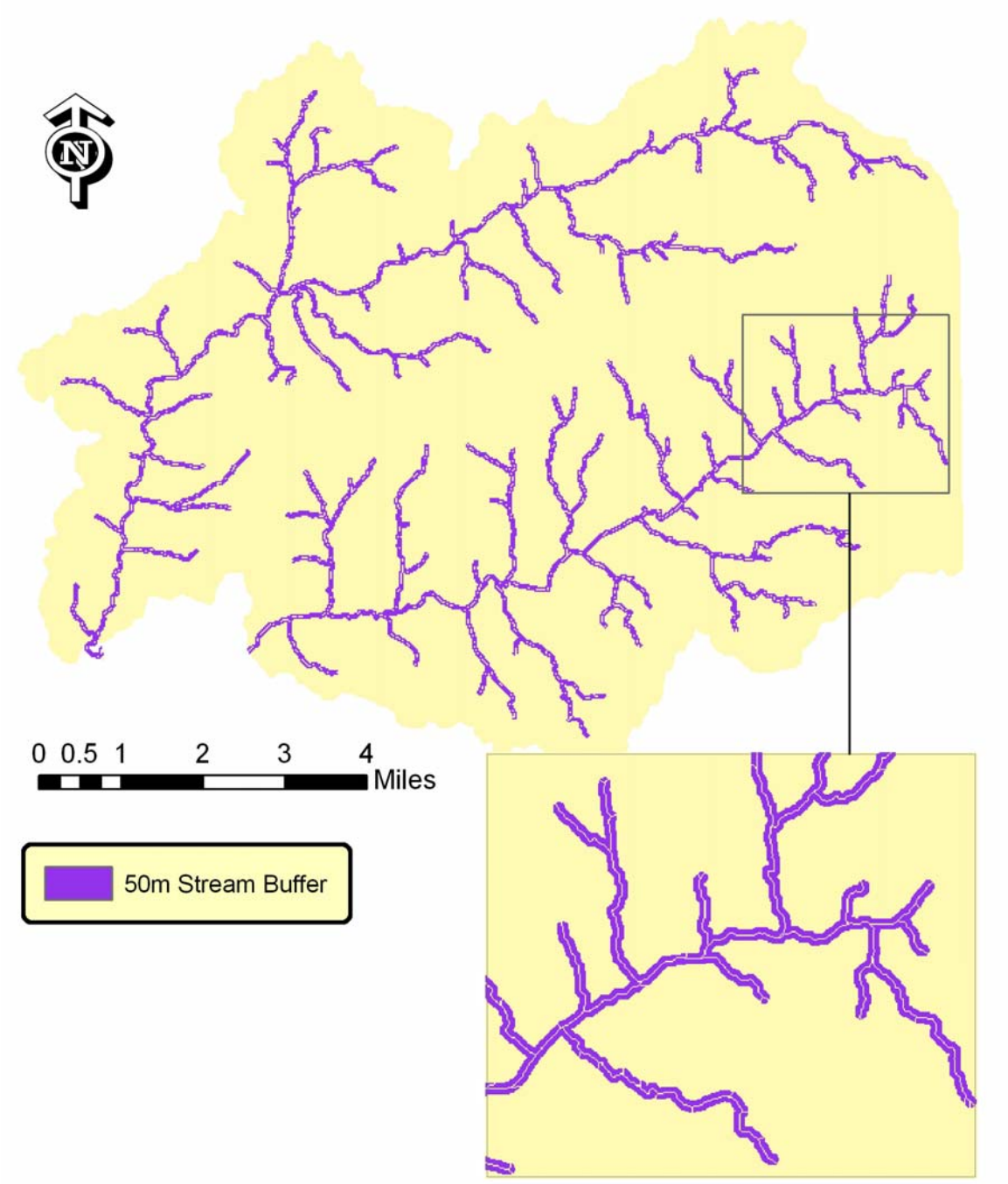


Figure 2.6.1 Fifty meter stream buffer for streams within the study area. Cells with highly concentrated flow were removed from the buffer area.

Table 2.6.1 Landcover reclassification for riparian quality.

Landcover	Riparian Boolean Grid
High Biomass Pasture	False
Low Biomass Pasture	False
Brushy Rangeland	True
Urban	False
Wheat/beans	False
Forest	True
Bare	False
Water	True
Stream	True

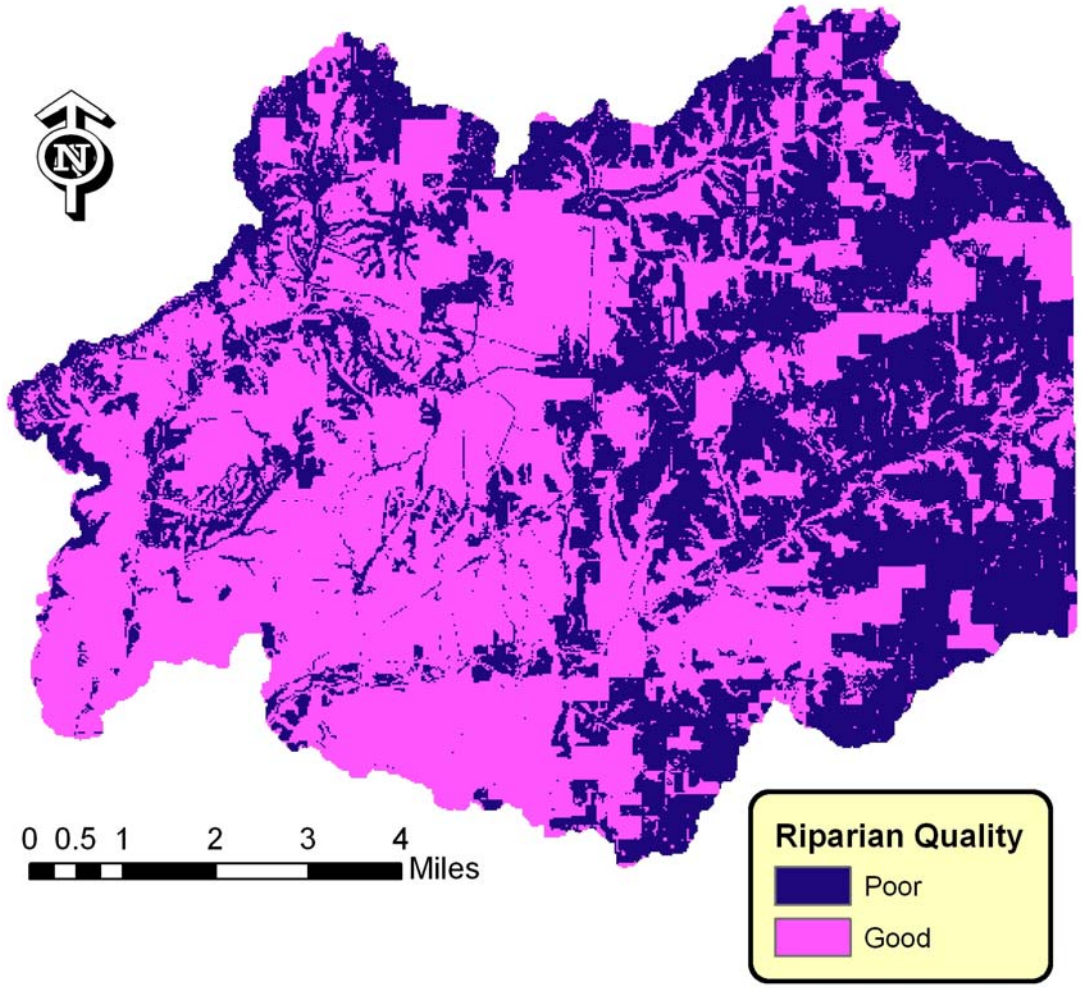


Figure 2.6.2 Landcover reclassified using Table 2.6.1. Good indicates landcovers such as forest, which are desirable in riparian zones.

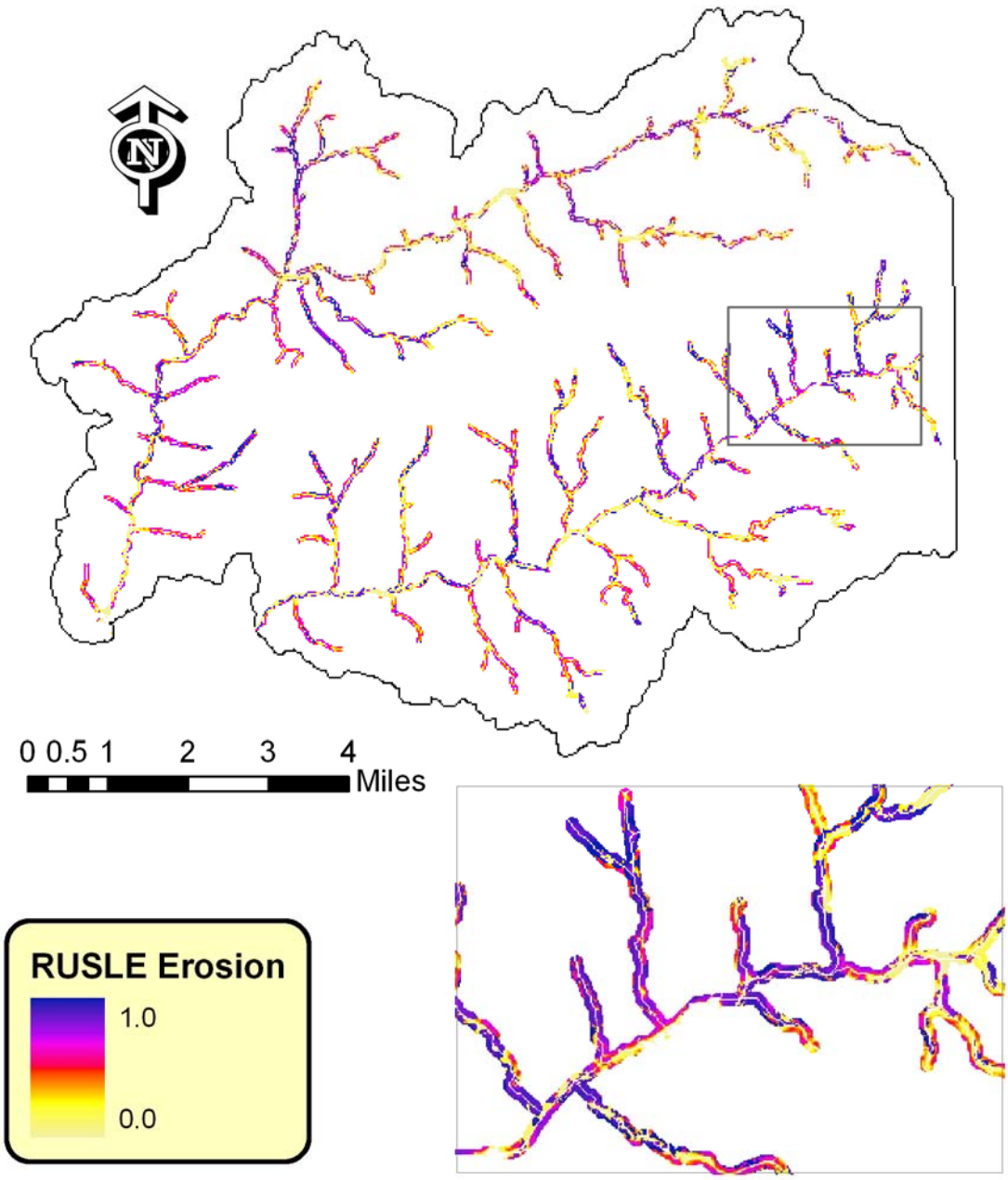


Figure 2.6.3 Normalized RUSLE erosion based index for the study area within a 50 meter stream buffer.

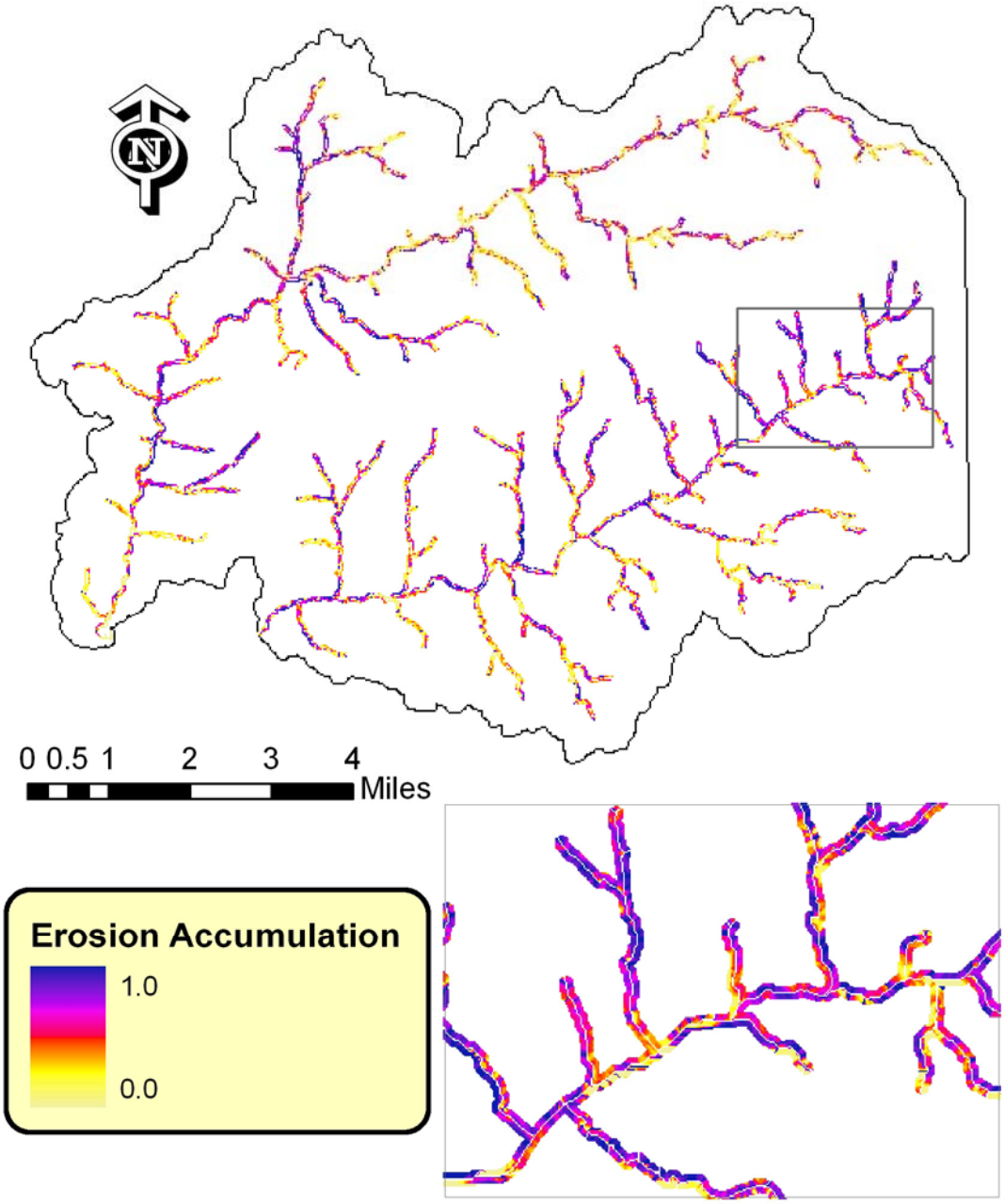


Figure 2.6.4 Normalized RUSLE erosion accumulation from adjacent areas index for the study area within a 50 meter stream buffer.

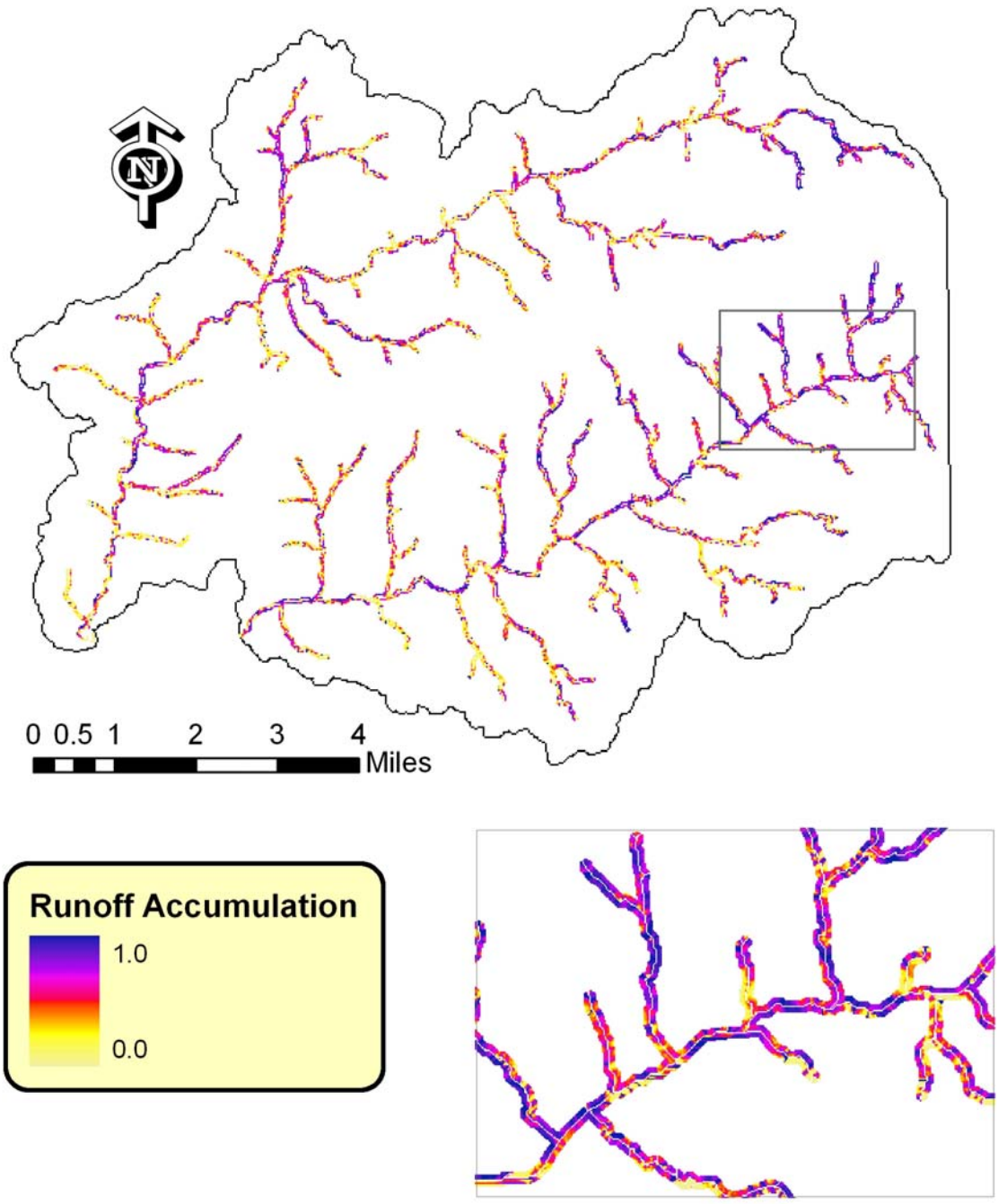


Figure 2.6.5 Normalized runoff accumulation from adjacent areas index for the study area within a 50 meter stream buffer.

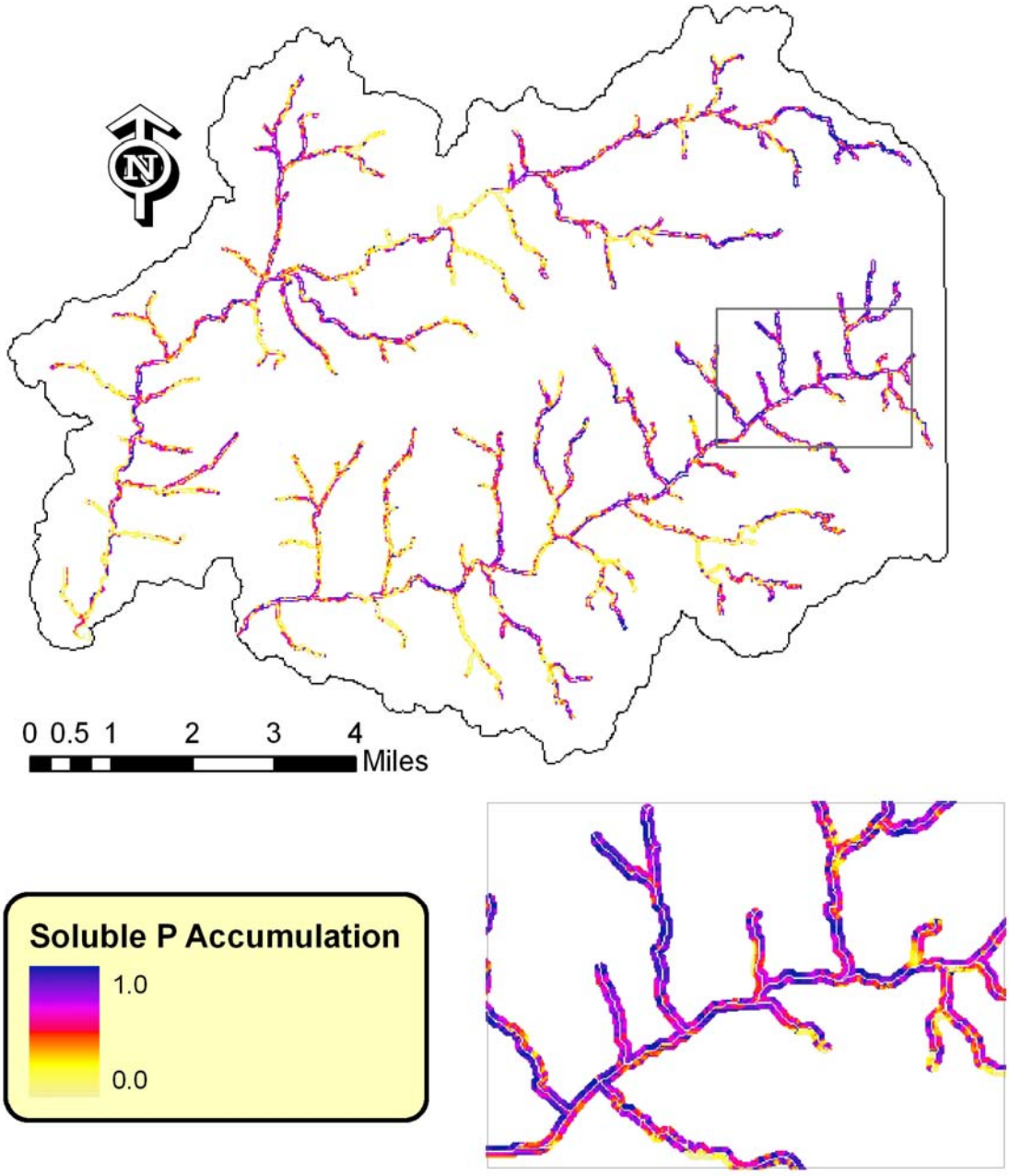


Figure 2.6.6 Normalized soluble phosphorus accumulation from adjacent areas index for the study area within a 50 meter stream buffer.

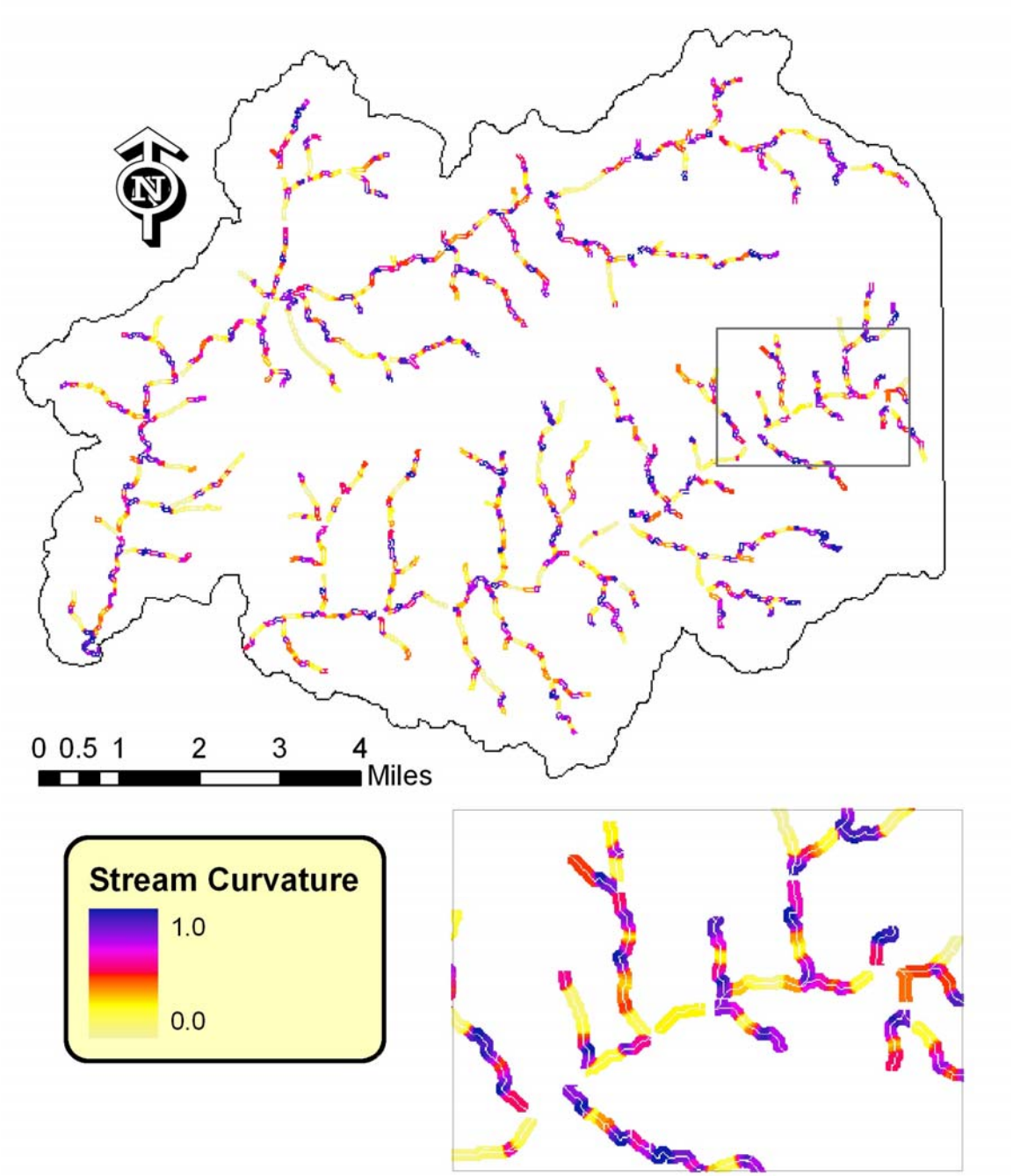


Figure 2.6.7 Normalized stream curvature based index for the study area within a 50 meter stream buffer.

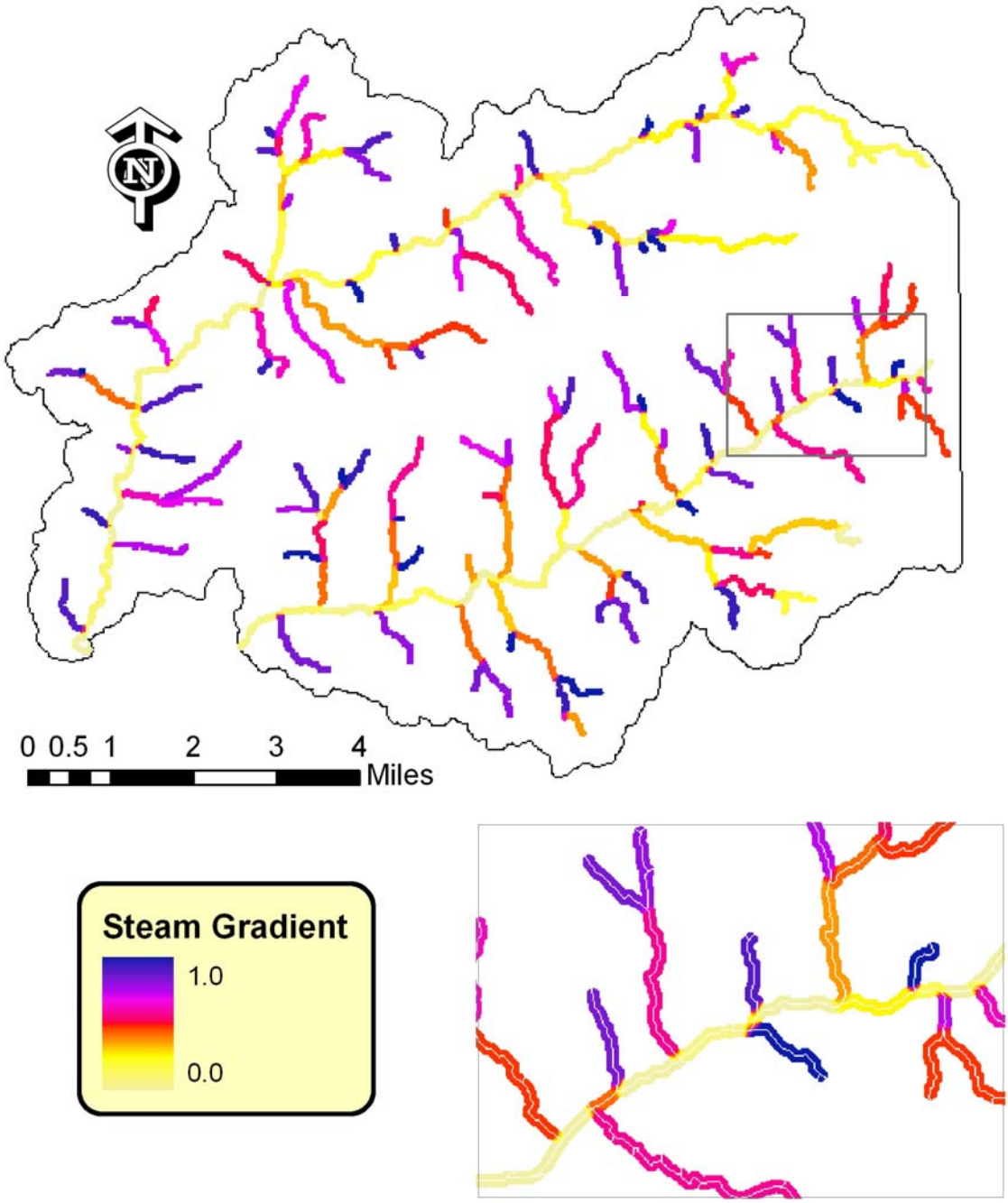


Figure 2.6.8 Normalized stream gradient based index for the study area within a 50 meter stream buffer.

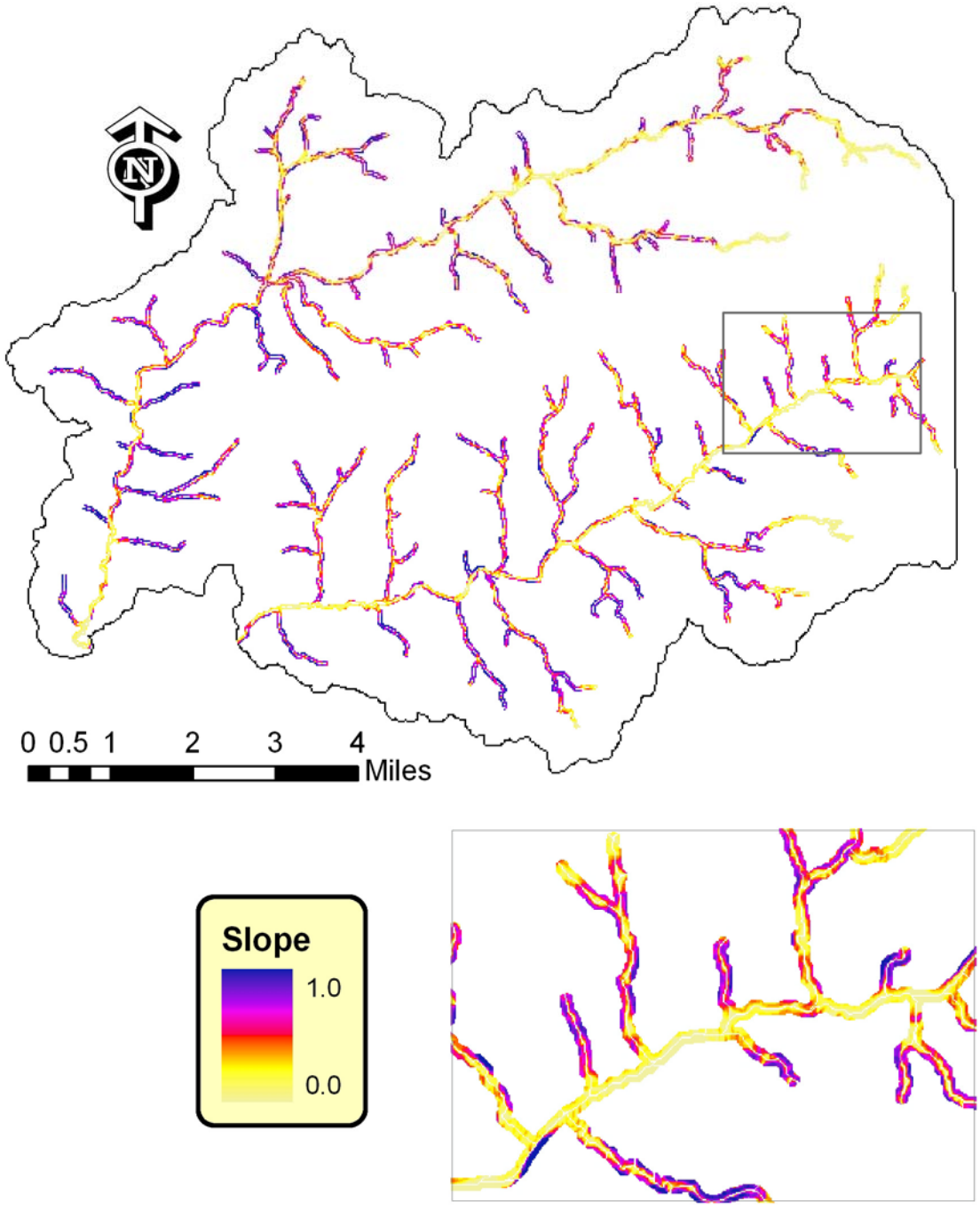


Figure 2.6.9 Normalized slope based index for the study area within a 50 meter stream buffer.

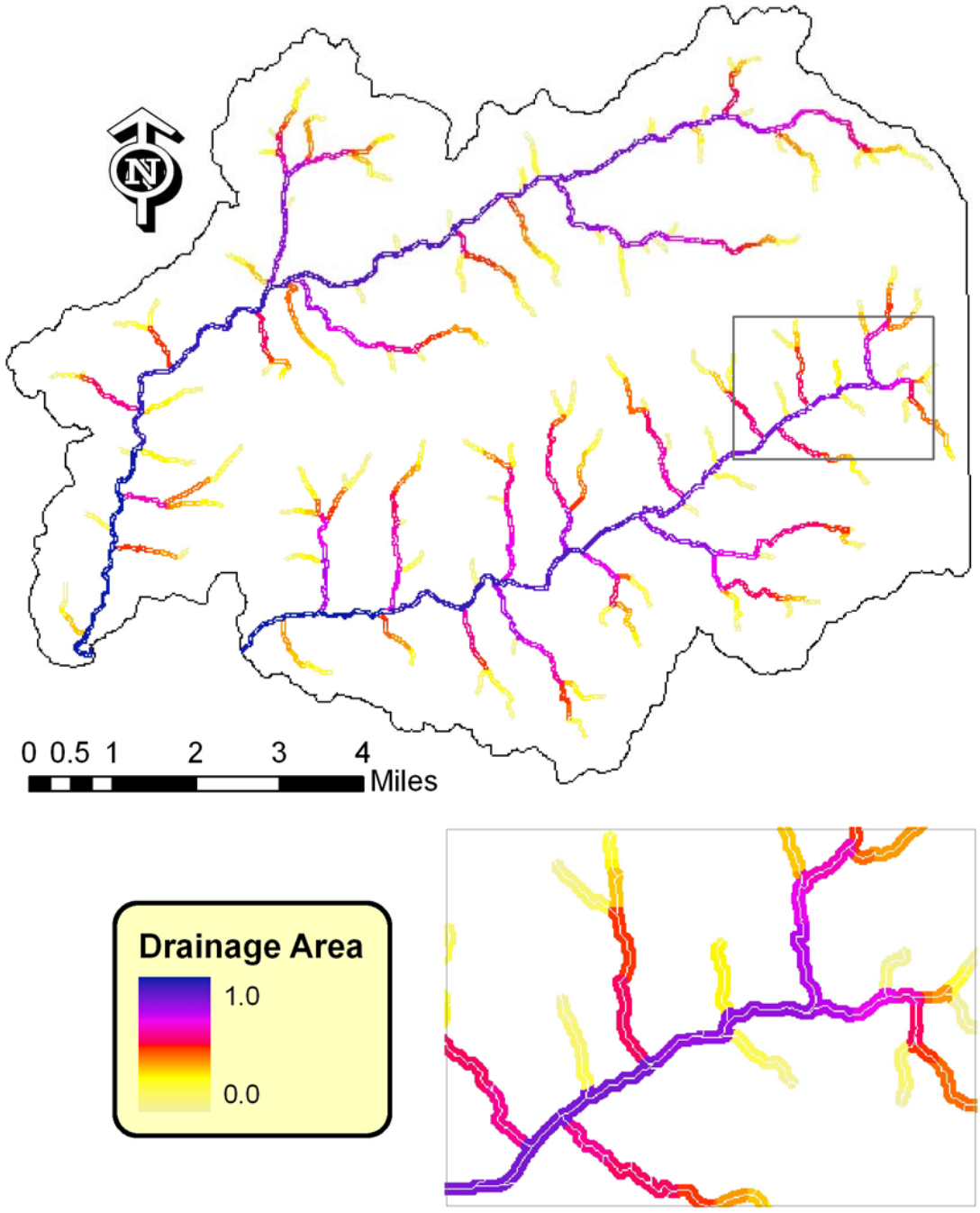


Figure 2.6.9 Normalized drainage area based index for the study area within a 50 meter stream buffer.

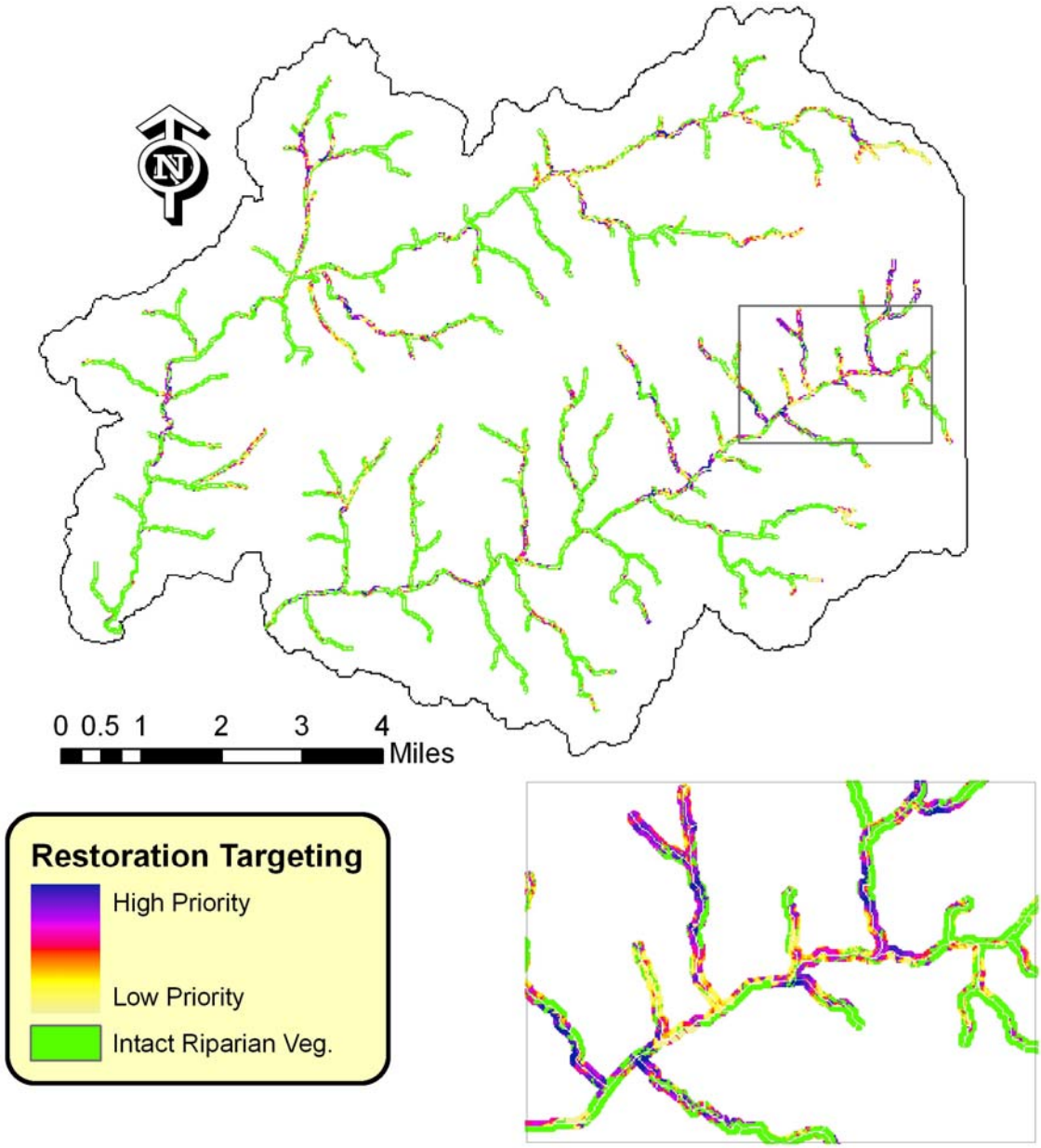


Figure 2.6.10 Targeting degraded riparian corridors, based on uniform factor weights.

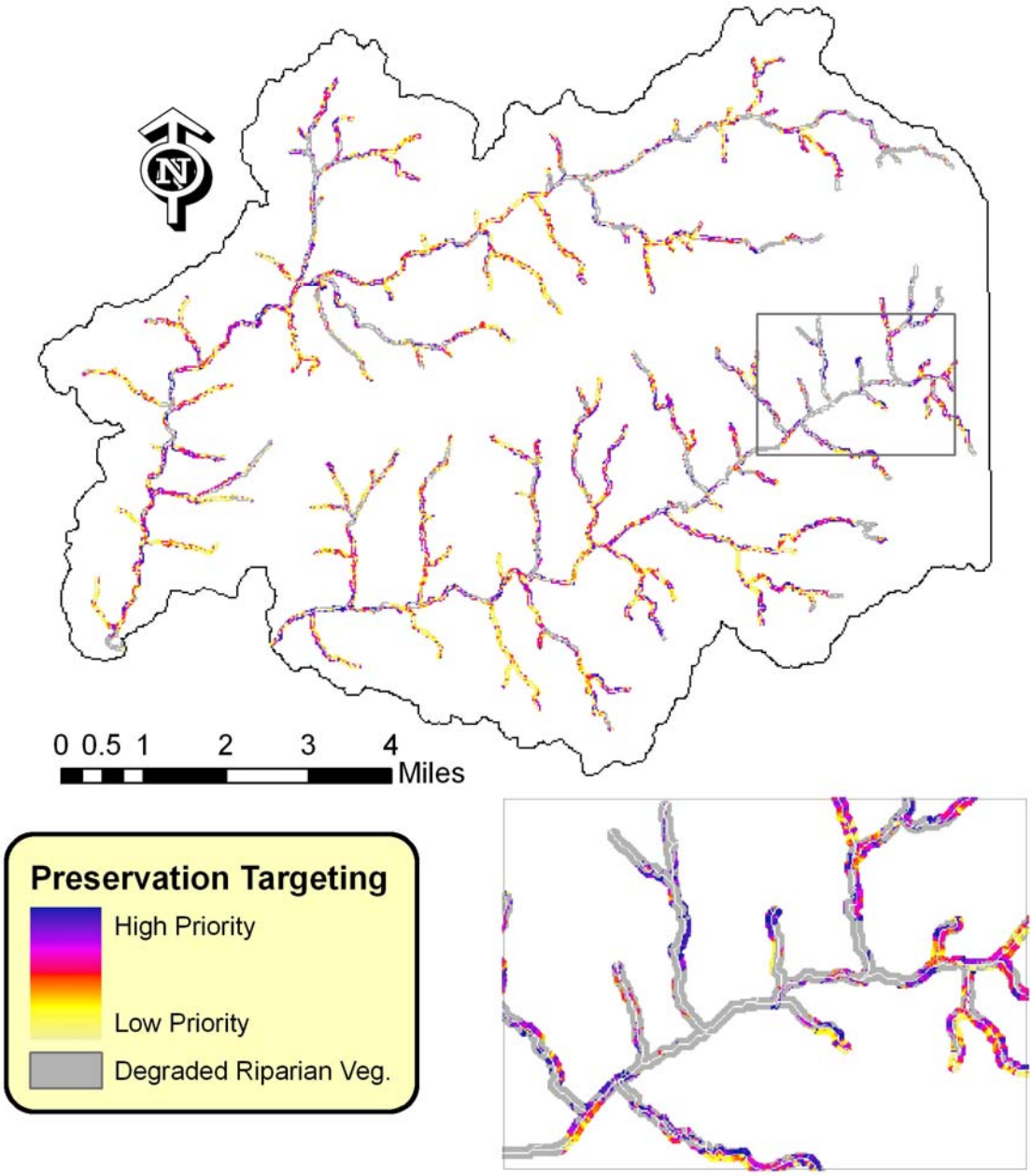


Figure 2.6.11 Targeting well vegetated riparian corridors for preservation, based on uniform factor weights.

Table 2.6.2 Correlation coefficient between factor grids in the study area.

Layer	Drainage Area	Curvature	Current Vegetation	Runoff Acc.	Stream Gradient	Buffer Slope	Soluble P Acc.	Erosion	Erosion Acc.
Drainage Area	1.00	0.06	-0.02	0.06	-0.75	-0.33	0.07	-0.08	0.06
Curvature	0.06	1.00	0.00	-0.06	-0.06	-0.13	-0.02	-0.09	-0.10
Current Vegetation	-0.02	0.00	1.00	-0.09	0.04	0.09	-0.13	-0.16	-0.08
Runoff Acc.	0.06	-0.06	-0.09	1.00	-0.15	-0.27	0.85	0.25	0.81
Stream Gradient	-0.75	-0.06	0.04	-0.15	1.00	0.47	-0.18	0.12	-0.04
Buffer Slope	-0.33	-0.13	0.09	-0.27	0.47	1.00	-0.35	0.34	-0.07
Soluble P Acc.	0.07	-0.02	-0.13	0.85	-0.18	-0.35	1.00	0.26	0.76
Erosion	-0.08	-0.09	-0.16	0.25	0.12	0.34	0.26	1.00	0.38
Erosion Acc.	0.06	-0.10	-0.08	0.81	-0.04	-0.07	0.76	0.38	1.00

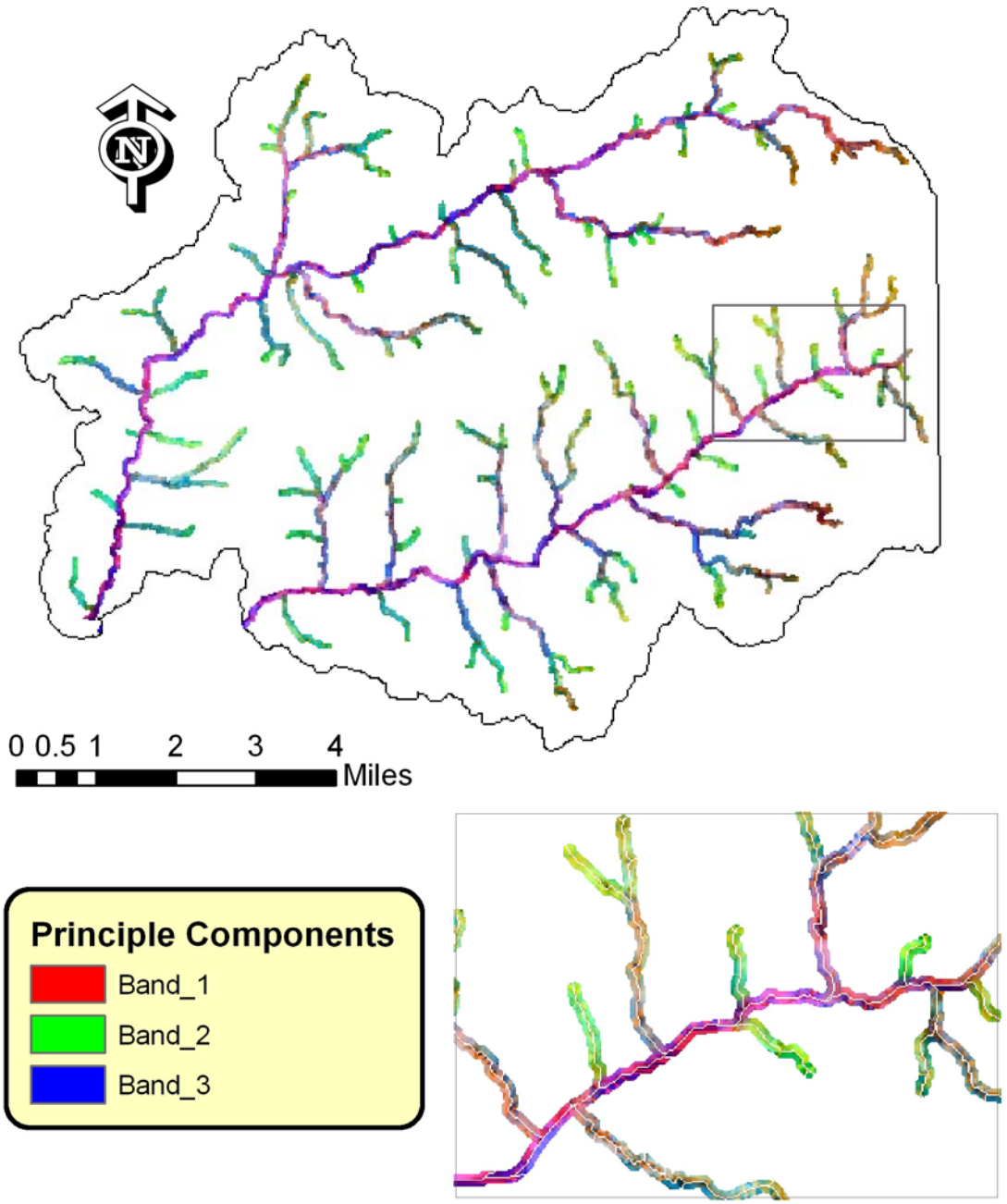


Figure 2.6.12 Principle components analysis of eight factors resulting in three bands. Similar colors represent similar factor values.

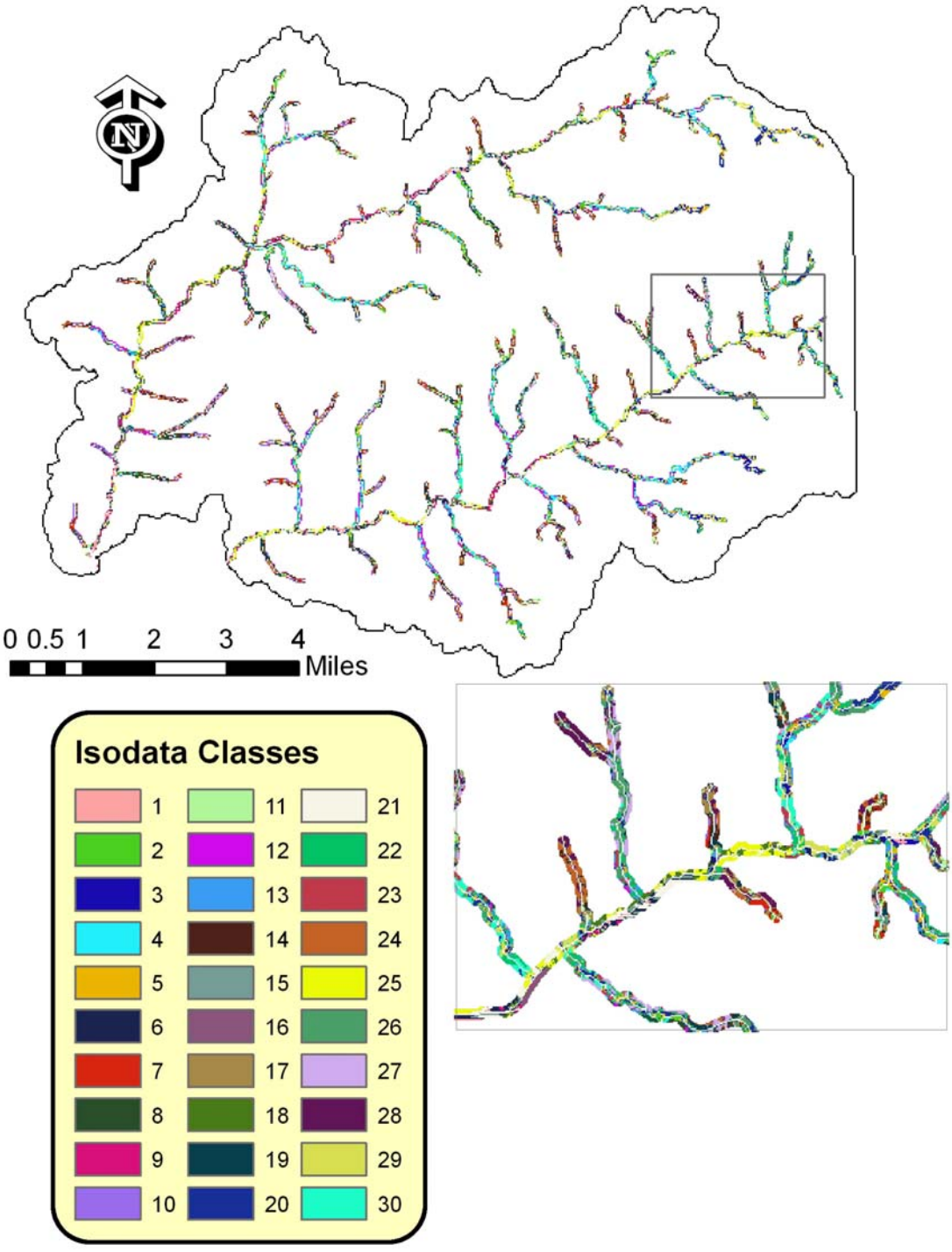


Figure 2.6.13 Isodata clustering results. Categories represent zones with similar characteristics as defined by factors.

Table 2.6.3 Isodata clustering classes for average factor value.

Isodata Class	Drainage Area	Erosion Acc.	Erosion	Soluble P Acc.	Stream Gradient	Runoff Acc.	Curvature	Buffer Slope	Total Indicator
1	0.91	0.08	0.08	0.15	0.17	0.10	0.64	0.81	2.93
2	0.38	0.17	0.15	0.22	0.67	0.18	0.69	0.61	3.08
3	0.57	0.15	0.12	0.36	0.34	0.21	0.77	0.83	3.34
4	0.64	0.17	0.29	0.16	0.45	0.17	0.54	0.37	2.79
5	0.44	0.36	0.19	0.51	0.45	0.51	0.66	0.75	3.87
6	0.87	0.32	0.20	0.41	0.19	0.36	0.59	0.76	3.70
7	0.22	0.19	0.33	0.18	0.83	0.17	0.56	0.29	2.77
8	0.42	0.20	0.48	0.13	0.67	0.16	0.49	0.13	2.68
9	0.89	0.27	0.41	0.26	0.18	0.26	0.48	0.39	3.13
10	0.21	0.23	0.58	0.14	0.87	0.18	0.36	0.05	2.63
11	0.24	0.44	0.26	0.45	0.78	0.45	0.62	0.52	3.76
12	0.63	0.35	0.62	0.24	0.46	0.30	0.42	0.11	3.12
13	0.62	0.47	0.46	0.43	0.46	0.46	0.52	0.46	3.88
14	0.23	0.44	0.58	0.36	0.82	0.38	0.43	0.14	3.38
15	0.80	0.54	0.19	0.62	0.23	0.64	0.59	0.79	4.40
16	0.91	0.50	0.71	0.41	0.14	0.40	0.45	0.25	3.76
17	0.19	0.69	0.43	0.69	0.82	0.70	0.58	0.42	4.53
18	0.89	0.59	0.63	0.62	0.15	0.59	0.49	0.62	4.58
19	0.32	0.59	0.59	0.51	0.71	0.52	0.48	0.28	4.00
20	0.34	0.70	0.20	0.86	0.49	0.87	0.64	0.78	4.87
21	0.89	0.81	0.18	0.86	0.14	0.87	0.53	0.84	5.13
22	0.59	0.67	0.59	0.70	0.46	0.70	0.55	0.56	4.81
23	0.67	0.73	0.84	0.60	0.41	0.59	0.39	0.31	4.55
24	0.22	0.72	0.80	0.58	0.83	0.57	0.37	0.16	4.26
25	0.90	0.83	0.80	0.85	0.15	0.84	0.44	0.62	5.42
26	0.37	0.87	0.63	0.89	0.61	0.90	0.57	0.59	5.43
27	0.38	0.87	0.87	0.78	0.67	0.79	0.32	0.34	5.01
28	0.17	0.88	0.75	0.88	0.85	0.87	0.49	0.36	5.24
29	0.69	0.85	0.52	0.93	0.31	0.93	0.59	0.76	5.58
30	0.68	0.91	0.87	0.87	0.40	0.88	0.41	0.50	5.53

(3) Principal Findings and Significance

The primary objective of this research was to develop a framework to best utilize existing data to predict the optimal placement of riparian buffers within a basin. This research incorporated both methods from previous studies, and new novel approaches to optimally place buffers. Several findings of this research are listed below:

- Currently available models lacked either the spatial detail or spatial extent to quantitatively target riparian buffers at the basin scale.
- Flow accumulation was a valuable tool to characterize water and nutrient movement over the land surface and through riparian buffers.
- Simple models like the RUSLE can be used to easily estimate gridcell erosion at a basin scale.
- Extrapolation of load or runoff by landcover and soil can produce adequate gridcell level estimates for an entire basin.
- Stream curvature may be a valuable predictor of current stream bank instability and future stream migration. Buffer distance may need to be increased in these areas to allow for future stream movement.
- Ground truth data are essential to develop appropriate weighting factors, and will be required in future applications of these methods.
- Principles adapted from remotes sensing can utilize examples of high priority riparian buffer to find areas with similar characteristics in an entire basin.

(3.1) Utility of Models

Process based models can predict the optimal placement of riparian buffers within a small watershed. Even these small models are very complex and difficult to parameterize. Available models and or combinations of models lack either the spatial extent (field scale models) or the process detail (basin scale models) to simulate hundreds or thousands of possible riparian buffers within a basin. Even though we have a reasonable understanding of the processes governing the movement of water and nutrients across the land surface and through a riparian buffer, we lack the computational power and data with which to parameterize a model with a large spatial extent. Currently available basin scale models aggregate input GIS data to reduce complexity. Lost are the subtle yet important details of aspect and slope which determine how water moves across the land surface to the stream. Gridcell versions of basin scale models may recapture this information at the expense of tremendous computational requirements. For these reasons we decided to use simple GIS based models with no aggregation of input data.

(3.2) Quantitative Limitations

The method as detailed in this study is qualitative in nature. This is an important limitation, and the consequence of using GIS indicators in lieu of process based models. Each factor proposed is an indicator of riparian buffer functionality, but the exact relationship is unknown. EPA and the 319 program are under pressure from Congress to estimate water quality improvements to justify allocated funds. Watershed models are quantitative by nature and will provide a number with varying degrees of uncertainty. Simplification and aggregation of input data increase this uncertainty as field scale processes are ignored or consolidated to accommodate limited computational resources and limited data. At some point the uncertainty limits the utility of these model predictions.

It is possible to use the methods presented here in a quantitative manor. In this study all quantitative aspects for each factor were reduced by nonparametric transformations to make factors directly comparable. For example the soluble phosphorus flow accumulation was an estimate of how much soluble phosphorus passed through the riparian buffer at any given location. If we were able to estimate the removal efficiency of the riparian buffer, we could determine the soluble phosphorus load reduction from the buffer. The same could be done for many of the factors. Other factors such as curvature may require additional research to quantify their effects. Curvature is ignored in watershed models, as are many other possible significant processes. It is possible to estimate the effect of each factor on the whole and estimate an improvement in water quality. Similar to watershed models, the uncertainty contained within this estimate would be unknown.

The method presented here was intended to be flexible and allow the inclusion of other riparian factors of interest. The optimal placement of buffers depends upon what is the intended function or functions of the buffers. Riparian buffers were considered in this study for sediment and nutrient removal, but they have many other benefits. Wildlife use riparian buffers as corridors to increase landscape connectivity. Riparian forest in low order streams provide shade which decreases water temperature and provide woody debris which enriches stream habitat and influences stream morphology. Indicators for many of these valuable riparian buffer services can be derived from readily available GIS data. Many of these benefits while important are difficult to quantify.

(3.3) Riparian Buffer Classification

The classification of riparian areas can be a useful tool. Most riparian inventories are based on landcover alone or on expensive field surveys. Factors developed as indicators of riparian effectiveness can be used with remote sensing algorithms to define sites with similar characteristics. With a few examples of highly effective riparian buffers within a basin, that signature can be defined and similar areas located within the basin or ecoregion for preservation. Similar areas with degraded landcover could be located and targeted for restoration. The entire

riparian area within a basin could be classified into categories, and each category could be rated for riparian buffer efficiency or function based on ground truth data. The result would be map of all riparian zones including characteristics of each class and level of functionality. Other data such as habitat assessments could be extrapolated from a few survey sites, to an entire basin. This method could be very useful for the inventory and assessment of riparian buffers within a basin.

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Springs in Time: Comparison of Present and Historical Flows

Basic Information

Title:	Springs in Time: Comparison of Present and Historical Flows
Project Number:	2004OK29B
Start Date:	3/1/2004
End Date:	3/31/2006
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Research Category:	Ground-water Flow and Transport
Focus Category:	Conservation, Groundwater, Hydrology
Descriptors:	Arbuckle-Simpson aquifer, groundwater-surface water interactions, springs, dewatering, temporal change, biomonitoring, invertebrate fauna
Principal Investigators:	Aondover Tarhule, Elizabeth A. Bergey

Publication

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2. Tarhule, A; E. Bergey 2006, Springs in Time: Comparison of Present and and Historical Flows. Paper presented at the Annual Meeting of the Oklahoma Water Resources Board, Oklahoma City, Oklahoma, Febrary 2006.
3. Tarhule, A. Manuscript in preparation for "Journal of the American Water Resources Association."

Springs in time:

Comparison of present and historical flows

Final Report Submitted to:

OKLAHOMA WATER RESOURCES RESEARCH INSTITUTE

Start Date: March 1, 2004

End Date: March 31, 2006

Congressional District: 4th District

Focus Categories: COV, GW, HYDROL

Descriptors: **Aquifer, Groundwater, Oklahoma, Precipitation, Springs**

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Date: May 30, 2006

Abstract

The study reported here developed in response to published and anecdotal evidence suggesting some springs in Oklahoma either had dried up or were experiencing significantly diminished flow volumes in recent decades. Owing to scarcity of long-term data on spring flows however, the study focused primarily on groundwater levels based on time series of well level measurements which are longer in time, more reliable, and abundant over space. The change in focus is justified on the basis that groundwater level in aquifers is related to spring discharge. The study analyzed annual time series for 429 wells distributed throughout Oklahoma. The distribution-free, non-parametric Mann-Kendall trend test and Sen's slope estimates were used to investigate occurrence of trends in the groundwater level time series. Somewhat unexpectedly, the results indicate that 58% (248 wells) of wells are experiencing statistically significant (α=0.05) positive trends, 25% (109 wells) are experiencing significant negative trends, and 17% showed no change in groundwater elevation during the study period (1970-2003). On average, the trend magnitude for rising wells was 0.43ft/yr (median=0.328 ft/yr) compared to 0.992ft/yr (median=0.661 ft/yr) for declining wells. Consequently, groundwater level in many wells throughout Oklahoma has risen about 12 feet higher during the preceding 28 years but declined approximately 27 feet in other wells during the same time period. Most of the groundwater level decline is occurring in the Ogallala aquifer in the Oklahoma Panhandle. However, the eastern part of the aquifer is experiencing significant groundwater level rise. Groundwater rise predominates elsewhere in the state but especially in the western part between longitudes 98°-100° west. Wells with no change in groundwater level are sprinkled among those with upward water level rises and consequently display no coherent spatial pattern.

To investigate the possible cause of groundwater level change, we analyzed annual precipitation time series (1970-2004) at103 gauging sites throughout Oklahoma using the same trend procedure. The results indicate 16% of the annual precipitation time show statistically significant (α=0.05) positive trends but there is no clearly discernible pattern to their spatial distribution. The average magnitude of precipitation trend rise is 0.28 in./yr or 7.84 in. over 28 years, which represents a rise of 0.95%. Assuming aquifer specific yield of 5.4%, such precipitation increase could theoretically account entirely for the average observed groundwater level rise of 12 feet.

However, more rigorous analysis is needed to discover the specific causes and relative contributions of groundwater changes as well as their possible agricultural, recreational and economic impacts.

The study also surveyed invertebrates and fish in 23 Oklahoma springs because springs sustain unique ecosystems by virtue of their near constant temperature and flow discharge. Seven species of fish and three species of crayfish were found in the springs. The springs in the Arbuckle-Simpson aquifer had the greatest diversity of species but none of the sampled Central Oklahoma Aquifer springs had fish. No rare or spring-endemic crayfishes were found. With only 5 out 60 taxa, Sulphur spring is the most taxonomically unique of the study springs.

While many studies have reported previously on precipitation and runoff trends, this study is to our knowledge, among a very few that have examined widespread groundwater level change over such a large area. Critical outstanding issues are identified. It is suggested that further research is needed for developing a comprehensive and definitive reference source on Oklahoma's changing water resources

Keywords: Aquifer, Groundwater, Oklahoma, Precipitation, Springs.

Students supported by the project

Student Status	Number*	Disciplines
Undetrgraduate	4	Biology, Geography
M.S.	3	Biology, Geography
Ph.D.	2	Geography
Post Doc		
Total		

*** Includes students supported for any duration of time on project funds. For example, one Ph.D student was supported for two weeks.**

Publications

Faulkner, M; A.Tarhule and E. Bergey 2004. Springs in Time: Comparison of Present and Historical Flows. Paper presented at the Annual Meeting of the Southwestern Association of American Geographers, Stephen F. Austin University, Nacodoches, Texas, November 12-13.

Tarhule A. and E. Bergey (2006). Springs in Time: Comparison of Present and Historical Flows. Paper presented at the Annual Meeting of the Oklahoma Water Resources Board, Feb 06, Oklahoma City.

Tarhule, A. Groundwater level trends in Oklahoma. Manuscript in preparation for *Journal of the American Water Resources Association*.

Problem and Research Objectives

This is the final report of the study *Springs in time: comparison of present and historical flows.* The study builds upon the findings of an earlier OWRRI award to Dr. Elizabeth Bergey (Co-PI on this proposal) titled: *Springs in Peril: Have changes in groundwater input affected Oklahoma Springs?* (Bergey 2002). Bergey's study uncovered anecdotal evidence from landowners as well as published reports suggesting several springs draining major aquifers in Oklahoma or aquifers shared between Oklahoma and surrounding states had either gone dry or were experiencing significantly diminished flow volumes. For example, major changes in discharge have been reported for some springs in the Chickasaw National Recreation Area (OWRB 1990). In nearby Texas, up to one-half of springs no longer flow (Brune 1981) and additional springs are drying up (Clark Hubbs, personal communication).

Oklahoma springs have a very diverse fauna that includes both common, widespread species and spring specialists (Matthews et al. 1983). The study of spring faunas is important because of their intimate connection with groundwater and mineral resources, their interest to science, and their rarity. Several imperiled (G1-G2) animal species listed in the Natural Heritage Program and several species in the Oklahoma Department of Wildlife Conservation's Species of Greatest Conservation can be found in springs. The future success of these species relies heavily on the 'health' of springs and their groundwater sources.

Diminished spring discharge rates signify major changes in the groundwater aquifers that feed the affected springs. Knowledge concerning the magnitudes and spatial patterns of such changes is critical because groundwater is important to Oklahoma's economy, tourism, agriculture, and ecosystem health. For example, groundwater accounts for approximately 54% of total freshwater withdrawals in the state (Tortorelli, 2000). Most of the withdrawn groundwater

is used for irrigation (80%) and municipal and household water supply (17%; Tortorelli, 2000). Groundwater is also vital for wildlife and for maintaining the high-quality outdoors environment of Oklahoma. Groundwater feeds natural springs and river environments that constitute the central features at several recreational areas and state parks including Chickasaw National Recreation Area, Boiling Springs State Park, and Roman Nose State Park among many others).

More recently, there have been concerns that contentions over groundwater resources by competing stakeholder interests as well as proposals for extensive groundwater abstraction to supply major municipalities, such as the proposed Arbuckle-Simpson aquifer water sale (see, http://www.owrb.state.ok.us/studies/groundwater/arbuckle_simpson/arbuckle_study.php), may lower aquifer levels and adversely affect discharge into associated springs and streams. Even if impacted springs do not dry up entirely, these changes could affect the springs discharge, temperature and water quality significantly beyond the range of natural variability, which may, in turn, disrupt the unique spring-fed ecosystems that are highly susceptible to these variables (see also Mattson et al. 1995; P. Rakes, cited in Shute et al. 1997). There is a need, therefore, to analyze changes in spring flow volumes and aquifer levels in Oklahoma.

Study Objectives

The specific objectives of the present study are to:

(i) Determine whether spring discharge in five selected aquifers are experiencing negative trends;

(ii) Place short-term observations of spring flow and groundwater level changes in the context of long-term patterns of variability over space and time; and

(iii) Document further the faunal biodiversity of Oklahoma spring-fed streams and habitats to increase the knowledge status of these unique ecosystems and to identify species possibly susceptible to spring flow changes.

The information produced contributes to on-going efforts to document further Oklahoma's groundwater dynamics. The study aquifers selected initially for investigation are those identified from Dr. Bergey's research as experiencing declining flow rates including the Ogallala Formation, Trinity Group, Vamoosa Formation, and the Garber Sandstone/Wellington Formation. Additionally, it was also decided to add to the list of study aquifers the Simpson-Arbuckle aquifer because of the large-scale water sales plan that has been proposed for this aquifer. For reasons that will become apparent in this report however, the study has been extended to cover nearly all major aquifers throughout Oklahoma.

Methodology and Data

To investigate temporal changes in spring discharge [i.e. Objective (i) above], it was proposed originally to analyze the long-term (≈ 20 years) trends in instrumental time series records of spring discharge. The USGS spring database lists 609 springs throughout Oklahoma. However, no discharge measurement exists for the vast majority (76%) of these. Of the remaining springs with discharge records, the data is fragmentary, discontinuous, unsystematic, or comprises only *ad hoc* one time measurement. Upon further search, we discovered, and subsequently added to the database, previously undigitized spring discharge readings for more than 10 locations during the 1930s and early 1940 but these too were spotty and fragmented. Indeed, while discharge records of various lengths and completeness were found for over 60

springs, only two (Byrds Mill Spring; 46 years and Antelope Spring; 20 years) had discharge records sufficiently long and continuous to yield robust and reliable statistical trend parameters.

Given this data situation, it was apparent that even adding a few more flow measurements (as originally proposed) would still not produce definitive trend estimates (direction and magnitude). As an alternative, it was decided to analyze trends in well levels in the aquifers feeding the springs. The following section describes the rationale for this approach.

Springs, by definition, are points where groundwater flows to the surface. Springs occur primarily (although not exclusively) in areas underlain by soluble karstic rocks notably limestone, gypsum, or dolomite. In Oklahoma, two major types of springs dominate. The more common of these, contact springs, occur where the water table intersects the surface. Artesian springs - where the water reaches the surface under pressure from a confined or semi-confined aquifer also occur but are far less common. There does not exist at the present time a good inventory or database of Oklahoma spring geology and flow characteristics.

Springs that originate or discharge near the top of an aquifer may experience rhythmic flow fluctuations in response to the up and down movement of the groundwater table (Fig 1). In contrast, springs discharging near the base of an aquifer tend generally to experience steady flow characteristics including volume and temperature. These characteristics are very important for the unique fauna that springs foster and support. However, these springs may in fact be of only limited utility as indicators of groundwater level changes, both because they occur only in a few geologic environments and because they show only moderate response to aquifer head change.

The above considerations suggest that historical well level measurements, which are temporally longer and spatially better distributed than springs discharge measurement points, provide the best means for analyzing the temporal and spatial patterns of groundwater resources

change in Oklahoma. Consequently, water table elevations for selected wells were obtained from the USGS Oklahoma City Office. The selection criteria was that the wells should have continuous annual (i.e. at least one record per year) water level measurements for at least 20 years. To avoid possible complications introduced by intra-seasonal variations, only those wells with water level measurements in the winter months (December –March) when anthropogenic withdrawals are lowest are analyzed. Strictly speaking therefore, the data analyzed is the winter groundwater level elevations in Oklahoma. Finally, to address the problem of missing data, only those wells with no more than an arbitrarily set cutoff threshold of 15% consecutive missing values were selected. A total of 429 wells satisfied these criteria and were selected.

For a given watershed, changes in groundwater level or storage (ΔS) could be investigated by rearranging the water budget equation so that:

$$\Delta S = \text{Input} - \text{Output} \quad (1)$$

or

$$\pm\Delta S = P - \{E+R\} \quad (2)$$

Where P is precipitation, E is evapotranspiration and R is runoff. The above simplified version of the water budget equation assumes that groundwater inflows from, and outflows to, adjoining watersheds is negligible. In general, $\Delta S \rightarrow 0$ if the system is in dynamic equilibrium and the time period analyzed is sufficiently long ($\approx$ 10years). The conservative nature of this equation dictates that for a system without significant anthropogenic impacts, increasing or decreasing trends in groundwater storage are the results of corresponding trends in either water input into the basin (i.e. precipitation), or water output from the basin through evapotranspiration and/or runoff. Because precipitation data is more readily available and reliable than evapotranspiration and runoff data, we examine only for trends in precipitation as a possible cause of groundwater level

change. Thus, the time series of annual precipitation totals (1970-2005) were obtained from the Oklahoma Mesonet for 103 stations distributed throughout the state.

To investigate trends in the groundwater level and precipitation time series, we employed the Mann-Kendall test and Sen's slope estimate method (Salmi et al 2002). In common with non-parametric methods generally, the Mann-Kendall test requires no assumptions about the nature of the probability distribution characterizing the time series. Furthermore, by working only with the ranked values of the time series, it minimizes the impacts of single data errors or outlier events on trend direction and magnitude.

For a time series of annual values of length n, the test statistic, S, is calculated using the formula (Salmi et al 2002, p.9)

$$S = \sum_{k=1}^{n-1} \sum_{j=k+1}^{n} \mathrm{sgn}(x_j - x_k) \tag{3}$$

Where x_j and x_k are the annual values in years j and k, j<k, and

$$\mathrm{sgn}(x_j - x_k) = \begin{cases} 1 & if \quad x_j - x_k > 0 \\ 0 & if \quad x_j - x_k = 0 \\ -1 & if \quad x_j - x_k < 0 \end{cases} \tag{4}$$

If $n \leq 9$ the absolute value of S is compared directly to the theoretical distribution of S derived by Mann and Kendall (Gilbert, 1987). For $n \geq 10$, the variance of S is computed from:

$$\mathrm{VAR(S)} = \frac{1}{18}\left[n(n-1)(2n+5) - \sum_{p=1}^{q} t_p (t_p - 1)(2t_p + 5) \right] \tag{5}$$

Where q is the number of tied ranks and t_p is the number of points in the p^{th} group. Finally, the Z statistic is used to test for the significance of a trend. The statistic (Z) has a normal distribution and is calculated as (Salmi et al 2002):

$$Z = \begin{cases} \dfrac{S-1}{\sqrt{VAR(S)}} & if \quad S > 0 \\ 0 & if \quad S = 0 \\ \dfrac{S+1}{\sqrt{VAR(S)}} & if \quad S < 0 \end{cases} \tag{6}$$

For a two-tailed test, the null hypothesis is rejected if the absolute value of Z is greater than $Z_{1-\alpha/2,}$ where α is a specified significance or probability level.

The above procedure detects only the presence and statistical significance of a trend. Slope magnitude is then estimated using Sen's method. First, the slopes (Q_i) of all data value pairs are obtained as:

$$Q_i = \frac{x_j - x_k}{j - k} \tag{7}$$

Where j>k.

For a time series of length, n, eq. 7 above produces N=n(n-1)/2 slope estimates, Q_i and the median value of N is the Sen's slope estimate. The slopes obtained are comparable to those produced from linear regression modeling.

Spring Inventories

The methods used to sample springs were those used by Bergey (2002) and included the following components:

- Site description, including TRS coordinates, GPS readings, a site sketch, photos, local land use, modifications of the spring, and directions for re-finding the site.
- Discharge information (flow width, depths, and mean velocities). Velocity was measured with a Marsh-McBirney electromagnetic flow meter.

- An owner questionnaire to get information on land use changes, changes in discharge, and historical use of springs.
- Fish sampling, using seines or dipnets. Only one or two fish of each species were collected in springs with fish.
- Invertebrates sampling, using hand nets for qualitative sampling and a small corer for quantitative sampling. Samples were preserved in the field and returned to the laboratory for sample sorting and invertebrate identification.
- Invertebrate identification is ongoing.

Principal Findings and Significance

Figure 2 shows the location and distribution of the 429 wells analyzed for this study. A majority of the wells are concentrated in western Oklahoma, reflecting greater importance of groundwater in this semi-arid part of the state. Both rainfall and runoff are much more abundant in the sub-humid to humid eastern half of Oklahoma and consequently, there is less reliance on groundwater.

Figure 3 is a classification of the study wells in terms of length of records. The series ranged in length from 17 years to 38 years with an average of 28 years. The most recent year for which data is analyzed is 2003. Hence, the average well series covers the period from 1975-2003. Similarly, depth to water table (in feet below ground surface, ft.b.g.s.) shows also a wide range, from as little as 4 ft.b.g.s. to a maximum of 345 ft, with an average of 95 ft. In general, the deepest wells occur in the west, especially in the panhandle region, which experiences the driest climate and is therefore more dependent on groundwater to support a thriving irrigated agricultural sector.

Observed Changes in Groundwater Elevations

Application of the non-parametric Mann-Kendall trend test indicates that of the 429 wells analyzed, 58% (248 wells) showed statistically significant (α=0.05) positive trends or rise in groundwater elevations, 25% (109 wells) showed statistically significant negative trends or drop in groundwater elevations, and 17% (74 wells) experienced no change over the study period (Figure 4). Appendix A contains the complete output of the Mann-Kendall analysis. The above result was largely unanticipated. As stated previously, an important motivation for this study was to investigate anecdotal evidence and complaints about failing springs and falling ground water levels from landowners, which appeared to come from across the state. It came as a complete surprise therefore that from a random sample of wells across Oklahoma, the number of wells experiencing water level rises out number by a margin of better than 2:1 those experiencing water level declines. Table 1 summarizes the characteristics of the wells showing statistically significant water table rises and declines.

Table 1. Statistical characteristics of wells showing water elevation rise and decline.

	Wells Showing Water Level Rises	**Wells Showing Water Level Declines**
Number of Wells	248 (57.8%)	109 (25.4%)
Average Depth (ft. b.g.s.)	64.71	192.66
Median Depth (ft. b.g.s.)	45.92	201.25
Average Slope Magnitude	+0.429 ft/yr (5.15 in/yr)	-0.992 ft/yr (11.90 in/yr)
Median Slope Magnitude	+0.328 ft/yr (3.94 in/yr)	-0.661 ft/yr (7.93 in/yr)
Standard Deviation	0.363	0.847
Minimum Slope	+0.090 ft/yr (1.08 in/yr)	-0.067 ft/yr (0.804 in/yr)
Maximum Slope	+1.901 ft/yr (22.81 in/yr)	-3.984 ft/yr (47.81 in/yr)

Spatial distribution of Trends

Figure 5 presents the spatial distribution of groundwater level trends. The figure reveals that wells with statistically negative trends (red inverted triangles) occur predominantly in the Oklahoma panhandle although a few isolated wells elsewhere in the state show also negative trends. Rising wells (green, upright triangles) are concentrated largely in the area between longitudes 98° -100° west but also in the panhandle. Finally, there appears to be no spatial coherence in the distribution of wells with no change (open circles) in groundwater level during the study period. Indeed principal component analysis failed to segregate among the wells. In addition to the general pattern noted above, occurrence in close proximity of all three types of wells may indicate that well trends are function of water use intensity rather than climatic changes.

Trend Magnitudes and Temporal Changes

Table 1 indicates that for those wells in which the water table is rising, the rate of rise is about one-half foot per year compared to a decline of nearly a foot per year for wells with falling water tables. Over a twenty year period therefore, we would expect, on average, the water table to be 10 feet closer to the ground surface, and 20 feet deeper respectively for rising and declining wells (see section on precipitation trends below).

Figure 6 presents the distribution of trend magnitude for various slope class intervals only for those wells showing statistically significant water level rises or declines. About 63% are rising wells with slope magnitudes between +0.1 to +1.5 ft/yr compared to 25% declining wells with -0.1 to -1.5 ft/yr.

Furthermore, the average depth to the water table in rising wells is about 65 feet below ground surface, compared to 193 feet for declining wells (Table 1). Figure 7 illustrates further the relationship between slope magnitude and well depth.

Figures 8-10 provide illustrative examples of the three types of temporal patterns of water level changes in Oklahoma i.e. rise, drawdown, and no change. For the purpose of clarity only, both rising and falling walls were divided into moderate and severe rises/declines. Moderate rises/declines have trend magnitudes near the median value for the group (Table 1) and the wells grouped as severe have trend magnitudes near the high end for each type. It is important to note that the wells in Figs 8-10 were selected randomly within each specified trend range.

Figure 8a (median rising wells) shows a very linear and steady pattern of water level rise for all illustrative wells. For example, despite a few missing measurements, it is clear that well 9437 rose steadily from a depth of 68.6 ft.b.g.s. in 1965 to 56.4 ft.b.g.s. in 2000, a rise of 12 ft in 35 years (≈ 0.343 ft/year). The rate of rise in the other wells is comparable but this may be a function of the fact that they were all selected within a specified range of trend magnitudes.

Two observations could be made with respect to severe rising wells (Fig. 8b). First, they show more variability than the moderate rising wells. Thus, for many well time series, shorter scale upward spikes and downward dips are superimposed on the generally rising trends. Second, the rate of rise appears to have peaked in the late 1990s for some well time series and several wells in fact show relative declines after that period. It is not clear whether this marks a turning point from the pattern prevalent during the preceding 20 to 30 years, or whether is represents only short term fluctuations around the overall trend.

Similar patterns apply to declining wells (Fig. 9). The magnitude of water table drawdown is truly astonishing in some of these wells. For example, well 1111 dropped from 163

ft.b.g.s. in 1965 to 297 ft.b.g.s. in 2003 or 134 ft in all over 38 years (3.5 ft./year). While it is among the highest observed, such drawdown rate is clearly unsustainable in the long-run. Finally, Figure 10 shows several wells where the overall trend is not significantly different from zero ($\alpha = 0.05$), despite short term fluctuations.

Observed Groundwater Level Changes in Major Aquifers

It is important to identify the specific aquifers being tapped by the study wells and to discover the type and magnitude of groundwater level change in the various aquifers. Figure 11 overlays the major groundwater aquifers in Oklahoma on the study well locations (Fig. 2). Using GIS, the wells contained within each aquifer were identified and their characteristics summarized in Table 2.

Table 2 shows that aquifers experiencing water table rises are distributed widely throughout Oklahoma while the Ogallala is, essentially the only aquifer experiencing widespread falling water table. Unfortunately, the number of wells in some aquifers is too few to make definitive statements about the direction and magnitude of groundwater elevation change. Among those aquifers with at least five wells, the Garber-Wellington, Enid Isolated Terrace, Rush Springs, and Blaine Formation aquifers are experiencing the fastest rates of groundwater level rise.

The groundwater level changes in the Ogallala appear almost paradoxical but may hold the key to understanding groundwater dynamics throughout the state. On the one hand 96 of study wells show declines but an almost equal number (89) show statistically significant rises and the time series for 25 wells remained statistically flat during the study period.

Table 2. Summary of groundwater level change by aquifer

	Groundwater Level Rise						No change	Groundwater Level Decline					
Aquifer	**N**	**Average**	**Median**	**Stdev**	**Min**	**Max**	**N**	**N**	**Average**	**Median**	**Stdev**	**Min**	**Max**
Antlers	5	0.510	0.253	0.491	0.147	1.334	1	1	-1.300				
Arbukcle-Simpson	1	1.394											
Arkansas	1	0.076											
Blaine	13	0.615	0.353	0.453	0.082	1.562	1						
Canadian River	5	0.576	0.208	0.574	0.116	1.416	1						
Cimarron River	16	0.330	0.303	0.216	0.066	0.661	1						
Enid Isolated Terrace	7	0.837	0.893	0.342	0.165	1.312							
Garber-Wellington	5	1.125	0.923	0.812	0.296	2.265	4	2	-0.468				
Getty Sand	1	0.500											
North Canadian River	17	0.331	0.328	0.217	0.087	1.031	4						
North Fork (Red River)	16	0.448	0.365	0.341	0.137	1.614	7	1	-0.261				
Ogalalla	89	0.324	0.260	0.278	0.034	1.901	25	96	-1.057	-0.840	0.869	-0.984	-0.027
Roubidoux	1	0.317											
Rush Springs	16	0.771	0.742	0.438	0.110	1.753	3						
Tillman Terrace	11	0.485	0.450	0.209	0.168	0.812	1						
Vamoosa-Ada	2	1.196											
Washita River	1	0.352											

Again, wells with groundwater level rises are juxtaposed in spaced within the same aquifers with wells showing significant declines or no change over time. This suggests the cause of groundwater level change could be anthropogenic, rather than climatic. Furthermore, it may imply that the cones of depression are accentuated around well points. Over time, outward expansion and coalescence of the cones of depression will drive down the water table in the aquifer as a whole.

The above explanation is unsatisfactory however, with respect to rising water tables. Recharge mounds around well points make sense only if an artificial groundwater recharge program is being implemented, otherwise we must conclude that the water level rise is aquifer-wide. Three types of scenarios can theoretically account for this situation; (i) rising precipitation and groundwater recharge, (ii) declining runoff, and (iii) declining evapotranspiration. A possible anthropogenic cause could be decreased water withdrawals but investigating such scenario is beyond the scope of this study.

Several studies (Garbrecht and Rossel 2002; Garbrecht et al. 2004; Hu et al. 1998) have documented an upward trend in precipitation for the Great Plains as a whole including some watersheds in Oklahoma. Their results are consistent with larger studies for the continental USA obtained by Karl et al. (1996), Karl and Knight (1998), and Easterling et al. (2000) among many others. On the other hand, Zume and Tarhule (in press) found no statistically significant upward trends in the annual precipitation time series for Northwestern Oklahoma. This study employed the Mann-Kendall test to investigate trends in 103 annual precipitation time series throughout Oklahoma.

Observed Trends in Annual Precipitation Time Series

The distribution of the precipitation time series analyzed appears in Figure 12. The well-known east to west precipitation gradient is reproduced accurately in Fig. 12 reflecting generally excellent distribution of the precipitation gauging sites (except, perhaps, in the panhandle region). The Mann-Kendall trend test identified 27 sites (26%) with statistically significant (α=0.1) positive trends. Using the more stringent 0.05 criterion, 16 sites (15.5%) are statistically significant. Only one site, Buffalo, in Harper County (36°51′ N, 99°63′) had a statistically significant negative trend (α=0.1). Figure 13 plots the temporal pattern of precipitation variability for five illustrative sites. Notice only the time series at Gates has a statistically significant trend (superimposed). Finally, figure 14 plots the distribution of precipitation sites showing positive trends (α=0.1; green triangles). The average trend magnitude is 0.28 in. per year with a maximum of 0.39 at Pawhuska (Osage County, 36°40′ N, 96°21′ W).

Most precipitation sites showing upward trends appear to be in the northern half of the state and all four gauging sites analyzed for the panhandle have statistically significant upward trends. Recall that the panhandle is the area experiencing largely negative groundwater trends (Fig. 6), which supports further the earlier observation that the anthropogenic influence supersedes the climatic signal in the panhandle. Beyond these weak and highly generalized patterns, there appears to be no spatial coherence to the distribution of precipitation sites showing positive trends.

Possible Causes of Groundwater Changes

Falling groundwater levels in the Ogallala aquifer are not unusual and have been the subject of much research over the past four decades (Bittinger and Green 1980, McAda 1985,

Holmes and Petrulis 1988, Kromm and White 1992). There is general consensus among these studies that declining groundwater level in the Ogallala aquifer is the result of excessive groundwater extraction for irrigated agriculture. Thus, our study confirms continuing declining trends in the Oklahoma portion of the Ogallala. It is important to reiterate the point that on the eastern margin of the aquifer, groundwater levels are rising significantly. As precipitation trends have not increased over the same period, it may be assumed that changes in the intensity of groundwater exploitation and use are responsible for this trend but further investigation is needed to establish definitively both the cause and possible implications.

The magnitude of rising groundwater levels appears rather dramatic. As stated previously, the average groundwater level trend is 0.43 ft/yr (5.15 in/yr) or a rise of about 12 ft (3.67m) over 28 years. Simple calculations based on assumed values suggest such increase is entirely within the limits of natural variability. For example, the average precipitation trend is 0.28 in/yr or a total of 7.84 in during the study period (28 years). This increase represents 0.95% if the average annual precipitation is considered to be 30 in. Such precipitation increase is sufficient to account entirely for the groundwater level rise if aquifer storativity or specific yield is assumed to be 5.4%, which is on the low end of yield range.

It is important to point out the many qualifications and assumptions inherent in the above estimate. First, increased precipitation trends are isolated, not general, across the study area. Second, specific yield is estimated from observed precipitation and groundwater level trends and may not therefore represent actual yields. Third, and finally, other relevant variables including evapotranpiration, runoff, and baseflow trends have not been considered. Even so, the significance of the exercise is to draw attention to the fact that the observed groundwater level

changes could have been caused by small increases in precipitation. Further studies are needed to establish the specific causes.

Spring Inventories

Twenty-three springs were surveyed (Table 3). Thirteen of the springs were in the Arbuckle area and included springs in the following counties:

- Johnston County: 4 springs
- Pontotoc County: 5 springs
- Coal County: 2 springs (1 of these was a sulphur spring)
- Murray County: 1 spring.

Three springs were in sandstone areas east of Oklahoma City and were located in two counties:

- Lincoln County: 1 spring.
- Pottawatomie County: 2 springs.

The remaining seven springs were in Ellis County.

Spring characteristics

Arbuckle-Simpson springs are karst/limestone springs. Springs emanating from the same water source should have very similar mean annual temperatures and, indeed, most of the Arbuckle springs are 18.0 to 18.5 ^{o}C (Table 3). Exceptions result from water being warmed by retention in a small reservoir (Wildcat Spring) or through water exchange with the adjacent Pennington Creek in a stream-associated spring. The cooler temperatures of Sheep Creek Spring, the nearby Shipes Spring and Coal Spring may signal a different source within the aquifer or shallower source of water.

Table 3. Characteristics of Springs Surveyed in 2004-2005.

Aquifer	Code	Site Name	County	Month Sampled	Q (l/s)	T (°C)	pH	C (µS/cm)	Crayfish	Fish	Notes
Arbuckle-Simpson	SPR04-01	Lowrance Spring	Murray	Jun-04	90.62	18.3	7.2	160	Y	Y	
	SPR04-10	Sheep Creek Spring	Pontotoc	Jul-04	44.04	17.1	7.2	513	Y	Y	Fish were only below weir
	SPR04-06	Rutherford Spring	Johnston	Jul-04	15.53	18.3	6.9	660	N	Y	
	SPR04-08	Viola Spring	Johnston	Jul-04	11.38		7.1	1,580	Y	N	
	SPR04-03	Three Spring	Johnston	Jul-04	7.08	18.0	7.2	513	Y	Y	
	SPR04-04	Wolf Spring	Johnston	Jul-04	3.17	18.1	7.3	485	Y	Y	
	SPR04-02	Pennington Creek Spring	Johnston	Jun-04	2.34	20.6	7.2	544	Y	Y	Between channels of Pennington Creek
	SPR04-11	Shipes Spring	Pontotoc	Jul-04	2.33	17.0	7.2	522	Y	N	In yard
	SPR04-12	Wildcat Spring	Pontotoc	Aug-04	1.82	19.4	6.9	496	N?	Y	Dammed up; fish stocked?
	SPR04-05	Logsdon Spring	Pontotoc	Jul-04	0.67	18.1	7.2	604	Y	N	
	SPR05-01	Coal Cave Spring	Pontotoc	May-05	0.19	16.8	7.1	576	Y	Y	
	SPR04-09	Houghtubby Spring	Coal	Jul-04	0.08	18.5	7.1	620	N	N	
AVERAGE					**14.94**	**18.2**	**7.1**	**606**			
Sulphur spring	SPR04-07	Rotten Egg Spring	Coal	Jul-04	0.52	20.5	6.9	11,370	N	N	Sulphur spring
Central Oklahoma	SPR05-02	Doddehl Spring	Lincoln	May-05	0.50	14.8	6.6	207	N	N	Wooded
	SPR05-04	Nash Spring	Pottawatomie	May-05	0.15	16.6	7.3	725	Y	N	Drips into pool from bluff
	SPR05-03	Trevor Spring	Pottawatomie	May-05	0.08	16.2	6.0	101	Y	N	Crayfish in spring box
Average					**0.24**	**15.9**	**6.6**	**344**			
High Plains	SPR05-05	West Creek Spring	Ellis	Jun-05	NA*	24.4	8.1	635	Y	NA	Seeps along stream
	SPR05-07	Word Spring	Ellis	Jun-05	NA*	20.6	7.8	718	N	NA	Seeps in channel + a small hill slope seep
	SPR05-11	Dugger Spring	Ellis	Jun-05	NA*	25.0	8.1	693	Y	NA	Seeps along stream.
	SPR05-08	McCorkle Seep	Ellis	Jun-05	13.12	22.1	7.7	389	N	Y	Very large seep area
	SPR05-09	Bowman Seep	Ellis	Jun-05	5.00	25.9	7.8	700	N	N	Hill slope seep
	SPR05-10	Reininger Spring	Ellis	Jun-05	1.18	20.6	7.4	577	N	Y	Spring in lower floodplain
	SPR05-06	West Creek Seep	Ellis	Jun-05	0.13	27.9	7.6	944	N	Y	Large seep/wetland
Average					**4.86**	**23.8**	**7.8**	**665**			

The pH among most Arbuckle springs was similar (6.9 to 7.3 µS/cm; Table 3), as is expected because of the buffering by limestone. Conductivity of most springs ranged between 485 and 660 µS/cm. Lowrance Springs had a much lower conductivity (160 µS/cm), whereas Viola Spring had a much higher conductivity (1580 µS/cm). The conductivity of Rotten Egg Spring was well beyond the range of the other sampled Arbuckle springs; but this is a sulphur spring emanating from an apparent bore hole. The water temperature of this sulphur spring was higher than the other Arbuckle springs.

The sandstone springs associated with the Central Oklahoma Aquifer included two springs with somewhat acidic waters (pH 6.0-6.6; Table 3) and low conductivity. Nash Spring had higher pH and conductivity than the nearby Trevor Spring. Water temperatures of the sandstone springs tended to be lower than temperatures of the Arbuckle-Simpson limestone springs.

The sampled High Plains springs were of two types: linear springs along streams and hillslope seeps that typically drained into a nearby stream. Patches of water cress (*Nasturtium officinale*) were used to indicate spring upwellings in streamside springs.
High Plains springs were warmer than the springs from the Arbuckle-Simpson and Central Oklahoma Aquifers (means of 23.8, 18.4, and 15.9 ºC; respectively). Warmer spring temperatures may indicate a deeper source of water (Scott Christianson, personal communication), as would be expected in this portion of the High Plains Aquifer (Pete Thurmond, personal communication). These springs also had relatively high pH (7.4 to 8.1).

Spring fauna

Seven species of fish were found in the springs (Table 4). Arbuckle springs had the greatest diversity of species. The central stoneroller *Campostoma anomalum* and young bluegill *Lepomis macrochiris* were found in a spring pool within the lower floodplain of Pennington Creek, and one mid-sized, probably stocked, smallmouth bass *Micropterus salmoides* was observed in a concreted pool at Wolf Spring. The mosquitofish *Gambusia affinis* was especially widespread and abundant; other fish were darters, which comprised three species plus some individuals that were apparently hybrids. None of these fishes are rare or are spring specialists.

Spring discharge varied greatly, even between nearby springs. Discharge affects habitat 'space' and is related to the presence/absence of larger animals. Fish were present in 8 of the 23 springs (Table 3) and the discharge of springs with fish averaged 15.79 l/s. Crayfish were more frequently encountered, inhabiting 12 of 23 springs and the discharge of springs with crayfish averaged 15.89 l/s. The discharge of springs lacking both crayfish and fish averaged only 1.53 l/s (Table 3).

None of the sampled Central Oklahoma Aquifer springs had fish. These three streams were either distant from or well uphill from their corresponding mainstem streams, which are a common source of fish. The only fish seen in the isolated High Plains Aquifer springs were *Gambusia affinis*, which is the most common fish species in Oklahoma springs. The fish in streams with springs along the edges were not collected because these fish were not associated with the springs themselves.

At least three species of crayfishes were found (Table 4; collections of juveniles could not be identified). *Orconectes palmeri longimanus* is known only in Oklahoma and Arkansas, but is common within its range (G5, S5; NatureServe web site and Bergey et al 2005). Its presence in three springs in the Arbuckles may add two new county records (Coal and Pontotoc

Counties). *Orconectes virilis* is common throughout the Arbuckles. *Procambarus simulans* is common and fairly widespread in Oklahoma. No rare or spring-endemic crayfishes were found.

Occasionally, cave-adapted crustaceans are encountered in springs. Two of the surveyed Arbuckle springs had cave isopods. One spring is associated with a cave that has an identified population of cave isopods; the second spring is a new location. The two specimens from the second spring await identification by a taxonomic expert.

The identification of invertebrate samples is ongoing and not all groups have been identified. Identifications are provisional. Thus far, sixty taxa of invertebrates (exclusive of crayfish) have been identified. Several No beetles or worms have been identified and these two taxa, in particular, will increase the taxonomic list.

The springs in each aquifer were biologically diverse. Full data are given as an appendix and summarized in Table 5. More taxa were found in the Arbuckle-Simpson Aquifer than either of the other two aquifers; however, there were also more springs surveyed in this aquifer and the high aquifer diversity may be an artifact of the area sampled. Individual High Plains aquifer springs were the most diverse. Central Oklahoma Aquifer springs were apparently the least diverse, both at the aquifer-wide scale and at the single-spring scale.

Invertebrate assemblage composition was compared among springs using the ordination technique of Non-metric Multidimensional Scaling (NMDS). In this technique, samples (springs) are plotted on a graph and the distance between any two 'springs' indicates the similarity of their two assemblages. The most taxonomically unique spring is the sulphur spring in the Arbuckle-Simpson area. Only five taxa have been identified from this spring. Graphically, there is a clear difference among the groups of springs. The Central Oklahoma aquifer springs are the most distinct and are characterized by the absence of three groups that are typically found in Oklahoma springs: soldier flies (Stratiomyidae), flatworms (Planaria), and amphipods (especially *Hyalella* sp.). Other than the subterranean amphipods, no rare invertebrates have been identified in any of the springs.

Aquifer	Code	Site Name	County	Month Sampled	Q (l/s)	T (°C)	pH	C (µS/cm)	Crayfish	Fish	Notes
Arbuckle-Simpson	SPR04-01	Lowrance Spring	Murray	Jun-04	90.62	18.3	7.2	160	Y	Y	
	SPR04-10	Sheep Creek Spring	Pontotoc	Jul-04	44.04	17.1	7.2	513	Y	Y	Fish were only below weir
	SPR04-06	Rutherford Spring	Johnston	Jul-04	15.53	18.3	6.9	660	N	Y	
	SPR04-08	Viola Spring	Johnston	Jul-04	11.38		7.1	1,580	Y	N	
	SPR04-03	Three Spring	Johnston	Jul-04	7.08	18.0	7.2	513	Y	Y	
	SPR04-04	Wolf Spring	Johnston	Jul-04	3.17	18.1	7.3	485	Y	Y	
	SPR04-02	Pennington Creek Spring	Johnston	Jun-04	2.34	20.6	7.2	544	Y	Y	Between channels of Pennington Creek
	SPR04-11	Shipes Spring	Pontotoc	Jul-04	2.33	17.0	7.2	522	Y	N	In yard
	SPR04-12	Wildcat Spring	Pontotoc	Aug-04	1.82	19.4	6.9	496	N?	Y	Dammed up; fish stocked?
	SPR04-05	Logsdon Spring	Pontotoc	Jul-04	0.67	18.1	7.2	604	Y	N	
	SPR05-01	Coal Cave Spring	Pontotoc	May-05	0.19	16.8	7.1	576	Y	Y	
	SPR04-09	Houghtubby Spring	Coal	Jul-04	0.08	18.5	7.1	620	N	N	
AVERAGE					**14.94**	**18.2**	**7.1**	**606**			
Sulphur spring	SPR04-07	Rotten Egg Spring	Coal	Jul-04	0.52	20.5	6.9	11,370	N	N	Sulphur spring
Central Oklahoma	SPR05-02	Doddehl Spring	Lincoln	May-05	0.50	14.8	6.6	207	N	N	Wooded
	SPR05-04	Nash Spring	Pottawatomie	May-05	0.15	16.6	7.3	725	Y	N	Drips into pool from bluff
	SPR05-03	Trevor Spring	Pottawatomie	May-05	0.08	16.2	6.0	101	Y	N	Crayfish in spring box
AVERAGE					**0.24**	**15.9**	**6.6**	**344**			
High Plains	SPR05-05	West Creek Spring	Ellis	Jun-05	NA*	24.4	8.1	635	Y	NA	Seeps along stream
	SPR05-07	Word Spring	Ellis	Jun-05	NA*	20.6	7.8	718	N	NA	Seeps in channel + a small hill slope seep
	SPR05-11	Dugger Spring	Ellis	Jun-05	NA*	25.0	8.1	693	Y	NA	Seeps along stream.
	SPR05-08	McCorkle Seep	Ellis	Jun-05	13.12	22.1	7.7	389	N	Y	Very large seep area
	SPR05-09	Bowman Seep	Ellis	Jun-05	5.00	25.9	7.8	700	N	N	Hill slope seep
	SPR05-10	Reininger Spring	Ellis	Jun-05	1.18	20.6	7.4	577	N	Y	Spring in lower floodplain
	SPR05-06	West Creek Seep	Ellis	Jun-05	0.13	27.9	7.6	944	N	Y	Large seep/wetland
AVERAGE					**4.86**	**23.8**	**7.8**	**665**			

* Discharge (Q) is unavailable for springs located in the channel of flowing streams.

Table 4. Crayfish and fish species found during the 2004-2005 springs survey. (*O*. = *Orconectes*, *P*. = *Procambarus*, *G*. = *Gambusia*, *E*. = *Etheostoma*; *Etheostoma* sp. = unidentified specimens, possibly hybrids).

Site name	Crayfishes	Fishes
Lowrance Spring	*O. virilis*	*G. affinis, E. radiosum, E. gracile, Etheostoma* sp.
Pennington Crk spring	*O. virilis*	*G. affinis, Campostoma anomalum, E. spectabile, Lepomis macrochirus*
Three Springs	*P. simulans*	
Wolf Spring		*G. affinis, E. spectabile, Micropterus salmoides*
Logsdon Spring	unidentified juvenile	
Rutherford Spring		*G. affinis*
Rotten Egg Spring		
Viola Spring	*O. palmeri longimanus*	
Houghtubby Spring		
Sheep Creek Spring	*O. palmeri longimanus*	Fish blocked by weir; below weir: *C. anomalum, E. radiosum, Etheostoma* sp.
Shipes Spring	unidentified juvenile	
Wildcat Spring		a small reservoir: *G. affinis*
Coal Spring	*O. palmeri longimanus, P. simulans*	*E. radiosum*
Doddehl Spring		
Trevor Spring	*P. simulans*	
Nash Spring	*P. simulans*	
West Crk spring	unidentified juvenile	NA*
West Crk seep		*G. affinis*
Word Spring		NA*
McCorkle Spring		*G. affinis*
Bowman Seep		
Reininger Spring		*G. affinis*
Dugger Spring	unidentified juvenile	NA*

* fish were present in the contiguous stream and were not sampled

Table 5. Taxonomic diversity of macroinvertebrates in springs, based on identifications of select taxa.

Aquifer	# of springs	Taxa in aquifer	Mean taxa/spring	Range among springs
Arbuckle-Simpson	12	47	14.8	11-23
Central Oklahoma	3	22	12.7	10-15
High Plains	7	43	17.0	12-24
Arbuckle-Simpson (sulphur spr)	1		5	

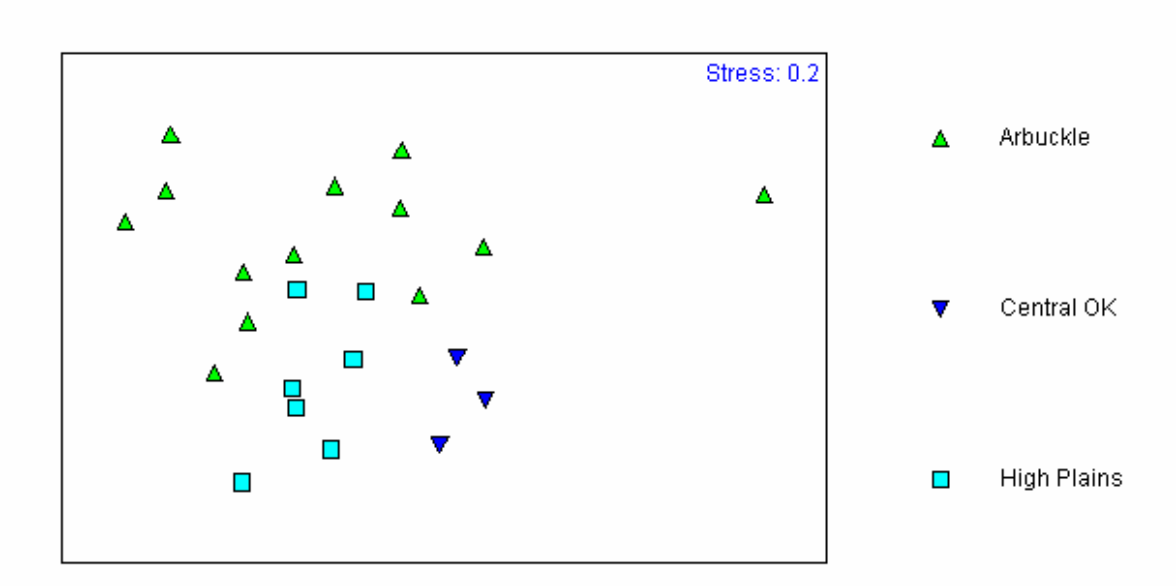

Figure 14. Non-metric multidimensional scaling ordination of invertebrate assemblages of 23 Oklahoma springs.

Summary and Conclusions

This study was motivated by anecdotal and other evidence that several springs in Oklahoma have experienced significantly diminished flow volumes or have ceased to flow altogether in recent years. Springs are a "window" into groundwater resources. As a result, adverse changes in spring flow dynamics foretell possible adverse impacts on groundwater

resources. Hence, the study was designed to analyze the spatial and temporal patterns of spring flow variability in Oklahoma. Insufficiency of long-term and continuous instrumental records of spring flows however compelled a shift of focus to groundwater level analysis for which data is more abundant. Because groundwater aquifers feed spring flows, this shift does not compromise the overarching goal of the study.

The major findings emerging from the study could be summarized as follows:

1. Analysis of trends for groundwater level time series in 429 wells throughout Oklahoma reveals that 58% are experiencing statistically significant upward trends (indicating groundwater table rise), 25% show statistically significant downward trends (indicating groundwater table decline), and 17% show no change. The average trend magnitude is +0.43 ft/yr (5.15 in/yr) for water table rise and -0.992 ft/yr (11.90 in/yr) for water table decline. Consequently the water table has risen, on average, 12 ft over the 28 year study period in some wells and declined nearly 28 ft in others.
2. Groundwater decline is occurring primarily in the panhandle region of the Ogallala aquifer. Elsewhere in the state, a few isolated wells show declining trends but with no coherent spatial pattern. Groundwater level rise is occurring along the eastern part of the Ogallala aquifer and indeed most of western Oklahoma. Wells with no change in groundwater level are interspersed, and sometimes in close proximity with wells showing either rises or declines. As a measure of the level of spatial mixing, principal component analysis failed to segregate among the different types of wells.
3. The mixed spatial distribution of wells showing rises or declines raises important questions about the possible cause of groundwater level variations. Analysis of precipitation times

series found that 26% (of 103 precipitation gauging sites) show statistically significant upwards trends. Only one site had a significant negative trend. However the precipitation sites showing positive trends are widely distributed and interspersed with stations showing no change over the study period.

4. The result of trend analysis presented here appear to contradict some previously published research suggesting that precipitation time series in Oklahoma is on the rise, like the rest of the great plains or Central United States. Because of the significance of precipitation to agriculture and other activities in Oklahoma, it is critical that a reliable and definitive estimate of the precipitation trends is established to facilitate water resources planning and management.

5. A precipitation increase on the order of 1% during the study period is sufficient theoretically to account for observed groundwater level rise if aquifer specific yield is assumed to be 5.4%. Such precipitation increase has in fact occurred at several stations throughout Oklahoma but the results cannot be assumed to be applicable generally because other intervening station series did not experience similar rise. Nevertheless, the important point to emphasize is that observed groundwater level rise in Oklahoma could be explained by natural precipitation increase without the need to invoke anthropogenic factors.

6. Seven species of fish were found in the springs but none are rare or spring specialists. Fish were present in 8 of the 23 springs sampled and crayfish in 12. Arbuckle springs had the greatest diversity of species. The discharge of springs containing fish averaged 15.79 l/s similar to the discharge in springs with crayfish (15.89 l/s). Springs with average discharge around 1.5 l/s had neither fish nor crayfish.

7. None of the sampled Central Oklahoma Aquifer springs had fish or crayfish. In fact, the only fish seen in the isolated High Plains Aquifer springs were *Gambusia affinis*, a very common fish species in Oklahoma springs.
8. At least three species of crayfishes were found, along with a few cave-adapted crustaceans. Two of the surveyed Arbuckle springs had cave isopods.
9. Sixty taxa of invertebrates (excluding crayfish) have been identified thus far and work continues on identifying others. The most taxa were found in the Arbuckle-Simpson Aquifer but individual High Plains aquifer springs were the most diverse and the Central Oklahoma Aquifer springs were apparently the least diverse. Sulphur spring in the Arbuckle-Simpson area is the most taxonomically unique spring.
10. The study has not disproved the claim that the discharge volumes in some springs may be declining. Rather it uncovered the interesting paradox that groundwater levels are declining in some aquifers even as they are rising in others. A follow up study that attributes these changes to specific causal mechanisms is solely needed to provide a comprehensive reference source for research as well as water resources planning and management in Oklahoma.

Directions for Future Research

Several important questions emanating from the study need to be investigated more rigorously and systematically. For example, there is a need for a comprehensive analysis of the causes of groundwater level dynamics in Oklahoma. Specifically;

(i) Are other hydroclimatic time series in Oklahoma (i.e. precipitation, stream discharge, baseflow, evapotranspiration, soil moisture index) generally experiencing positive or

negative trends? If so, what are the magnitudes, spatial patterns, and reasons for these changes?

(ii) To what extent are observed changes in groundwater levels in Oklahoma climatically induced or the result of anthropogenic processes and what is the relative contribution of both processes?

(iii) Is the observed change temporary or part of a more permanent trend?

(iv) Is the present trend unique or part of a low frequency oscillatory behavior in groundwater variability?

(v) What are the possible impacts of changes in groundwater storage on the unique spring-fed ecosystems that rely on the groundwater?

(vi) What are the impacts and long term implications of observed changes in Oklahoma's water resources on various agricultural, recreational, economic, and other sectors in Oklahoma?

Further research is needed for developing a comprehensive and definitive reference source on Oklahoma's changing water resources.

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compiled and analyzed the spring and well flow time series and developed several GIS layers. Joseph Zume and Daniel Sambu carried out further statistical and GIS analysis. We appreciate especially the landowners and managers who allowed access to springs. Additional funding for identifications and the 2005 sampling was provided by the Oklahoma Department of Wildlife Conservation.

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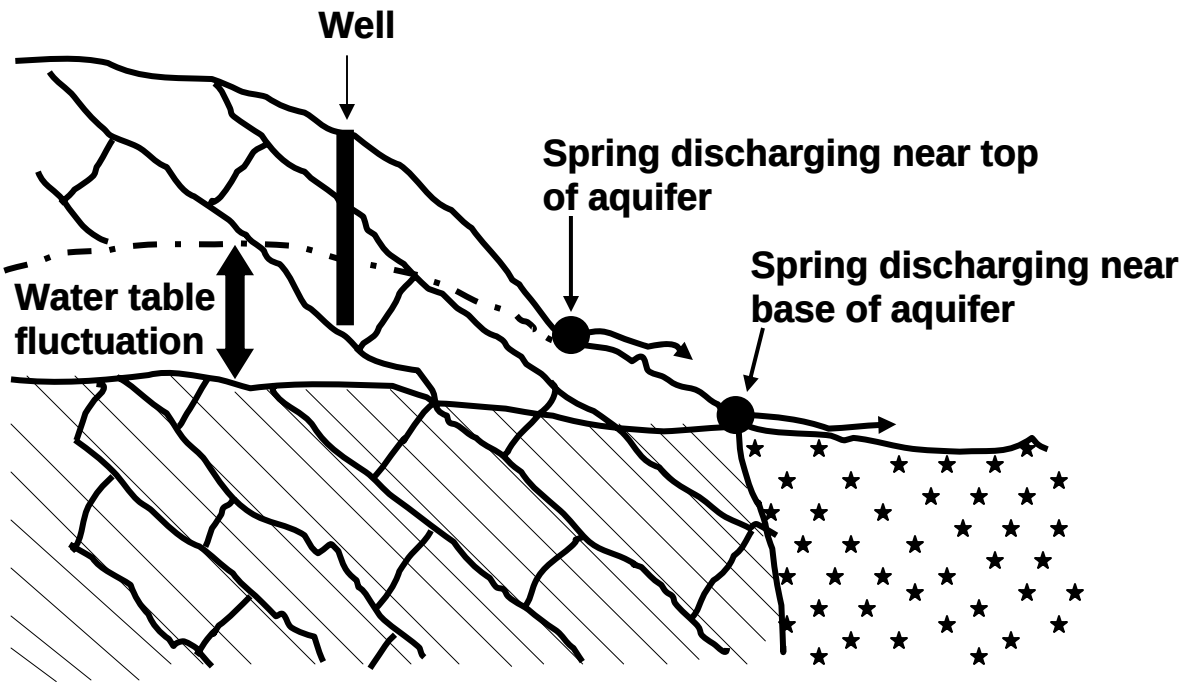


Figure 1. Conceptual illustration of aquifer water table fluctuations and changes in spring flow

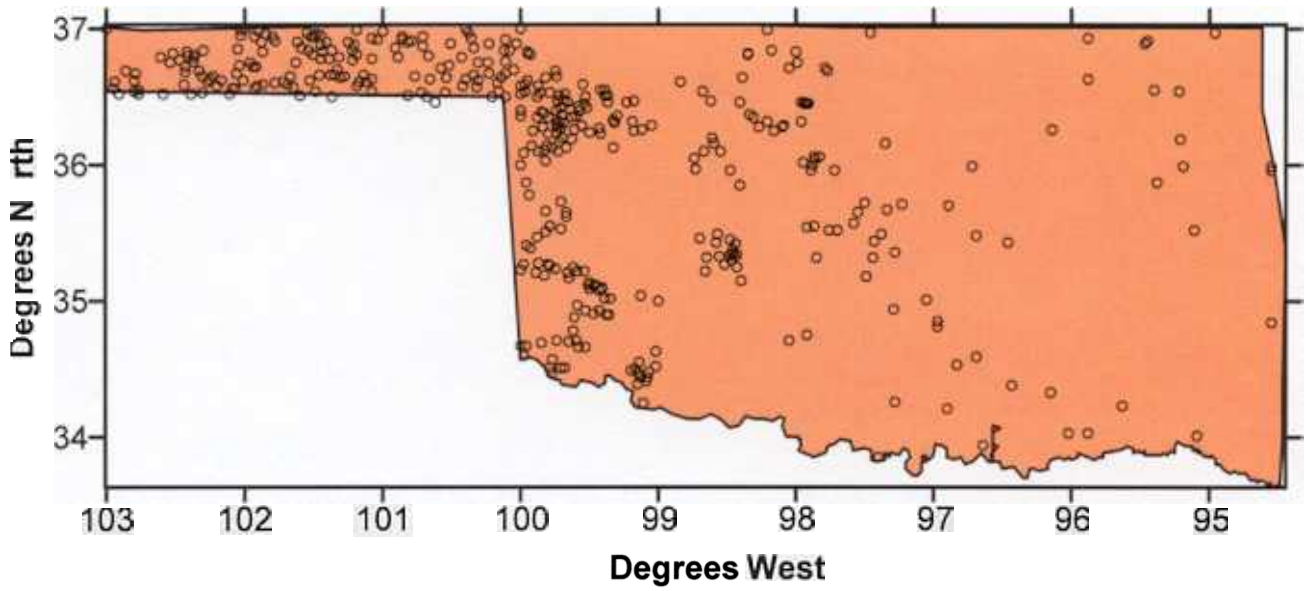


Figure 2. The locations the 429 well points analyzed

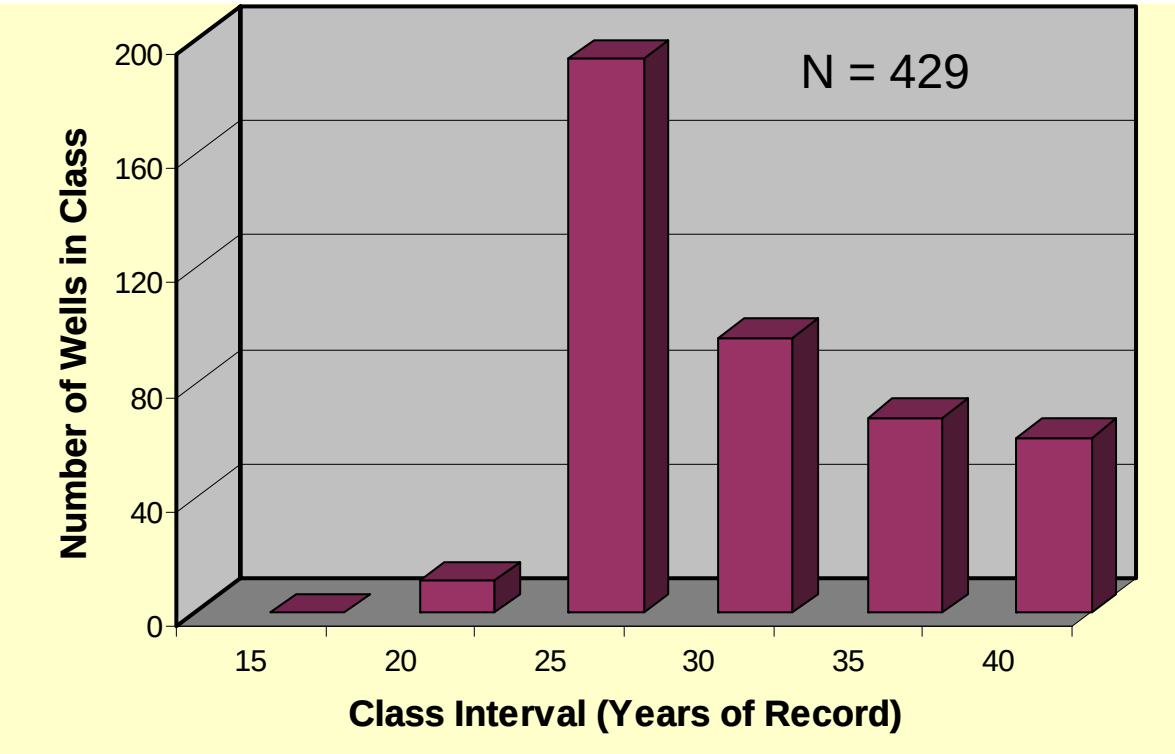


Figure 3. Length of annual well level time series analyzed and the frequency of wells in each class interval. The series ranged from 17 to 38 years with an average of 28 years.

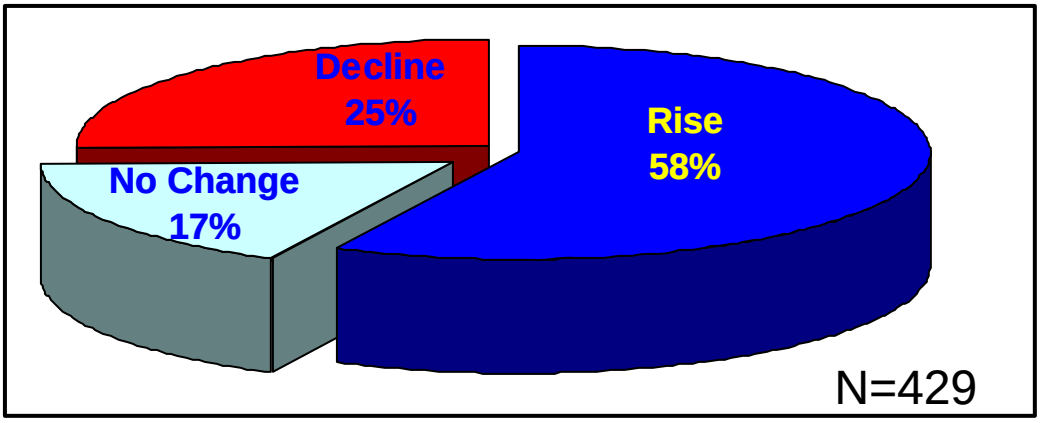


Figure 4. Percentage distribution of wells showing statistically significant (α=0.05 water level rise, decline, and no change. Total number of wells is 429.

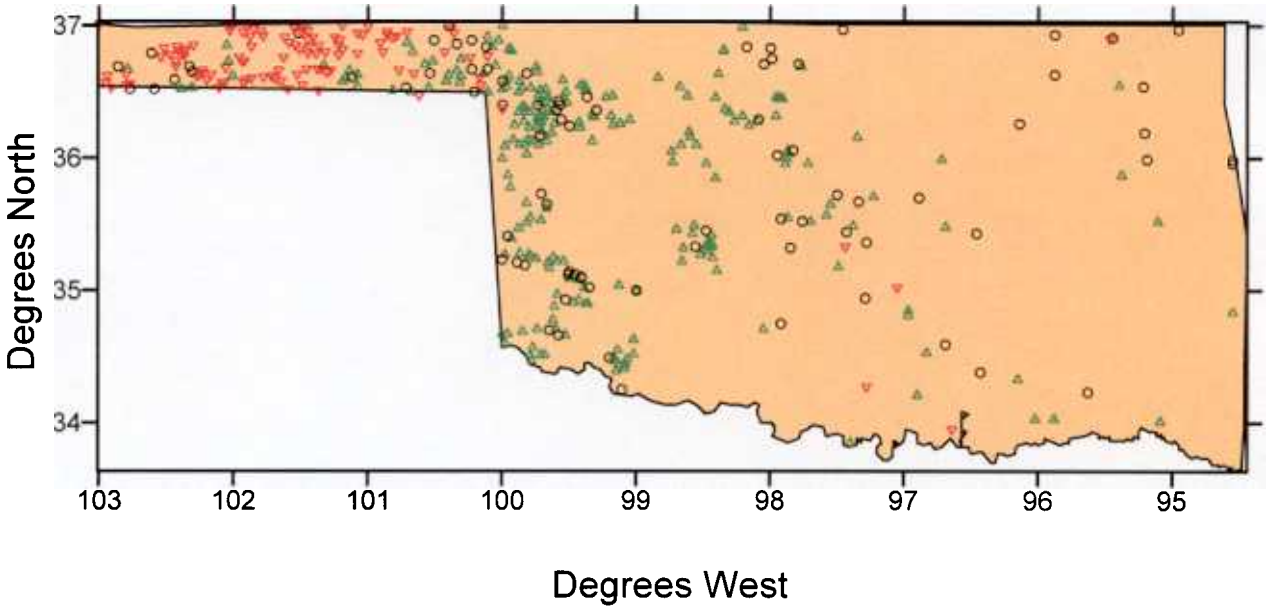


Figure 5. Spatial distribution of groundwater level trends. Inverted red triangles are wells showing statistically significant negative declines, upright green triangles are wells showing statistically positive rises, and open circles are the wells with no change in groundwater level during the study period.

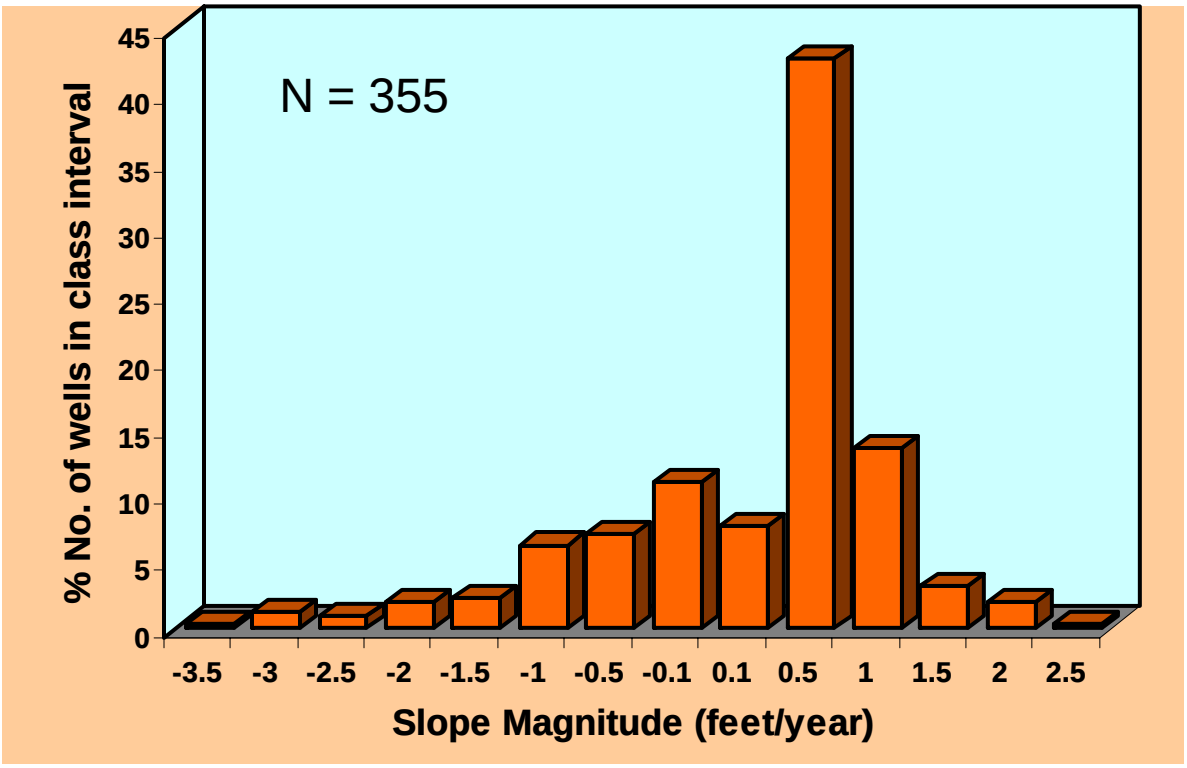


Figure 6. Number of wells in various slope magnitude classes. All wells are statistically significant at α=0.05. Total number of well time series is 355.

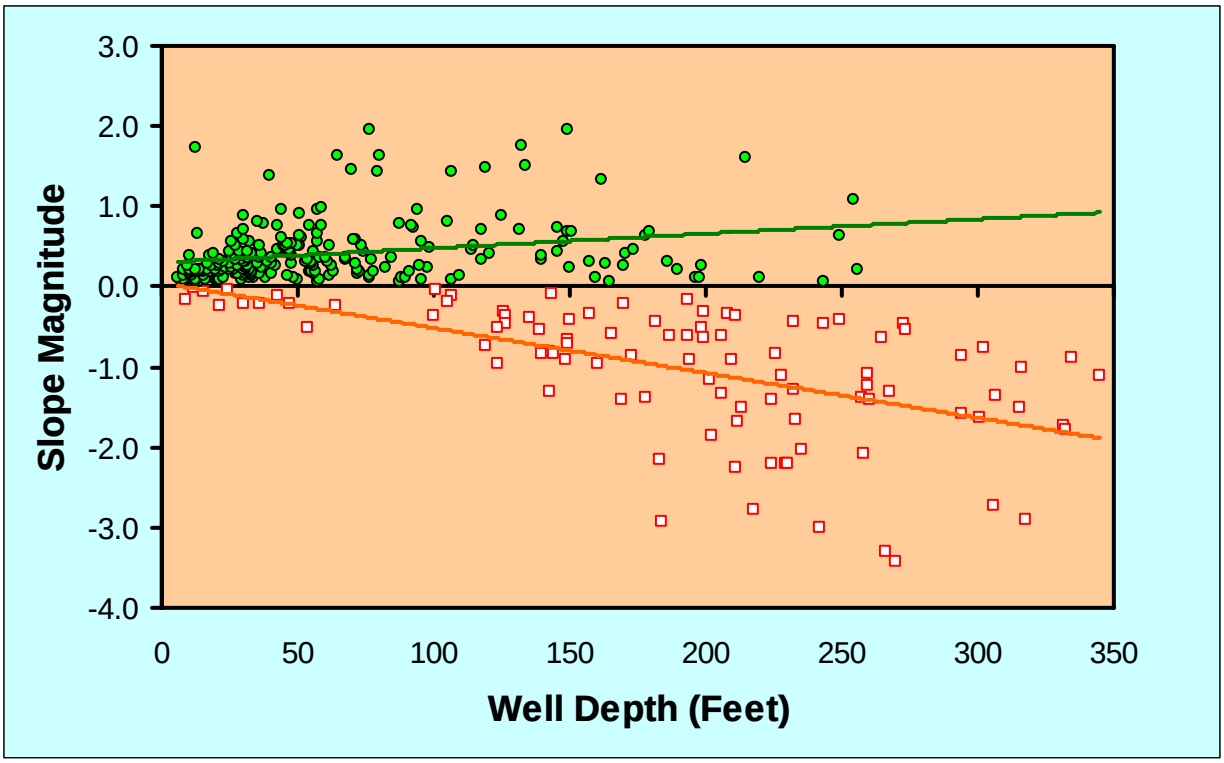


Figure 7. Relationship between slope magnitude and well depth. Notice most deep wells (open red squares) show negative trends and most wells showing positive trends (filled green circles) cluster around a depth of 45 feet below ground surface.

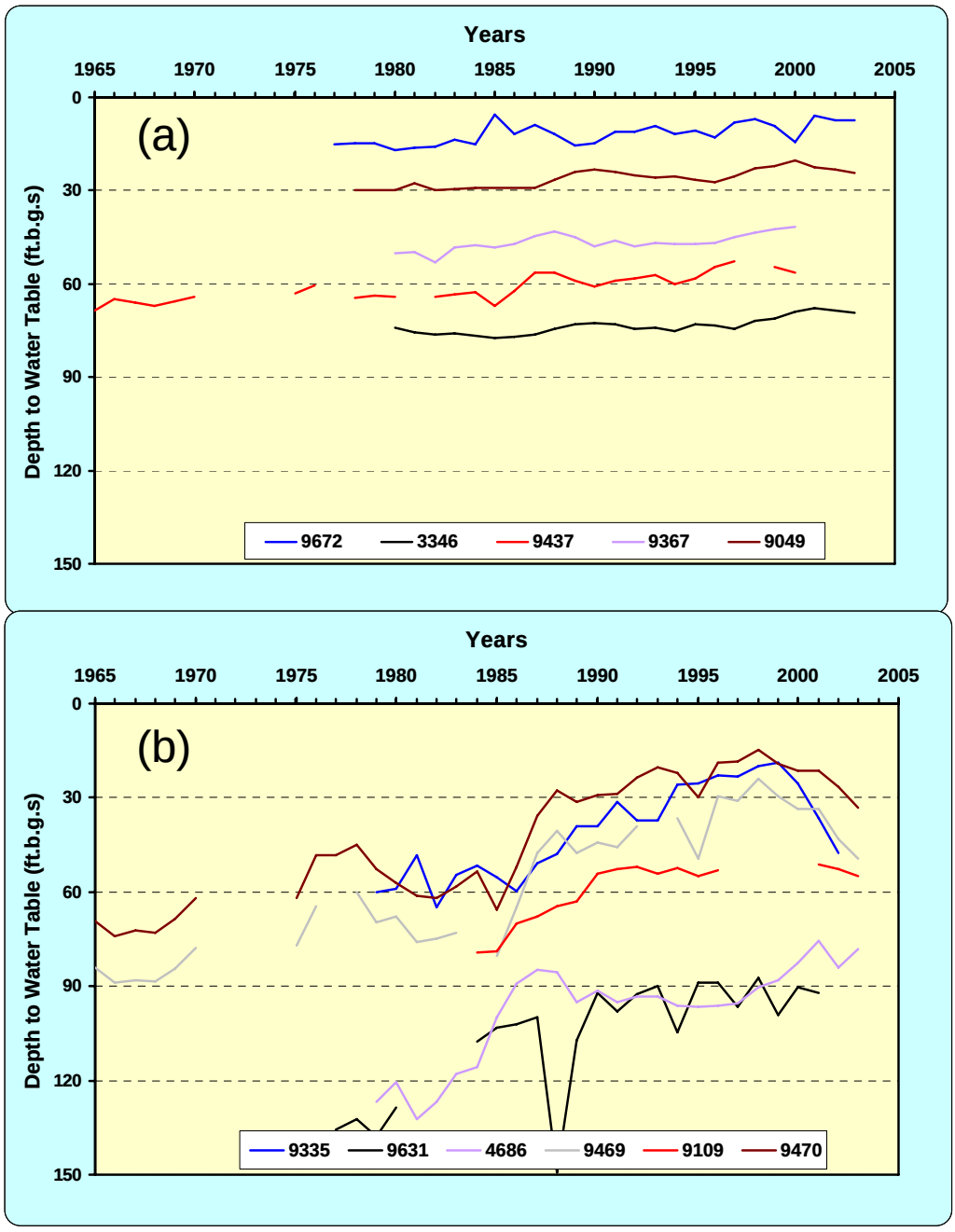


Figure 8. (a) Illustrative wells showing median slope positive magnitudes (i.e. about +0.328 ft/yr) and (b) Wells with high positive slope magnitudes.

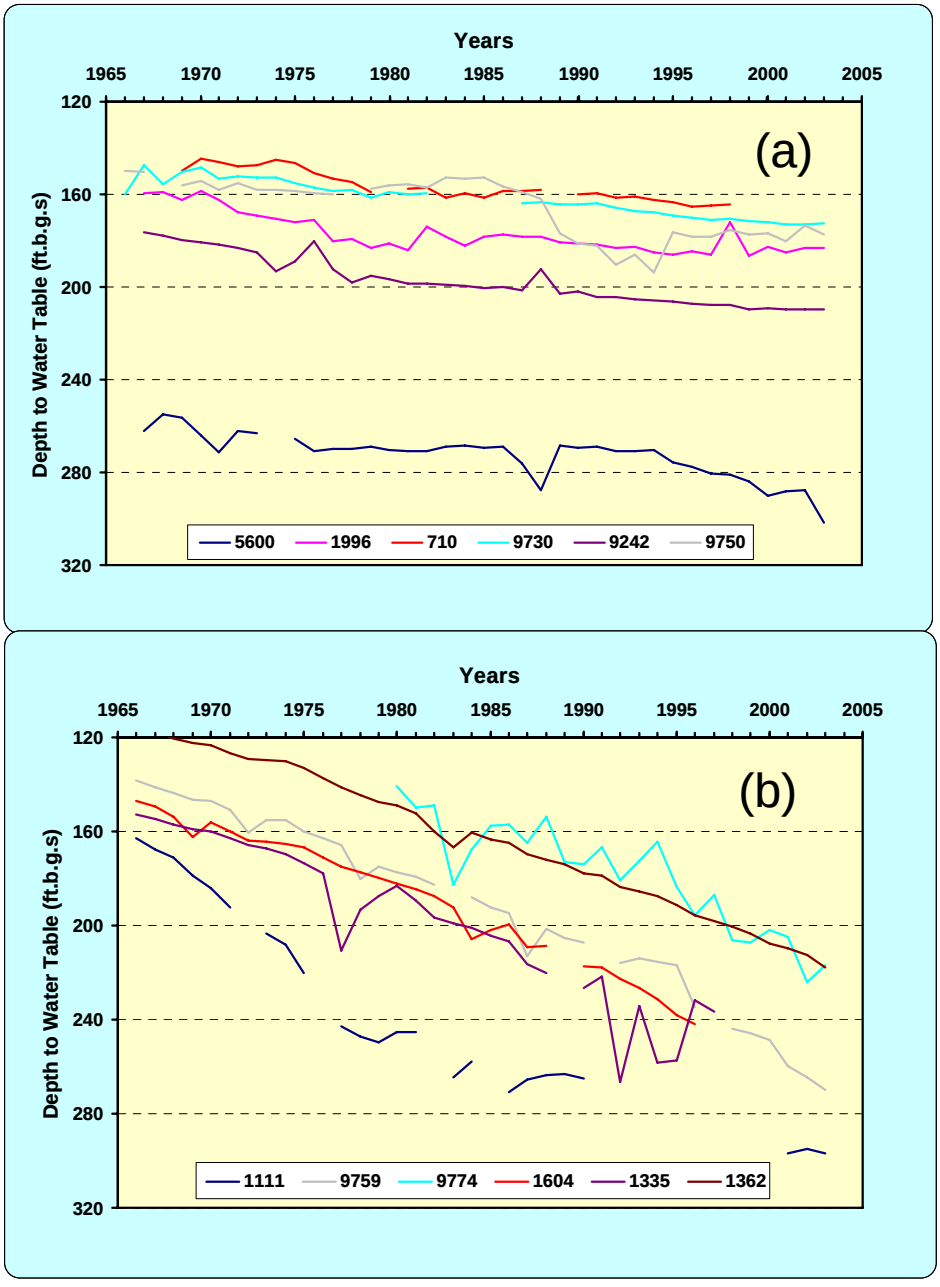


Figure 9. Illustrative wells showing median negative slope magnitudes (i.e. about -0.661ft/yr) and (b) Wells with high negative slope magnitudes.

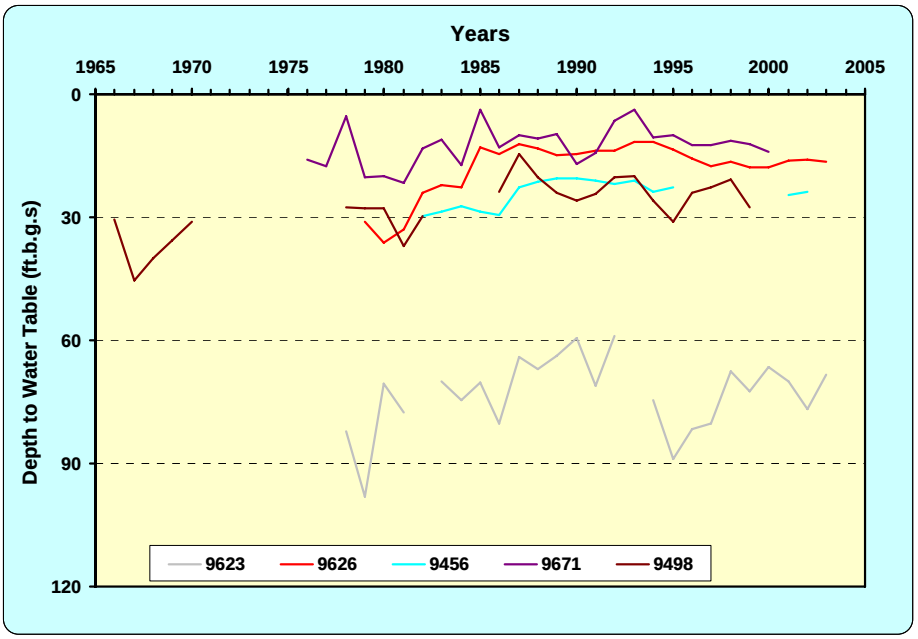


Figure 10. Illustrative wells showing no change in groundwater elevation during the study period.

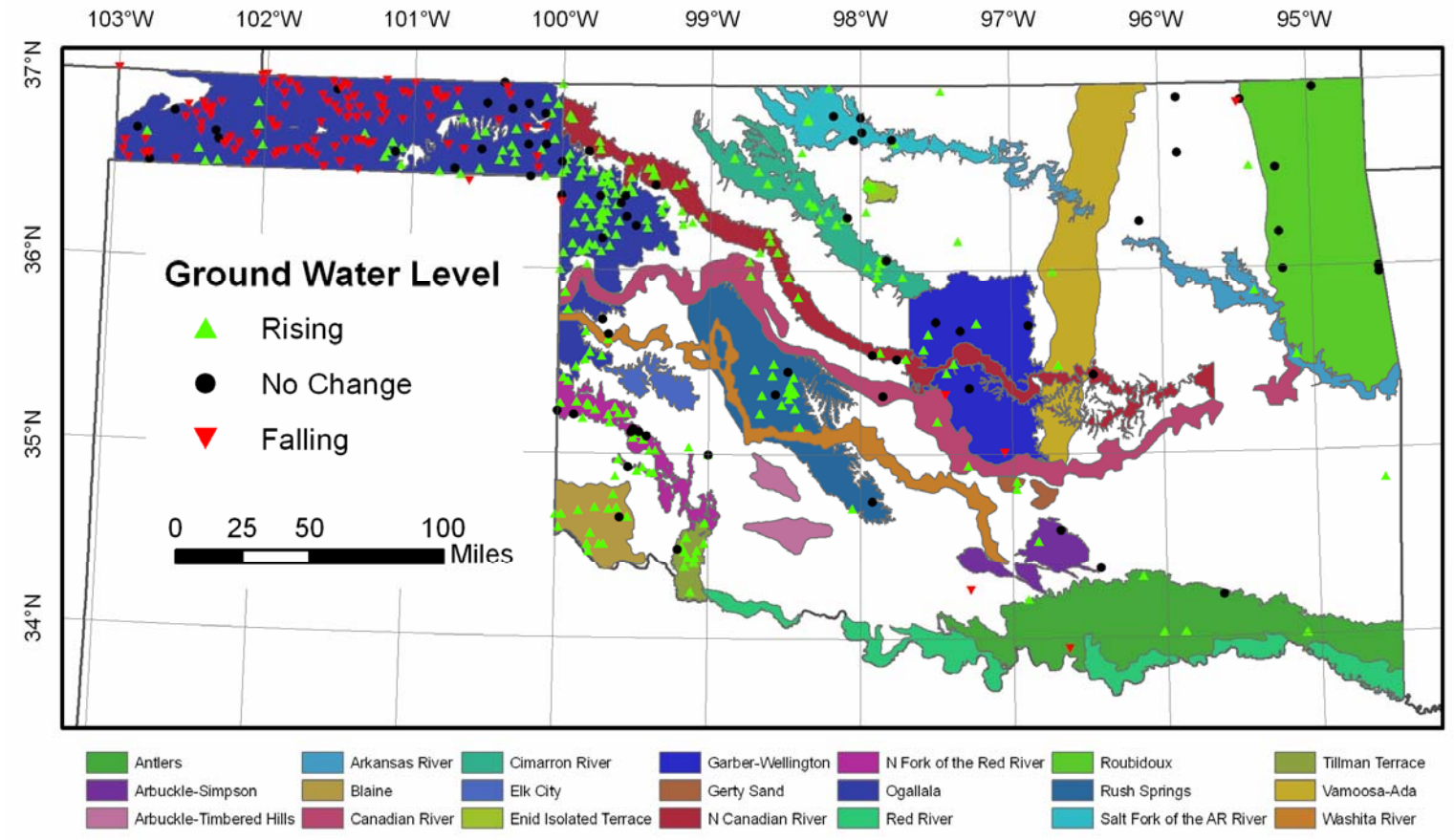


Figure 11. Major groundwater aquifers in Oklahoma and distribution of wells showing water level rise, decline, and no change.

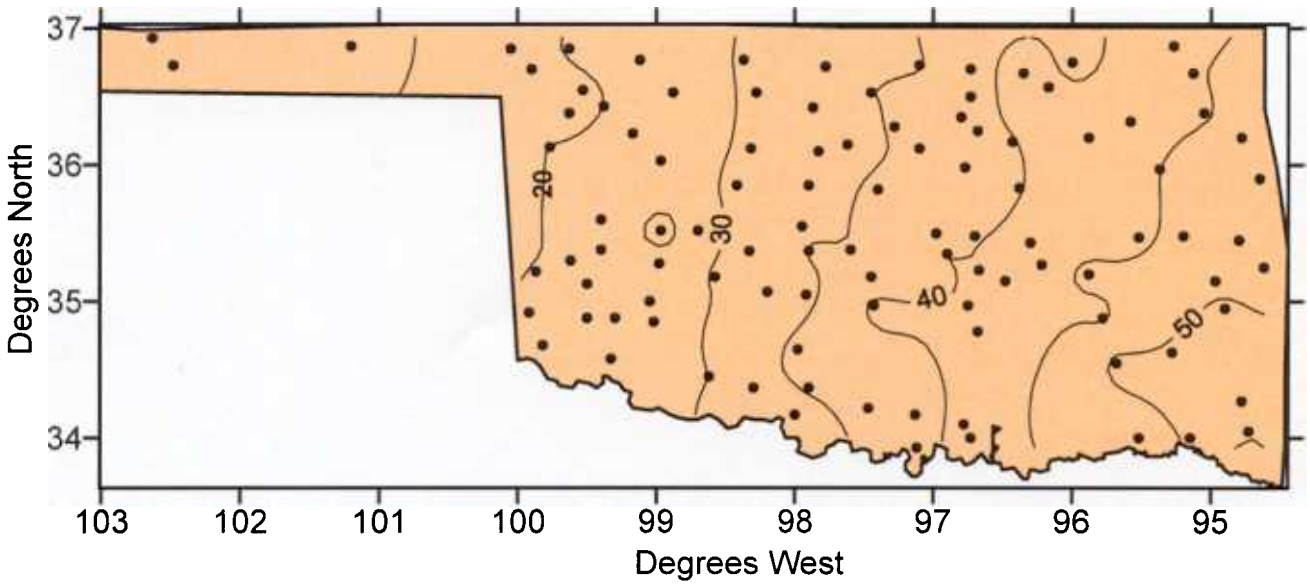


Figure 12. Distribution of the 103 precipitation gauging sites for which time series have been analyzed.

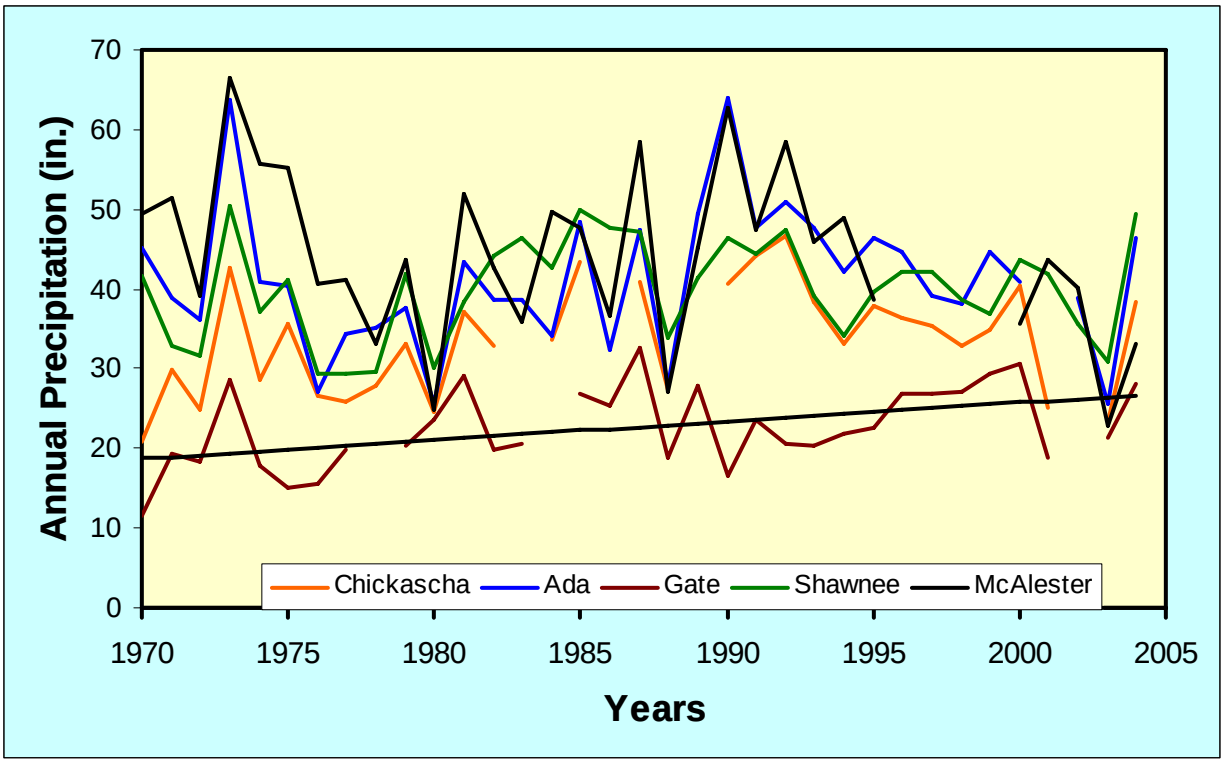


Figure 13. Temporal pattern of annual precipitation variability at five illustrative gauging sites.

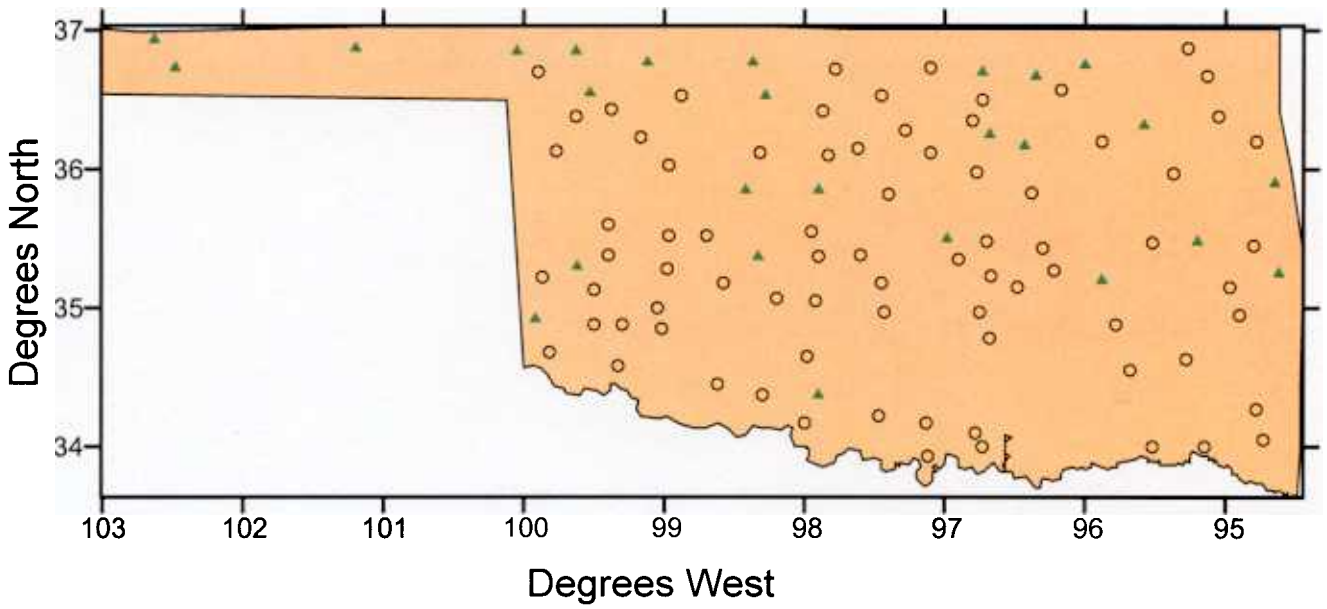


Figure 14. Distribution of precipitation gauging sites showing positive trends (green triangles).

APPENDIX A

Result of Mann-Kendall Analysis for Groundwater Level trends for Wells in Oklahoma

	Ground Water						M-K Trend		Sen's slope estimate											
S/NO	SITE_ID	Lat	Lon	First year	Last Year	n	Test Z	Signific.	Q	Q(final)	Label*	Qmin99	Qmax99	Qmin95	Qmax95	B	Bmin99	Bmax99	Bmin95	Bmax95
1	9569	33.85	-97.40	1980	2001	21	-2.929	**	-0.404	0.404	1	-0.674	-0.050	-0.608	-0.139	49.540	55.468	40.476	54.084	42.362
2	9595	34.01	-95.09	1976	2003	28	-2.509	*	-0.147	0.147	1	-0.299	0.017	-0.258	-0.026	74.168	77.475	70.549	76.536	71.437
3	9131	34.03	-96.02	1977	2003	27	-5.859	***	-1.334	1.334	1	-1.572	-0.883	-1.506	-0.971	167.264	171.549	164.179	170.193	164.443
4	9174	34.03	-95.88	1976	2003	27	-3.919	***	-0.253	0.253	1	-0.373	-0.130	-0.344	-0.151	29.450	32.525	26.940	32.009	27.599
5	9504	34.21	-96.90	1977	2003	26	-2.601	`	-0.223	0.223	1	-0.532	-0.002	-0.442	-0.050	31.631	38.393	25.824	36.110	27.148
6	9803	34.25	-99.11	1977	2003	27	-1.876	+	-0.168	0.168	2	-0.419	0.074	-0.369	0.012	31.882	37.208	26.134	35.815	28.036
7	9010	34.33	-96.15	1976	2003	28	-2.509	*	-0.595	0.595	1	-1.460	0.044	-1.207	-0.148	46.228	74.148	33.808	66.419	37.322
8	9811	34.39	-99.15	1974	2003	29	-6.622	***	-0.387	0.387	1	-0.469	-0.297	-0.451	-0.329	40.756	42.586	38.965	42.035	39.714
9	9812	34.41	-99.09	1975	2003	28	-6.342	***	-0.804	0.804	1	-0.926	-0.639	-0.901	-0.683	61.745	64.108	58.243	63.667	59.332
10	9818	34.44	-99.13	1965	2003	36	-4.931	***	-0.472	0.472	1	-0.658	-0.281	-0.609	-0.336	28.085	30.951	25.448	30.087	26.200
11	9816	34.44	-99.08	1968	2003	32	-4.849	***	-0.397	0.397	1	-0.535	-0.232	-0.477	-0.274	28.640	31.334	26.723	30.166	27.542
12	9823	34.47	-99.15	1981	2003	22	-2.116	*	-0.514	0.514	1	-1.119	0.123	-0.952	-0.051	28.884	43.164	9.959	40.217	15.060
13	9494	34.47	-99.78	1978	2000	23	-2.007	*	-0.284	0.284	1	-0.454	0.075	-0.432	-0.018	79.735	84.175	71.592	83.756	73.975
14	9824	34.48	-99.07	1974	2003	28	-2.904	**	-0.410	0.410	1	-0.641	-0.064	-0.593	-0.169	29.585	34.772	19.991	33.624	23.784
15	9828	34.50	-99.17	1974	2003	29	-4.671	***	-0.812	0.812	1	-1.080	-0.619	-1.004	-0.681	42.253	48.973	37.787	46.917	39.025
16	9464	34.51	-99.79	1976	2003	26	-2.094	*	-0.315	0.315	1	-0.634	0.059	-0.564	-0.049	21.680	28.908	10.556	27.160	14.318
17	9495	34.51	-99.68	1965	2000	30	-4.372	***	-0.293	0.293	1	-0.405	-0.159	-0.371	-0.195	34.523	36.408	32.127	35.771	32.770
18	9463	34.51	-99.71	1965	2003	35	-5.341	***	-0.240	0.240	1	-0.340	-0.162	-0.310	-0.183	16.732	18.643	15.267	18.070	15.600
19	9829	34.52	-99.02	1974	2003	29	-3.452	***	-0.239	0.239	1	-0.425	-0.082	-0.382	-0.120	18.944	23.091	15.562	22.083	16.311
20	9599	34.53	-96.83	1976	2003	28	-2.490	*	-1.394	1.394	1	-3.035	0.053	-2.663	-0.393	119.961	156.477	82.843	149.966	97.656
21	9831	34.55	-99.14	1975	2003	27	-3.544	***	-0.450	0.450	1	-0.702	-0.216	-0.629	-0.276	34.270	40.108	28.406	38.230	30.122
22	9465	34.57	-99.77	1976	2003	27	-2.377	*	-0.736	0.736	1	-1.406	0.116	-1.243	-0.124	44.671	61.459	22.849	56.081	30.469
23	9466	34.60	-99.98	1966	2003	33	-2.619	**	-0.082	0.082	1	-0.199	-0.002	-0.165	-0.023	34.218	36.542	32.734	35.870	33.253
24	9835	34.63	-99.02	1977	2003	27	-4.525	***	-0.689	0.689	1	-0.904	-0.446	-0.831	-0.524	34.433	39.632	28.596	37.835	30.647
25	9497	34.66	-99.53	1965	2003	35	-3.139	**	-0.353	0.353	1	-0.608	-0.102	-0.523	-0.161	38.264	42.500	33.154	41.392	33.990
26	9469	34.67	-100.00	1965	2003	32	-5.497	***	-1.642	1.642	1	-1.994	-1.156	-1.910	-1.290	90.719	95.010	83.709	93.812	84.045
27	9470	34.67	-99.96	1965	2003	35	-5.709	***	-1.562	1.562	1	-1.881	-1.106	-1.824	-1.261	74.682	77.841	63.144	76.364	67.779
28	9471	34.69	-99.85	1965	2002	31	-4.521	***	-1.333	1.333	1	-1.785	-0.839	-1.685	-0.970	67.570	73.923	59.512	71.931	61.336
29	9499	34.70	-99.65	1978	2003	23	-1.717	+	-0.349	0.349	2	-0.753	0.105	-0.654	0.033	26.210	35.451	11.064	32.777	13.235
30	4686	34.71	-98.05	1979	2003	25	-3.947	***	-1.753	1.753	1	-2.375	-0.690	-2.275	-0.899	143.397	156.238	111.521	154.331	116.635
31	9472	34.71	-99.74	1965	2003	35	-3.380	***	-0.974	0.974	1	-1.657	-0.328	-1.474	-0.446	75.634	85.178	65.828	84.076	68.336
32	9501	34.71	-99.60	1976	2003	26	-3.086	**	-0.818	0.818	1	-1.348	-0.176	-1.243	-0.339	50.030	63.551	35.094	60.534	38.300
33	9436	34.78	-99.62	1966	2003	32	-3.487	***	-0.657	0.657	1	-1.018	-0.231	-0.935	-0.355	41.755	48.239	33.003	46.627	35.054
34	9424	34.81	-96.97	1975	2003	28	-3.536	***	-0.293	0.293	1	-0.430	-0.087	-0.396	-0.154	75.096	78.921	69.433	78.192	71.245
35	9557	34.84	-94.55	1980	2003	24	-4.589	***	-1.112	1.112	1	-1.755	-0.686	-1.594	-0.760	61.234	78.559	49.343	74.029	51.479
36	9425	34.85	-96.97	1975	1999	24	-3.349	***	-0.500	0.500	1	-0.853	-0.160	-0.762	-0.224	36.892	44.748	28.791	42.921	30.279
37	9437	34.88	-99.61	1965	2000	29	-5.346	***	-0.340	0.340	1	-0.467	-0.243	-0.438	-0.270	67.691	70.281	65.677	69.586	66.399
38	9438	34.90	-99.36	1980	2003	24	-4.093	***	-0.622	0.622	1	-0.879	-0.286	-0.815	-0.390	39.271	45.357	29.816	43.939	32.984
39	9439	34.90	-99.38	1980	1999	20	-4.804	***	-0.418	0.418	1	-0.558	-0.261	-0.503	-0.308	37.415	40.868	33.326	39.717	34.571
40	9442	34.91	-99.47	1965	2003	29	-4.408	***	-0.252	0.252	1	-1.495	-0.115	-0.597	-0.136	32.730	70.083	29.299	43.140	29.777
41	9444	34.93	-99.43	1980	2002	23	-4.014	***	-0.195	0.195	1	-0.275	-0.095	-0.265	-0.109	30.900	32.852	28.551	32.564	28.862
42	9591	34.94	-97.29	1976	2003	27	-1.960	+	-0.206	0.206	2	-0.481	0.075	-0.411	0.001	12.240	18.515	4.920	17.257	6.823
43	9448	34.97	-99.59	1980	2003	24	-2.332	*	-0.239	0.239	1	-0.390	0.014	-0.360	-0.034	33.105	36.578	25.928	35.875	27.314
44	9834	35.00	-99.00	1965	2003	35	-3.665	***	-0.159	0.159	1	-0.267	-0.068	-0.243	-0.093	14.356	15.809	12.670	15.629	13.306
45	9451	35.02	-99.39	1980	2001	22	-2.425	*	-0.404	0.404	1	-0.679	0.034	-0.621	-0.093	39.092	45.965	28.147	44.382	31.753
46	9450	35.02	-99.35	1980	2003	24	-1.860	+	-0.137	0.137	2	-0.321	0.066	-0.272	0.019	29.763	34.540	24.160	33.408	25.391
47	9554	35.04	-99.13	1976	2003	26	-2.535	*	-0.067	0.067	1	-0.157	0.006	-0.141	-0.015	19.903	21.764	18.117	21.372	18.541
48	9454	35.08	-99.43	1980	2003	23	-2.535	*	-0.343	0.343	1	-0.705	0.005	-0.619	-0.103	25.421	34.072	15.494	32.411	18.942
49	9458	35.09	-99.50	1980	2002	23	-2.324	*	-0.478	0.478	1	-1.024	0.076	-0.874	-0.077	48.226	63.895	33.277	59.682	37.398

50	9461	35.11	-99.47	1980	2000	21	-2.325	*	-0.346	0.346	1	-0.687	0.040	-0.583	-0.081	32.519	39.649	22.016	37.674	25.281
51	9133	35.15	-98.40	1974	2003	27	-3.461	***	-0.723	0.723	1	-1.139	-0.359	-1.037	-0.450	85.553	93.002	78.972	90.572	81.000
52	9090	35.17	-99.65	1980	2003	24	-3.150	**	-0.431	0.431	1	-0.757	-0.073	-0.696	-0.190	22.205	29.773	12.318	28.520	15.305
53	9593	35.18	-97.49	1976	2003	27	-2.168	*	-0.208	0.208	1	-0.396	0.037	-0.341	-0.028	12.058	16.557	5.694	15.553	7.628
54	9091	35.19	-99.83	1980	2003	23	-1.796	+	-0.436	0.436	2	-0.909	0.217	-0.829	0.013	49.681	60.486	32.329	59.240	37.948
55	9096	35.21	-99.66	1980	2003	22	-4.568	***	-0.675	0.675	1	-0.895	-0.461	-0.849	-0.533	37.325	42.442	31.299	41.327	33.307
56	9842	35.22	-98.66	1979	2003	24	-6.573	***	-1.448	1.448	1	-1.699	-1.201	-1.631	-1.271	104.558	110.995	97.357	109.185	99.207
57	9098	35.22	-99.54	1980	2003	24	-4.390	***	-0.505	0.505	1	-0.779	-0.237	-0.714	-0.277	49.747	56.702	41.748	55.072	42.766
58	9100	35.22	-99.59	1981	2003	23	-3.222	**	-0.311	0.311	1	-0.472	-0.064	-0.441	-0.133	72.124	76.254	66.027	75.721	67.832
59	9108	35.24	-99.75	1980	2003	24	-4.490	***	-0.455	0.455	1	-0.599	-0.266	-0.561	-0.315	53.142	56.218	48.148	55.367	49.667
60	9109	35.25	-99.62	1980	2003	18	-3.864	***	-1.614	1.614	1	-2.303	-0.441	-2.161	-0.839	100.235	113.959	69.290	110.828	81.758
61	9138	35.25	-98.43	1974	2003	27	-3.357	***	-0.760	0.760	1	-1.320	-0.240	-1.123	-0.402	83.560	93.159	77.620	89.705	79.616
62	9114	35.26	-99.79	1980	2003	24	-4.242	***	-0.522	0.522	1	-0.758	-0.210	-0.680	-0.304	32.368	38.152	23.143	35.887	25.827
63	3899	35.27	-98.52	1974	2003	26	-2.998	**	-0.604	0.604	1	-1.036	-0.137	-0.917	-0.280	48.338	57.862	38.209	55.433	42.044
64	9119	35.27	-99.98	1980	2003	23	-4.888	***	-0.385	0.385	1	-0.489	-0.265	-0.458	-0.313	60.805	62.795	58.816	62.188	59.576
65	1918	35.27	-99.80	1980	2003	24	-2.902	**	-0.341	0.341	1	-0.575	-0.040	-0.521	-0.153	40.710	44.947	34.895	44.014	37.436
66	9120	35.28	-99.87	1980	2003	23	-3.433	***	-0.193	0.193	1	-0.449	-0.064	-0.378	-0.091	47.250	52.359	44.431	50.901	45.095
67	4093	35.30	-98.47	1975	2000	24	-3.696	***	-0.857	0.857	1	-1.481	-0.410	-1.362	-0.519	108.160	123.926	97.202	121.079	99.865
68	9844	35.32	-98.65	1979	2003	25	-2.966	**	-0.110	0.110	1	-0.176	-0.022	-0.158	-0.048	6.020	7.739	3.733	7.276	4.398
69	9145	35.33	-98.45	1978	2003	25	-4.648	***	-1.065	1.065	1	-1.465	-0.645	-1.392	-0.743	102.328	109.514	94.752	107.751	96.531
70	4039	35.34	-98.48	1977	2003	26	-3.703	***	-0.632	0.632	1	-0.988	-0.221	-0.889	-0.311	100.363	109.559	89.696	106.904	92.063
71	9147	35.34	-98.43	1974	2003	26	-2.513	*	-0.500	0.500	1	-1.132	0.067	-0.916	-0.097	70.910	81.979	64.653	77.050	65.340
72	4017	35.35	-98.46	1974	2003	27	-2.585	**	-0.703	0.703	1	-1.280	-0.029	-1.097	-0.185	121.047	136.335	103.312	130.918	106.686
73	9151	35.38	-98.46	1974	2003	28	-3.536	***	-0.767	0.767	1	-1.406	-0.361	-1.241	-0.477	138.138	150.982	131.007	147.950	132.077
74	9122	35.39	-99.93	1980	2003	23	-3.169	**	-0.220	0.220	1	-0.377	-0.069	-0.329	-0.098	21.370	24.320	19.118	23.545	19.558
75	9123	35.41	-99.96	1980	2003	23	-1.770	+	-0.068	0.068	2	-0.188	0.054	-0.157	0.013	9.552	11.823	7.220	11.483	8.247
76	9156	35.42	-98.44	1974	2003	29	-4.485	***	-1.103	1.103	1	-1.506	-0.617	-1.430	-0.686	93.147	101.004	85.396	99.992	86.568
77	9157	35.43	-98.58	1974	2003	29	-4.335	***	-0.207	0.207	1	-0.310	-0.116	-0.287	-0.132	91.123	92.882	89.450	92.531	89.777
78	9608	35.44	-97.43	1975	2003	27	-1.918	+	-1.620	1.620	2	-4.129	0.475	-3.553	0.105	223.040	290.416	161.825	277.743	174.535
79	9847	35.46	-98.70	1979	2003	25	-5.395	***	-0.914	0.914	1	-1.187	-0.673	-1.107	-0.757	53.557	61.118	46.609	58.946	49.114
80	9335	35.47	-99.88	1979	2002	24	-4.738	***	-1.901	1.901	1	-2.576	-1.144	-2.358	-1.350	66.562	77.015	56.029	72.378	59.427
81	9558	35.48	-96.69	1980	2003	22	-4.906	***	-0.587	0.587	1	-0.777	-0.400	-0.714	-0.446	77.825	82.878	73.317	81.570	74.218
82	9611	35.49	-97.38	1979	2003	24	-3.795	***	-0.522	0.522	1	-0.791	-0.206	-0.718	-0.290	46.479	53.877	37.477	51.860	39.996
83	9160	35.49	-98.57	1974	2003	29	-2.532	*	-0.186	0.186	1	-0.458	0.003	-0.361	-0.050	68.931	74.176	65.589	71.949	66.638
84	9647	35.51	-99.82	1980	2003	24	-3.920	***	-0.174	0.174	1	-0.280	-0.087	-0.249	-0.106	22.170	25.190	20.002	24.337	20.545
85	9672	35.52	-95.11	1977	2003	27	-3.794	***	-0.317	0.317	1	-0.443	-0.133	-0.409	-0.190	18.984	22.615	14.680	21.728	16.114
86	9164	35.52	-97.70	1977	2003	25	-2.103	*	-0.152	0.152	1	-0.337	0.035	-0.300	-0.009	7.573	11.371	4.349	10.694	4.904
87	9649	35.53	-99.71	1980	2003	24	-4.390	***	-0.197	0.197	1	-0.290	-0.097	-0.274	-0.135	13.712	15.789	10.757	15.386	11.901
88	9650	35.55	-99.79	1980	2003	23	-4.595	***	-0.343	0.343	1	-0.485	-0.224	-0.442	-0.262	65.637	69.179	62.481	68.106	63.383
89	9167	35.55	-97.87	1977	2003	27	-2.064	*	-0.183	0.183	1	-0.401	0.080	-0.343	-0.010	11.517	14.968	6.790	13.981	8.312
90	9624	35.57	-97.58	1976	2003	23	-2.060	*	-0.296	0.296	1	-0.682	0.075	-0.568	-0.025	69.058	77.764	58.637	74.982	61.069
91	9328	35.62	-99.67	1976	2002	23	-4.067	***	-0.352	0.352	1	-0.533	-0.187	-0.486	-0.234	10.852	12.460	8.738	12.121	9.302
92	9619	35.65	-97.55	1976	2000	25	-5.142	***	-0.923	0.923	1	-1.157	-0.715	-1.082	-0.773	102.366	107.675	97.496	106.042	99.165
93	9657	35.66	-99.82	1980	2003	24	-3.226	**	-0.121	0.121	1	-0.226	-0.029	-0.198	-0.052	61.893	64.875	60.065	64.067	60.583
94	9622	35.71	-97.23	1977	1999	21	-2.084	*	-2.265	2.265	1	-3.900	0.202	-3.424	-0.143	88.796	127.435	17.541	117.400	27.557
95	9664	35.78	-99.94	1980	2002	21	-3.657	***	-0.301	0.301	1	-0.563	-0.107	-0.448	-0.153	42.364	48.580	38.326	46.754	39.101
96	5131	35.85	-98.41	1976	2003	26	-5.181	***	-0.461	0.461	1	-0.730	-0.290	-0.600	-0.331	26.388	32.927	22.065	29.659	23.021
97	9666	35.87	-99.96	1980	2003	21	-5.103	***	-0.116	0.116	1	-0.154	-0.080	-0.143	-0.092	7.290	8.357	6.210	8.028	6.593
98	9838	35.87	-95.38	1976	2000	25	-3.014	**	-0.076	0.076	1	-0.142	-0.015	-0.131	-0.028	7.878	9.347	6.558	9.154	6.762
99	9127	35.96	-98.48	1977	2003	27	-4.503	***	-0.345	0.345	1	-0.454	-0.193	-0.427	-0.234	54.105	55.939	51.349	55.456	52.035
100	9515	35.96	-97.89	1975	1999	24	-2.084	*	-0.196	0.196	1	-0.410	0.050	-0.379	-0.018	13.150	18.695	6.534	18.136	8.122
101	9512	35.96	-97.72	1975	2003	28	-2.016	*	-0.066	0.066	1	-0.195	0.025	-0.147	-0.002	19.157	21.975	16.655	21.008	17.599
102	9288	35.97	-98.73	1976	2003	26	-5.995	***	-1.416	1.416	1	-1.667	-1.185	-1.589	-1.255	109.407	113.880	105.855	112.374	107.042
103	9631	35.99	-96.72	1977	2001	23	-4.226	***	-1.805	1.805	1	-2.660	-0.804	-2.459	-1.095	148.765	166.041	120.428	162.671	127.713
104	9303	36.00	-100.00	1980	2003	23	-4.331	***	-0.370	0.370	1	-0.532	-0.214	-0.471	-0.278	85.671	89.029	84.046	87.560	84.370

105	3307	36.00	-100.00	1980	2003	24	-4.589	***	-0.297	0.297	1	-0.541	-0.157	-0.449	-0.186	150.876	156.405	147.612	154.428	148.295
106	3302	36.00	-100.00	1980	2003	23	-4.729	***	-0.260	0.260	1	-0.332	-0.180	-0.315	-0.209	98.290	100.113	96.209	99.560	96.955
107	9521	36.00	-97.88	1975	2003	27	-3.210	**	-0.220	0.220	1	-0.347	-0.072	-0.319	-0.113	16.900	20.183	12.774	19.413	14.148
108	9522	36.02	-97.95	1975	2003	27	-1.939	+	-0.123	0.123	2	-0.261	0.034	-0.221	0.000	25.520	29.109	20.857	28.331	21.993
109	9297	36.03	-99.82	1980	2003	24	-3.027	**	-0.234	0.234	1	-0.375	-0.065	-0.344	-0.130	24.365	26.655	22.180	26.079	22.804
110	9524	36.04	-97.86	1975	2003	28	-4.129	***	-0.463	0.463	1	-0.677	-0.225	-0.625	-0.297	36.731	42.483	29.928	41.384	32.055
111	9289	36.05	-98.74	1979	2003	25	-6.096	***	-0.935	0.935	1	-1.065	-0.731	-1.035	-0.812	61.733	63.586	59.301	63.095	60.513
112	9526	36.06	-97.87	1975	2003	24	-2.060	*	-0.090	0.090	1	-0.198	0.018	-0.173	-0.003	8.040	10.459	5.132	9.798	5.756
113	9301	36.09	-99.98	1980	2003	22	-4.342	***	-0.379	0.379	1	-0.537	-0.194	-0.500	-0.217	172.232	174.275	170.011	173.800	170.213
114	9306	36.10	-99.73	1980	2003	24	-5.680	***	-0.579	0.579	1	-0.797	-0.490	-0.768	-0.522	153.419	156.168	152.169	155.765	152.679
115	9128	36.10	-98.55	1978	2003	24	-5.531	***	-0.557	0.557	1	-0.688	-0.418	-0.663	-0.473	34.352	36.431	32.237	35.961	32.986
116	9291	36.10	-98.67	1979	2003	22	-3.752	***	-0.328	0.328	1	-0.450	-0.150	-0.429	-0.188	27.594	29.115	25.317	28.781	26.147
117	9302	36.10	-99.87	1980	2002	21	-2.992	**	-0.270	0.270	1	-0.470	-0.062	-0.427	-0.099	68.334	70.686	66.201	70.214	66.688
118	3012	36.11	-99.83	1980	2003	24	-3.970	***	-0.200	0.200	1	-0.331	-0.100	-0.290	-0.132	25.868	28.748	23.572	27.835	24.338
119	2990	36.13	-99.77	1980	2003	24	-5.707	***	-0.700	0.700	1	-0.897	-0.493	-0.848	-0.529	159.708	164.814	155.342	163.556	156.192
120	9383	36.13	-99.67	1980	2003	22	-3.779	***	-0.676	0.676	1	-0.921	-0.237	-0.848	-0.454	180.685	183.573	176.580	182.856	178.421
121	9293	36.13	-99.33	1980	2002	21	-5.559	***	-0.461	0.461	1	-0.554	-0.336	-0.528	-0.361	63.513	65.011	62.277	64.633	62.663
122	9315	36.14	-99.81	1980	2003	23	-4.912	***	-0.510	0.510	1	-0.721	-0.132	-0.660	-0.196	116.450	120.038	112.922	118.993	113.602
123	9313	36.14	-99.92	1980	2003	23	-4.490	***	-0.425	0.425	1	-0.678	-0.207	-0.598	-0.250	170.030	174.899	167.408	173.470	167.990
124	9130	36.16	-98.60	1976	2003	25	-3.854	***	-0.343	0.343	1	-0.504	-0.147	-0.441	-0.195	22.727	24.584	19.230	23.891	20.253
125	9636	36.16	-97.35	1977	1999	21	-3.536	***	-0.088	0.088	1	-0.255	-0.033	-0.176	-0.044	8.754	12.626	7.371	10.648	7.582
126	9322	36.19	-99.63	1980	2003	22	-5.583	***	-0.648	0.648	1	-0.758	-0.512	-0.731	-0.560	181.634	183.583	179.641	183.173	180.452
127	9323	36.20	-99.70	1980	2003	24	-5.432	***	-0.754	0.754	1	-0.925	-0.545	-0.882	-0.602	62.854	65.800	60.682	64.933	60.938
128	9571	36.20	-98.61	1979	2003	25	-5.513	***	-0.447	0.447	1	-0.539	-0.330	-0.520	-0.355	27.786	29.820	25.222	29.229	26.031
129	3043	36.23	-99.80	1980	2003	23	-4.676	***	-0.545	0.545	1	-0.713	-0.339	-0.685	-0.402	52.740	56.886	47.854	55.982	49.034
130	9867	36.23	-99.42	1980	2001	22	-5.418	***	-0.495	0.495	1	-0.604	-0.370	-0.588	-0.383	121.701	124.162	119.276	123.735	119.574
131	4315	36.24	-99.74	1980	2002	22	-3.869	***	-0.399	0.399	1	-0.547	-0.199	-0.509	-0.258	15.971	19.174	10.647	18.051	12.172
132	9334	36.25	-99.91	1980	2003	23	-3.856	***	-0.500	0.500	1	-0.658	-0.275	-0.617	-0.345	74.790	76.973	70.996	76.304	72.178
133	9575	36.25	-98.16	1965	2003	35	-5.368	***	-0.495	0.495	1	-0.618	-0.352	-0.589	-0.378	22.520	25.213	18.832	24.800	19.506
134	9390	36.25	-99.18	1977	2003	27	-4.836	***	-0.262	0.262	1	-0.353	-0.189	-0.330	-0.210	198.052	199.336	196.974	198.917	197.170
135	9871	36.26	-99.12	1977	2003	25	-4.321	***	-0.350	0.350	1	-0.616	-0.197	-0.522	-0.233	36.034	38.957	33.732	37.999	34.325
136	9367	36.26	-99.61	1980	2000	21	-3.714	***	-0.313	0.313	1	-0.598	-0.112	-0.511	-0.166	51.164	56.038	48.742	53.963	49.451
137	3097	36.27	-99.71	1980	2003	24	-3.349	***	-0.213	0.213	1	-0.314	-0.067	-0.270	-0.109	55.973	58.377	52.327	57.586	53.483
138	9870	36.27	-99.43	1980	2003	24	-2.332	*	-0.163	0.163	1	-0.253	0.032	-0.227	-0.046	43.924	45.234	40.765	45.056	41.963
139	9412	36.28	-98.10	1975	2003	27	-4.044	***	-0.661	0.661	1	-1.018	-0.242	-0.915	-0.406	28.236	36.784	19.409	34.707	22.355
140	3143	36.28	-99.84	1980	2003	24	-3.795	***	-0.575	0.575	1	-0.824	-0.331	-0.757	-0.402	49.790	56.025	44.453	54.251	45.972
141	9578	36.28	-98.27	1977	2003	26	-3.306	***	-0.202	0.202	1	-0.430	-0.059	-0.353	-0.088	20.347	26.389	16.974	24.167	17.658
142	9345	36.28	-99.73	1980	2003	24	-3.150	**	-0.103	0.103	1	-0.182	-0.026	-0.162	-0.047	37.830	38.827	36.852	38.618	37.230
143	9872	36.29	-99.05	1978	2000	23	-5.233	***	-0.304	0.304	1	-0.380	-0.225	-0.362	-0.246	79.587	80.420	78.718	80.184	78.923
144	9366	36.30	-99.74	1979	2002	24	-2.902	**	-0.151	0.151	1	-0.270	-0.022	-0.230	-0.061	38.872	40.720	37.437	40.237	37.954
145	5385	36.31	-99.32	1980	2003	23	-4.067	***	-0.289	0.289	1	-0.346	-0.169	-0.319	-0.214	34.539	35.816	31.542	35.304	32.684
146	9353	36.31	-99.69	1980	2003	24	-2.606	**	-0.190	0.190	1	-0.360	0.000	-0.319	-0.045	55.108	57.087	52.605	56.745	53.404
147	9580	36.32	-98.21	1976	2003	27	-3.169	**	-0.559	0.559	1	-0.867	-0.134	-0.791	-0.273	31.106	38.561	19.770	37.005	24.518
148	9314	36.32	-97.96	1977	2003	24	-3.894	***	-0.255	0.255	1	-0.397	-0.119	-0.359	-0.148	22.573	26.205	19.320	25.225	20.066
149	9876	36.32	-99.19	1975	2000	21	-3.839	***	-0.183	0.183	1	-0.320	-0.089	-0.290	-0.117	8.343	9.277	6.760	9.059	7.364
150	9356	36.32	-99.72	1980	2003	24	-3.597	***	-0.120	0.120	1	-0.173	-0.050	-0.157	-0.070	32.643	33.495	31.582	33.281	31.890
151	9877	36.33	-99.33	1980	2003	24	-3.001	**	-0.257	0.257	1	-0.409	-0.045	-0.377	-0.109	10.027	12.301	6.115	11.730	7.327
152	9581	36.35	-98.31	1976	2003	27	-5.003	***	-0.610	0.610	1	-0.726	-0.428	-0.690	-0.498	31.370	34.113	26.276	33.255	28.391
153	9364	36.35	-99.63	1980	2003	24	-3.845	***	-0.487	0.487	1	-0.737	-0.242	-0.662	-0.320	51.610	54.662	48.494	53.817	49.596
154	9362	36.35	-99.80	1980	2003	23	-3.328	***	-0.162	0.162	1	-0.288	-0.048	-0.262	-0.077	74.322	75.644	73.166	75.362	73.349
155	3244	36.35	-99.88	1981	2003	23	-2.271	*	-0.093	0.093	1	-0.198	0.012	-0.163	-0.011	218.528	220.834	216.110	220.069	216.669
156	9880	36.36	-99.30	1975	2003	28	-1.719	+	-0.060	0.060	2	-0.128	0.033	-0.116	0.008	3.830	4.948	3.036	4.784	3.302
157	9583	36.37	-98.34	1978	2003	26	-4.408	***	-0.562	0.562	1	-0.734	-0.274	-0.685	-0.345	36.232	40.460	28.271	39.447	30.033
158	9368	36.37	-99.70	1980	2001	21	-2.265	*	-0.111	0.111	1	-0.205	0.034	-0.187	-0.017	22.114	23.480	19.937	23.202	20.701
159	9372	36.38	-99.83	1980	2003	24	-4.343	***	-0.154	0.154	1	-0.268	-0.068	-0.252	-0.088	72.566	73.928	71.514	73.692	71.863

160	9884	36.39	-99.85	1978	2003	25	-4.816	***	-0.242	0.242	1	-0.370	-0.131	-0.348	-0.175	30.408	31.561	28.895	31.344	29.602
161	9378	36.41	-99.64	1980	2003	24	-3.497	***	-0.175	0.175	1	-0.281	-0.070	-0.258	-0.094	15.284	16.792	13.580	16.364	13.957
162	9887	36.42	-99.52	1978	2003	25	-4.461	***	-0.439	0.439	1	-0.549	-0.208	-0.514	-0.294	44.678	46.270	41.786	45.632	42.879
163	9380	36.42	-99.71	1980	2003	22	-2.820	**	-0.267	0.267	1	-0.532	-0.031	-0.452	-0.082	55.603	60.015	52.110	58.783	52.883
164	5558	36.43	-99.58	1981	2003	23	-1.902	+	-0.189	0.189	2	-0.420	0.046	-0.361	0.005	10.248	16.068	3.915	14.875	5.022
165	9417	36.45	-97.92	1975	2003	29	-6.434	***	-1.312	1.312	1	-1.569	-0.956	-1.514	-1.073	54.510	61.323	43.927	59.673	47.692
166	9418	36.45	-97.93	1975	2003	29	-5.534	***	-0.954	0.954	1	-1.198	-0.683	-1.125	-0.730	61.995	68.162	55.527	66.455	56.180
167	9419	36.45	-97.94	1975	2003	29	-5.984	***	-0.908	0.908	1	-1.147	-0.686	-1.084	-0.761	60.043	65.686	55.673	64.337	56.980
168	9415	36.45	-97.94	1975	2003	29	-5.854	***	-0.893	0.893	1	-1.141	-0.627	-1.069	-0.703	56.539	62.244	51.329	60.588	52.584
169	9616	36.45	-97.91	1975	2003	28	-6.263	***	-0.859	0.859	1	-1.058	-0.647	-1.018	-0.698	39.358	43.963	33.969	43.070	35.133
170	5552	36.45	-99.54	1980	2003	24	-4.341	***	-0.441	0.441	1	-0.588	-0.254	-0.531	-0.302	29.747	33.475	25.516	31.906	26.586
171	9422	36.46	-97.94	1975	2003	27	-6.546	***	-0.770	0.770	1	-0.890	-0.665	-0.852	-0.706	44.667	48.183	42.647	47.055	43.356
172	5523	36.46	-99.23	1978	2003	24	-4.886	***	-0.707	0.707	1	-0.890	-0.397	-0.848	-0.509	50.926	54.734	43.093	53.786	46.163
173	3270	36.46	-99.66	1980	2003	24	-3.771	***	-0.271	0.271	1	-0.401	-0.111	-0.355	-0.142	64.602	67.744	61.528	66.580	62.288
174	9423	36.46	-97.96	1975	2003	29	-3.940	***	-0.165	0.165	1	-0.244	-0.064	-0.230	-0.089	10.104	12.316	7.594	11.940	8.230
175	9587	36.46	-98.41	1976	2003	27	-2.335	*	-0.080	0.080	1	-0.177	0.020	-0.146	-0.022	8.260	10.725	5.747	10.001	6.651
176	9387	36.47	-99.73	1980	2003	23	-5.388	***	-0.467	0.467	1	-0.611	-0.357	-0.565	-0.388	101.641	103.583	99.887	103.040	100.468
177	5514	36.47	-99.18	1978	2003	26	-4.608	***	-0.306	0.306	1	-0.445	-0.170	-0.396	-0.210	68.918	71.809	65.499	70.990	66.505
178	9848	36.47	-98.62	1979	2003	25	-2.336	*	-0.093	0.093	1	-0.209	0.010	-0.183	-0.018	6.536	9.249	4.022	8.768	4.804
179	3266	36.48	-99.69	1980	2003	23	-3.514	***	-0.245	0.245	1	-0.430	-0.070	-0.398	-0.099	65.415	69.711	61.545	69.062	62.273
180	9031	36.50	-100.11	1968	2003	34	-4.240	***	-0.649	0.649	1	-0.921	-0.361	-0.851	-0.440	147.369	153.103	141.275	151.247	143.403
181	45	36.50	-100.68	1977	2003	20	-5.548	***	-0.435	0.435	1	-0.776	-0.292	-0.706	-0.340	252.321	257.635	248.514	256.674	250.147
182	9895	36.50	-99.53	1980	2003	24	-3.398	***	-0.356	0.356	1	-0.588	-0.116	-0.506	-0.185	35.744	38.854	31.142	37.813	32.795
183	3296	36.50	-99.88	1980	2003	23	-4.701	***	-0.207	0.207	1	-0.284	-0.128	-0.271	-0.149	192.784	194.712	191.153	194.384	191.682
184	9897	36.51	-99.39	1978	2001	24	-5.135	***	-0.431	0.431	1	-0.530	-0.310	-0.501	-0.330	53.125	54.217	51.430	53.954	51.695
185	9352	36.51	-99.72	1980	2002	21	-4.620	***	-0.368	0.368	1	-0.506	-0.194	-0.462	-0.227	140.451	143.310	138.073	142.425	138.499
186	9896	36.51	-99.37	1977	2000	23	-2.747	**	-0.130	0.130	1	-0.354	-0.019	-0.285	-0.038	33.220	35.312	31.969	34.313	32.198
187	9015	36.51	-100.82	1968	2003	33	-3.409	***	-0.084	0.084	1	-0.121	-0.016	-0.110	-0.046	242.776	243.586	241.184	243.319	241.953
188	3348	36.52	-100.00	1980	2003	24	-5.383	***	-0.353	0.353	1	-0.432	-0.255	-0.412	-0.277	122.676	124.640	120.686	124.113	121.150
189	9398	36.52	-99.68	1980	2003	24	-2.753	**	-0.213	0.213	1	-0.409	-0.021	-0.341	-0.065	33.087	37.834	28.872	35.851	30.000
190	9186	36.52	-102.39	1967	2003	34	-4.344	***	-0.100	0.100	1	-0.162	-0.048	-0.146	-0.061	104.429	105.550	103.606	105.336	103.898
191	9018	36.53	-100.55	1967	1999	31	-3.535	***	-0.284	0.284	1	-0.449	-0.124	-0.402	-0.153	224.367	228.138	221.034	227.261	221.622
192	9399	36.53	-99.86	1981	2003	23	-4.912	***	-0.254	0.254	1	-0.321	-0.170	-0.306	-0.192	174.217	176.004	172.110	175.601	172.667
193	9400	36.53	-99.79	1981	2001	20	-4.220	***	-0.121	0.121	1	-0.171	-0.074	-0.158	-0.092	47.740	48.940	46.681	48.645	47.125
194	9192	36.53	-102.31	1968	2003	36	-3.065	**	-0.060	0.060	1	-0.110	-0.008	-0.097	-0.020	193.110	193.755	191.811	193.594	192.104
195	9852	36.54	-98.68	1977	2003	25	-3.620	***	-0.472	0.472	1	-0.793	-0.164	-0.683	-0.274	37.966	44.902	31.733	42.376	34.368
196	3346	36.54	-99.97	1980	2003	24	-3.944	***	-0.336	0.336	1	-0.463	-0.162	-0.442	-0.201	81.681	85.126	77.768	84.678	78.634
197	9687	36.54	-101.08	1966	2003	38	-3.118	**	-0.054	0.054	1	-0.104	-0.011	-0.092	-0.022	12.232	13.110	11.226	12.930	11.467
198	9898	36.55	-99.39	1978	2003	26	-4.673	***	-0.511	0.511	1	-0.674	-0.265	-0.642	-0.370	46.346	48.978	42.715	48.427	44.576
199	9669	36.55	-95.40	1979	2003	25	-2.499	*	-0.297	0.297	1	-0.619	0.007	-0.527	-0.087	23.189	30.820	15.467	28.897	18.337
200	9899	36.55	-99.42	1978	2002	23	-3.937	***	-0.242	0.242	1	-0.400	-0.104	-0.358	-0.137	31.010	33.583	29.783	32.835	30.046
201	9025	36.57	-100.32	1981	2003	23	-4.067	***	-0.128	0.128	1	-0.177	-0.057	-0.157	-0.075	161.255	162.558	159.733	161.969	160.105
202	911	36.58	-101.19	1966	2003	36	-5.353	***	-0.399	0.399	1	-0.580	-0.250	-0.523	-0.288	133.425	137.830	129.820	136.632	130.747
203	107	36.58	-100.42	1967	1999	31	-3.161	**	-0.225	0.225	1	-0.631	-0.027	-0.467	-0.054	224.554	232.045	221.359	228.599	221.894
204	9409	36.58	-99.87	1980	2003	24	-2.134	*	-0.105	0.105	1	-0.205	0.019	-0.177	-0.020	13.106	15.505	9.868	15.020	10.855
205	9473	36.59	-99.97	1980	2003	21	-2.808	**	-0.161	0.161	1	-0.274	-0.010	-0.247	-0.051	58.483	61.680	55.340	61.031	56.310
206	9900	36.59	-99.55	1980	2003	20	-2.888	**	-0.153	0.153	1	-0.272	-0.021	-0.250	-0.050	9.377	11.148	7.725	10.900	8.264
207	9208	36.59	-102.44	1967	2003	31	-1.938	+	-0.068	0.068	2	-0.128	0.025	-0.115	0.001	103.469	104.254	100.770	103.979	101.599
208	9703	36.61	-101.17	1966	2003	36	-4.045	***	-0.419	0.419	1	-0.592	-0.152	-0.549	-0.220	177.545	181.637	171.038	180.628	173.065
209	9857	36.61	-98.84	1977	2002	25	-4.694	***	-0.387	0.387	1	-0.666	-0.173	-0.612	-0.227	45.397	51.635	41.697	50.195	42.482
210	9710	36.62	-102.01	1967	2003	35	-3.579	***	-0.290	0.290	1	-0.406	-0.089	-0.375	-0.144	211.404	214.090	205.906	213.308	207.798
211	9034	36.62	-100.31	1980	2003	24	-5.432	***	-0.210	0.210	1	-0.387	-0.102	-0.344	-0.116	153.735	159.081	150.074	157.770	150.528
212	9475	36.62	-99.95	1977	2000	24	-3.077	**	-0.182	0.182	1	-0.320	-0.035	-0.294	-0.083	37.311	40.863	34.278	40.160	35.287
213	9711	36.63	-101.08	1966	2003	36	-2.561	*	-0.391	0.391	1	-0.777	0.003	-0.696	-0.101	147.396	155.729	139.700	153.850	142.402
214	9035	36.63	-100.40	1967	2003	33	-5.843	***	-0.080	0.080	1	-0.113	-0.054	-0.103	-0.059	85.684	86.630	84.980	86.340	85.146

215	9005	36.64	-98.39	1975	2002	25	-3.200	**	-0.150	0.150	1	-0.287	-0.043	-0.241	-0.076	7.894	10.512	5.569	9.622	6.268
216	9038	36.64	-100.14	1967	2003	36	-5.218	***	-0.054	0.054	1	-0.071	-0.036	-0.068	-0.041	10.363	10.761	9.941	10.690	10.049
217	9478	36.65	-99.74	1978	2003	21	-5.647	***	-1.031	1.031	1	-1.249	-0.748	-1.176	-0.858	53.450	59.477	45.371	57.359	48.646
218	9724	36.66	-101.14	1966	1999	29	-3.133	**	-0.238	0.238	1	-0.327	-0.068	-0.298	-0.124	96.932	98.002	94.043	97.683	94.827
219	9041	36.66	-100.34	1981	2003	23	-3.330	***	-0.053	0.053	1	-0.077	-0.025	-0.072	-0.034	58.123	58.748	57.351	58.624	57.677
220	9222	36.67	-102.79	1966	2003	38	-7.330	***	-0.242	0.242	1	-0.275	-0.215	-0.268	-0.222	149.823	150.416	149.320	150.354	149.424
221	9482	36.69	-99.75	1978	2003	24	-2.704	**	-0.341	0.341	1	-0.560	-0.015	-0.498	-0.069	19.553	25.571	9.311	23.977	11.014
222	9431	36.69	-97.77	1975	2003	29	-2.007	*	-0.224	0.224	1	-0.496	0.085	-0.429	-0.007	18.221	24.110	10.425	22.683	13.092
223	9726	36.70	-101.33	1966	2003	37	-8.253	***	-0.888	0.888	1	-0.956	-0.790	-0.938	-0.812	127.544	129.071	125.500	128.733	125.897
224	9049	36.70	-100.05	1978	2003	26	-4.585	***	-0.306	0.306	1	-0.441	-0.160	-0.401	-0.186	34.342	37.454	30.445	36.770	31.213
225	9051	36.71	-100.58	1980	2002	23	-3.169	**	-0.116	0.116	1	-0.209	-0.024	-0.180	-0.060	90.792	93.413	88.292	92.749	89.343
226	9052	36.73	-100.52	1968	2003	36	-5.489	***	-1.280	1.280	1	-1.803	-0.673	-1.709	-0.777	118.668	132.245	100.038	131.054	103.353
227	9232	36.73	-102.04	1967	2003	36	-2.002	*	-0.098	0.098	1	-0.251	0.033	-0.211	-0.004	189.632	193.077	187.231	192.175	187.762
228	9054	36.75	-100.31	1968	2002	35	-2.983	**	-0.050	0.050	1	-0.100	-0.007	-0.088	-0.016	84.400	85.880	83.530	85.517	83.658
229	9061	36.80	-100.67	1980	2003	24	-3.597	***	-0.300	0.300	1	-0.492	-0.127	-0.433	-0.157	32.857	37.513	28.454	35.831	29.092
230	9006	36.81	-98.35	1975	2003	27	-3.794	***	-0.244	0.244	1	-0.369	-0.083	-0.334	-0.133	12.055	14.967	9.617	14.306	10.694
231	9486	36.81	-99.93	1972	2003	28	-3.222	**	-0.087	0.087	1	-0.164	-0.019	-0.146	-0.035	8.465	9.994	6.892	9.450	7.088
232	9487	36.82	-99.95	1978	2003	24	-3.249	**	-0.230	0.230	1	-0.375	-0.039	-0.350	-0.093	28.481	31.327	23.404	30.691	25.022
233	9007	36.82	-98.35	1975	2003	28	-3.892	***	-0.222	0.222	1	-0.331	-0.085	-0.303	-0.116	11.276	13.492	8.493	12.559	8.975
234	9488	36.83	-99.95	1980	2001	20	-3.246	**	-0.360	0.360	1	-0.560	-0.037	-0.515	-0.148	34.030	38.150	24.126	37.327	27.759
235	9064	36.84	-100.08	1967	2003	36	-5.912	***	-0.150	0.150	1	-0.187	-0.113	-0.178	-0.120	57.194	57.900	56.100	57.726	56.251
236	9258	36.85	-102.05	1967	2003	36	-2.343	*	-0.047	0.047	1	-0.260	0.010	-0.181	-0.012	200.508	207.223	199.452	205.006	199.676
237	9069	36.87	-100.71	1967	2003	37	-1.962	*	-0.034	0.034	1	-0.080	0.015	-0.073	0.000	134.697	135.636	133.695	135.493	133.980
238	9072	36.89	-100.03	1968	2003	36	-5.244	***	-0.299	0.299	1	-0.434	-0.212	-0.405	-0.228	24.082	26.651	22.065	25.899	22.432
239	9080	36.92	-100.11	1980	2003	23	-5.757	***	-0.958	0.958	1	-1.155	-0.745	-1.101	-0.825	66.702	71.226	60.926	70.004	63.001
240	9509	36.97	-97.46	1975	2003	25	-1.729	+	-0.053	0.053	2	-0.115	0.026	-0.095	0.005	33.595	35.071	31.544	34.690	32.115
241	25024	36.99	-98.21	1975	2003	28	-3.004	**	-0.087	0.087	1	-0.169	-0.019	-0.150	-0.037	8.133	9.152	7.377	8.945	7.641
242	9483	37.00	-100.00	1980	2002	21	-4.137	***	-0.282	0.282	1	-0.490	-0.138	-0.428	-0.167	60.748	65.523	57.465	64.259	58.141
243	3327	37.00	-100.00	1981	2003	22	-2.596	**	-0.057	0.057	1	-0.179	0.000	-0.148	-0.022	20.687	23.161	19.270	22.530	19.835
244	9496			1965	2001	35	-4.260	***	-0.439	0.439	1	-0.601	-0.225	-0.576	-0.288	28.439	31.276	24.364	30.829	26.165
245	9552			1976	1996	20	-2.368	*	-0.364	0.364	1	-0.901	0.028	-0.719	-0.108	29.479	37.509	24.995	34.716	26.538
246	9511			1975	2003	28	-2.035	*	-0.089	0.089	1	-0.196	0.023	-0.172	-0.007	4.538	7.640	1.425	7.025	2.247
247	9644	34.23	-95.63	1980	2003	22	-1.044		-0.065	0.065	2	-0.237	0.124	-0.173	0.082	11.735	15.413	6.226	14.361	7.206
248	9505	34.38	-96.43	1979	2003	25	-1.285		-0.054	0.054	2	-0.176	0.064	-0.132	0.040	8.108	11.469	4.792	10.375	5.604
249	9825	34.49	-99.20	1974	2003	29	-1.632		-0.106	0.106	2	-0.276	0.096	-0.243	0.040	12.523	16.034	7.672	15.445	8.785
250	9638	34.59	-96.69	1977	2003	26	-0.529		-0.135	0.135	2	-0.770	0.386	-0.630	0.223	69.855	85.759	59.042	81.930	61.263
251	9498	34.66	-99.58	1976	1999	21	-1.419		-0.233	0.233	2	-0.575	0.188	-0.472	0.093	30.565	39.289	20.493	36.463	23.256
252	9427	34.75	-97.92	1979	2003	22	1.608		0.068	-0.068	2	-0.060	0.212	-0.020	0.180	11.025	13.843	7.119	12.945	8.021
253	9447	34.93	-99.53	1980	2003	24	0.174		0.009	-0.009	2	-0.289	0.351	-0.227	0.290	9.008	15.925	-1.057	14.562	0.933
254	9833	35.00	-99.00	1977	2003	26	-1.499		-0.087	0.087	2	-0.250	0.094	-0.203	0.037	15.613	19.681	10.897	18.480	12.542
255	9460	35.10	-99.41	1980	2003	24	-1.116		-0.078	0.078	2	-0.280	0.115	-0.237	0.058	28.223	33.225	22.468	32.417	24.089
256	9462	35.12	-99.51	1980	2002	17	-1.319		-0.218	0.218	2	-0.744	0.360	-0.537	0.181	31.188	44.152	16.280	38.587	22.263
257	9088	35.12	-99.46	1980	2003	24	-1.092		-0.118	0.118	2	-0.425	0.278	-0.350	0.161	23.902	31.132	12.908	29.280	16.189
258	9089	35.14	-99.50	1980	2003	24	-0.521		-0.068	0.068	2	-0.331	0.253	-0.240	0.144	20.065	26.743	11.842	24.218	14.503
259	9106	35.21	-99.89	1980	2003	24	-1.563		-0.151	0.151	2	-0.362	0.130	-0.315	0.050	13.943	19.589	6.688	18.338	9.015
260	9101	35.23	-100.00	1980	2003	23	-1.162		-0.159	0.159	2	-0.507	0.237	-0.404	0.122	43.974	53.092	32.756	51.052	36.107
261	9430	35.32	-97.85	1979	2003	24	0.719		0.055	-0.055	2	-0.171	0.321	-0.130	0.248	6.535	11.260	-0.440	10.548	1.867
262	9146	35.33	-98.56	1979	2003	24	-1.166		-0.183	0.183	2	-0.600	0.227	-0.455	0.114	72.942	79.262	64.073	77.018	66.981
263	9271	35.36	-97.28	1979	2003	22	-0.959		-0.086	0.086	2	-0.333	0.103	-0.269	0.066	9.170	13.404	5.126	12.199	5.899
264	9671	35.43	-96.46	1976	2000	25	-1.565		-0.235	0.235	2	-0.594	0.195	-0.487	0.077	18.431	27.222	6.582	24.179	10.000
265	9154	35.45	-98.48	1974	2003	28	-1.324		-0.091	0.091	2	-0.346	0.122	-0.282	0.066	134.629	139.304	131.728	137.693	132.317
266	9163	35.52	-97.76	1977	2003	26	-0.882		-0.077	0.077	2	-0.317	0.133	-0.267	0.079	11.611	15.418	7.736	14.647	8.819
267	9166	35.54	-97.92	1978	2003	26	-1.059		-0.061	0.061	2	-0.209	0.123	-0.175	0.055	18.988	20.944	15.508	20.623	16.704
268	2687	35.65	-99.67	1976	2003	25	-0.934		-0.053	0.053	2	-0.290	0.199	-0.245	0.117	30.573	36.502	24.286	35.552	26.145
269	9621	35.67	-97.34	1976	2003	28	0.138		0.009	-0.009	2	-0.124	0.183	-0.098	0.156	25.395	29.070	20.564	28.488	21.407

270	9559	35.70	-96.89	1980	2003	21	-1.057		-0.060	0.060	2	-0.562	0.177	-0.273	0.091	7.152	22.736	0.967	13.481	2.679
271	9623	35.72	-97.50	1976	2003	25	-1.518		-0.319	0.319	2	-1.240	0.277	-0.933	0.140	80.659	103.250	64.997	95.271	67.743
272	9658	35.73	-99.71	1976	2003	25	-0.304		-0.002	0.002	2	-0.040	0.030	-0.030	0.021	7.198	8.160	6.286	7.846	6.570
273	9000	35.96	-94.55	1980	2003	23	-1.109		-0.198	0.198	2	-0.777	0.422	-0.585	0.246	60.280	69.748	40.686	66.092	46.551
274	9172	35.99	-95.19	1979	2003	24	-1.141		-0.079	0.079	2	-0.353	0.168	-0.284	0.078	12.663	18.119	7.599	16.400	9.498
275	9001	35.99	-94.55	1980	2003	24	0.149		0.021	-0.021	2	-0.259	0.253	-0.166	0.200	12.302	19.215	6.006	16.427	7.496
276	9527	36.06	-97.83	1975	2003	25	-1.425		-0.105	0.105	2	-0.291	0.098	-0.235	0.034	9.476	13.357	4.646	12.481	6.173
277	3054	36.17	-99.72	1980	2003	23	-0.396		-0.049	0.049	2	-0.527	0.297	-0.350	0.233	120.114	130.155	109.967	126.532	111.887
278	9589	36.19	-95.21	1979	2003	25	0.070		0.002	-0.002	2	-0.156	0.185	-0.121	0.120	34.542	38.518	29.242	37.467	31.450
279	9869	36.24	-99.50	1980	2003	24	-0.670		-0.051	0.051	2	-0.207	0.178	-0.180	0.095	158.078	160.962	154.524	160.261	155.717
280	9626	36.26	-96.14	1979	2003	25	-0.981		-0.293	0.293	2	-0.863	0.199	-0.747	0.138	25.790	38.177	9.883	36.074	11.576
281	9873	36.29	-99.56	1980	2003	24	-1.514		-0.086	0.086	2	-0.160	0.039	-0.143	0.020	129.952	131.167	127.523	130.849	127.880
282	9413	36.29	-98.09	1975	2003	28	-0.494		-0.047	0.047	2	-0.276	0.150	-0.231	0.106	5.803	11.636	0.363	10.395	1.422
283	9882	36.36	-99.60	1977	1999	23	-0.792		-0.067	0.067	2	-0.234	0.247	-0.194	0.159	27.637	29.777	22.624	29.290	23.904
284	9885	36.40	-99.57	1980	2003	23	-1.268		-0.080	0.080	2	-0.279	0.131	-0.197	0.065	4.500	8.727	2.287	6.824	2.921
285	9375	36.40	-99.74	1980	2003	24	-0.397		-0.075	0.075	2	-0.318	0.280	-0.248	0.181	101.244	105.110	95.698	103.939	97.276
286	3251	36.40	-100.00	1980	2003	24	0.695		0.023	-0.023	2	-0.132	0.129	-0.090	0.095	54.101	57.293	51.588	56.463	52.380
287	9891	36.46	-99.37	1978	2003	25	-1.611		-0.079	0.079	2	-0.166	0.046	-0.139	0.017	12.116	13.551	9.600	12.983	10.183
288	9014	36.50	-100.21	1967	2003	33	0.759		0.014	-0.014	2	-0.041	0.073	-0.024	0.052	169.874	171.222	168.955	170.785	169.275
289	2041	36.52	-102.77	1967	2000	34	1.334		0.594	-0.594	2	-0.701	2.037	-0.315	1.697	151.894	165.550	130.478	160.607	135.649
290	9017	36.53	-100.72	1980	2003	24	0.670		0.006	-0.006	2	-0.026	0.034	-0.015	0.027	211.540	212.292	210.852	212.081	211.022
291	9278	36.54	-95.22	1979	2003	24	-1.415		-0.048	0.048	2	-0.153	0.060	-0.127	0.027	2.460	4.180	0.695	3.832	1.136
292	9410	36.58	-100.00	1980	2000	20	-1.006		-0.043	0.043	2	-0.236	0.084	-0.148	0.060	111.320	115.400	108.693	113.371	109.137
293	1156	36.61	-101.12	1966	2003	29	-0.694		-0.100	0.100	2	-0.518	0.293	-0.385	0.153	139.715	149.046	132.829	146.575	134.915
294	9839	36.63	-95.88	1979	2003	24	-1.463		-0.218	0.218	2	-0.493	0.180	-0.430	0.064	25.006	31.542	12.106	30.403	15.700
295	9477	36.64	-99.82	1977	2003	25	-0.257		-0.017	0.017	2	-0.120	0.107	-0.103	0.080	8.128	10.476	5.402	10.032	6.004
296	9037	36.64	-100.54	1967	2003	35	0.682		0.006	-0.006	2	-0.012	0.030	-0.007	0.023	12.654	12.942	12.320	12.881	12.403
297	2117	36.65	-102.31	1967	1993	23	0.317		0.021	-0.021	2	-0.204	0.260	-0.134	0.181	165.416	168.802	163.029	167.957	164.184
298	9042	36.67	-100.11	1967	2003	37	-0.105		-0.003	0.003	2	-0.049	0.042	-0.036	0.032	19.750	20.629	18.970	20.289	19.175
299	9045	36.67	-100.23	1969	2003	35	0.966		0.028	-0.028	2	-0.050	0.104	-0.032	0.088	57.165	59.354	55.533	58.905	56.053
300	2275	36.69	-102.33	1967	2003	36	1.553		0.054	-0.054	2	-0.050	0.142	-0.020	0.117	137.643	140.720	136.203	139.739	136.541
301	9227	36.69	-102.86	1967	2003	36	1.526		0.160	-0.160	2	-0.069	0.408	-0.021	0.320	23.153	28.497	16.526	27.730	19.183
302	9433	36.71	-97.79	1975	2002	25	-1.331		-0.115	0.115	2	-0.356	0.098	-0.312	0.045	13.033	18.545	7.597	17.206	9.348
303	9432	36.71	-98.05	1975	2000	20	0.000		-0.002	0.002	2	-0.177	0.378	-0.137	0.208	9.765	14.068	3.468	13.151	5.893
304	9434	36.75	-97.99	1975	2003	28	-1.620		-0.097	0.097	2	-0.306	0.072	-0.250	0.020	11.232	15.696	7.321	14.518	8.865
305	9245	36.79	-102.61	1967	2003	35	-0.199		-0.018	0.018	2	-0.257	0.180	-0.195	0.118	210.087	213.978	206.478	213.063	207.695
306	9435	36.83	-98.00	1975	2003	29	-1.257		-0.127	0.127	2	-0.361	0.120	-0.310	0.067	14.013	19.289	8.329	17.868	9.253
307	9008	36.84	-98.18	1975	2003	28	-1.225		-0.023	0.023	2	-0.090	0.036	-0.070	0.023	4.127	5.722	3.191	5.264	3.407
308	9063	36.84	-100.12	1980	2003	24	-0.347		-0.008	0.008	2	-0.082	0.071	-0.056	0.047	26.300	28.353	24.099	27.685	24.849
309	9066	36.86	-100.34	1980	2003	23	0.661		0.009	-0.009	2	-0.033	0.078	-0.019	0.057	67.019	67.890	65.479	67.657	65.984
310	9073	36.89	-100.51	1980	2003	23	-0.264		-0.030	0.030	2	-0.288	0.309	-0.191	0.233	20.350	25.256	12.160	23.381	14.064
311	9074	36.89	-100.23	1970	2003	28	1.028		0.057	-0.057	2	-0.051	0.120	-0.041	0.099	189.529	192.574	187.851	192.277	188.483
312	9602	36.91	-95.45	1979	2003	24	-1.612		-0.099	0.099	2	-0.228	0.074	-0.200	0.050	6.279	9.760	1.915	8.994	2.505
313	9841	36.93	-95.88	1979	2003	25	-1.542		-0.109	0.109	2	-0.366	0.080	-0.277	0.041	10.886	18.340	5.207	15.915	6.402
314	1874	36.94	-101.52	1966	2000	29	-1.594		-0.553	0.553	2	-1.090	0.413	-0.979	0.143	243.215	254.853	219.576	251.958	225.941
315	9627	36.97	-94.96	1979	2003	25	0.000		-0.002	0.002	2	-0.193	0.135	-0.140	0.109	3.116	7.440	-0.217	5.958	0.475
316	9087	37.00	-100.40	1980	2003	23	-1.004		-0.013	0.013	2	-0.038	0.023	-0.029	0.011	7.100	7.655	6.210	7.490	6.526
317	9840			1977	2003	27	-0.396		-0.011	0.011	2	-0.113	0.106	-0.090	0.074	12.611	15.357	9.460	14.810	10.318
318	9040			1965	1990	24	-0.422		-0.007	0.007	2	-0.099	0.083	-0.049	0.051	76.345	77.667	74.996	76.907	75.572
319	9813			1974	2003	28	1.028		0.036	-0.036	2	-0.075	0.128	-0.039	0.101	5.320	7.654	3.053	6.726	3.933
320	9456	35.09	-99.41	1980	2002	17	-1.277		-0.261	0.261	2	-0.918	0.210	-0.699	0.135	30.625	45.287	17.030	40.901	19.718
321	9588	33.94	-96.64	1978	2003	26	6.436	***	1.299	-1.299	3	1.169	1.432	1.190	1.387	94.946	98.584	91.721	98.083	92.585
322	9170	34.26	-97.28	1976	2003	28	2.667	**	0.607	-0.607	3	0.014	1.170	0.158	0.997	27.874	39.285	17.706	37.309	20.079
323	9639	35.01	-97.05	1978	2001	22	4.230	***	0.526	-0.526	3	0.247	0.805	0.300	0.736	35.974	43.590	29.841	41.935	31.234
324	9270	35.32	-97.44	1980	2003	22	4.173	***	0.410	-0.410	3	0.189	0.593	0.218	0.548	123.351	125.989	120.089	125.684	120.794

325	3257	36.36	-100.00	1980	2003	24	2.257	*	0.108	-0.108	3	-0.027	0.196	0.025	0.169	57.926	61.612	55.632	60.114	56.349
326	9085	36.46	-100.62	1967	2003	36	4.632	***	0.110	-0.110	3	0.065	0.151	0.078	0.143	102.842	103.880	101.916	103.529	102.094
327	9386	36.50	-101.37	1977	2003	27	4.858	***	0.615	-0.615	3	0.341	0.831	0.426	0.776	245.925	250.185	242.246	248.604	243.330
328	9385	36.51	-101.60	1977	2003	26	3.659	***	0.470	-0.470	3	0.190	0.905	0.248	0.810	217.290	221.740	207.826	220.933	210.207
329	9189	36.52	-102.59	1968	2003	35	1.704	+	0.111	-0.111	2	-0.052	0.251	-0.014	0.220	54.355	56.937	52.482	56.443	52.799
330	1996	36.52	-102.91	1967	2003	37	5.677	***	0.643	-0.643	3	0.368	0.835	0.452	0.763	165.320	171.525	160.986	169.226	162.792
331	2074	36.52	-102.11	1967	2003	37	7.520	***	1.012	-1.012	3	0.788	1.221	0.840	1.160	274.541	278.821	270.554	277.497	271.892
332	9020	36.54	-100.16	1967	2003	37	6.017	***	0.099	-0.099	3	0.065	0.139	0.072	0.132	38.522	39.290	37.957	39.151	37.999
333	2019	36.54	-102.80	1967	2003	37	3.754	***	0.546	-0.546	3	0.144	0.947	0.243	0.885	111.261	116.365	106.351	115.322	107.906
334	871	36.54	-101.49	1966	2003	31	7.512	***	0.988	-0.988	3	0.849	1.205	0.885	1.170	168.190	170.032	166.454	169.167	166.630
335	9198	36.55	-102.77	1969	2003	33	4.603	***	0.601	-0.601	3	0.198	0.945	0.312	0.887	98.104	103.261	93.880	102.381	94.463
336	1988	36.56	-102.95	1966	1999	32	5.287	***	0.417	-0.417	3	0.231	0.583	0.270	0.545	185.206	189.576	182.185	188.645	182.752
337	9202	36.56	-102.09	1970	2003	33	7.794	***	1.228	-1.228	3	1.050	1.436	1.074	1.415	261.143	265.763	255.337	265.266	255.805
338	9691	36.56	-101.93	1966	2003	37	8.671	***	1.602	-1.602	3	1.515	1.715	1.534	1.683	144.373	145.000	142.893	144.858	143.432
339	9201	36.56	-102.07	1969	2001	28	6.740	***	1.671	-1.671	3	1.445	1.799	1.497	1.772	271.722	276.973	268.770	275.958	269.120
340	9695	36.57	-101.65	1966	2003	38	8.775	***	1.487	-1.487	3	1.266	1.690	1.299	1.660	161.325	167.639	156.355	166.654	157.004
341	9207	36.58	-102.20	1967	2003	33	7.314	***	0.843	-0.843	3	0.780	0.944	0.797	0.899	302.894	303.916	301.809	303.728	302.078
342	812	36.58	-101.91	1966	2003	35	8.252	***	1.543	-1.543	3	1.382	1.718	1.422	1.680	147.135	149.435	144.500	148.550	145.378
343	949	36.59	-101.87	1966	2003	37	8.331	***	1.309	-1.309	3	1.177	1.466	1.215	1.434	150.663	152.781	147.871	152.175	148.513
344	988	36.59	-101.69	1966	1996	29	6.021	***	1.678	-1.678	3	0.898	2.262	1.165	2.056	188.311	204.589	180.823	197.382	182.461
345	9032	36.60	-100.17	1967	2003	33	2.976	**	0.027	-0.027	3	0.005	0.044	0.012	0.040	10.313	10.631	10.125	10.512	10.170
346	9702	36.60	-101.78	1966	2003	32	7.136	***	0.257	-0.257	3	0.239	0.275	0.243	0.269	18.282	18.701	17.921	18.588	18.056
347	9210	36.61	-102.94	1966	2001	34	3.529	***	0.050	-0.050	3	0.015	0.090	0.026	0.078	96.415	96.869	95.437	96.756	95.744
348	2127	36.61	-102.27	1967	2001	34	4.151	***	0.394	-0.394	3	0.170	0.633	0.240	0.555	225.177	228.483	220.715	227.920	221.671
349	953	36.61	-101.72	1966	2003	35	7.172	***	1.384	-1.384	3	0.900	1.797	1.030	1.710	246.866	259.475	236.957	255.877	238.654
350	9213	36.62	-102.80	1967	2002	35	3.366	***	0.298	-0.298	3	0.086	0.574	0.131	0.509	102.465	106.767	98.666	105.706	99.391
351	2146	36.62	-102.17	1967	2003	37	5.715	***	1.150	-1.150	3	0.864	1.385	0.937	1.330	295.380	300.747	291.212	298.941	292.042
352	9708	36.62	-101.53	1970	2003	32	7.703	***	1.850	-1.850	3	1.762	1.961	1.788	1.930	134.370	136.202	131.899	135.501	132.703
353	9036	36.63	-100.66	1967	2003	37	7.390	***	0.180	-0.180	3	0.164	0.194	0.167	0.190	98.640	98.902	98.242	98.827	98.316
354	5600	36.63	-102.25	1967	2003	36	5.244	***	0.661	-0.661	3	0.389	0.938	0.456	0.870	260.551	264.505	255.604	263.178	257.485
355	1120	36.64	-101.31	1966	2003	36	7.832	***	1.326	-1.326	3	0.875	1.786	0.966	1.698	185.150	198.045	174.436	195.359	176.580
356	9039	36.65	-100.77	1967	1999	33	7.066	***	0.383	-0.383	3	0.240	0.502	0.272	0.475	170.173	174.040	168.287	173.259	168.848
357	1135	36.65	-101.28	1966	2003	38	3.332	***	0.385	-0.385	3	0.107	0.630	0.173	0.577	182.715	188.773	177.362	187.197	178.154
358	9219	36.65	-102.23	1967	2001	35	7.328	***	0.846	-0.846	3	0.655	1.036	0.719	0.973	261.434	265.007	257.435	263.438	258.136
359	9715	36.65	-101.67	1966	2003	36	6.866	***	1.075	-1.075	3	0.846	1.273	0.921	1.217	212.508	218.330	208.864	216.436	209.853
360	9720	36.66	-101.46	1966	2003	31	7.750	***	2.344	-2.344	3	1.914	2.951	2.004	2.836	234.274	246.815	219.418	244.296	221.846
361	1106	36.66	-101.38	1966	1997	31	5.287	***	2.683	-2.683	3	1.166	3.726	1.433	3.388	233.888	269.678	220.780	262.630	222.469
362	1111	36.66	-101.35	1966	2003	24	6.005	***	3.984	-3.984	3	2.685	5.385	3.087	5.188	175.749	201.010	162.932	191.558	163.856
363	9221	36.67	-102.06	1967	2000	25	1.985	*	0.054	-0.054	3	-0.019	0.203	0.001	0.154	114.873	116.304	113.101	115.899	114.252
364	9223	36.68	-102.43	1967	2003	37	2.668	**	0.175	-0.175	3	0.005	0.406	0.080	0.323	131.665	136.091	128.030	133.485	129.828
365	1178	36.72	-101.94	1966	1999	33	7.856	***	0.549	-0.549	3	0.508	0.606	0.521	0.590	175.603	176.595	174.414	176.239	174.748
366	9730	36.72	-101.92	1966	2003	35	7.343	***	0.669	-0.669	3	0.593	0.728	0.615	0.713	149.164	150.893	147.710	150.408	148.060
367	9230	36.72	-102.43	1967	2003	36	6.907	***	0.836	-0.836	3	0.660	0.963	0.714	0.924	108.857	113.612	106.847	112.078	107.292
368	9734	36.75	-101.63	1966	1999	32	3.260	**	0.085	-0.085	3	0.028	0.119	0.047	0.111	61.595	62.253	60.875	62.080	61.102
369	9053	36.75	-100.24	1967	1995	23	2.483	*	0.253	-0.253	3	-0.007	0.390	0.050	0.361	12.556	19.402	10.591	17.940	10.821
370	9733	36.75	-101.96	1966	2002	37	6.997	***	0.530	-0.530	3	0.480	0.597	0.497	0.584	118.600	119.831	117.265	119.438	117.543
371	9237	36.75	-102.54	1968	2003	36	7.015	***	1.142	-1.142	3	0.789	1.568	0.870	1.453	196.794	204.501	190.065	202.576	191.297
372	9055	36.76	-100.11	1968	2003	34	5.841	***	0.579	-0.579	3	0.437	0.721	0.479	0.700	142.528	146.637	139.121	145.404	139.655
373	2231	36.76	-102.37	1967	2003	36	8.350	***	0.626	-0.626	3	0.457	0.819	0.480	0.787	98.794	103.230	94.743	102.510	95.089
374	2300	36.77	-102.47	1967	2003	37	6.212	***	1.346	-1.346	3	0.983	1.792	1.071	1.711	172.855	180.699	161.141	178.923	162.740
375	1421	36.77	-101.22	1966	2003	37	7.860	***	1.385	-1.385	3	1.234	1.563	1.280	1.503	128.864	132.545	126.444	131.419	126.915
376	9736	36.78	-101.08	1966	2003	38	6.286	***	0.587	-0.587	3	0.433	0.685	0.473	0.667	99.105	103.179	96.973	102.042	97.327
377	1354	36.78	-101.39	1966	2002	36	8.377	***	2.318	-2.318	3	2.204	2.387	2.231	2.370	131.945	133.615	130.369	133.151	130.820
378	1356	36.78	-101.45	1966	2001	35	7.527	***	2.390	-2.390	3	2.056	2.576	2.160	2.543	149.520	156.861	145.694	154.742	146.258
379	9059	36.79	-100.43	1967	2002	35	2.812	**	0.038	-0.038	3	0.005	0.072	0.012	0.061	21.999	22.464	21.433	22.346	21.640

380	2325	36.79	-102.37	1967	2003	37	4.329	***	0.348	-0.348	3	0.186	0.609	0.221	0.559	126.484	130.453	120.101	129.670	121.252
381	9058	36.79	-100.89	1967	1999	32	7.119	***	0.362	-0.362	3	0.275	0.425	0.297	0.404	113.739	115.896	112.474	115.315	112.844
382	1375	36.79	-101.32	1966	2003	34	5.071	***	0.395	-0.395	3	0.226	0.555	0.273	0.522	111.966	115.789	108.558	115.025	109.223
383	2319	36.80	-102.41	1967	2003	34	3.959	***	0.405	-0.405	3	0.138	1.000	0.200	0.771	170.813	178.015	160.255	176.550	163.434
384	9746	36.81	-101.51	1966	1999	33	2.727	**	0.168	-0.168	3	0.015	0.311	0.069	0.284	183.831	186.137	180.040	185.351	180.374
385	2354	36.81	-102.36	1967	2003	37	5.271	***	0.559	-0.559	3	0.336	0.793	0.381	0.743	161.971	167.733	157.384	166.364	158.453
386	1412	36.81	-101.19	1966	1996	31	7.037	***	2.170	-2.170	3	1.889	2.417	1.939	2.355	116.020	118.888	111.136	118.095	111.993
387	9251	36.82	-102.52	1967	2003	35	2.486	*	0.086	-0.086	3	-0.005	0.165	0.013	0.143	121.263	123.308	120.194	123.003	120.468
388	2305	36.83	-102.42	1967	1996	28	2.648	**	0.451	-0.451	3	0.013	1.049	0.163	0.813	204.277	213.459	198.531	209.700	200.264
389	9750	36.83	-101.87	1966	2003	36	4.427	***	0.827	-0.827	3	0.428	1.208	0.557	1.095	150.442	154.983	142.006	153.816	145.148
390	1335	36.83	-101.44	1966	1997	31	7.037	***	3.003	-3.003	3	2.741	3.479	2.786	3.342	147.920	151.875	142.824	151.221	144.176
391	9256	36.84	-102.30	1967	1999	30	3.818	***	0.295	-0.295	3	0.090	0.570	0.156	0.492	184.452	188.022	180.200	186.585	181.729
392	1387	36.84	-101.22	1966	2003	38	7.769	***	0.934	-0.934	3	0.788	1.052	0.835	1.018	116.958	119.757	115.073	118.709	115.759
393	1316	36.84	-101.54	1966	2003	38	8.649	***	2.043	-2.043	3	1.830	2.199	1.876	2.162	186.396	191.973	183.527	190.697	184.004
394	1362	36.84	-101.37	1966	2003	38	8.750	***	2.794	-2.794	3	2.689	2.872	2.712	2.855	110.972	113.092	109.192	112.774	109.597
395	572	36.86	-100.91	1968	2003	36	6.633	***	0.630	-0.630	3	0.380	0.861	0.412	0.809	126.631	132.911	121.775	132.176	122.560
396	1584	36.86	-101.59	1966	2002	35	7.498	***	1.362	-1.362	3	1.192	1.470	1.244	1.447	176.894	180.610	174.980	179.600	175.487
397	1679	36.88	-101.21	1966	2003	37	7.770	***	0.873	-0.873	3	0.780	0.966	0.802	0.946	111.558	113.479	109.684	113.034	110.174
398	9759	36.88	-101.45	1966	2003	35	8.095	***	3.215	-3.215	3	2.907	3.544	2.974	3.471	132.805	137.549	128.317	136.061	129.902
399	9601	36.89	-95.47	1979	2003	24	2.209	*	0.046	-0.046	3	-0.007	0.104	0.004	0.089	0.124	1.463	-1.391	1.159	-1.076
400	1675	36.90	-101.25	1966	2003	35	4.062	***	0.200	-0.200	3	0.080	0.285	0.107	0.268	125.820	128.731	123.273	128.500	123.830
401	613	36.90	-100.82	1967	1990	23	4.860	***	0.330	-0.330	3	0.243	0.411	0.277	0.385	149.830	151.088	148.520	150.612	148.983
402	9767	36.90	-101.88	1966	2003	36	6.415	***	1.070	-1.070	3	0.743	1.369	0.836	1.297	211.882	218.695	204.575	216.778	206.357
403	9766	36.90	-101.62	1966	1999	30	4.210	***	1.140	-1.140	3	0.517	1.732	0.677	1.595	220.090	231.103	211.256	227.227	212.763
404	1604	36.90	-101.41	1966	1996	30	7.529	***	3.015	-3.015	3	2.728	3.291	2.826	3.230	144.130	146.881	138.873	146.670	139.656
405	9077	36.91	-100.79	1967	2003	37	6.840	***	0.216	-0.216	3	0.157	0.262	0.169	0.249	160.176	161.740	159.496	161.422	159.552
406	1536	36.91	-101.78	1966	2003	38	6.789	***	1.378	-1.378	3	0.779	1.736	1.032	1.641	213.378	229.370	208.343	221.843	209.468
407	9079	36.92	-100.86	1968	2003	34	5.841	***	0.579	-0.579	3	0.437	0.721	0.479	0.700	143.686	147.511	140.563	146.361	141.055
408	9774	36.92	-101.04	1980	2003	24	4.886	***	3.097	-3.097	3	2.146	3.753	2.453	3.591	99.531	122.030	83.258	114.283	87.959
409	9081	36.93	-100.36	1967	2003	37	7.953	***	0.243	-0.243	3	0.209	0.273	0.218	0.264	56.017	56.903	55.624	56.677	55.753
410	9777	36.93	-101.08	1966	2003	35	6.078	***	2.749	-2.749	3	2.088	3.480	2.306	3.287	73.174	86.212	56.028	80.272	59.892
411	9083	36.94	-100.71	1980	2003	24	6.027	***	0.284	-0.284	3	0.195	0.402	0.218	0.368	186.094	188.121	183.320	187.595	184.274
412	710	36.94	-100.86	1967	1998	29	5.609	***	0.631	-0.631	3	0.424	0.794	0.480	0.742	145.078	149.831	142.019	148.740	143.120
413	1531	36.94	-101.80	1966	2001	36	4.699	***	1.274	-1.274	3	0.527	1.873	0.684	1.728	225.999	242.239	213.183	237.753	215.120
414	1745	36.94	-101.93	1966	2002	37	7.599	***	1.757	-1.757	3	1.412	1.992	1.536	1.932	271.519	279.237	265.298	276.229	267.005
415	9762	36.94	-101.50	1966	2000	33	2.185	*	1.903	-1.903	3	-0.651	2.870	0.195	2.657	212.180	240.151	204.440	228.850	206.685
416	9789	36.95	-102.03	1966	2003	37	6.291	***	0.465	-0.465	3	0.378	0.558	0.407	0.533	221.235	222.326	219.064	222.237	219.683
417	9786	36.95	-101.45	1980	2003	21	6.130	***	2.174	-2.174	3	2.044	2.360	2.072	2.310	147.839	150.968	143.062	150.476	144.157
418	1771	36.96	-101.85	1966	2003	38	5.533	***	1.382	-1.382	3	0.760	2.114	0.873	1.892	250.795	266.559	235.060	264.183	238.781
419	9792	36.96	-101.48	1966	2003	34	8.183	***	2.289	-2.289	3	2.137	2.522	2.175	2.444	176.480	180.000	174.456	178.959	175.149
420	9086	36.97	-100.38	1980	2003	24	4.962	***	0.172	-0.172	3	0.126	0.257	0.140	0.229	40.961	42.158	38.735	41.836	39.513
421	1855	36.97	-101.55	1966	2003	34	3.736	***	0.406	-0.406	3	0.129	0.700	0.178	0.627	227.400	233.363	221.990	232.051	223.567
422	9798	36.98	-101.00	1966	2003	36	7.205	***	1.060	-1.060	3	0.862	1.197	0.919	1.171	78.512	82.709	74.996	81.757	75.524
423	9799	36.98	-101.90	1967	2002	34	6.641	***	1.500	-1.500	3	1.109	1.874	1.181	1.782	261.395	271.230	256.861	269.155	257.369
424	1895	36.99	-101.19	1966	2003	37	6.056	***	1.006	-1.006	3	0.725	1.340	0.810	1.269	118.256	123.945	108.844	123.338	111.427
425	9800	36.99	-102.03	1970	2003	34	7.412	***	1.114	-1.114	3	0.967	1.275	1.009	1.235	183.356	185.891	179.750	185.029	180.523
426	9177	37.00	-102.00	1967	2003	37	3.440	***	0.277	-0.277	3	0.052	0.535	0.093	0.482	78.278	81.384	74.368	80.678	75.709
427	9242	37.00	-103.00	1967	2003	37	7.979	***	0.762	-0.762	3	0.615	1.009	0.633	0.969	184.039	188.266	177.708	187.673	178.581
428	9259	37.00	-102.00	1980	2001	21	5.949	***	1.405	-1.405	3	1.194	1.596	1.277	1.537	135.224	139.252	133.176	137.504	133.666
429	9769			1970	2003	33	5.996	***	0.476	-0.476	3	0.363	0.560	0.400	0.541	168.513	171.470	165.900	170.444	166.434

APPENDIX B

Result of Mann-Kendall Analysis for Annual Precipitation Trends in Oklahoma

Annual Precipitation Time Series									M-K Test estimate												
S/NO	Site Name	COUNTY	LAT	LON	ELEV (ft)	First year	Last Year	n	Test Z	Signific.	slope Est	Label	Qmin99	Qmax99	Qmin95	Qmax95	B	Bmin99	Bmax99	Bmin95	Bmax95
1	ADA	PONTOTOC	34.78	-96.68	1014	*1970*	*2004*	*34*	0.623		0.083		-0.387	0.459	-0.231	0.383	39.455	48.315	32.937	45.261	34.106
2	ALTUS	JACKSON	34.58	-99.33	1379	*1970*	*2004*	*32*	1.038		0.119		-0.199	0.514	-0.125	0.416	25.198	30.864	20.091	30.058	21.099
3	ALTUS DAM	KIOWA	34.88	-99.30	1524	*1970*	*2004*	*31*	1.632		0.251		-0.152	0.684	-0.059	0.577	24.278	31.087	16.509	29.241	18.976
4	ANADARKO	CADDO	35.07	-98.20	1167	*1970*	*2004*	*29*	0.769		0.067		-0.238	0.367	-0.138	0.322	29.727	34.768	24.440	34.364	24.659
5	ARDMORE	CARTER	34.17	-97.13	879	*1970*	*2004*	*30*	0.571		0.106		-0.382	0.512	-0.245	0.420	34.316	41.153	26.102	38.280	27.233
6	ARNETT	ELLIS	36.13	-99.77	2464	*1970*	*2003*	*33*	1.286		0.117		-0.105	0.399	-0.051	0.321	22.702	26.914	19.599	25.959	20.456
7	BARNSDALL	OSAGE	36.57	-96.17	769	*1970*	*2000*	*30*	1.320		0.228		-0.237	0.729	-0.097	0.558	37.218	44.436	29.256	42.102	31.807
8	BARTLESVILLE PHILLI	OSAGE	36.75	-96.00	714	*1970*	*2004*	*33*	1.999	*	0.278	1	-0.130	0.613	0.014	0.484	33.538	38.686	26.888	37.272	29.950
9	BILLINGS	NOBLE	36.53	-97.45	999	*1970*	*2004*	*35*	0.596		0.057		-0.276	0.439	-0.187	0.349	33.827	38.242	26.377	36.196	27.544
10	BOISE CITY 2 E	CIMARRON	36.73	-102.48	4143	*1970*	*2004*	*32*	2.044	*	0.174	1	-0.041	0.416	0.010	0.343	15.576	18.621	11.472	17.530	12.645
11	BRISTOW	CREEK	35.83	-96.38	822	*1970*	*2004*	*30*	1.570		0.226		-0.196	0.601	-0.075	0.481	36.505	44.610	29.091	41.644	31.875
12	BROKEN BOW 1 N	MCCURTAIN	34.05	-94.73	474	*1970*	*2004*	*31*	1.088		0.176		-0.266	0.580	-0.139	0.515	47.884	55.664	39.148	52.770	40.204
13	BUFFALO	HARPER	36.85	-99.63	1794	*1970*	*2004*	*35*	-1.903	+	-0.243	1	-0.565	0.131	-0.484	0.009	27.334	33.715	23.931	31.889	24.568
14	BURBANK	OSAGE	36.70	-96.73	974	*1970*	*2004*	*32*	1.962	*	0.329	1	-0.084	0.667	-0.004	0.578	31.194	36.261	26.295	35.640	28.650
15	CARNEGIE 2 ENE	CADDO	35.18	-98.58	1480	*1970*	*2003*	*31*	1.632		0.301		-0.198	0.776	-0.044	0.632	25.529	33.093	18.552	29.986	21.391
16	CARTER TOWER	MCCURTAIN	34.27	-94.78	1299	*1970*	*2004*	*31*	0.714		0.096		-0.477	0.449	-0.306	0.369	48.338	62.246	42.248	57.322	44.108
17	CHATTANOOGA 3 NE	COMANCHE	34.45	-98.62	1153	*1970*	*2004*	*33*	0.418		0.054		-0.270	0.407	-0.191	0.296	28.263	34.099	22.483	32.654	24.880
18	CHECOTAH	MCINTOSH	35.47	-95.52	639	*1970*	*2003*	*34*	0.504		0.101		-0.377	0.493	-0.233	0.392	41.938	52.315	34.640	48.636	36.660
19	CHEROKEE	ALFALFA	36.77	-98.37	1179	*1970*	*2004*	*32*	2.481	*	0.380	1	-0.013	0.668	0.094	0.572	24.052	30.659	20.288	29.058	21.556
20	CHICKASHA EXP STN	GRADY	35.05	-97.92	1084	*1970*	*2004*	*31*	1.428		0.250		-0.148	0.559	-0.058	0.476	29.910	36.765	23.772	34.603	26.111
21	CLAREMORE 2 ENE	ROGERS	36.32	-95.58	587	*1970*	*2004*	*35*	2.017	*	0.298	1	-0.100	0.579	0.009	0.517	37.600	43.801	31.846	41.492	33.214
22	CLINTON	CUSTER	35.52	-98.97	1609	*1970*	*2004*	*32*	1.378		0.161		-0.189	0.589	-0.085	0.470	27.708	33.431	21.360	31.132	22.858
23	COMANCHE	STEPHENS	34.37	-97.90	979	*1970*	*2004*	*31*	2.108	*	0.339	1	-0.066	0.697	0.043	0.609	31.190	38.749	25.095	36.669	26.500
24	CORDELL	WASHITA	35.28	-98.98	1539	*1970*	*2004*	*34*	1.067		0.091		-0.270	0.489	-0.131	0.388	29.019	34.269	20.755	32.166	23.348
25	CUSHING	PAYNE	35.98	-96.77	949	*1970*	*2004*	*34*	0.771		0.080		-0.206	0.361	-0.132	0.304	34.875	39.212	30.865	38.384	31.313
26	DAISY 4 ENE	ATOKA	34.55	-95.68	754	1970	2004	32	0.146		0.02		-0.603	0.466	-0.461	0.353	50.343	63.058	40.953	59.303	42.220
27	EL RENO 1 N	CANADIAN	35.55	-97.95	1314	1970	2004	30	0.785		0.13		-0.347	0.519	-0.218	0.429	31.489	40.451	25.743	38.492	27.229
28	ELK CITY	BECKHAM	35.38	-99.40	1969	1970	2004	29	1.557		0.20		-0.110	0.590	-0.035	0.511	24.278	29.690	18.064	28.501	19.099
29	ENID	GARFIELD	36.42	-97.87	1244	1970	2004	29	0.431		0.07		-0.347	0.428	-0.245	0.344	30.331	36.940	25.522	36.158	26.391
30	ERICK	BECKHAM	35.22	-99.87	2059	1970	2004	33	0.728		0.07		-0.204	0.347	-0.142	0.272	23.646	28.311	19.258	26.582	21.025
31	FANSHAWE	LEFLORE	34.95	-94.90	544	1970	2004	31	1.258		0.25		-0.212	0.695	-0.109	0.550	48.071	55.562	40.367	52.061	42.965
32	FARGO	ELLIS	36.38	-99.63	2109	1970	2004	34	1.275		0.10		-0.105	0.314	-0.039	0.259	22.951	26.158	19.094	24.620	20.048
33	FORT SUPPLY DAM	WOODWARD	36.55	-99.53	2029	1970	2004	31	2.685	**	0.26	1	0.011	0.509	0.094	0.452	20.620	24.195	15.086	23.611	16.020
34	FREEDOM	WOODS	36.77	-99.12	1524	1970	2004	30	1.713	+	0.16	1	-0.121	0.363	-0.036	0.317	23.272	27.012	19.561	25.853	20.762
35	GATE	BEAVER	36.85	-100.05	2249	1970	2004	32	3.032	**	0.27	1	0.035	0.504	0.078	0.445	17.857	20.543	13.784	19.741	14.870
36	GUTHRIE	LOGAN	35.82	-97.40	1109	1970	2004	32	1.589		0.30		-0.156	0.608	-0.039	0.549	28.590	40.350	24.918	36.884	26.067
37	HAMMON 3 SSW	ROGER MILLS	35.60	-99.40	1819	1970	2004	33	1.550		0.17		-0.130	0.501	-0.029	0.428	23.602	28.180	19.203	27.428	20.086
38	HANNA	MCINTOSH	35.20	-95.88	678	1970	2001	32	1.930	+	0.31	1	-0.134	0.742	-0.003	0.627	38.686	48.084	33.081	45.268	34.917
39	HEALDTON	CARTER	34.22	-97.47	733	1970	2004	33	1.162		0.16		-0.257	0.430	-0.123	0.381	32.891	42.095	27.449	38.616	27.669
40	HELENA 1 SSE	ALFALFA	36.53	-98.28	1349	1970	2004	34	2.461	*	0.31	1	-0.013	0.589	0.063	0.524	25.630	31.256	21.306	29.951	22.358
41	HENNESSEY 4 ESE	KINGFISHER	36.10	-97.83	1149	1970	2004	31	1.564		0.19		-0.120	0.485	-0.044	0.423	29.326	36.352	24.028	34.519	26.154
42	HOBART MUNICIPAL A	KIOWA	35.00	-99.05	1551	1970	2004	30	1.463		0.17		-0.146	0.558	-0.060	0.457	25.379	29.045	20.671	28.072	21.284
43	HOLLIS	HARMON	34.68	-99.82	1620	1970	2004	30	0.321		0.05		-0.287	0.397	-0.211	0.328	24.806	29.918	19.444	28.796	20.512
44	HOLLOW	CRAIG	36.87	-95.27	909	1970	2003	29	0.544		0.09		-0.347	0.514	-0.278	0.353	39.210	49.357	32.872	48.048	35.892
45	HOOKER	TEXAS	36.87	-101.20	2994	1970	2004	30	1.820	+	0.17	1	-0.086	0.381	-0.024	0.342	14.781	18.722	11.026	17.927	11.617
46	HUGO	CHOCTAW	34.00	-95.52	569	1970	1998	29	1.407		0.32		-0.326	0.821	-0.123	0.662	40.542	49.675	34.942	49.162	36.807
47	JEFFERSON	GRANT	36.72	-97.78	1044	1970	2004	35	1.179		0.13		-0.250	0.478	-0.138	0.369	32.871	37.154	25.979	35.125	28.239
48	KANSAS 1 ESE	DELAWARE	36.20	-94.78	1179	1970	2004	33	0.031		0.02		-0.521	0.486	-0.373	0.348	48.142	57.662	39.175	54.705	40.278
49	KINGFISHER 2 SE	KINGFISHER	35.85	-97.90	1099	1970	2004	33	2.417	*	0.27	1	-0.015	0.510	0.045	0.446	28.230	32.929	24.347	31.669	25.200
50	KINGSTON	MARSHALL	34.00	-96.73	819	1970	2004	31	0.714		0.13		-0.402	0.684	-0.217	0.560	38.697	48.422	31.960	45.738	33.489
51	KONAWA	SEMINOLE	34.97	-96.75	974	1970	2004	34	0.445		0.06		-0.403	0.431	-0.287	0.328	38.234	47.296	31.427	45.419	33.337
52	LAVERNE	HARPER	36.70	-99.90	2099	1970	2004	33	1.565		0.11		-0.077	0.314	-0.029	0.264	19.286	23.573	17.066	22.604	17.762
53	LOOKEBA 2 ENE	CADDO	35.37	-98.33	1424	1970	2004	32	1.832	+	0.29	1	-0.125	0.632	-0.028	0.538	27.068	35.179	21.870	32.928	22.340
54	MADILL	MARSHALL	34.10	-96.78	709	1970	2004	32	1.184		0.22		-0.286	0.526	-0.171	0.439	36.926	46.192	31.202	43.541	32.208
55	MANGUM	GREER	34.88	-99.50	1594	1970	2004	32	1.216		0.16		-0.197	0.477	-0.121	0.392	25.444	30.318	21.353	28.707	22.550

56	MANNFORD 6 NW	PAWNEE	36.17	-96.43	829	1970	2004	33	2.402	*	0.32	1	-0.035	0.703	0.035	0.611	33.619	40.213	26.613	39.176	28.589
57	MARAMEC	PAWNEE	36.25	-96.68	944	1970	2002	32	2.092	*	0.25	1	-0.096	0.624	0.029	0.523	34.273	39.022	29.971	36.669	31.670
58	MARIETTA	LOVE	33.93	-97.12	844	1970	2003	33	1.286		0.27		-0.233	0.624	-0.113	0.515	30.930	41.048	26.347	37.816	27.872
59	MARLOW 1 WSW	STEPHENS	34.65	-97.98	1249	1970	2004	35	1.108		0.15		-0.237	0.493	-0.136	0.434	34.607	39.810	26.306	39.509	27.713
60	MARSHALL	LOGAN	36.15	-97.62	1044	1970	2004	31	1.632		0.18		-0.129	0.458	-0.039	0.386	28.740	33.414	23.547	32.966	25.568
61	MCALESTER MUNI AP	PITTSBURG	34.88	-95.78	759	1970	2004	31	-1.581		-0.33		-0.815	0.297	-0.734	0.145	50.760	59.244	39.109	58.057	42.294
62	MCCURTAIN 1 SE	HASKELL	35.15	-94.97	658	1970	2004	34	1.038		0.14		-0.355	0.577	-0.203	0.444	48.143	53.585	37.512	51.976	39.780
63	MEEKER	LINCOLN	35.50	-96.98	924	1970	2001	28	2.153	*	0.27	1	-0.050	0.567	0.030	0.511	32.816	38.173	27.780	36.624	28.751
64	MORAVIA 2 NNE	BECKHAM	35.13	-99.50	1689	1970	2004	29	0.807		0.12		-0.235	0.453	-0.157	0.350	24.996	30.714	18.436	29.412	20.586
65	MUTUAL	WOODWARD	36.23	-99.17	1864	1970	2004	32	0.276		0.02		-0.218	0.278	-0.142	0.202	25.986	30.158	20.391	28.559	22.397
66	NORMAN 3 S	CLEVELAND	35.18	-97.45	1108	1970	2004	29	1.069		0.19		-0.265	0.618	-0.117	0.559	36.115	41.901	28.050	40.643	29.167
67	OKEENE	BLAINE	36.12	-98.32	1209	1970	2004	33	-0.294		-0.05		-0.324	0.321	-0.252	0.244	29.571	35.660	25.153	33.838	26.186
68	OKEMAH	OKFUSKEE	35.43	-96.30	934	1970	2004	33	1.379		0.20		-0.186	0.590	-0.088	0.479	36.652	43.616	30.433	41.861	33.097
69	OKLAHOMA CITY ROG	OKLAHOMA	35.38	-97.60	1303	1970	2003	32	0.276		0.03		-0.331	0.420	-0.248	0.342	35.326	41.716	27.430	41.092	28.839
70	PAWHUSKA	OSAGE	36.67	-96.35	834	1970	2004	34	2.402	*	0.39	1	-0.019	0.821	0.111	0.717	35.838	42.809	28.771	40.205	30.388
71	PAWNEE	PAWNEE	36.35	-96.80	834	1970	1998	23	1.637		0.43		-0.231	0.843	-0.100	0.697	32.370	39.735	26.870	39.082	27.791
72	PERRY	NOBLE	36.28	-97.28	1024	1970	2004	33	0.930		0.14		-0.220	0.471	-0.120	0.399	32.602	38.691	25.586	37.232	26.920
73	PONCA CITY MUNI AP	KAY	36.73	-97.10	998	1970	2004	35	0.511		0.07		-0.334	0.555	-0.231	0.448	32.938	40.163	25.067	38.819	27.081
74	PRAGUE	LINCOLN	35.48	-96.70	1009	1970	2004	35	0.028		0.01		-0.310	0.371	-0.202	0.287	40.034	46.370	33.823	43.505	35.280
75	PURCELL	MCCLAIN	34.97	-97.43	1042	1970	2001	30	1.641		0.31		-0.234	0.839	-0.093	0.697	35.604	46.308	28.162	43.958	30.391
76	RALSTON	PAWNEE	36.50	-96.73	824	1970	2004	34	0.964		0.14		-0.284	0.468	-0.171	0.398	35.795	42.217	30.319	40.251	31.467
77	REGNIER	CIMARRON	36.93	-102.63	4019	1970	2003	31	2.210	*	0.17	1	-0.033	0.385	0.038	0.344	12.536	15.531	10.294	14.747	10.621
78	ROOSEVELT	KIOWA	34.85	-99.02	1464	1970	2004	33	0.728		0.08		-0.196	0.350	-0.128	0.291	27.549	32.314	23.044	31.155	24.416
79	SALLISAW 2 NE	SEQUOYAH	35.45	-94.80	659	1970	2004	31	0.340		0.08		-0.459	0.455	-0.275	0.401	45.776	57.118	38.135	51.914	39.665
80	SAYRE	BECKHAM	35.30	-99.62	1899	1970	2004	35	1.875	+	0.16	1	-0.073	0.433	-0.005	0.368	21.923	26.428	18.603	25.104	19.660
81	SEMINOLE	SEMINOLE	35.23	-96.67	864	1970	2004	35	0.383		0.03		-0.378	0.454	-0.222	0.350	39.555	47.234	30.949	43.795	33.108
82	SHAWNEE	POTTAWATOMIE	35.35	-96.90	1049	1970	2002	33	0.945		0.10		-0.278	0.440	-0.166	0.357	39.315	46.443	32.430	43.347	32.931
83	SPAVINAW	MAYES	36.38	-95.05	684	1970	2003	31	0.544		0.11		-0.335	0.474	-0.237	0.398	40.529	50.546	34.730	49.858	36.412
84	SPIRO	LEFLORE	35.25	-94.62	494	1970	2004	33	1.658	+	0.21	1	-0.158	0.658	-0.063	0.562	44.736	49.382	35.344	48.232	36.733
85	STILLWATER 2 W	PAYNE	36.12	-97.10	894	1970	2004	33	0.759		0.14		-0.249	0.505	-0.142	0.381	32.744	40.914	28.700	38.046	29.823
86	STILWELL	ADAIR	35.90	-94.65	999	1970	2001	30	1.748	+	0.32	1	-0.224	0.896	-0.062	0.733	44.254	54.629	34.375	51.462	38.231
87	TALOGA	DEWEY	36.03	-98.97	1704	1970	2004	35	1.236		0.13		-0.125	0.358	-0.055	0.323	26.412	31.219	22.022	29.400	22.618
88	TULSA INTL AP	TULSA	36.20	-95.88	649	1970	2001	31	-0.850		-0.11		-0.599	0.332	-0.478	0.226	42.268	47.962	34.050	47.139	35.137
89	TUSKAHOMA	PUSHMATAHA	34.63	-95.28	599	1970	2004	32	0.924		0.22		-0.314	0.770	-0.192	0.588	43.301	55.836	35.923	53.080	38.166
90	UNION CITY 1 SE	CANADIAN	35.37	-97.90	1254	1970	2004	34	-0.623		-0.08		-0.469	0.260	-0.362	0.176	37.547	44.818	31.122	42.012	32.629
91	VALLIANT 3 W	MCCURTAIN	34.00	-95.15	478	1970	2004	33	0.480		0.11		-0.414	0.645	-0.279	0.557	50.023	60.007	38.116	58.029	40.097
92	VINITA 2 N	CRAIG	36.67	-95.13	734	1970	2001	29	-0.338		-0.06		-0.578	0.370	-0.446	0.255	43.656	51.456	38.035	50.441	40.460
93	VINSON	HARMON	34.92	-99.92	1944	1970	2004	34	2.002	*	0.18	1	-0.067	0.479	0.001	0.399	21.430	26.495	17.943	25.287	19.028
94	WAGONER	WAGONER	35.97	-95.37	589	1970	1999	30	0.464		0.04		-0.418	0.508	-0.268	0.411	45.485	49.134	35.821	48.082	37.111
95	WALTERS	COTTON	34.37	-98.30	1004	1970	2004	29	-0.581		-0.12		-0.504	0.326	-0.400	0.191	34.397	40.728	28.571	38.918	30.327
96	WATONGA	BLAINE	35.85	-98.42	1549	1970	1998	29	1.932	+	0.33	1	-0.135	0.795	-0.016	0.659	25.960	32.695	19.789	30.954	21.554
97	WAURIKA	JEFFERSON	34.17	-98.00	874	1970	2004	30	-0.357		-0.03		-0.430	0.254	-0.310	0.176	30.585	39.312	25.884	36.852	26.342
98	WAYNOKA	WOODS	36.53	-98.88	1449	1970	2004	33	1.100		0.12		-0.144	0.401	-0.100	0.345	25.397	31.783	19.257	31.100	20.189
99	WEATHERFORD	CUSTER	35.52	-98.70	1641	1970	2004	29	0.581		0.08		-0.312	0.456	-0.231	0.362	26.624	34.342	20.504	32.302	23.056
100	WEBBERS FALLS	MUSKOGEE	35.48	-95.20	549	1970	2001	28	1.758	+	0.35	1	-0.173	0.821	-0.037	0.706	40.765	52.029	33.138	49.390	35.210
101	WETUMKA 3 NE	HUGHES	35.27	-96.22	709	1970	2004	35	1.591		0.24		-0.171	0.623	-0.067	0.536	38.975	46.149	32.971	43.541	34.721
102	WEWOKA	SEMINOLE	35.15	-96.48	829	1970	2003	31	0.884		0.14		-0.342	0.606	-0.238	0.484	37.670	46.641	30.253	44.640	31.592
103	WOODWARD	WOODWARD	36.43	-99.38	1899	1970	2003	32	0.454		0.05		-0.217	0.357	-0.143	0.273	23.150	27.386	19.322	26.037	20.430

Appendix C

Preliminary taxonomic list of macroinvertebrates from 23 Oklahoma springs (in part)

		Year	2004	2004	2004	2004	2004	2004	2004	2004	2004
		County	Murray	Johnston	Johnston	Johnston	Johnston	Johnston	Pontotoc	Pontotoc	Pontotoc
		site number	1	2	3	4	6	8	5	10	11
Group	Family	Genus									
Diptera	Ceratopogonidae	*Atrichopogon*	x				x				
		Bezzia/Palpomyia	x						x		
		Ceratopogon									
		Culicoides	x			x		x			
		Dasyhelea									
		Forcipomyia									
		Mallochohelea									
		Probezzia	x	x							
		Serromyia	x	x	x						
		Stilobezzia									
	Chironomidae	Chironominae	x	x	x	x	x		x		
		Orthocladinae	x			x		x		x	x
		Tanypodinae	x	x	x	x				x	x
		Tanytarsini	x	x	x	x				x	x
	Culicidae	*Anopheles*		x	x			x	x	x	x
		Culex									
	Dixidae	*Dixa*						x			
		Dixella	x			x			x	x	x
	Empididae	*Hemerodromia*									
	Psychodidae	*Psychoda*	x								
	Ptychopteridae	*Ptychoptera*									
	Simuliidae	*Simulium*	x								
		Twinnia									
	Stratiomyidae	*Myxosargus*				x	x	x	x		x
		Odontomyia/Hedriodiscus	x								
		Stratiomys				x					
	Tabanidae	*Chrysops*			x	x					
	Tipulidae	*Holorusia*								x	
		Limonia									
		Pseudolimnophila							x		
		Tipula	x	x			x	x			
		Ulomorpha									

		Year	2004	2004	2004	2004	2004	2004	2004	2004	2004
		County	Murray	Johnston	Johnston	Johnston	Johnston	Johnston	Pontotoc	Pontotoc	Pontotoc
		site number	1	2	3	4	6	8	5	10	11
Group	Family	Genus									
Hemiptera	Belostomatidae	*Belostoma/Abedus*	x								
	Corixidae	*Trichocorixa*	x		x	x	x	x	x	x	
		unid. (small)									
	Gerridae	*Aquarius*	x	x	x	x	x	x	x	x	
		Gerris		x	x			x			
		Limnoporus									
		Rheumatobates									
		Trepobates		x	x	x	x	x	x	x	x
	Hebridae	*Merragata*									
	Hydrometridae	*Hydrometra*									
	Macroveliidae	*Oravelia*									
	Mesoveliidae	*Mesovelia*	x								
	Notonectidae	*Notonecta*			x			x			
	Veliidae	*Microvelia*			x	x	x	x	x	x	x
		Platyvelia	x	x		x					
		Rhagovelia		x			x	x			
Odonata	Coenagrionidae	*Argia*									
		Enallagma			x						
		Enallagma/Ishnura			x						
	Lestidae	*Archilestes*			x						
Amphipoda	Hyalidae	*Hyalella*	x	x	x		x	x	x	x	
Isopoda	Asellidae	*Lirceus sp*									
Gastropoda	Lymnaeidae	*Fossaria*			x		x				
		Radix auricularia	x	x							
	Physidae	*Physella*	x	x	x	x	x			x	x
	Planorbidae	*Gyraulus*			x						
Tricladida	Dugesiidae	*Dugesia dortocephala*								x	x
		Dugesia sp.A	x			x	x	x	x		x

		Year	2004	2005	2004	2004	2005	2005	2005
		County	Pontotoc	Pontotoc	Coal	Coal	Lincoln	Pottawatomie	Pottawatomie
		site number	12	1	7	9	2	3	4
Group	Family	Genus							
Diptera	Ceratopogonidae	*Atrichopogon*							
		Bezzia/Palpomyia	x	x		x		x	
		Ceratopogon							
		Culicoides	x						
		Dasyhelea							x
		Forcipomyia		x					
		Mallochohelea	x	x					
		Probezzia							
		Serromyia		x			x		
		Stilobezzia							
	Chironomidae	*Chironominae*	x	x	x	x	x	x	x
		Orthocladinae	x	x		x	x	x	x
		Tanypodinae	x	x		x	x	x	x
		Tanytarsini	x	x	x	x	x	x	x
	Culicidae	*Anopheles*		x					x
		Culex						x	x
	Dixidae	*Dixa*							
		Dixella		x					
	Empididae	*Hemerodromia*							
	Psychodidae	*Psychoda*							x
	Ptychopteridae	*Ptychoptera*							
	Simuliidae	*Simulium*					x		
		Twinnia							
	Stratiomyidae	*Myxosargus*							
		Odontomyia/Hedriodiscus				x			
		Stratiomys			x				
	Tabanidae	*Chrysops*							
	Tipulidae	*Holorusia*				x			
		Limonia							x
		Pseudolimnophila							
		Tipula		x		x		x	x
		Ulomorpha							

		Year	2004	2005	2004	2004	2005	2005	2005
		County	Pontotoc	Pontotoc	Coal	Coal	Lincoln	Pottawatomie	Pottawatomie
		site number	12	1	7	9	2	3	4
Group	Family	Genus							
Hemiptera	Belostomatidae	*Belostoma/Abedus*							
	Corixidae	*Trichocorixa*				x			
		unid. (small)						x	
	Gerridae	*Aquarius*				x		x	
		Gerris	x	x				x	
		Limnoporus		x			x		
		Rheumatobates							
		Trepobates	x	x					
	Hebridae	*Merragata*	x						
	Hydrometridae	*Hydrometra*							
	Macroveliidae	*Oravelia*							
	Mesoveliidae	*Mesovelia*	x		x				
	Notonectidae	*Notonecta*							
	Veliidae	*Microvelia*		x		x	x	x	x
		Platyvelia							
		Rhagovelia							
Odonata	Coenagrionidae	*Argia*			x		x	x	
		Enallagma							
		Enallagma/Ishnura							
	Lestidae	*Archilestes*							
Amphipoda	Hyalidae	*Hyalella*	x						
Isopoda	Asellidae	*Lirceus sp*						x	x
Gastropoda	Lymnaeidae	*Fossaria*							
		Radix auricularia							
	Physidae	*Physella*	x	x			x	x	x
	Planorbidae	*Gyraulus*						x	
Tricladida	Dugesiidae	*Dugesia dortocephala*							
		Dugesia sp.A							

		Year	2005	2005	2005	2005	2005	2005	2005
		County	Ellis	Ellis	Ellis	Ellis	Ellis	Ellis	Ellis
		site number	5	6	7	8	9	10	11
Group	Family	Genus							
Diptera	Ceratopogonidae	*Atrichopogon*							
		Bezzia/Palpomyia				x			
		Ceratopogon			x	x			
		Culicoides							
		Dasyhelea				x			
		Forcipomyia							
		Mallochohelea							
		Probezzia				x			
		Serromyia		x					
		Stilobezzia				x			
	Chironomidae	*Chironominae*	x		x	x	x		x
		Orthocladinae	x	x	x	x			x
		Tanypodinae	x	x	x	x	x	x	x
		Tanytarsini	x	x	x	x	x	x	x
	Culicidae	*Anopheles*	x			x		x	
		Culex			x				
	Dixidae	*Dixa*							
		Dixella	x		x	x	x	x	
	Empididae	*Hemerodromia*	x						
	Psychodidae	*Psychoda*			x		x		
	Ptychopteridae	*Ptychoptera*					x		
	Simuliidae	*Simulium*	x		x	x			
		Twinnia			x				
	Stratiomyidae	*Myxosargus*							
		Odontomyia/Hedriodiscus				x			
		Stratiomys			x	x	x		
	Tabanidae	*Chrysops*	x	x					x
	Tipulidae	*Holorusia*							
		Limonia							
		Pseudolimnophila							
		Tipula			x				
		Ulomorpha						x	x

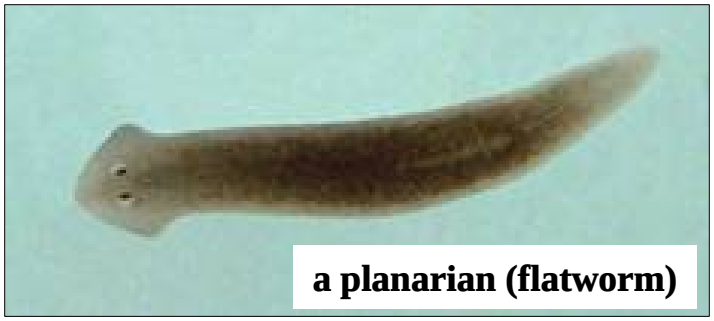
		Year	2005	2005	2005	2005	2005	2005	2005
		County	Ellis	Ellis	Ellis	Ellis	Ellis	Ellis	Ellis
		site number	5	6	7	8	9	10	11
Group	Family	Genus							
Hemiptera	Belostomatidae	*Belostoma/Abedus*		x	x	x	x		x
	Corixidae	*Trichocorixa*							x
		unid. (small)							
	Gerridae	*Aquarius*		x	x	x			x
		Gerris		x					x
		Limnoporus							
		Rheumatobates							x
		Trepobates			x	x			x
	Hebridae	*Merragata*				x			
	Hydrometridae	*Hydrometra*				x			
	Macroveliidae	*Oravelia*						x	
	Mesoveliidae	*Mesovelia*				x			
	Notonectidae	*Notonecta*							
	Veliidae	*Microvelia*	x	x	x	x	x	x	x
		Platyvelia					x		
		Rhagovelia	x						
Odonata	Coenagrionidae	*Argia*	x	x	x		x	x	
		Enallagma							
		Enallagma/Ishnura							
	Lestidae	*Archilestes*			x				
Amphipoda	Hyalidae	*Hyalella*	x	x	x	x	x	x	x
Isopoda	Asellidae	*Lirceus sp*							
Gastropoda	Lymnaeidae	*Fossaria*	x	x			x		
		Radix auricularia							
	Physidae	*Physella*	x	x	x	x	x	x	x
	Planorbidae	*Gyraulus*		x				x	
Tricladida	Dugesiidae	*Dugesia dortocephala*							
		Dugesia sp.A	x	x	x	x	x	x	x

Supplementary Images: Sampling Springs during the study

Supplementary Images: Oklahoma springs as aquatic refugees for biota and fauna

watercress

aquatic sowbug



a planarian (flatworm)

amphipods

Supplementary Images: Images of Oklahoma springs

Evaluation of Chemical and Biological Loading to the Blue River

Basic Information

Title:	Evaluation of Chemical and Biological Loading to the Blue River
Project Number:	2004OK30B
Start Date:	3/1/2004
End Date:	9/30/2005
Funding Source:	104B
Congressional District:	OK - 4th
Research Category:	Water Quality
Focus Category:	Nutrients, Water Quality, Surface Water
Descriptors:	E coli, water quality, TMDL, nutrients, Blue River
Principal Investigators:	Guy W. Sewell

Publication

1. Sanner, K; D. Menton, S. Pokarel, and G. Sewell 2005, "Chemical and Biological Monitoring of Blue River", Oklahoma Research Day, University of Central Oklahoma, November 11, Page 73.

Title: Evaluation of Chemical and Biological Loading to Blue River.

Principal Investigators:

Guy W. Sewell, Ph.D.
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Problem and Research Objectives:

Oklahoma's abundant water resources are adequate to provide for the current needs of the State's citizens, but for future use, these resources need to be managed properly and protected from degradation. A baseline assessment of natural or background biological loading is needed to evaluate water quality standards, and to serve as a baseline for the detection of, and for evaluating any degradation of water quality.

This project addresses one of the Priority Water Research Topics for 2004 as outlined in the call for proposals (#4),

> Quantitative relationship between runoff from wildlife habitats and in-stream bacterial concentration to distinguish between risks from human and natural contamination in setting water quality bacteriological standards.

and provides data for 2 other priority topics (#2, #5).

> Development of a phosphorus index that quantitatively relates field application of phosphorus fertilizer (e.g., chicken litter) to phosphorus loads in downstream receiving streams and lakes.
>
> Quantification of effectiveness of riparian zones to remove nutrients, sediment, and pathogens from runoff.

Blue River represents both a water and natural resource to Oklahoma (See Figure 1). Segments of the Blue River were listed in 1998 as impaired due to nutrients and noxious aquatic plants. While the river was not listed as impaired in the Oklahoma 2002 assessment report for these pollutants the need to assess and protect this resource remains.

ECU staff and students measured and evaluated total coliforms, E. coli, phosphorus, ammonia, nitrate and other parameters along the course of Blue River, monthly, over a one-year period. Four Oklahoma Department of Wildlife Conservation designated public access points were evaluated (6730, 6726, 6727, 6728), plus 7 additional locations (See Figure 2). Two of these seven sample locations (6732, 6731) were designated as the upper river section. The five remaining sample locations (6729, 6719, 6722, 6724, 6725) were designated as the lower river section. ODWC and ECU staff characterized land use patterns along the river course and evaluated daily usage in the public access points (See Table 1).

The major objectives of the project were as follows:

- Define bacterial (total coliform and E coli) load at sample locations
- Relate loading to upstream land use
- Evaluate bacterial loading in relationship to other water quality parameters
- Evaluate bacterial loading in relationship to human usage
- Define river discharge@time for sample locations
- Evaluate bacterial survival

Methodology:

A. Determination of total coliforms and *E coli* in water, and sample collection procedures: Total coliform and E coli quantification was determined through the use of Hach's m-ColiBlue24® Membrane Filtration method (EPA Approved* Method 10029) for the simultaneous detection of total coliforms and *E. coli.*

B. Nitrate: Total nitrate was determined by means of the Hach Water Analysis Handbook Method 8192, the Cadmium Reduction Digestion Method (0.01 to 0.5 mg/L range)

C. Ammonium: Total ammonium nitrogen was determined by means of the Hach Water Analysis Handbook Salicylate, the PhosVer 3, Acid persulfate Digestion Method (0.05 to 1.5 mg/L range)

D. Phosphorus: Total phosphorus was determined by means of the Hach Water Analysis Handbook Method 8190, the PhosVer 3, Acid persulfate Digestion Method (0.02 to 3.5 mg/L range)

F. Stream Velocity, Discharge and Cross Sectional Area Determinations:
Data and Calculations for average stream velocity and discharge was attempted using methods outlined in Fetter (2001), including the direct measurement of velocity with current meters, use of the Manning Equation, and determination of cross sectional area. Evaluation of the data indicated non-reproducible results. Access issues, low flow and equipment problems prevented obtaining reliable results. Flow determinations were dropped from the project final evaluation.

G. Land-use Determinations and Human Impact: ODWC and ECU personnel carried out land-use characterizations, by direct visualization linked to GPS referencing, and through the use of aerial photographs. ODWC personnel quantify fisheries usage (human daily recreational area usage numbers) to allow for human impact studies during times or low and high usage. Trout Stocking Schedule: Blue River 2004 Jan. 5, 8, 14, 22, 28; Feb. 5, 11, 19,25; Mar. 4, 10, 18, 23.

H. Loading and Decay Determinations: Bacterial and chemical loading determinations were to be conducted through the use of discharge and concentration data analysis at paired and multiple sampling locations. The lack of reliable flow data prevented this activity. Temperature impact on bacterial isolate survival was conducted in laboratory experiments.

Principal Findings and Significance:

Biological and Chemical Loading:

Biological and chemical assessment of Blue River samples indicates good to marginal water quality with a general trend of decreasing quality as the river travels to the South and East. Sections of the river overlaying the Arbuckle-Simpson Aquifer, designated as the upper river section, and the Blue River Wildlife Management Area (BRWMA), which appear to have significant base flow discharge from the aquifer, tend to have more desirable characteristics, subject to some fluctuation apparently related to rainfall events and seasonal variations. E. coli numbers have ranged from non-detect to approximately 100 cfu/ml, with average recoveries in the 0-2 cfu/ml, in the upper river (Table 2) and BRWMA samples (Table 3). Total coliform numbers in the upper river and BRWMA samples have ranged from non-detect to approximately 200 cfu/ml, with average recoveries of 38 to 57 cfu/ml, in the upper river and 18.5 to 27.5 cfu/ml in the BRWMA samples.

Total coliform number peaked during the winter months in upper river samples (Figure 3), while E coli counts remained relatively stable over the project period (Figure 4). In the BRWMA samples, total coliforms also showed elevated numbers during the winter months (Figure 5), while E coli counts seemed to increase at different locations at different times with no discernable pattern (Figure 6).

In the lower river samples, E. coli numbers have ranged from non-detect to approximately 12 cfu/ml, with average recoveries in the 0.41-2.4 cfu/ml range. Total coliform numbers in the lower river samples have ranged from 7.7 to approximately 700 cfu/ml, with average recoveries of 22.3 to 128 cfu/ml (Table 4).

Total coliform number trended higher during the Spring-Summer months in lower river samples, with the exception of the southernmost sample (6725) which showed dramatic increases on several occasions (Figure 7). E coli counts appeared to increase during the Spring months but did not produce a strong pattern (Figure 8).

In general the biological loading of the river seems to be relatively light in the upper river, with a decrease in water quality as the river moves from NW to SE. Bacterial counts decreased somewhat as the river enters the BRWMA and then begin to rise down-river of the wildlife management area (Figure 9). The sample location consistently showing the highest biological loading was 6725 which is near Smith-Lee Oklahoma, immediately up-river from the point where the Blue River discharges into the Red River system.

Evidence of nitrogen and phosphate loading to the watershed has been noted, but identification of the possible sources and relationships to land use showed no discernable pattern (Tables 2-4). Figures 10 and 11 show nitrate and phosphorus concentrations in BRWMA samples. Nitrogen

loading decreases over 2004 and shows some increase in the early spring of 2005. Phosphorus levels in BRWMA show no discernable pattern between locations, and neither the nitrogen or phosphorus results from BRWMA samples show a clear linkage to runoff loading conditions as evaluated from USGS stream discharge data from the same time period (Figure 12). Upper river and lower river locations showed a similar lack of discernable patterns.

The relationships between chemical and biological indicators were also evaluated. Figure 13 shows the relationship of average biological and chemical concentrations in BRWMA samples. No clear relationship between biological and chemical parameters is noted. Upper and Lower river samples also showed no relationship.

Land-use Determinations and Human Impact:

ODWC and ECU personnel carried out land-use characterizations, by direct visualization and through the use of aerial photographs. Based on aerial photographs, with the possible exception of location 6732, all sample locations appeared as either associated with undeveloped or grazing agriculture use. Sample location 6732 in Connerville maybe impacted by human activities associated with the nearby community. Sample location 6731, approximately 5 river-miles South of 6732, is intensively used pastureland with a high density of livestock. Sample locations 6730, 6726, 6727, 6728 (up river to down river respectively) represent BRWMA land under low use or undeveloped. These observations may explain the decrease seen in biological indicators as the river enters the BRWMA (Figure 9), and before they increase as the river leaves the BRWMA. While the potential impact of the communities of Armstrong, Durant and Blue cannot be excluded, aerial and visual inspection of the land directly up stream of the lower river sample sites does not identify any differences in land use, which appears to be low density grazing agriculture and/or woodlands.

Tracking of visitors to the BRWMA by the Oklahoma Department of Wildlife Conservation offered the opportunity to quantify the impact of human activities in and near the river on water quality, namely in the form of biological indicators. Figures 14 and 15 show the relationship between human activities as defined as ODWC tracked user days in the BRWMA, and average total coliform and E. coli counts for upper river sample locations and BRWMA sample locations. No correlation to user day was observed in the average total coliform or E. coli data.

Microbial Isolate Laboratory Studies:

Collaborative efforts with the University of Oklahoma to identify the strains of the recovered environmental isolates seems to suggest an unexpected predominance of strains related to human and animal pathogens. Forty-five presumptive *E. coli* river isolates were taken from the Blue River and classified using triplex PCR (See Appendix A). Isolates were characterized as follows: 0 class A, 1 class B1, 19 class B2, and 25 class D. The latter two classes contain the uropathogens and enteric pathogens, respectively, and these strains are the predominant forms of *E. coli* found in the river system between the months of February and May 2005. *Escherichia coli* is classified into four major groups, including types A (commensal strains), B1, B2, and D. The triplex PCR method, described by Clermont et. al., establishes a dichotomous key that can classify novel strains into the four major lineages. The method employs the amplification of 2 genes and a DNA fragment, including *chuA*, *yjaA*, and TSPE4.C2, respectively. The apparent predominance of pathogenic organisms in relation to the relative lack of the expected indicators strains (Type A- commensal strains) is a cause for concern. An indicator test should be

conservative in its protective determinations. If the indicator is itself a potential human pathogen, the test is no longer a conservative estimator of risk.

Following classification of all strains, 16s ribosomal sequencing was performed to examine phylogenetic relationships of 5 selected isolates (See Appendix B). Blast results indicated that isolate **6724-1** (group D) was most similar by 16S sequence to *Shigella sonnei* (citrate positive on GN2, citrate negative on IMViC); **6719-2** (group D) was most similar by 16S sequence to *Citrobacter freundii* (citrate positive on GN2, citrate positive on IMViC, no sheen on EMB agar); **6730-3** (group B2) was most similar by 16S sequence to *E. coli* CFT073 (not tested further); **6730-2** (group D) was most similar by 16S sequence to *E. coli/Shigella* (not tested further); **BTI-1** (group D) was most similar by 16S sequence to *E. coli* CFT073 (citrate positive on GN2, not tested on IMViC). However, a cluster alignment phylogram indicated that all five of the 16S sequences were most similar to *Citrobacter*. Differential tests, including IMViC and Biolog GN2 Microplates, were used on several strains to better understand the diversity of Blue River. On citrate medium (IMViC), seven of nine isolates tested were citrate positive. Overall, eight of eleven strains tested appeared to belong to the *Shigella* (1 or 2 isolates) or *Citrobacter* (6 or 7 isolates) genera. Thus, the majority of the tested strains of the presumptive *E. coli* (coliform) isolates were mistakenly classified as *E. coli,* and instead belonged to other genera of Enterobacteriaceae some of which (*Citrobacter*) are thought to be normal environmental inhabitants not associated with fecal contamination. Interestingly while the results of the triplex PCR studies suggests that the current methodologies based on E. coli as an indicator are under representing risk, these results suggest that misidentification of normal environmental strains as fecal source E. coli may over estimate risk. While neither of these studies is conclusive because of the low number of isolates used, they do suggest that a revaluation of our biological indicator strategy for water quality determinations is needed, at least for these rural surface waters in undeveloped locations. The result also suggest that a comparison of cultural and molecular characterization of know fecal source, and urban/rural surface water source presumptive E. coli isolates would be useful.

Additional studies were conducted to determine whether patterns of persistence or survival were present within strains of *Escherichia coli* from the same phylogenetic group (See Appendix C, D). Blue River isolates as well as laboratory E. coli strains were exposed to starvation conditions in nanopure water for prolonged periods of time. These conditions were used to simulate the oligotrophic, ground water fed conditions found in the BRWMA. Weekly platings on MacConkey agar for a period of forty six days was used to determine the efficiency of the individual strains to survive the stressful conditions. Overall, the results showed that certain river strains were able to remain at fairly consistent viable numbers over the 50 day course of the experiment, while others endured a general decline of approximately 2 log units; however, no patterns that would encompass entire phylogenetic groups were noticed. Never the less the degree of survival of some of the strains under these conditions was surprising.

REFRENCES CITED

Fetter, C.W. 2001. Elements of the Hydrologic Cycle. *In* Applied Hydrology 4th ed. Upper Saddle River, NJ, Prentice Hall, pp. 24-65.

Table 1: Sample Sites

Sample Site Designation	Latitude	Longitude	Description	Geography	River Section
6719	34.19696	96.44772	WNW Folsen E1940RD bridge	Sandy, Slow	Lower
6722	34.02647	96.29957	NE Durant, SE Armstrong E2060RD bridge	Slow, Muddy	Lower
6724	33.94862	96.14759	South Bokchito N3860RD bridge	Slow, Muddy	Lower
6725	33.89097	96.02530	Smith-Lee Hwy 70E bridge	Muddy, wide channel	Lower
6726	34.34953	96.59912	Wilderness Area	Falls, Rocky Wide deep channel	BRWMA
6727	34.32192	96.59597	Low-water bridge	Flowing rocky	BRWMA
6728	34.31869	96.58859	Near Camp site 17	Flowing, rocky	BRWMA
6729	34.27410	96.56545	NNW Milburn South Cheadle Falls E1890RD bridge	Rocky-Sandy Shallow, Slow	Lower
6730	34.36035	96.59085	Hwy 7 Handicap Access point	Wide, Slow Deep	BRWMA
6731	34.40491	96.61062	Tower Rd, E1800RD Frank Esterling Bridge	Rocky-Sandy Flowing	Upper
6732	34.44857	96.62267	Harris Ranch Road, Connorville, E1770RD bridge	Rocky, Wide Flowing	Upper

Table 2: Summary Sample Parameters, Upper River Sample Locations

Location	***6732***			***6731***	
Parameter	**Range**	**Average**		**Range**	**Average**
Nitrate (mg/L)	0 – 1.7	0.7		0.1 – 1.1	0.47
Ammonia (mg/L)	0 - 0.12	0.036		0 - 0.15	0.041
Phosphorus (mg/L)	0-0.5	0.22		0.12 -0.33	0.17
Chloride (mg/L)	10 - 20	12.9		10 - 20	12.9
Turbidity (FAU)	1 - 24	14		1 - 24	13
Total Coliforms (CFU/ml)	14.6 - 200	57.4		1.5 - 101	38
E. coli (CFU/ml)	0.5 – 1.2	0.86		0.1 - 2	0.88

Sample locations are ordered upstream to downstream.

Table 3: Summary Sample Parameters, Blue River Wildlife Management Area Sample Locations.

Location	***6730***			***6726***			***6727***			***6728***	
Parameter	**Range**	**Average**		**Range**	**Average**		**Range**	**Average**		**Range**	**Average**
Nitrate (mg/L)	0.1 – 0.9	.43		0.1 – 1.6	0.51		0 – 1.1	0.53		0 – 1.3	0.36
Ammonia (mg/L)	0 – 1.43	0.2		0 – 0.17	.092		0 – 2.18	0.26		0 – 2.26	0.26
Phosphorus (mg/L)	0 – 2.16	0.32		0 – 1.12	0.36		0 – 1.33	0.29		0 – 0.55	0.2
Chloride (mg/L)	10 – 150	27.1		20 – 65	20		10 - 20	11.7		10 – 20	14.2
Turbidity (FAU)	0 – 18	6.1		0 - 11	5.5		0 - 9	3.9		0 - 11	4.6
Total Coliforms (CFU/ml)	0.9 - 92	27.5		0.1 - 95	27.1		1.4 - 81	22.2		0.4 - 37	18.5
E. coli (CFU/ml)	0 – 1.2	0.39		0 – 0.9	0.275		0 – 0.9	0.29		0 - 1	0.32

Sample locations are ordered upstream to downstream.

Table 4: Summary Sample Parameters, Lower River Sample Locations.

Location	***6729***			***6719***			***6722***			***6724***			***6725***	
Parameter	**Range**	**Average**		**Range**	**Average**		**Range**	**Average**		**Range**	**Average**		**Range**	**Average**
Nitrate (mg/L)	0 – 1.3	0.38		0 – 1.3	0.6		0 – 1.7	0.65		0.2 – 2.9	1.22		0 – 3.8	1.23
Ammonia (mg/L)	0 – 0.14	0.052		0 – 0.13	0.035		0 – 0.2	0.068		0 – 0.27	0.098		0 – 0.45	0.1
Phosphorus (mg/L)	0.04 – 0.55	0.23		0.09 – 0.6	0.26		0.07 – 0.6	0.265		0.33 – 0.65	0.5		0.12 – 2.83	1.0
Chloride (mg/L)	10 – 15	12.5		10 – 20	13.3		10 – 15	12.5		10 – 20	13		10 – 25	13.3
Turbidity (FAU)	0 - 16	7		4 - 12	6.3		5 - 44	17.3		2 - 92	23.6		7 - 46	18.4
Total Coliforms (CFU/ml)	8.5 - 39	22.3		16 - 69	37.5		7.7 - 68	24.5		10.9 - 194	37.4		9.1 - 709	128
E. coli (CFU/ml)	0.2 – 0.8	0.41		0.2 - 2	1.1		0.2 - 12	1.9		0.2 - 3	0.96		0 - 12	2.4

Sample locations are ordered upstream to downstream.

Figure 1. Map showing the location of the Blue River in Oklahoma.

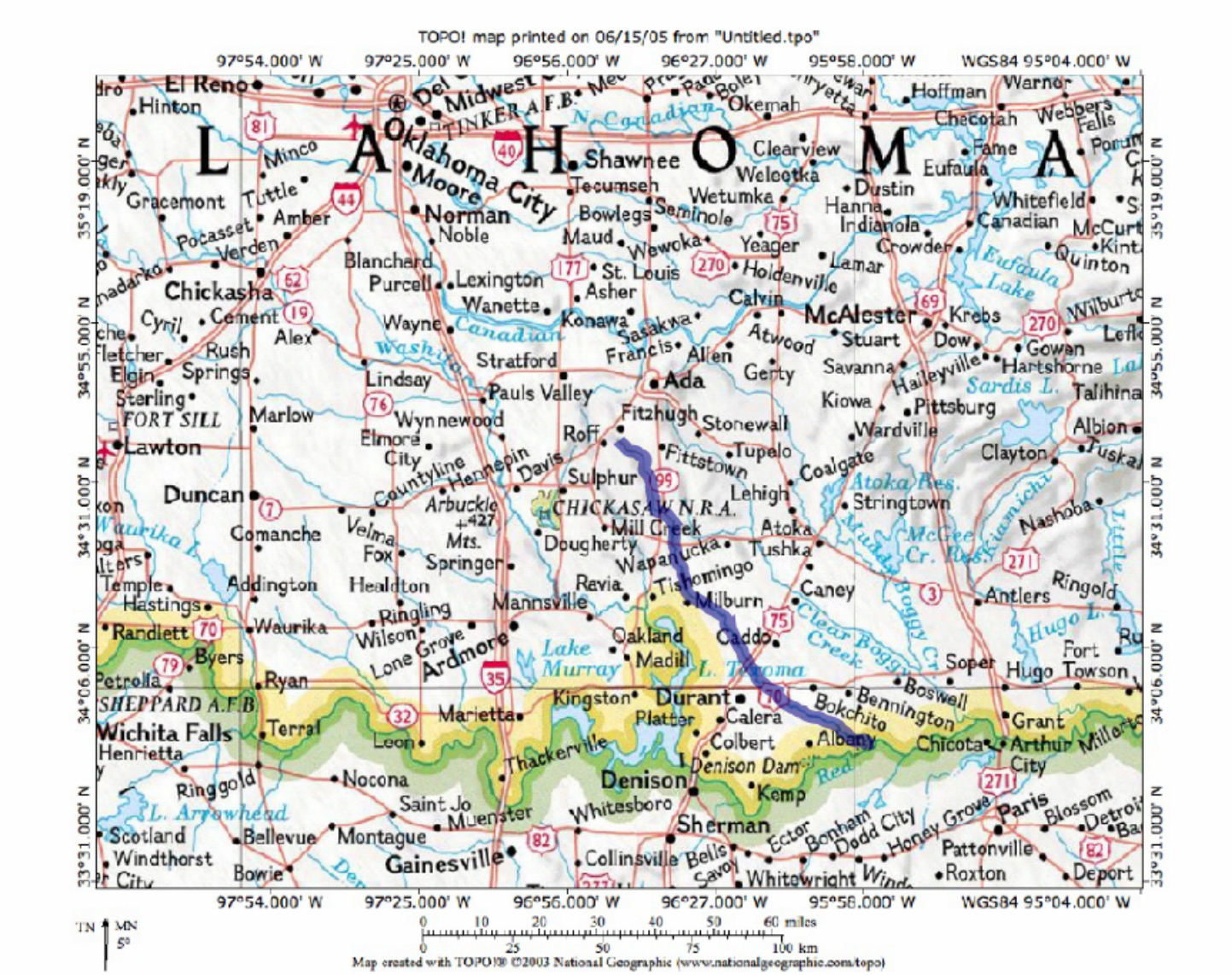


Figure 2. Map showing location of sampling points along the Blue River.

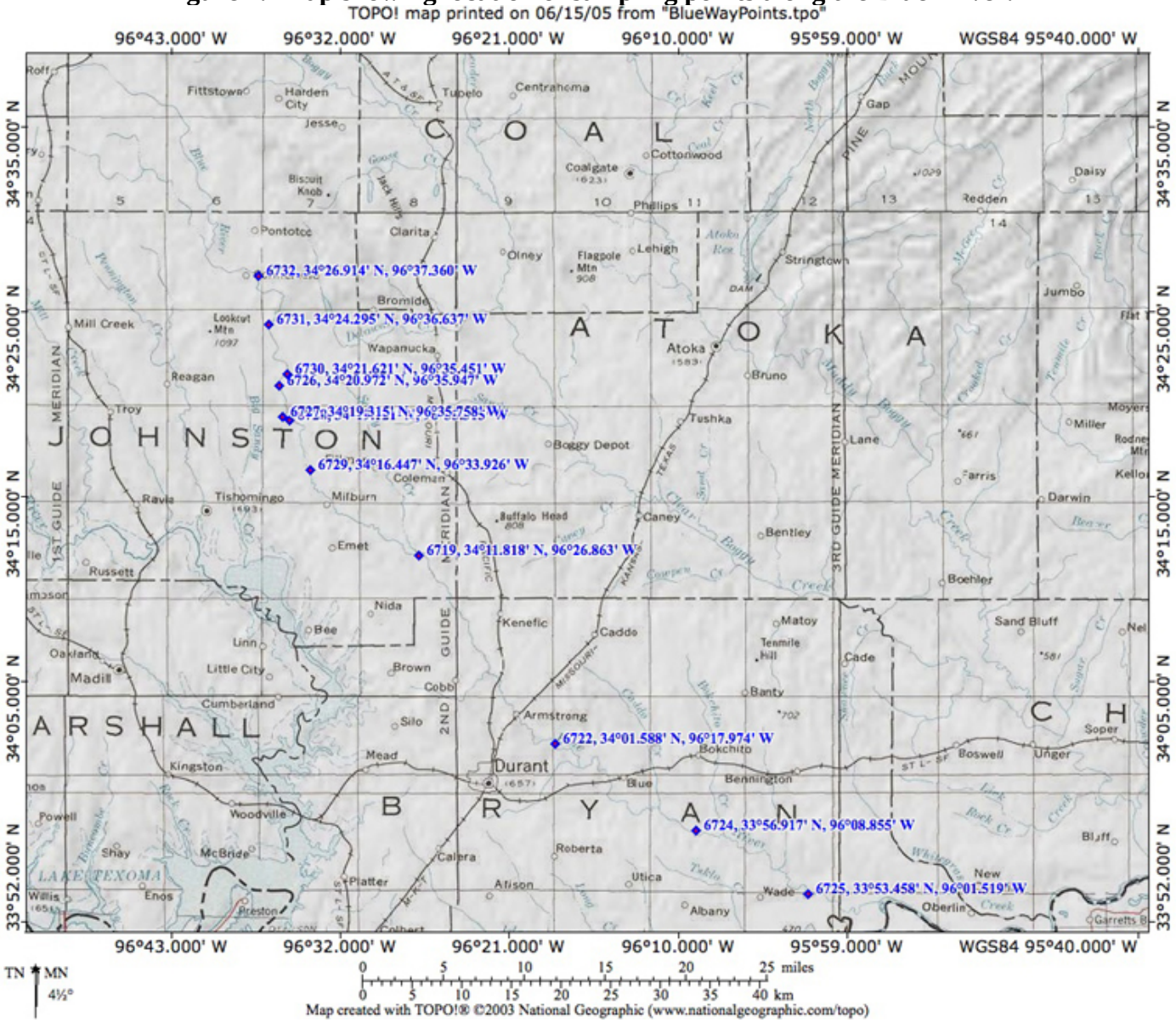


Figure 3: Total Coliforms-Upper River Section

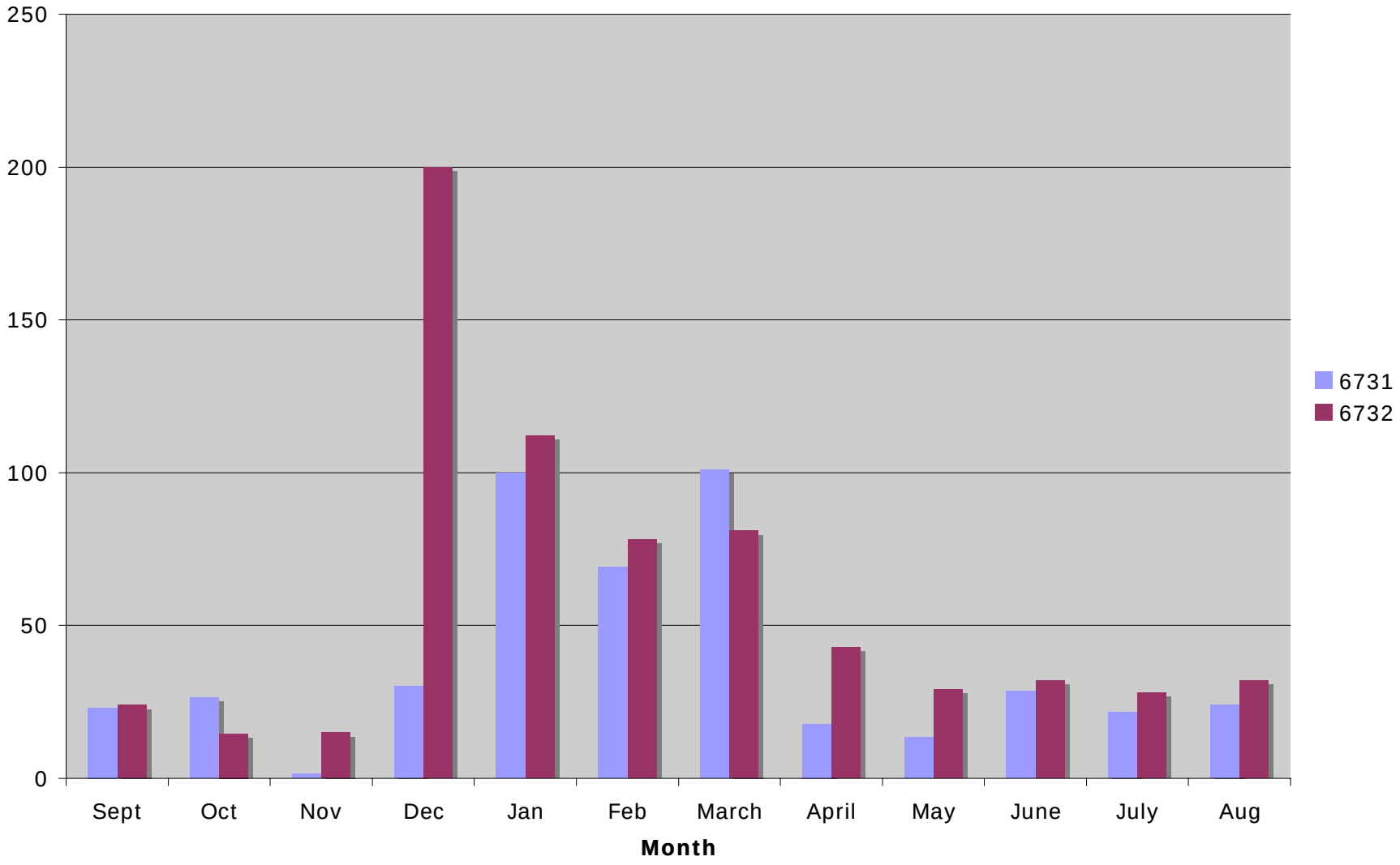


Figure 4: E. coli-Upper River Section

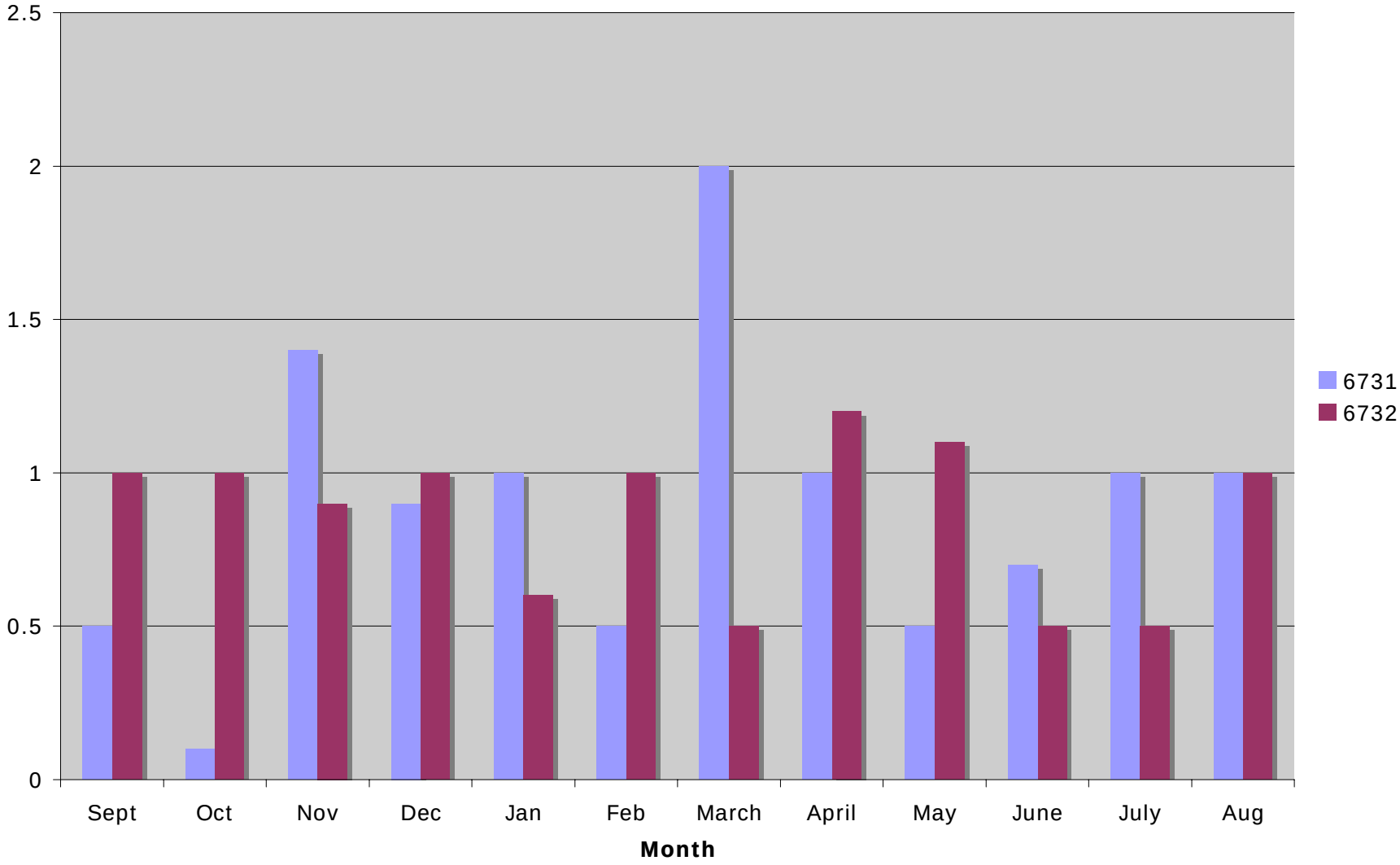


Figure 5: Total Coliforms-BRWMA

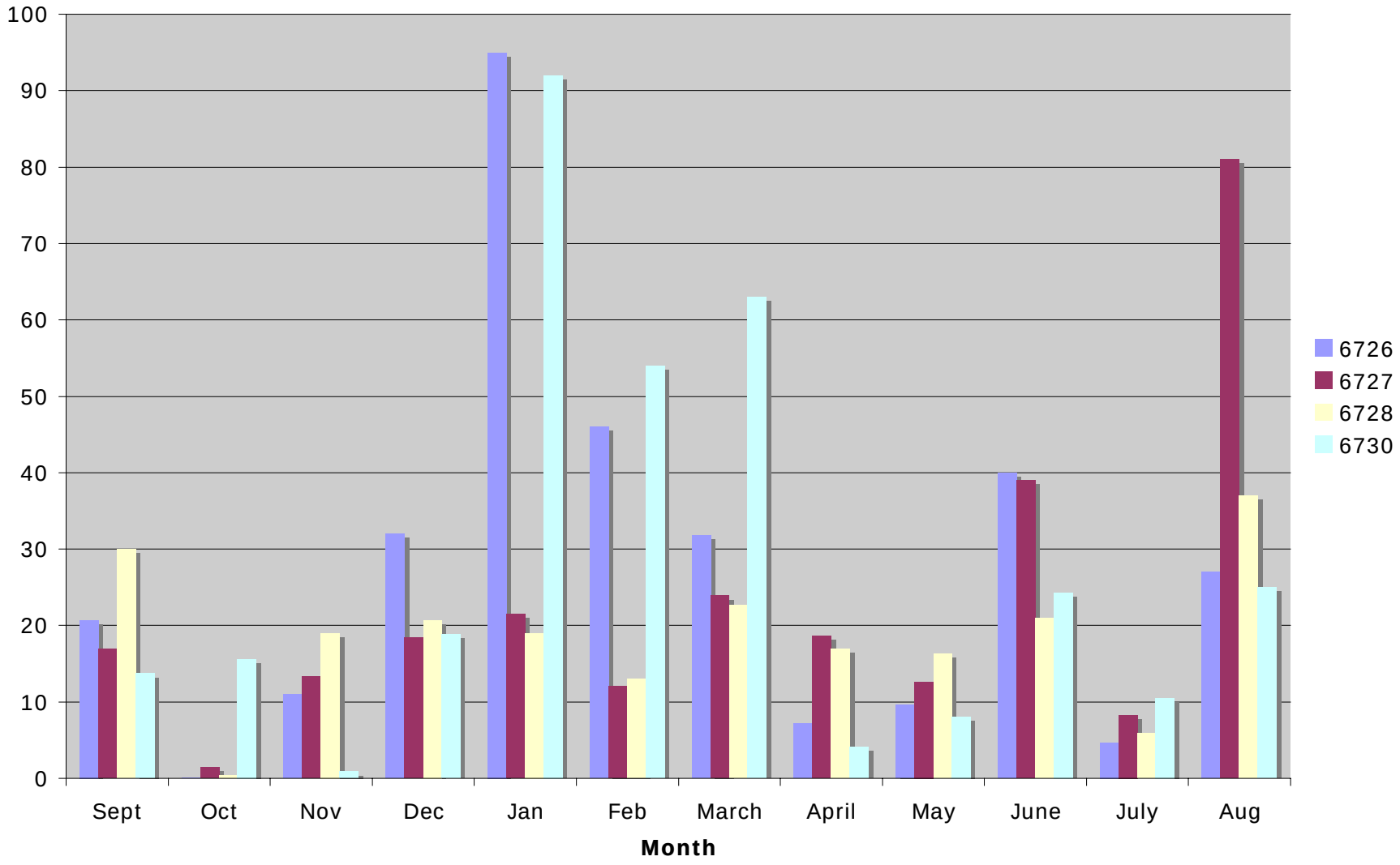


Figure 6: E. coli-BRWMA

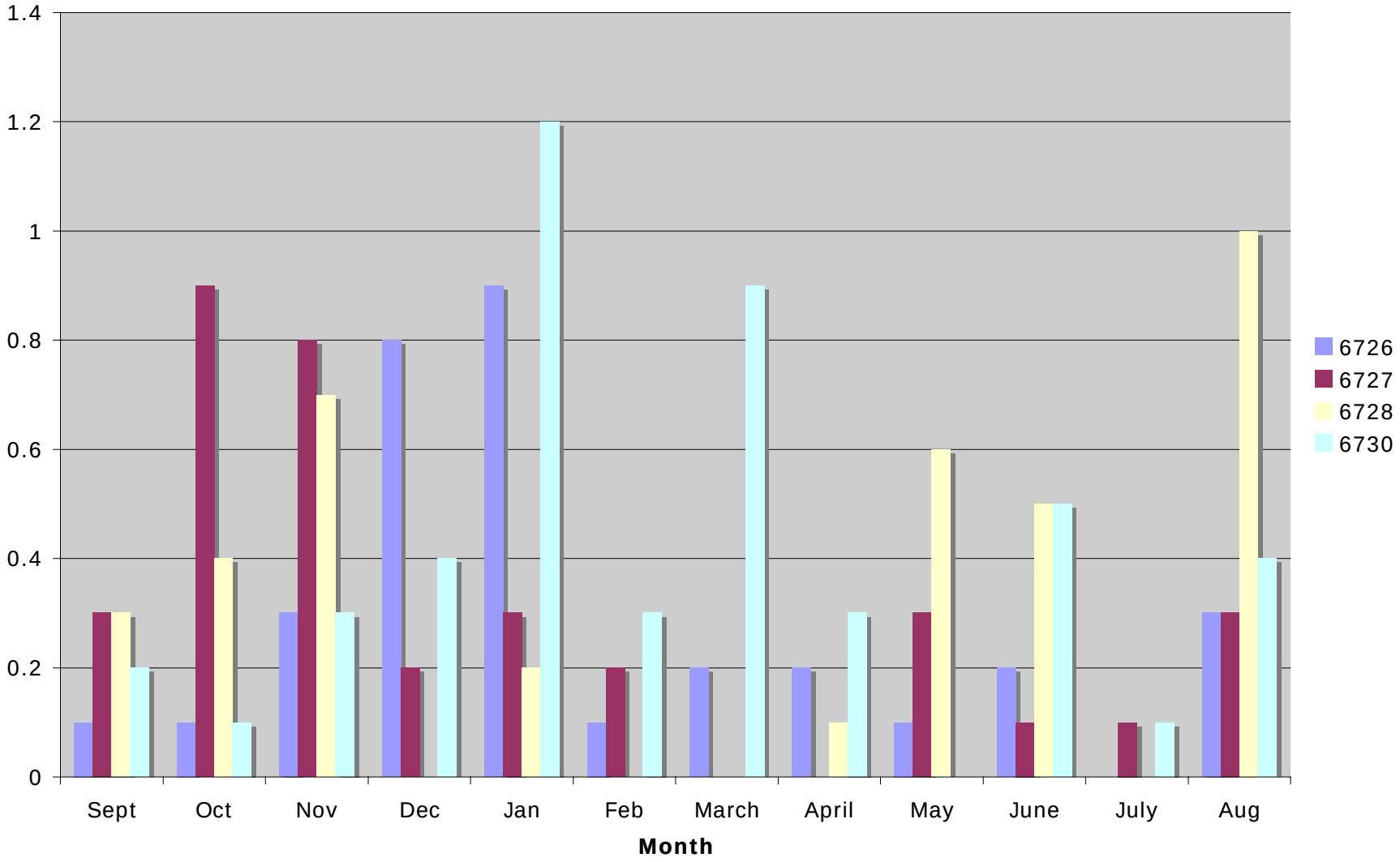


Figure 7: Total Coliforms-Down River Section

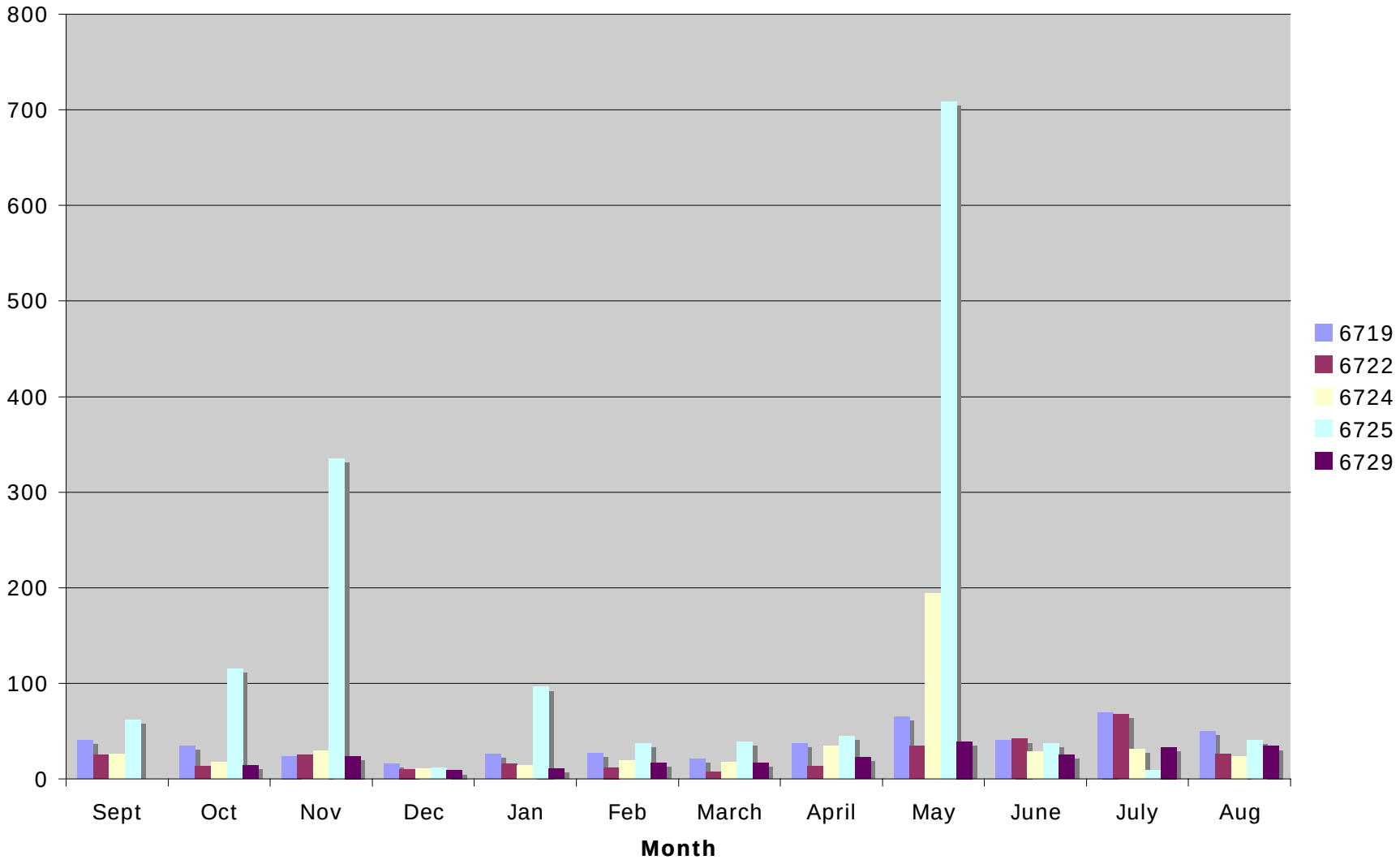


Figure 8: E. coli-Lower River Section

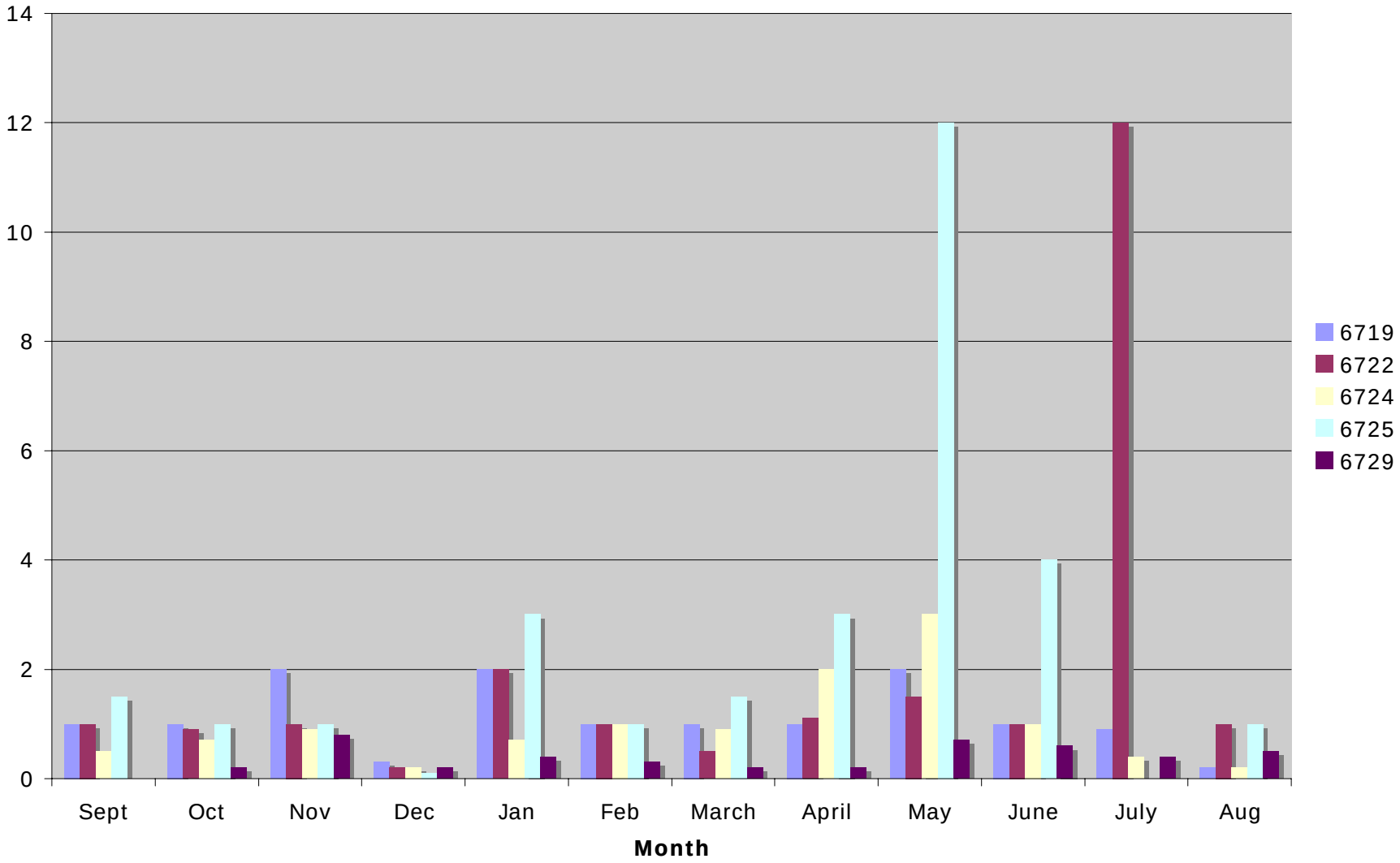


Figure 9: Average Total Coliforms and E. coli vs Location

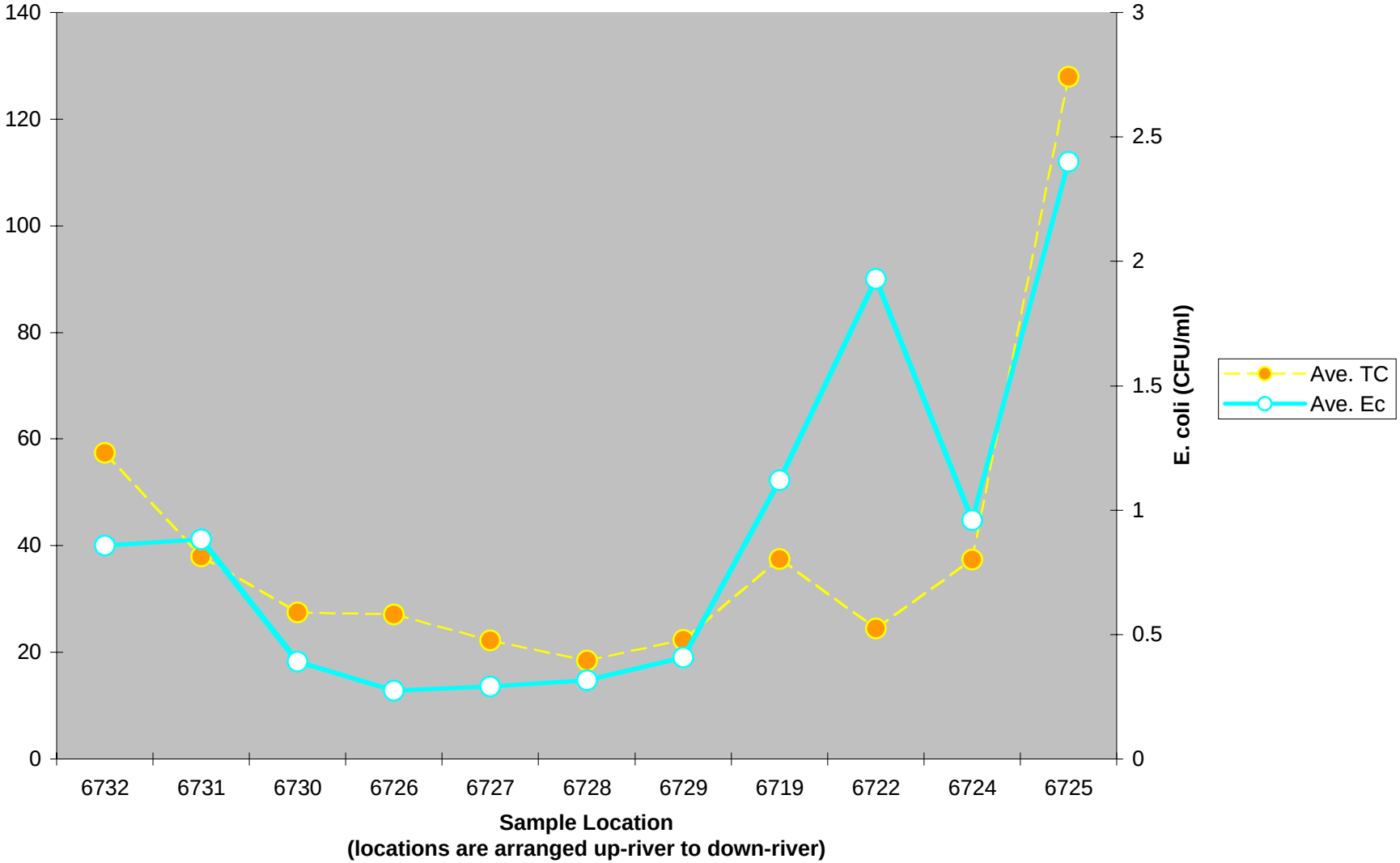


Figure 10: Nitrate vs Month-BRWMA

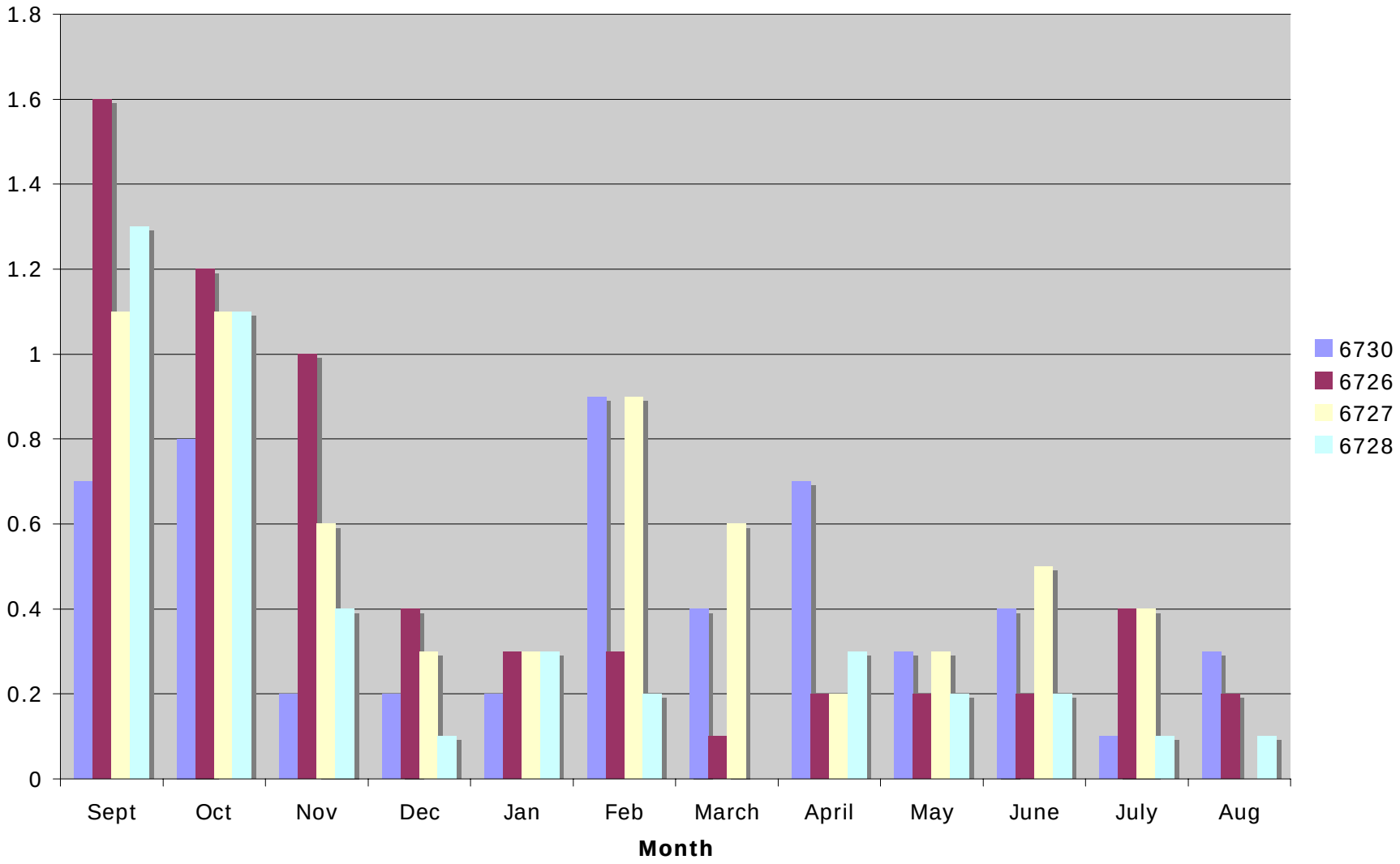


Figure 11: Phosphorus vs Month

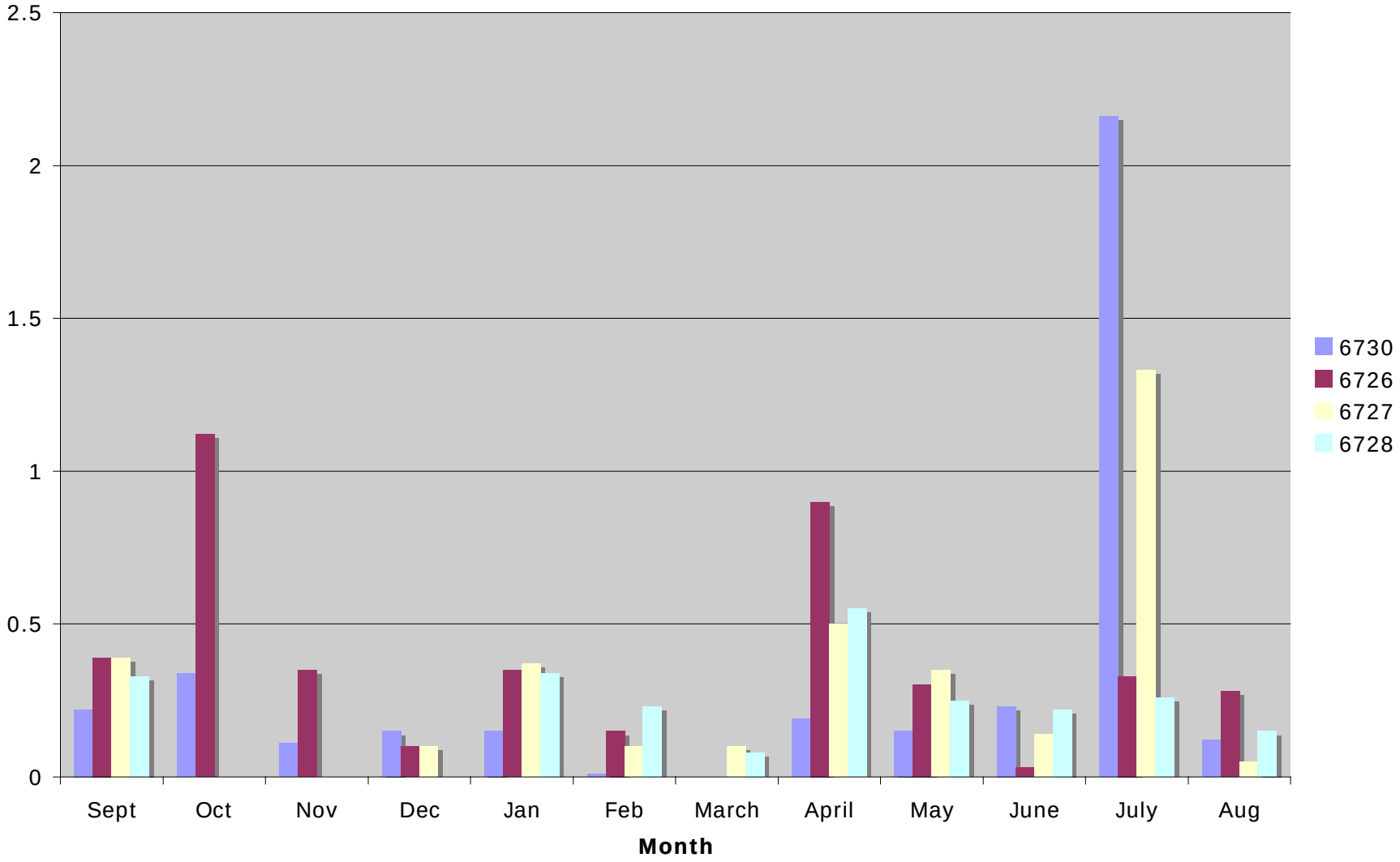


Figure 12: USGS Blue River Discharge Data

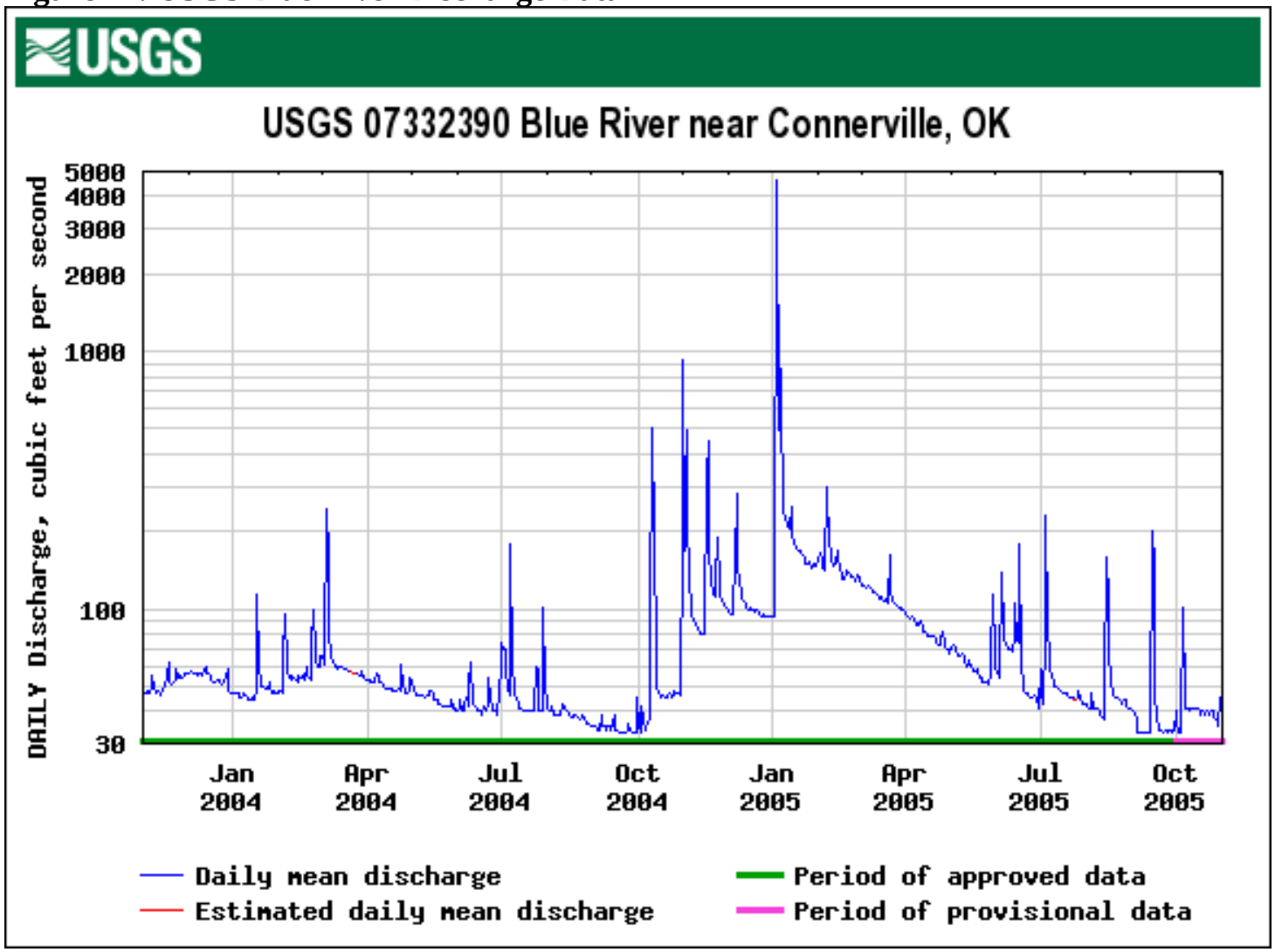


Figure 13: Nutrients, Total Coliforms, E. coli per Month

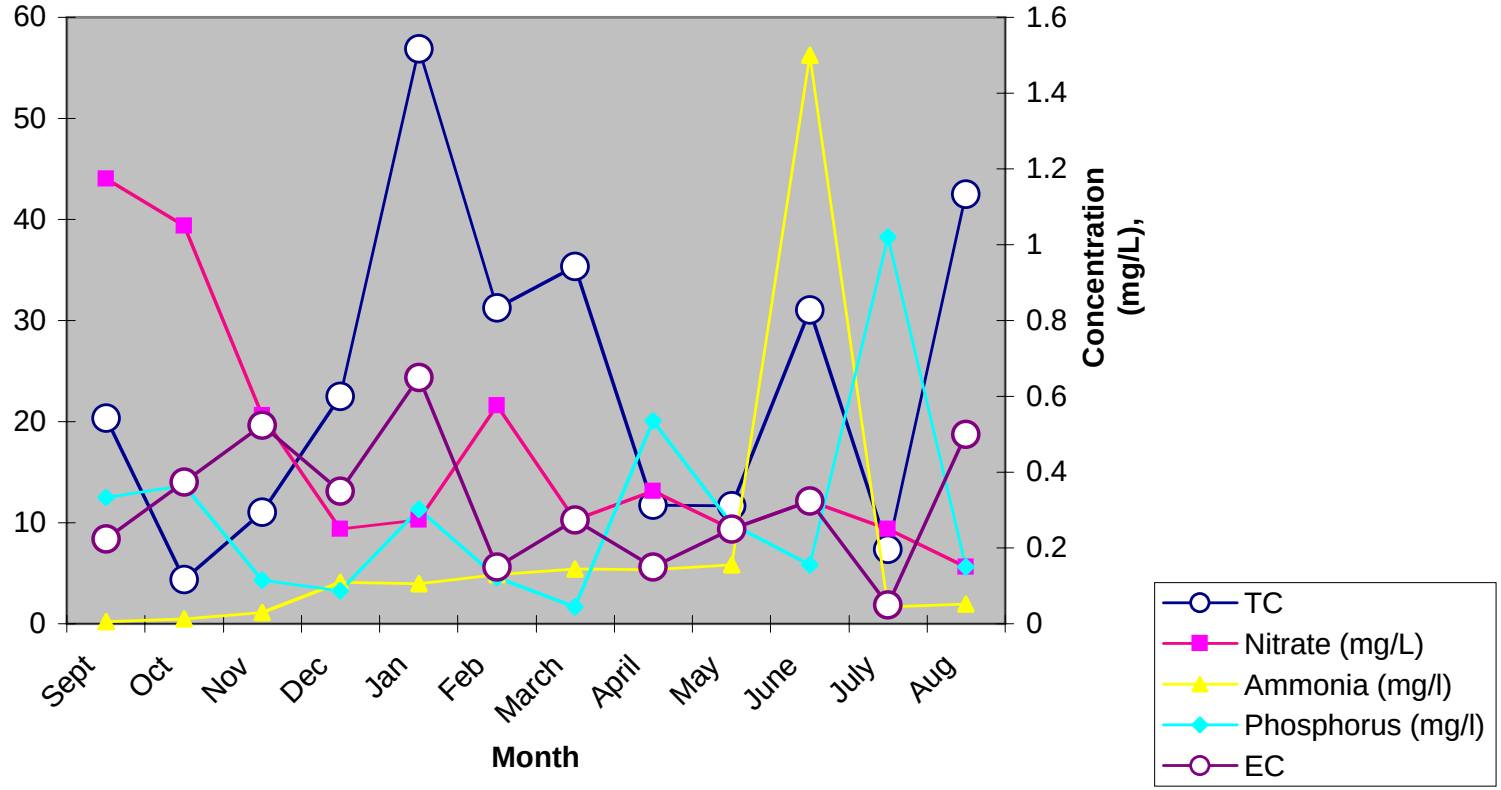


Figure 14: Impact of User Days on Total Coliform Counts

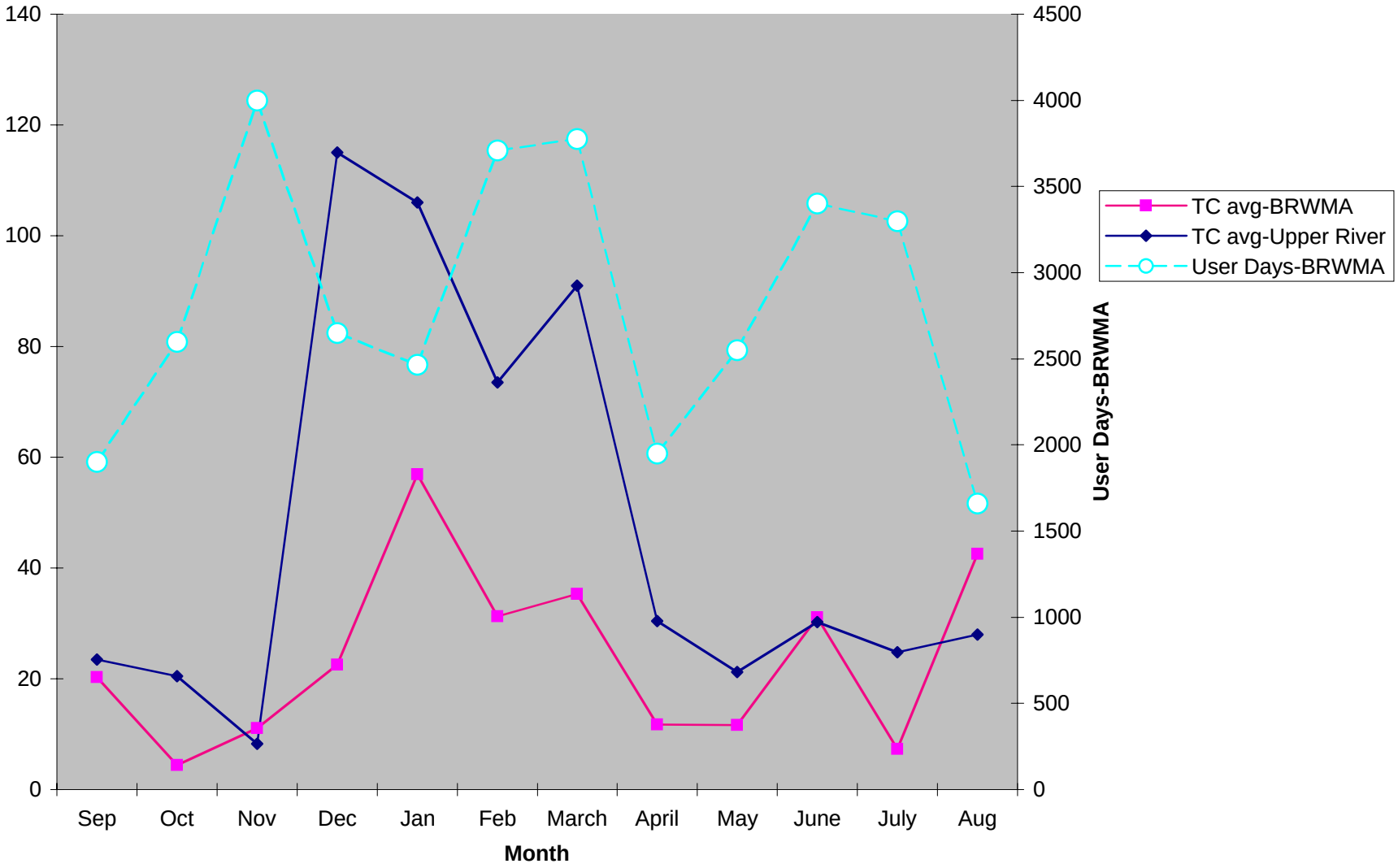
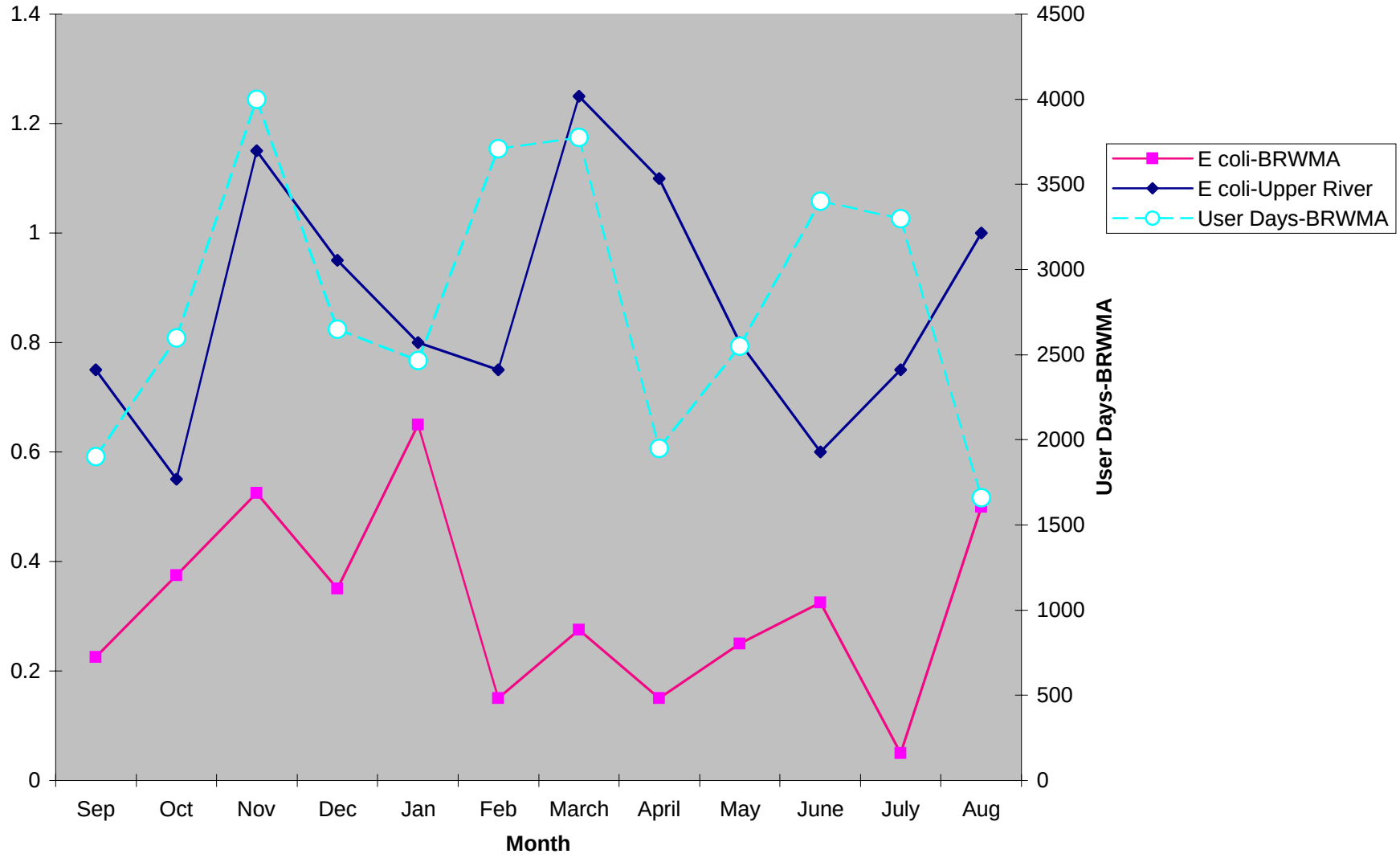


Figure 15: Impact of User Days on E. coli Counts



Appendix A

Classification of Novel *Escherichia Coli* River Strains using Triplex PCR

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Committee Member Dr. Tyrrell Conway

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Committee Member Dr. Michael McInerney

ABSTRACT

***Escherichia coli* is classified into four major groups, including types A, B1, B2, and D (12, 21). The triplex PCR method, described by Clermont et. al., establishes a dichotomous key that can classify novel strains into the four major lineages. The method employs the amplification of 2 genes and a DNA fragment, including *chuA*, *yjaA*, and TSPE4.C2, respectively. Forty-five presumptive *E. coli* river isolates were taken from the Blue River system near Tishomingo, Oklahoma, and classified using triplex PCR. Isolates were characterized as follows: 0 class A, 1 class B1, 19 class B2, and 25 class D. Since the latter two classes contain the uropathogens and enteric pathogens, respectively, these strains are the predominant forms of *E. coli* found in the river system between the months of February and May 2005. Local livestock are thought to be a potential mechanism of transmission of the waterborne pathogens.**

INTRODUCTION

As one of the first bacterial genomes to be sequenced, *Escherichia coli* is among the best understood microorganisms (3). *E. coli* is frequently used as a host strain in molecular biology and industry, however, some strains are associated with medical pathologies, making *E. coli* an important focus of biomedical research as well (1, 15). The ubiquity of *E.coli* is astounding, probably because it is the predominant facultative anaerobe in the gastrointestinal tract of mammals (9). Nutritional studies have indicated that *E. coli* colonizes the mucosal layer of the gastrointestinal tract (7). In addition to gastrointestinal research, several studies have elucidated how *E. coli* is transmitted in nature.

Understanding the mode of transmission of microorganisms may potentially aid in the prevention or control of biological warfare. At the least, it may aid in comprehending the pathogeneses of waterborne disease. The Center for Disease Control reported 73, 000 annual cases of infection caused by *E. coli* O157:H7 between 1982 and 2002, 9% of which were waterborne infections (19). Identifying *E. coli* strain types present in nature is the first step in understanding the mechanisms of transmission. Therefore, the purpose of this study is to classify *E.coli* isolates found in an Oklahoma river system to create class phylogenies.

Escherichia coli strains can be classified into four main lineages, including types A, B1, B2, and D (12, 21). Most commensal strains belong to groups A and B1, while groups B2 and D contain pathogenic strains (2, 5, 13, 18). Clermont, et. al. described a rapid method to determine the class of *E. coli* based on a triplex PCR protocol (8). The three candidate markers featured in the triplex PCR include two genes, *chuA* and *yjaA*, and a DNA fragment known as TSPE4.C2 (8). The *chuA* gene encodes an outer membrane heme/hemoglobin receptor in *E. coli* O157:H7 (4, 16, 23). *YjaA* is a gene with unknown function that is found in the K-12 genome (3). Finally, the TSPE4.C2 marker is a fragment of unknown function that was selected from Clermont's clonal library (8). Based on the origin and fragment size of each triplex marker, a dichotomous key was developed to classify unknown strains (Fig. 1). Figure 2 displays Clermont's banding results of each strain class. Due to its efficiency, Clermont's triplex PCR method was employed to place river isolates into phylogenetic groups.

MATERIALS AND METHODS

Bacterial strains. Forty-eight novel *E. coli* strains were examined for classification. The K-12, ECOR-26, F-18, and O157:H7 strains were used as controls since each strain represents one of the four major lineages found within the species, A, B1, B2, and D, respectively. Control strains

were provided by Dr. Tyrrell Conway. The remaining 48 isolates were obtained from the Blue River system by Dr. Guy Sewell and were kindly donated to this project. River isolates were collected from a total of 13 different sites, between the GPS coordinates of 96°37.167'W to 96°33.833'W and 34°17.833'N to 34°22.000'N (Fig. 3).

Isolation of river strains. Strains were isolated using a MicroFunnel™ Filter Unit (Pall Life Sciences, East Hill, NY) following manufacturer's protocol. River isolates were grown on m-ColiBlue24 broth (Hach Company, Loveland, Colorado) and stored in a Tupperware container while in transport. Upon arrival, individual isolates were streaked onto gram negative selective MacConkey agar plates.

Genomic DNA isolations. Genomic DNA was extracted using a DNeasy® Tissue Kit (Qiagen, Inc., Valencia, Calif.) under manufacturer's recommendations for isolating DNA from gram-negative bacteria.

Oligonucleotide Primers and PCR Assay. PCR was performed using a modified version of the triplex PCR protocol described by Clermont et. al. (8). Each reaction mixture had a total volume size of 25 µl and included 17.2 µl of purified H_2O, 2.5 µl of 10X buffer (supplied with *Taq* polymerase), 1.0 µl of $MgCl^{2+}$ (supplied with *Taq* polymerase), 2.0 µl of dNTPs (Invitrogen, Carlsbad, California) at a concentration of 10 mM, 0.2 µl of each primer pair (Invitrogen, Carlsbad, California), 0.1 µl of Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, California), and 1.0 µl of genomic DNA. The amount of DNA sample and water was frequently adjusted to produce optimal results. Standard PCR protocol was observed with the exception that all three primer pairs were assayed for simultaneously. The primer pairs used were ChuA.f

(5′-GACGAACCAACGGTCAGGAT-3′) and ChuA.r (5′-TGCCGCCAGTACCAAAGACA-3′), YjaA.f (5′-TGAAGTGTCAGGAGACGCTG-3′) and YjaA.r (5′-ATGGAGAATGCGTTCCTCAAC-3′), and TspE4C2.f (5′-GAGTAATGTCGGGGCATTCA-3′) and TspE4C2.r (5′-CGCGCCAACAAAGTATTACG-3′) (8). Each primer set generates a 279-, 211-, and 152-bp fragment, respectively (8). PCR reactions were carried out using an Applied Biosystems GeneAMP® PCR System 9700 thermal cycler. Amplification conditions began with an initial denaturation step of 94°C for 5 min, followed by 25 repetitions of the following cycle: 30 sec at 95°C, 30 sec at 50°, 1 min at 72°, followed by a final extension of 10 min at 72°. PCR products were then migrated on a 1% agarose gel and visualized on a UVP Epi Chem II Darkroom transilluminator.

RESULTS

PCR grouping results. A total of 48 river isolates were examined using triplex PCR, 45 of which were classified using the dichotomous key established by Clermont et. al (Table 1). Twenty-five strains were classified as group D, with 8 samples exhibiting the *chuA* marker only (subgroup 1) and 16 samples exhibiting both the *chuA* and TSPE4.C2 marker (subgroup 2). One strain 6737-2-10 contained a very faint TSPE4.C2 band after being assayed twice, and therefore could not be differentiated between subgroup 1 or 2, however, both subgroups indicate a group D strain. Also, 6 of the group D subgroup 2 strains contained a band located between 500 and 600 bp in addition to the expected genetic marker bands. Nineteen strains were classified as B2, with 18 samples exhibiting all three DNA markers (subgroup 1) and 1 sample exhibiting only the *chuA* and *yjaA* bands (subgroup 2). One strain was classified as B1. No isolates were identified

as class A. Three samples, 6719-1, 6727-2-10, and 6727-4-10 were deemed unclassifiable due to inconsistency in banding patterns after many attempts.

DISCUSSION

Assay techniques. Triplex PCR experiments were initially run using bacterial colonies, however, inconsistency in results lead to the employment of genomic DNA. Utilizing genomic DNA produced repeatable results and generally produced a clearer banding pattern. Also, reaction conditions did not have to be adjusted as frequently since DNA sample concentrations could be controlled. To illustrate DNA source disparities, control samples from each representative class were run using genomic DNA as opposed to the original bacterial isolates (Fig. 4). The ECOR-26 strain seen in lane 4 shows an additional band between 500 and 600 bp that was not visualized using bacterial colonies under the same reaction conditions. Also, notice that the control samples mirror the results obtained by Clermont et. al. (Fig. 2 and 4).

Six group D river isolates exhibited the same band between 500 and 600 bp found in the ECOR-26 control sample. However, reaction conditions varied the intensity or presence of the band. For example, sample 6730-2 was classified as a member of group D, subgroup 2. Two different trial runs confirmed the classification, yet only one gel displayed the extra band (Fig. 5). Altering the concentration of the genomic DNA used as well as the extension time may have contributed to the presence or absence of the band.

Although the annealing temperature of the reaction was as low as 50˚C, the reaction conditions were a likely cause of the additional band. Perhaps the band should be excised and sequenced to eliminate any uncertainty for future work.

Finally, faint bands were occasionally visualized around 100 bp (Fig. 7). These bands are also seen in Clermont's example, and are likely to be primer dimers (Fig. 2).

PCR grouping results. Clermont described a dichotomous tree to determine classification between four major lineages of *E. coli*, yet both groups B2 and D could be identified through 2 different banding patterns (Fig. 2). Thus, for this experiment, strains classified as group B2 were deemed subgroup 1 if all three genetic markers were present (Fig. 2; lane 7) or subgroup 2 if only the *chuA* and *yjaA* markers were present (Fig. 2; lane 6). Group D organisms were deemed subgroup 1 if the only band present was *chuA* (Fig. 2; lane 4) or subgroup 2 if both the *chuA* and TSPE4.C2 markers were simultaneously present (Fig. 2; lane 5). Fig. 6 displays a gel containing subgroups 1 and 2 of class B2 as well as group D subgroup 2.

The most obvious aspect of the phylogenetic analysis is the overwhelming presence of groups B2 and D and lack of groups B1 and A. As mentioned previously, most commensal strains belong to groups A and B1, while groups B2 and D contain virulent strains (2, 5, 13, 18). Thus, since only one commensal strain was identified, the samples indicate that the predominant form of *E. coli* located in the Blue River system belongs to pathogenic groups.

According to Clermont's dichotomous tree, the presence of only the *yjaA* band indicates group A *E. coli*. The K-12 strain that was used as a control in this experiment duplicated this banding pattern, yet no river isolates were identified as group A (Fig. 4). To eliminate any concern about the lack of group A in the future, a K-12 control sample should be run with all river isolate samples. Kuhnert et. al. identified another method to rapidly confirm the presence of K-12 strains through PCR analysis of the *rfb* cluster (15). Employment of this method would verify the phylogenetic analysis results using triplex PCR.

All river isolates should be streaked onto a gram-negative selective media such as MacConkey agar to eliminate other types of strains. Both *Wolinella succinogenes* and *Campylobacter jejuni* contain the *chuA* gene, and thus, could be classified as group D *E. coli* if there was not an initial screen for *E. coli* (17).

Finally, Clermont analyzed 230 strains of *E. coli*, only 2 of which were classified incorrectly (8). Two strains indicated a group A banding pattern, although they originated from a group B1 lineage. They attributed the discrepancy to either an intermediate genetic base or the possibility that the markers could be located in regions closer together in these strains than to the regions studied by their method (8). Thus, although Clermont's utilization of the dichotomous tree produced 99.1% accuracy, it left room for error. However, this confused classification occurred in an ECOR 70 strain which has been questioned as a genetic hybrid, so it is statistically unlikely that any of the river strains would cause such a discrepancy. Also, Clermont misidentified a group B1 specimen as a group A specimen, whereas no group A organisms were identified in this study.

Predominance of enteric ***E. coli*** **groups**. The abundance of group B2 and D river isolates suggests that these strains contain survival mechanisms not seen in commensal *E. coli*. In Rhodes and Kator's study, it was determined that the two prevalent factors that inhibit the growth of *E. coli* in estuarine environments are water temperature and autochthonous microbiota (20). Rhodes found that *E. coli* flourished in higher temperatures, especially around 37˚C (20). In addition, the months of April, May, and June witnessed the highest microflagellate densities because these times corresponded to periods in which seasonal water temperatures increased (20). Therefore, it is possible that groups B2 and D may more readily adapt to climactic changes or contain enzymes that are less sensitive to temperature changes than commensal strains. In this

study, river isolates were collected in the months of February and May. A more thorough characterization of *E. coli* populations in the Blue River system would include samples taken from all seasons of the year. Such a sampling would indicate whether or not enteric *E. coli* predominate throughout the year or if domination only correlated with water temperatures.

Potential means of transmission. Previous studies suggest a variety of mechanisms by which enteric *E. coli* could be transmitted into a river environment. While fecal coliforms often suggest contaminants from sewage or a cesspool, studies in Hawaiian river systems found that the predominate source of enteric bacteria was derived from land run-off (10). However, the sampling locations in this study were not located near any major housing developments. Secondly, in a series of studies by Hazen in conjunction with various federal agencies, it was reported that several locations, including Nigeria, Hawaii, New Guinea, Puerto Rico Sierra Leone, and the Ivory Coast, maintain water sources that contain high levels of *E. coli* in the complete absence of any known fecal contaminant (11). Further investigation by Hazen suggested that in pristine regions of the tropics, samples of *E. coli* were found 15 m above the ground in tree epiphytes in the absence of fecal sources (11). While these studies suggest insightful mechanisms of *E. coli* transmission, the area around Tishomingo, Oklahoama, has a temporal and topographical environment that is distinctly different from regions in the tropics.

Livestock is the most likely vector for group B2 and group D *E. coli* transmission in local bodies of water of near Tishomingo, Oklahoma. Cattle have been identified as the chief source of human infection with enterohaemorrhagic *E. coli* (6). Other animals, such as sheep, goats, water buffaloes, and deer are also potential sources of enteric microorganisms (6). Furthermore, studies indicate that pigs and poultry do not transmit the pathogen (6). Cattle are a likely host of

enteric *E. coli* since the O157:H7 strain does not cause disease in cattle (24). Also, several livestock farms are located in the Tishomingo, Oklahoma, area (online directory).

A study by Scott et. al. involved measuring the life span of *E. coli* O157:H7 in water. They found that the inoculum survived up to 109 days in water (22). Also, *E. coli* samples collected from inoculated cattle survived up to 10 weeks longer than laboratory cultures (22). Thus, passage through the gastrointestinal tract of cattle may increase the survivability of *E. coli* in low-nutrient conditions (22).

Since *E. coli* is avirulent to cattle and studies have shown that *E. coli* survivability may increase post-gastrointestinal colonization, local cattle should be studied as a source of enteric *E. coli* in Oklahoma's Blue River system. In addition, temperature fluctuations as well as other environmental conditions will contribute to understanding the pathogenesis of enteric organism.

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FIGURES AND TABLES

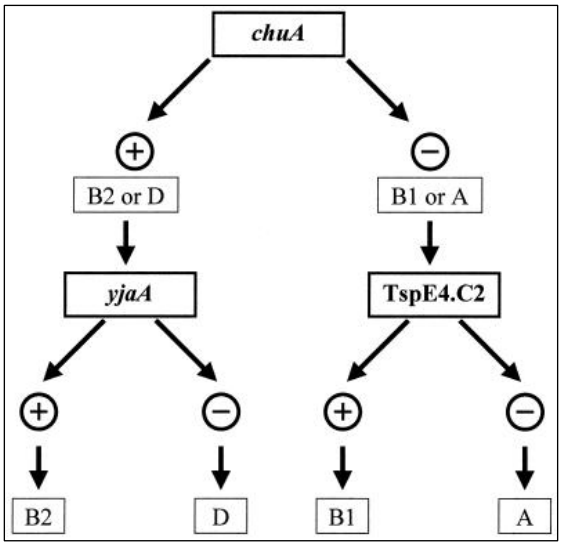

FIG. 1. Dichotomous decision tree taken from the Clermont paper that describes the phylogenetic classification of each group based on the presence or absence of the *chuA*, *yjaA*, and TSPE4.C2 markers.

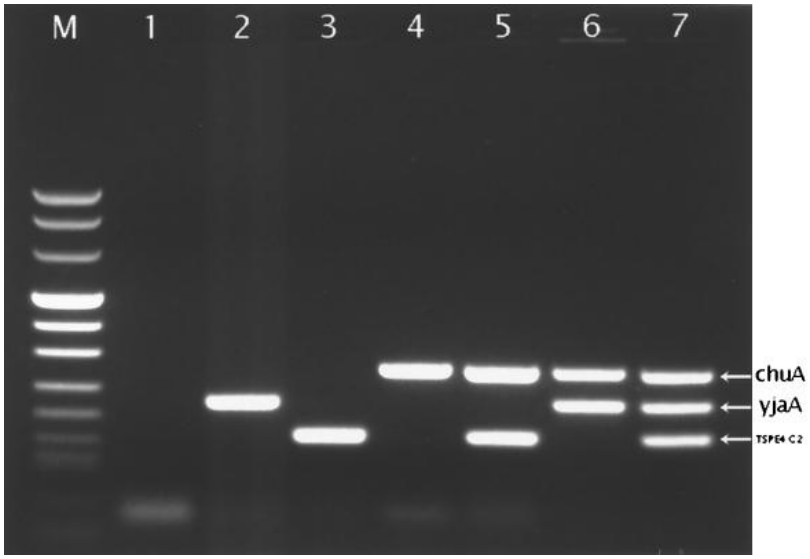

FIG 2. Triplex PCR profiles specific for *E. coli* phylogenetic groups. Each combination of *chuA* and *yjaA* gene and DNA fragment TSPE4.C2 amplification allowed phylogenetic group determination of a strain. Lanes 1 and 2, group A; lane 3, group B1; lanes 4 and 5, group D; lanes 6 and 7, group B2. Lane M contained markers (Clermont).

FIG 3. Topographical map of *E. coli* sampling locations near Tishomingo, Oklahoma, provided by Dr. Guy Sewell.

TABLE 1. Triplex PCR groupings of novel *E. coli* river strains. The class D strains marked with a * symbol indicates the presence of a band located between 500-600 bp in addition to the standard *chuA* and/or *yjaA* bands. The designation of ½ for class D indicates that a faint *yjaA* band was present, however, it was too faint to determine the subclass.

Strain Name	Location Collected	Date Collected	Strain Class	Sub Class
K-12 (Control)	Conway lab	n/a	A	
F-18 (Control)	Conway lab	n/a	B2	1
Ecor 26 (Control)	Conway lab	n/a	B1	1
EDL (Control)	Conway lab	n/a	D	1
6719-2-10	6719	5/12/2005	D	2
6722-1-10	6722	5/12/2005	D	2
6722-2-10	6722	5/12/2005	D	1
6722-3-10	6722	5/12/2005	B2	1
6722-4-10	6722	5/12/2005	B2	1
6724-1-10	6724	5/12/2005	D	2*
6724-2-10	6724	5/12/2005	B2	1
6724-3-10	6724	5/12/2005	B2	1
6725-1-10	6725	5/12/2005	D	1
6725-2-10	6725	5/12/2005	D	2*
6725-3-10	6725	5/12/2005	D	2*
6726-1-10	6726	2/20/2005	B2	1
6727 Test	6727	2/20/2005	B2	1
6727-1-10	6727	2/20/2005	D	2
6727-3-10	6727	2/20/2005	B2	1
6728-1-10	6728	5/12/2005	B1	1
6728-2-10	6728	5/12/2005	B2	1
6728-3-10	6728	5/12/2005	B2	1
6728-4-10	6728	5/12/2005	B2	1
6728-5-10	6728	5/12/2005	D	2
6728-6-10	6728	5/12/2005	D	2
6828-4-10	6828	5/12/2005	B2	1
6730-1-10	6730	5/12/2005	B2	1
6730-2-10	6730	5/12/2005	D	2*
6730-3-10	6730	5/12/2005	B2	1
6731-1-10	6731	5/12/2005	D	2
6731-2-10	6731	5/12/2005	B2	1
6731-3-10	6731	5/12/2005	B2	2
6731-4-10	6731	5/12/2005	D	1
6732-1-10	6732	5/12/2005	D	2
6732-2-10	6732	5/12/2005	D	1
6732-4-10	6732	5/12/2005	B2	1
6732-5-10	6732	5/12/2005	B2	1
6737-2-10	6737	5/12/2005	D	½
C-4-10	Unknown	5/12/2005	B2	1
BTI 1	Unknown	2/26/2005	D	2

BTI 2	Unknown	2/26/2005	D	2
BTI 3	Unknown	2/26/2005	D	1
BTI 4	Unknown	2/26/2005	D	1
BTI 5	Unknown	2/26/2005	D	1
BTI 6	Unknown	2/26/2005	D	1
BTI 7	Unknown	2/26/2005	D	1
BTI 8	Unknown	2/26/2005	D	2*
BTI 9	Unknown	2/26/2005	D	2*
BTI 10	Unknown	2/26/2005	D	2

Appendix B

Bacterial Diversity of the Blue River

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Keywords: *Escherichia coli*, Blue River, *Citrobacter*, 16s

Forty-nine river samples were collected from the Blue River, the source of drinking water for Ada, OK. The fecal coliform assay was used to isolate *Escherichia coli,* and these samples were classified according to the Clermont method. The Clermont method uses triplex PCR to amplify three genes to yield unique banding patterns. Each pattern determines the *E. coli* phylogenetic class: A, B1, B2, or D. Following classification of all strains, 16s ribosomal sequencing was performed to examine phylogenetic relationships. Sequencing results indicated that many strains were mistakenly classified as *E. coli,* but instead belonged to other genera of Enterobacteriaceae. Differential tests, including the IMViC battery of tests and Biolog GN2 Microplates, were used on several strains to better understand the diversity of Blue River. Most strains tested appear to belong to the *Shigella* or *Citrobacter* genera, and this data should shed light on the ecological niche present in Blue River.

INTRODUCTION

Enterobacteriaceae is a large family of bacteria encompassing the genera of *Shigella, Citrobacter, Salmonella,* and *Escherichia* (3). Enterobacteriaceae are small gram-negative rods that grow aerobically or are facultatively anaerobic. They are not spore forming, not acid-fast, and are mostly capable of reducing nitrates to nitrites (3). Members of the family Enterobacteriaceae are found in diverse habitats, including the human intestine, food, water, feces, and urine (3).

Many members of the Enterobacteriaceae family show similar characteristics. Of particular interest in this study are the genera of *Escherichia* and *Citrobacter.* For many years, organisms belonging in the *Citrobacter* genus were classified as *Escherichia*. *Citrobacter* organisms are primarily classified by the ability to grow on citrate as the sole carbon source, whereas *Escherichia* can not. Additionally, *Citrobacter* species are not capsulated; many organisms of *Escherichia* do contain capsules or microcapsules (3). *Escherichia coli* is probably the best understood organism on the planet, and has long been known as one of the predominant occupants of the microbial, commensal flora of the human intestine (18). *Citrobacter* is typically isolated from water or food (3).

In the current study we were interested in examining Enterobacteriaceae in The Blue River, the source of drinking water for Ada, Oklahoma (14). Knowing the bacterial diversity in the water could be an important issue in drinking water purity and useful for future studies. In a study on the bacterial persistence in drinking water taken in western Oregon, *Citrobacter freundii* made up over 60% of collected samples and the *Enterobacter* genus made up 30% of samples. Both of these genera belong to the Enterobacteriaceae family (13). The study concluded that many

bacteria are able to escape from the action of chlorine by adhering to solid particles (13). Knowing bacterial contamination in other water sources could help compare and prevent similar problems in Oklahoma.

Working in conjunction with Guy Sewell of East Central University, forty-nine water samples from different locations were collected from The Blue River. The fecal coliform assay was used to presumptively identify *Escherichia coli,* and only these isolates were saved for use in the remainder of the experiment. We initially employed the rapid and simple determination of *E. coli* classes as determined by the Clermont group (5). This triplex PCR assay amplifies three genes: *chuA*, *yjaA*, and *TSPE4.C2*. *ChuA* is a gene involved in heme transport, *yjaA*, a gene identified through genome sequencing of *E. coli* K-12, and lastly, *TSPE4.C2*, is a DNA fragment (5). The assay uses a banding pattern from these amplified genes to classify unknown *E. coli* strains into the four main phylogenetic groups that were established by Herzer: A, B1, B2, and D (5,11).

Following classification, 16s rRNA analysis was performed on five strains. 16s rRNA analysis is commonly used to distinguish between different species. This region is highly conserved and can be used to establish phylogenies (4,10). Sequencing confirmed that most strains had been mistakenly identified as *E. coli,* but were instead members of other genera in the Enterobacteria family. The Biolog GN2 Microplate™ test panel was used for further confirmation among initial B1, B2, and D phylogenetic classes. Finally, samples were streaked onto eosin methylene blue (EMB) agar and the IMViC battery of tests (Indole, Methyl Red, Voges-Proskauer, and Citrate) was run to help further identify bacterial genera. Knowing the bacterial diversity of the

river at different sites can help identify locations of fecal contamination, and generate a better understanding of this ecological niche.

Materials and Methods

Coliform Isolation, Classification, and Sequencing: Water samples were collected from Blue River, Ada, OK, by Guy Sewell. *E. coli* was isolated from these river samples using the Hach® Coliform: Membrane Filtration assay. m-ColiBlue24® broth was used to differentiate fecal coliform colonies, which turned blue. The membrane filter apparatus was used according to the manufacturer's instruction (15). With the intact membrane, the petri dish was vacuumed, inverted, and incubated at 35°C for 24 hours. Presumptive *E. coli* colonies were selected by their blue color.

As previously mentioned, the Clermont assay for classification of *E. coli* was used (5). The primer pairs for the three genes are as follows: *ChuA*.1 (59-GACGAACCAACGGTCAGGAT-39) and *ChuA*.2 (59-TGCCGCCAGTACCAAAGACA-39), *YjaA*.1 (59-TGAAGTGTCAGGAGACGCTG-39) and *YjaA*.2 (59-ATGGAGAATGCGTTCCTCAAC-39), and TspE4C2.1 (59-GAGTAATGTCGGGGCATTCA-39) and TspE4C2.2 (59-CGCGCCAACAAAGTATTACG-39), which generate 279-, 211-, and 152-bp fragments, respectively. PCR was performed using Platinum® *Taq* DNA polymerase (Invitrogen, Carlsbad, CA) in 50μL reactions in a GeneAmp® PCR system 9700 thermocycler (Applied Biosystems, Foster City, CA). PCR was run for 35 cycles at the following temperatures: 94°C for 2:00 minutes, 94.0°C for :30 seconds, 50.0°C for :30 seconds, 72.0°C for :30 seconds, 72.0°C for 10:00 minutes, and samples were held at 4.0°C upon completion. 16s PCR used the following primer sets: insert sequences here. 16s PCR was performed at the following conditions: 94°C for

5:00 minutes, 94.0°C for :30 seconds, 54.0°C for 1:00 minute, 72.0°C for 2:00 minutes, 72.0°C for 20:00 minutes, and samples were held at 4.0°C upon completion.

Separation of gene fragments was accomplished via agarose electrophoresis using 1% agarose gels made with 1X Tris-Borate-EDTA and run at 65V for approximately 1 hour. All samples were run with a standard 100 base pair DNA ladder (1μg/μL) (Invitrogen Corp., Carlsbad, CA). .05μL of ethidium bromide was used per mL of melted agarose or buffer solution. Agarose gels were visualized under UV light using an EpiChemi II Darkroom (UVP, Inc., Uplands, CA).

The primers (Invitrogen Corp., Carlsbad, Calif.) used for amplifying the 16S rRNA genes were as follows: forward, 8f (5_ AGAGTTTGATCCTGGCTCAG 3_) and reverse, 805r (5_ GACTACCAGGGTATCTAATCC 3_) (8). 16s PCR product was prepared for sequencing using a QIAquick® PCR Purification Kit Cat. No. 28104 (Qiagen, Inc., Valencia, CA) following the manufacturer's instruction (16). In a 96 well microplate, 4μL of purified PCR product was added to 2μL of both 7mmol 16s forward and reverse primers. Sequencing was performed by Dr. Bruce Roe's laboratory, Stephen Research and Technology Center, University of Oklahoma, Norman, OK. Analysis of homologous sequences was determined using ClustalW (6,19).

Differential Tests: The GN2 Microplate™ test panel was used to identify a broad range of carbon utilization from river strains tested in this experiment. The tests were performed in duplicate. The strains were streaked onto tryptic soy agar plates and 500mM thioglycate was used for the experiment. The plates were incubated between 18 and 24 hours and the experiment was conducted according to the manufacturer's instruction (16).

The IMViC battery of tests was performed according to the Microbiology Laboratory Theory and Application text (12). All media and reagents were prepared and received as a gift from the University of Oklahoma, Department of Microbiology, Norman, OK. Eosin Methylene Blue Agar plates were prepared according the manufacturer's label (Merck, Darmstadt, Germany) to isolate gram-negative enteric rods.

RESULTS

In order to better understand the bacterial population of the Blue River, we initially set out to classify different strains of *E. coli* from unique sites. The fecal coliform assay was employed in this study, and as it is extensively used for the quick determination and isolation of *E. coli* from water samples (15). Based on the fecal coliform assay results, forty-nine samples of *E. coli* were collected for this study. Classification was based on the banding pattern of the three amplified genes (5). Figure 1. is an illustration of the dichotomous tree used to determine classification. Of the forty-nine strains originally classified, only nineteen were classified as B2 and two were classified as B1 strains. The remaining twenty-eight samples were classified as class D. Please refer to Table 1. for a complete listing of strains and classification.

Following classification, the 16s rRNA gene was sequenced from five strains to examine phylogenetic relationships. This gene has long been used for two primary purposes: to determine strain relatedness and to detect pathogenic bacteria (4,10). Sequencing data showed some intriguing results. Basic Local Alignment Search Tool (BLAST) searches were performed on 16s sequences, and results show close associations with *Citrobacter* or *Shigella* genera, rather than *E. coli* (1). Table 2. shows the two highest scores of BLAST from generated sequences. A phylogram was generated from ClustalW that is displayed as Figure 2 (6, 19). Additionally, Entrez Gene searches were performed for *chuA*, *yjaA*, and *TSPE4.C2* (9). *TSPE4.C2* yielded no

other matches; however, *chuA* and *yjaA* both showed homology in other organisms. Other than *E. coli,* the most probable match for *yjaA* was the *Shigella* genus.

Differential tests were performed at this point to confirm sequencing. Biolog GN2 Microplate™ test panels were performed for five strains (3-class D, 1-class B1, 1-class B2) and two controls (1-class A, 1-class D). Three of these same strains were also sequenced: 6719-2, BTI-1, and

> Because the five strains did not all appear to be *E. coli,* we decided to test six additional strains with the IMViC test to add to the presumptive identification and to identify other strains. The IMViC battery of tests is typically used to distinguish between members of Enterobacteriaceae (12). The methyl red (M.R.) test shows a positive red color change when the pH is lowered due to mixed acid fermentation end products. The vogues-proskauer test also shows a positive red color change when acid end products are converted to acetoin and 2,3-butanediol. Typically used to differentiate between *Salmonella* and *Shigella*, the indole test detects the presence of tryptophanase, an enzyme that hydrolyzes tryptophan to pyruvic acid. Finally, the citrate test determines the utilization of citrate as the sole carbon source (12). IMViC trials were performed on ten strains, and that data is summarized in Table 5. All strains were indole positive. Additionally, all strains were positive for M.R., with the exception of river strain 6719-2; this strain was positive with the V.P., but all other strains tested negative. Only one strain, 6724-1, did not utilize citrate. Finally, the results from EMB agar plates are displayed in Table 6. EMB agar plates yield a green sheen for gram-negative enteric bacteria. The only strain that did not display the green sheen was 6719-2.

6724-1. A complete listing with duplicate data is shown as Table 3. Citrate utilization information collected from the Biology GN2 Microplate is displayed in Table 4. Neither *E. coli* control utilized citrate as a sole carbon source, but the five river strains tested from classes B1, B2, and D all were capable of utilizing citrate.

Based on data collected from sequencing and differential tests, we were able to presumably identify strains in the following genera. Strain 6719-2 is the only strain that did not belong in the Enterobacteriaceae family. 6719-2 did not exhibit a green sheen on EMB agar nor match any expected IMViC results, and remained unidentified. However, 6719-2 was like *Citrobacter* in that it was able to utilize citrate as a sole carbon source. Strains 6730-3 and BTI-1 were likely *E. coli.* Strain 6730-2 either belonged to the *Escherichia* or *Shigella* genera. Strain

6724-1 was seemingly *Shigella.* Finally, strains C4, 6722-3, 6722-4, 6725-1, 6726-1, and 6728-1 all belonged to *Citrobacter.* A total of six strains were identified as *Citrobacter*, which created the majority and corresponded with the phylogram results.

DISCUSSION

Public concern for the quality of water reached national attention in the 1970's. The Environmental Protection Agency (EPA) enacted the law that later became known as The Clean Water Act. Although many amendments have since been instigated, the EPA continues to ensure that public water sources maintain biological integrity. Preserving this high quality of water includes minimizing pollution to ensure the continued proliferation of organisms in natural habitats (20). The results of this study contribute to the comprehensive knowledge of the microbial population present in Blue River, Ada, OK. Knowing the bacterial diversity of the river may shed light on natural pollution (from fecal contamination) and safety measures necessary prior to human consumption.

For the last century, physicians and public health officials have used the fecal coliform assay to detect fecal contamination of beverages, food, and water (7). The fecal coliform assay has been adapted in recent years to specifically and quickly test for the presence of *E. coli*, indicating a contamination. A recent publication, though, draws attention to the misconceptions and errors associated with the current fecal coliform assay. Other species within Enterobacteriaceae including *Citrobacter, Enterobacter,* and *Klebsiella* yielded a false-positive result as *E. coli* (7). This same assay was used to falsely identify river isolates as *E. coli.*

We classified our isolates according to the Clermont method. Interestingly, the samples amplified according to the banding pattern proposed by the French scientists. Most samples

were classified as B2 or D strains. Previous studies indicate that most commensal *E. coli* strains of the intestine often are class A, whereas pathogenic strains are usually class B2 or D (2,5). The animals in the natural habitat surrounding Blue River contaminate the river daily with their feces; this increases the likelihood of type B2 or D strains of *E. coli*. The banding pattern for B2 and D classes of *E. coli* involve amplification of the *yjaA* and *chuA* genes (5). Results from Entrez Gene searches, though, showed other organisms that contain these same genes. For instance, *Shigella* organisms also contain the *yjaA* gene. The phylogram generated from 16s sequencing showed a closer association between all of the river strains and *Citrobacter* or *Shigella* rather than *Escherichia.*

Sequence comparison confirmed that these genes could have amplified non-specifically in other organisms of the Enterobacteriaceae family using the Clermont primers. BLAST results showed few matches with *E. coli* and *Citrobacter,* and river strains 6730-2 and BTI-1 also showed matches of lower scores with *Shigella*. Moreover, the phylogram results indicated the closest association with *Citrobacter freundii.* The Fukushima group determined an alternative to 16s sequencing that allows for better differentiation at the species level. They noted that it was especially difficult to distinguish between *Shigella* and *E. coli*, and suggested amplification of the *gyrB* gene (10). Amplification of the *gyrB* gene could be a useful tool should sequencing of the river strains be continued.

According to the Bergey's Manual of Discriminative Bacteriology, the *Escherichia* genus should be M.R. positive, V.P. negative, indole positive, and citrate negative. The *Citrobacter* genus should be M.R. positive, V.P. negative, indole positive or negative, and citrate positive. *Shigella* organisms are the same as *Citrobacter* except for the inability to utilize citrate (3). Every isolate

tested with the IMViC series, excluding the *E. coli* control and 6724-1, was able to utilize citrate as a sole carbon source. This is an indication of *Citrobacter.* Although 6724-1 did not utilize citrate during the IMViC test, it did utilize citrate on the Biolog GN2 Microplate. Sequencing data from 6724-1 indicates that it might belong in the *Shigella* genus, but it should be unable to utilize citrate. River strain 6719-2 showed interesting results from the IMViC tests. The strain was citrate positive, V.P. positive, M.R. negative, and indole positive. 6719-2 also did not show a green sheen on EMB agar; the strain is, therefore, probably not in the Enterobacteriaceae family. However, sequencing data showed the closest association with *Citrobacter.* This does not correspond with differential test results. Finally, river strain BTI-1 showed a close BLAST score with *E. coli;* BTI-1 did not utilize citrate on the Biolog GN2 Microplate, and probably is a true *E. coli.*

Although forty-eight samples were collected and originally determined to be *E. coli*, we discovered the true composition of the river to be much more diverse. Many strains that were isolated are likely *Citrobacter* or *Shigella* genera. Overall, there did not appear to be any correlation between Clermont classification and genera. The fecal coliform assay yielded inaccurate results, and the strains should be evaluated further. *E. coli* is probably among the group of river strains, but was isolated much less frequent than we expected. Future directions of the study will be to further analyze the remaining river strains with both Biolog GN2 Microplates and the IMViC battery of tests. Sequencing may also be performed on the remaining strains. All 16s PCR product is purified for the remaining strains and stored at 4°C at the Stephenson Research and Technology Center, Norman, OK. Additionally, it could be of interest to establish phylogenetic relationships using the *gyrB* gene as suggested by the Fukushima group. We hope

that the results of this study will be useful information for Guy Sewell and other scientists who work with Blue River.

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FIGURES AND TABLES

Table 1. Complete Classification of *E. coli* strains

Strain Name	Location Collected	Date Collected	Strain Class
K-12 (Control)	Conway lab	n/a	A
F-18 (Control)	Conway lab	n/a	B2
Ecor 26 (Control)	Conway lab	n/a	B1
EDL (Control)	Conway lab	n/a	D
6719_2_10		5/12/2005	D
6722_1_10		5/12/2005	D
6722_2_10		5/12/2005	D
6722_3_10		5/12/2005	B2
6722_4_10		5/12/2005	B2
6724_1_10		5/12/2005	D
6724_2_10		5/12/2005	B2
6724_3_10		5/12/2005	B2
6725_1_10		5/12/2005	D
6725_2_10		5/12/2005	D
6725_3_10		5/12/2005	D
6726		2/20/2005	D
6726_1_10		2/20/2005	B2
6727 Test		2/20/2005	B2
6727_1_10		2/20/2005	D
6727_2_10		2/20/2005	D
6727_3_10		2/20/2005	B2
6727_4_10		2/20/2005	B1
6728_1_10		5/12/2005	B1
6728_2_10		5/12/2005	B2
6728_3_10		5/12/2005	B2
6728_4_10		5/12/2005	B2
6728_5_10		5/12/2005	D
6728_6_10		5/12/2005	D
6828_4_10		5/12/2005	B2
6730		5/12/2005	B2
6730_1_10		5/12/2005	B2
6730_2_10		5/12/2005	D
6730_3_10		5/12/2005	B2
6731_1_10		5/12/2005	D
6731_2_10		5/12/2005	B2
6731_3_10		5/12/2005	B2

6731_4_10		5/12/2005	D
6732_1_10		5/12/2005	D
6732_2_10		5/12/2005	D
6732_4_10		5/12/2005	B2
6732_5_10		5/12/2005	B2
6737_2_10		5/12/2005	D
C-4_10		5/12/2005	B2
BTI 1	Unknown	2/26/2005	D
BTI 2	Unknown	2/26/2005	D
BTI 3	Unknown	2/26/2005	D
BTI 4	Unknown	2/26/2005	D
BTI 5	Unknown	2/26/2005	D
BTI 6	Unknown	2/26/2005	D
BTI 7	Unknown	2/26/2005	D
BTI 8	Unknown	2/26/2005	D
BTI 9	Unknown	2/26/2005	D
BTI 10	Unknown	2/26/2005	D

Figure 1. Dichotomous Tree Used to Determine *E. coli* Classification

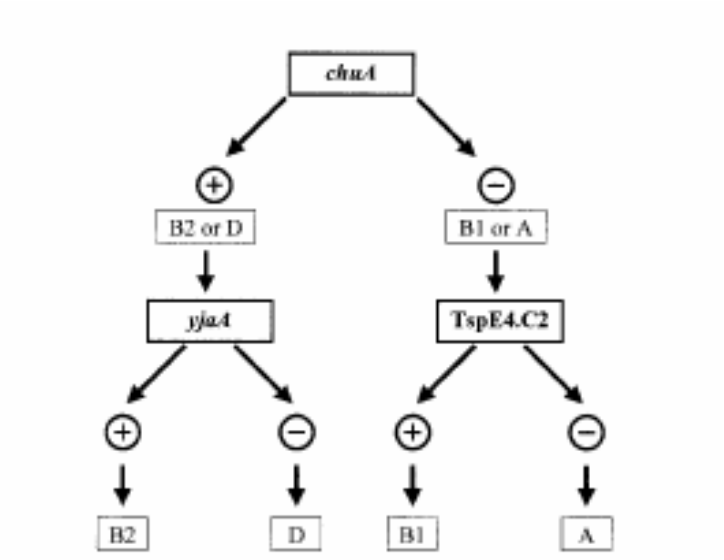
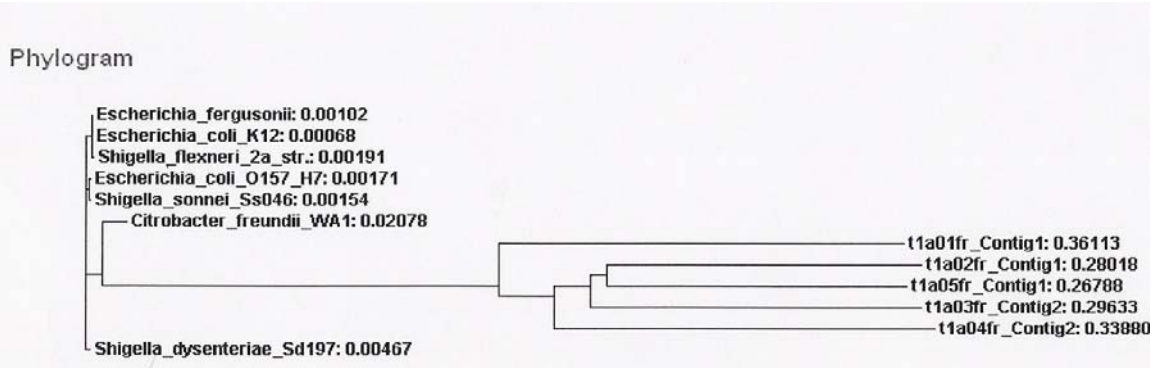


Figure 2. Phylogram Results



Key: t1a01fr=**6724-1**, t1a02fr=**6719-2**, t1a03fr=**6730-3**, t1a04fr=**6730-2**, and t1a05fr=**BTI-1**

Table 2. Sequencing/BLAST Results					
Strain	6724-1	6719-2	6730-3	6730-2	BTI-1
Hit 1	Uncultured gamma proteobacterium clone: ribosomal RNA gene, partial sequence	*Citrobacter freundii* strain 7-16S	Uncultured bacterium clone: 16S ribosomal RNA gene, partial sequence	Uncultured bacterium clone:16S ribosomal RNA gene, partial sequence	Uncultured bacterium clone: 16S ribosomal RNA gene, partial sequence
Hit 2	*Shigella sonnei* 16S rRNA gene	Uncultured bacterium clone: 16S ribosomal RNA gene, partial sequence	*Escherichia coli* CFT073	Uncultured gamma proteobacterium clone: 16S ribosomal RNA gene, partial sequence	*Escherichia coli* CFT073

Table 4. GN2 Microplate™ Citrate Utilization Chart							
Strain	MG1655 (Class A, *E. coli* Control)	6719-2 (D)	BTI –1 (D)	6724-1 (D)	EDL (Class D, *E. coli* Control)	6728-1 (B1)	6722-3 (B2)
Citrate Utilized	No	Yes	No	Yes	No	Yes	Yes

Table 5. IMViC Battery Test Results									
Strain	6726-1	C4 (D)	6722-3 (B2)	6722-4 (B2)	EDL (Class D, *E. coli* Control)	6728-1 (B1)	6724-1 (D)	6719-2 (D)	6725-1 (D)
Indole	+	+	+	+	+	+	+	+	+
Methyl-Red	+	+	+	+	+	+	+	-	+
Vogues-Proskauer	-	-	-	-	-	-	-	+	-
Citrate Utilized	+	+	+	+	-	+	-	+	+

Table 6. EMB Streaking Results		
Strain	Class	Green Sheen
BTI-1	D	yes
C4	B2	yes
6719-1	D	yes
6719-2	D	no
6722-1	D	yes
6722-3	B2	yes
6722-4	B2	yes
6724-1	D	yes
6725-1	D	yes
6725-2	D	yes
6725-3	D	yes
6726	D	yes (dark sheen)
6726-1	B2	yes
6728-1	B1	yes

Appendix C

Survival and Persistence of Phylogentically Grouped *Escherichia coli* Strains in Nanopure Water

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5-12-2006

This study was designed to determine whether patterns of persistence or survival were present within strains of *Escherichia coli* from the same phylogenetic group. Natural river isolates as well as laboratory strains were exposed to starvation conditions in nanopure water for prolonged periods of time, which is known to elicit a RpoS global response. Weekly platings on MacConkey agar for a period of forty six days were used to determine the efficiency of the individual strains to survive the stressful conditions. During the course of experimentation it was noted that white, Lac$^-$ colonies appeared on the plates. Overall, the results showed that certain strains were able to remain at fairly consistent viable numbers over the course of the experiment, while others endured a general decline; however, no patterns that would encompass entire phylogenetic groups were noticed, and the results showed that there was more of a direct correlation between the ability of a strain to express virulence factors and its aptitude of survival. Proposed microarray analysis of individual strains during experimentation could be used to provide a model of how diverse *E. coli* strains regulate genetic machinery during the starvation.

Introduction

Escherichia coli has a highly variable genome that allows for differential colonization of hosts (22). It is also frequently associated with various intestinal (diarrhea) and extra-intestinal (bacteremia and neonatal meningitis) diseases (16). Phylogenetic analyses have shown that this species is composed of five main classes (A, B1, B2, D and E) (18, 32, 59). Other studies have shown there is a distinct evolutionary relationship between the species and pathogenicity (6, 10, 17, 18). For the most part, virulent extra-intestinal strains fall mainly into group B2, and to a minor degree group D (6, 10, 36, 53). Also, severe diarrhea-causing strains belong mainly to class E, while most commensal strains fit into class A; however, all phylogenetic classes have strains capable of producing virulence factors. At present, several phylogenetic grouping techniques exist, including multilocus enzyme electrophoresis (32, 58) and ribotyping (4, 5, 15); however, both of these protocols are time-consuming and complex. Studies have suggested certain genes or fragments of DNA can be used as specific phylogenetic markers (4, 9). Three markers have been used for this purpose: (I) *chuA*, involved in heme transport in the *E. coli* EDL933:H7 (47, 64, 69); (II) *yjaA*, the function of which is unknown, but was found to be in the genome of K-12 (8); and (III) TspE4.C2, a random DNA fragment from the genetic library formed by subtracting a phylogenetic class A ECOR strain from *E. coli* C5 of class B2 (9). Phylogenetic classification of *E. coli* strains can help to understand evolutionary relationships that may help to elucidate not only pathogenicity and virulence factors but also other physiological factors, such as growth and survival mechanisms.

E. coli is mainly noted as a normal flora of the gastrointestinal tract of most animals, including humans. However, the species is quite ubiquitous and found to survive in a variety of environments (11, 68). While most studies are geared towards the colonization, competition or growth habits of the species in various models or utilizing various substrates, relatively few have focused on the actual ability of *E. coli* to survive over periods of time within stressful environments. Some recent studies have shown the survival of *E. coli* in freshwater (25, 11), marine environments including sediments (3, 21), as well as in a drinking water system (68). However, none focus on the survivability of differing *E. coli* strains under the extremely nutrient depleted environment of purified water over prolonged periods of time.

E. coli, like many other free-living bacteria, lives in environments that may change rapidly and often with respect to nutrient and environmental conditions. To survive these stresses associated with starvation for prolonged periods of time, *E. coli* have developed highly specialized metabolic states. These cells must undergo diverse morphological and physiological changes that are involved in a complex regulatory network, which induces the expression of various stationary-phase-response genes (28, 39). This complex and physiologically extensive response system is often under the control of a master regulator, which in the case of *E. coli* is the *rpoS* gene product σ^s (27, 30, 37). This is an alternative sigma factor of the normal that can partially displace the normal σ^{70} subunit of RNA polymerase under many forms of stress (29). The σ^s protein accumulates in the cell during stationary phase or in response to stress conditions that directly regulate the transcription of approximately 100 different genes, many of which are of unknown function (33, 38, 57). This form of global regulation allows enhanced resistance to a variety of stresses, including starvation, near-UV irradiation, changes in osmolarity or pH, oxidation and heat (27, 31, 34, 35, 42, 45, 48, 49). Therefore, the RpoS regulon is more noted for its diversity than its uniformity among certain bacterial species.

The RpoS regulon controls a large diverse set of genes, resulting in a diverse group of phenotypes compiling from the numerous possible allelic combinations (38). The gene seems to be easily mutated but may not always induce the same phenotype, due to the variability of promoters that σ^s recognizes in addition to the overlap with the σ^{70} subunit (41). In addition, no particular region within the gene seems to show a high frequency of mutation, as transposon insertions and mutations occur at a variety of locations within *rpoS* (43). RpoS can be regulated at the level of transcription, post-transcription and translation, which adds to the diversity of the genes controlled by the cascade (43). This global regulator controls such genes as *bolA*, which codes for the cell to make a morphological conformation into a much more compact, spherical form as witnessed during stationary phase growth, helping the *E. coli* cells to conserve energy (56). In addition, this particular regulon has control over several virulence factors, including *csgA*, which encodes for surface fibers called curli that aid in adhesion to the GI tract material (1, 26).

In addition to the RpoS regulon, other genes have been found that are necessary to improve the survival capabilities of *E. coli* strains under long exposure to starvation conditions. These include a family of universal stress proteins, such as UspA, which is a small cytoplasmic protein that is unique in its ability to respond to a variety of stresses (28). *E. coli* lacking an active copy of *uspA* are subject to excess growth inhibition when subjected to starvation or other stressful conditions (51, 52). These gene systems often overlap and interact with the master regulatory RpoS system to elicit distinctive responses to various stresses.

When bacterial cells are subjected to stationary-phase inducing growth environments, some cells are able to acquire beneficial or so-called adaptive mutations (13). These mutations are often quite different from those found in rapidly dividing cells and can only be noticed when the conditions are growth limiting (20, 54). The specific mechanisms and regulation of these particular mutations are of considerable interest because they signify systems for understanding the relationship between the surroundings and the genome, particularly whether and how stressful conditions can possibly induce genetic alteration. In the "directed mutation" model (12), stressful conditions were attributed to stimulate either the induction or preservation of these mutations selected for in specific genes. In the "hypermutation" model (24, 50, 54, 63), mutation rates are suspected to increase throughout the genome, producing mostly deleterious effects in the hopes of producing something advantageous to survival. In these mutations is often the increased expression of DNA polymerase IV, which is known for its high rate of error (69). Once again, this protein is under the control of the sigma factor RpoS, which can have a large

effect on mutations that arise in cells during non-lethal selection (13). DNA PolIV accumulates mutations in cells under adverse conditions in an effort to produce mutations that will allow the survival of their descendants.

While RpoS is not the only system that is involved in *E. coli* stress response, it is a global regulator that is often involved in some way to induce expression of the numerous genes responsible for the ability of strains to react to the ever-changing environmental conditions with each eliciting a unique response. Overall, the complex of genes with their numerous possible alleles involved in the RpoS signal transduction pathway can have an additive effect to produce quite varying mechanisms to deal with starvation conditions. Once again, it is a system where diversity is the only constant.

Materials and Methods

Bacterial strains and isolation conditions. A representative laboratory stock culture from each of the four main phylogenetic groups (A, B1, B2 and D) of *E. coli* was provided by various members of the Conway Lab group: MG1655 (K12), F-18, ECOR 26 and EDL933, respectively. Additionally, strains from each of the B1, B2 and D classes (6732-2, 6728-1, 6725-3; 6731-2, 6731-3, 6726-1, 6730-3; 6728-5, 6724-2, 6731-4) were chosen for experimentation from numerous isolates from the Blue River in southern Oklahoma provided by Dr. Guy Sewell. The strain designations were based on the location on the river where the strains were collected. Strains of different location were chosen for each of the different classes to lower the risk of the strains being identical. The various strains were originally isolated at 37°C on Luria-Bertani agar and later on Difco MacConkey agar to avoid contamination that complicated results on Luria-Bertani.

PCR Amplification. As a means to determine the phylogenetic class of the unknowns, triplex PCR as described by Clermont et al was performed (14). First, the four reference strains, which were of known phylogenetic classes, were used to determine whether the triplex PCR technique could efficiently identify their particular classes. Once the reference strains were correctly identified, the triplex PCR protocol were refined to produce the most definitive banding patterns. PCR was performed with a standard protocol, using a 25µl mixture consisting of 2.5µl 10X buffer, 2.0µl of a deoxynucleotide triphosphate mixture (10mM each), 1.0µl $MgCl_2$ (50mM), 0.1µl of platinum *Taq* DNA polymerase (Invitrogen cat no. 10297-018; lot no. 1263576), 0.2µl of each of the three primers (ChuA, YjaA and TspE4C2) both forward and reverse, a single colony isolated on MacConkey agar of the specific strain of interest, along with 18.2µl of nanopure water to a total reaction volume of 25µl. The PCR was carried out using an Applied Biosystems GeneAmp PCR System 9700 thermal cycler in 100µl PCR tubes under the following conditions: denaturation for 5 min at 94°C; 30 cycles of 30s at 94°C, 30s at 55°C, and 30s at 72°C; a final extenstion step of 7 min at 72°C; and the tubes then remained at 4°C until they were removed from the cycler. The primer pairs were as follows:
ChuA.F (5'-GACGAACCAACGGTCA GGAT-3')
ChuA.R (5'-TGCCGCCAGTACCAAA GACA-3')
YjaA.F (5'-TGAAGTGTCAGGAGAC GCTG-3')
YjaA.R (5'-ATGGAGAATGCGTTCCT CAAC-3')
TspE4C2.F (5'-GAGTAATGTCGGGG CATTCA-3')
TspE4C2.R (5'-CGCGCCAACAAAGT ATTACG-3')

These primers generate PCR fragments of 279, 211 and 152 base pairs, respectively. Each of the isolated river strains were then subjected to this standard PCR protocol to determine into which phylogenetic group each of the chosen strains fit. This procedure was performed in cooperation with fellow Conway lab personnel, Serena Freeman and Erin Goranson.

Agarose gel electrophoretic analysis of PCR products. 1% agarose gels were formed using 1X TBE buffer and 10mg/ml stock solution EtBr (0.05µl/ ml buffer). 5ml of tracking dye was added to the 25µl total volume PCR products. Then, 12µl of this mixture was added to a lane of the gel for each of the strains to be tested, along with a lane containing 12µl of the Invitrogen 100 bp DNA ladder (cat no. 15628-019; lot no. 1289697). Gels were run at 70mV using a BioRad Power Pac 300 as the power supply until the tracking dye reached the halfway point of the gel. Gels were then viewed in the Ultra-Violet Products Epi Chemi II Darkroom. Pictures of the gels were taken using the UVP LabWorks Image Acquisition and Analysis Software to illustrate the specific banding patterns of the added reference or isolate strains to make a final determination of which phylogenetic class the specific strain would fall.

Overnight cultures and re-suspension of cells in nanopure water. The following protocol was carried out for each of the reference and isolate *E. coli* strains over varying durations. A single colony isolated on MacConkey agar was chosen for each of the strains and aseptically transferred into 5ml of Luria-Bertani broth containing 0.1% by volume glucose in normal test tubes with plastic caps to allow gases to be exchanged with the environment. These tubes were allowed to incubate overnight in the Barnstead/Lab-Line MaxQ 5000 shaker at 37°C and 250 revolutions per minute. Then, 0.5ml of the overnight cultures were transferred to a 50ml batch culture containing the same media, which were allowed to incubate in the New Brunswick Scientific Innova 44 shaker at 37°C and 250rpm. The optical density at 260nm of the batch culture was taken as often as deemed necessary, and the culture was removed when the OD was as close to 0.8 as possible. 30ml of the remaining batch culture was then removed and added to a 50ml Corning centrifuge tube. This was centrifuged at 12,000rpm and 10°C for a period of 7 min in the Beckman Coulter Allegra 21R centrifuge using the F0850 rotor. After centrifugation, the supernatant was discarded with care not to disturb the pelleted cells. The cellular pellet was then re-suspended in the original 30ml volume of nanopure water, as a means to remove any of the original LB growth media. This procedure was repeated three times. After the final re-suspension, 5ml of the washed cells suspended in nanopure water were added to each of three 15ml Corning centrifuge tubes, leaving 15ml of re-suspended cells in the original 50ml centrifuge tube, which was saved for later in the experimentation and serve as a control. Each of these three tubes was assayed separately, resulting in the experiment being performed in triplicate.

Original serial dilution. Immediately, 100µl from each of the three tubes containing the re-suspended cells for each strain was added to a separate 1.5ml Sorensen SafeSeal microcentrifuge tube containing 900µl of nanopure water. A separate ten-fold serial dilution, taking 100µl of the previous dilution tube and adding it to a tube containing 900µl of nanopure water was performed for each of the three strains to produce a series ranging from 10^{-1} to 10^{-7} dilutions as compared to the original bacterial concentrations after re-suspension.

Original plating. To determine an original concentration of the cells to use as a reference for the remainder of the experimentation, the day zero dilutions were plated for each of the strains in triplicate. Judging that an optical density of 0.8 corresponds to approximately 10^8

cells/ml, the 10^{-5}, 10^{-6} and 10^{-7} dilutions of the above series were chosen to be plated. 100µl aliquots of each of the chosen dilutions for each of the three tubes for every strain was plated on a separate MacConkey agar plate using the spread plate technique, which corresponds to another 10 fold dilution, as compared to the dilution of the tube the aliquot was removed from. Therefore, plates ranging from 10^{-6}–10^{-8} final dilutions were prepared and were incubated at 37°C for a 24hr period. A representative plate for each of the three tubes for each strain was chosen, with the colonies being counted and logged with care to note the color of the enumerated colonies.

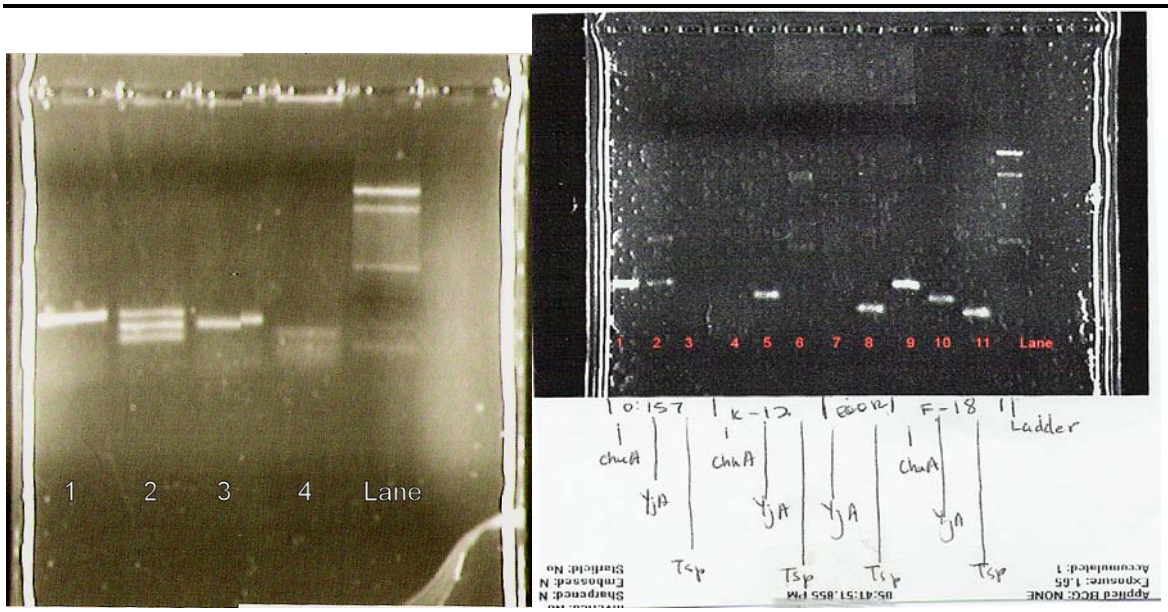
Protocol for short-term persistence under starvation conditions. At first, only the four reference strains were tested. Here, the three experimental tubes of cell suspensions in nanopure water for each strain remained at room temperature. A time point was taken daily, consisting of the above serial dilution and plating protocols, only using LB agar containing 0.1% glucose as the media. With each time point, a representative plate was chosen and the number of colonies, dilution factor, tube number, strain and time elapsed was recorded. This procedure was followed daily for a period of 11 days to determine the change in bacterial numbers. Here, there were no control tubes with which to compare the final results. After short-term persistence patterns were catalogued, the protocol was slightly changed to determine the persistence of the differing *E. coli* phylogenetic classes over a longer duration.

Protocol for long-term persistence under starvation conditions. The three centrifuge tubes containing re-suspended cells for experimentation now remained closed for the duration of approximately one week at room temperature. Each week the tubes were vortexed at low speed to make sure that the cells were uniformly suspended and another time-point was taken, utilizing the above serial dilution and plating protocols. The dilutions utilized and plated were varied as necessary to fit the changing trends of the bacterial concentrations as time progressed. The numbers of red, white and total colonies were once again noted along with the number of days since the start of experimentation. This procedure was followed for approximately a six week period to determine whether any varying trends existed for the different *E. coli* phylogenetic groups. The control tubes were never touched except to open the screw caps every two weeks to allow the exchange of gases. Then on the day of the final time point for the experimental tubes, the control was also plated using the same protocol to determine if any differences were to be found between the control and experimental tubes for each strain.

Re-Isolation, Gram Staining and Triplex PCR. The final time point plates consisting of both red and white colonies were taken and used for isolation. A red colony from each plate was chosen and isolation streaked on its own separate MacConkey agar plate. The same was done for all white colonies for all strains. The plates were allowed to incubate for a 24hr period at 37°C and checked the following day to ensure that only a single color of colonies appeared on each plate. A single colony was chosen from each of the isolated plates and was subjected to a standard Gram Stain as a presumptive determination of whether the red and white colonies were both *E. coli*. Then, another colony of each color was chosen for each strain. The above triplex PCR and agarose gel electrophoresis protocols were then performed to determine whether or not the banding patterns matched for both the red and white colonies of the same strain and those from the original isolates.

Results and Discussions

Triplex PCR determination of phylogenetic classes. The four laboratory strains, which are of known phylogenetic class, were originally used to determine whether this method of PCR would be effective in determining the proper class designations.



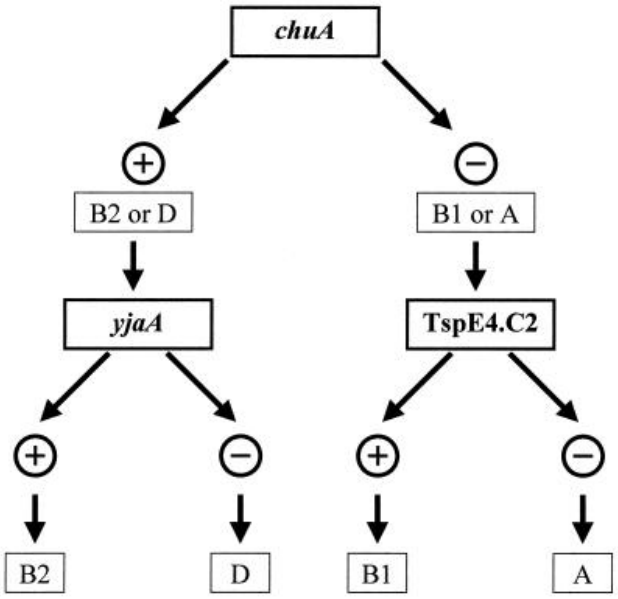
Gel A. Gel B.

Figure 1. Agarose gels of Triplex PCR products used to determine the efficacy of the phylogenetic classification of known laboratory *E. coli* strains. (A) Agarose gel containing PCR products for EDL933, F-18, K-12 and ECOR 26, respectively. (B) Agarose gel of single gene PCR products that shows a more definitive banding pattern for the TspE4.C2 fragment for ECOR 26 in lane 8.

The PCR for the amplification of the ChuA and YjaA genes as well as the TspE4.C2 DNA fragment generate 279, 211 and 152bp products, respectively. Therefore, it can be deduced that Lane 1 contains only the ChuA gene product, Lane 2 contains all three possible products, Lane 3 contains only the YjaA gene product, and Lane 4 has a faint band corresponding to the TspE4.C2 fragment product, with a more reliable representation of this band in the second gel in Lane 8.

Lane	Lab Strain	ChuA	YjaA	TspE4.C2
1	EDL933	+	-	-
2	F-18	+	+	+
3	K-12	-	+	-
4	ECOR 26	-	-	+

(A)



(B)

Figure 2. (A) Table showing the results of the triplex PCR amplification. (B) Dichotomous tree used to determine the phylogenetic class of an *E. coli* strain by amplification of the ChuA and YjaA target gene products and TspE4.C2 DNA fragment during triplex PCR.

Clermont et al. Appl Environ Microbiol. 2000 October; 66(10): 4555–4558.

Based on the above flowchart, it can be determined that *E. coli* EDL933 was of class D, F-18 of class B2, K-12 of class A, and ECOR 26 of class B1. This shows that each of the laboratory strains was classified correctly, validating the use of triplex PCR to determine the class designation of the provided river isolates.

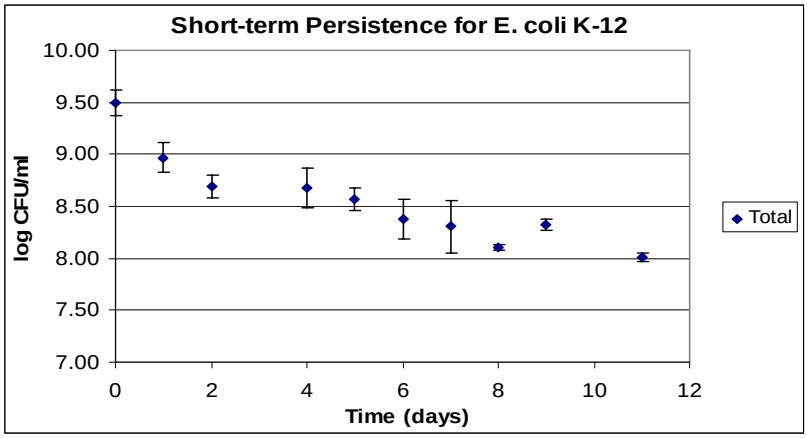
All *E. coli* river isolates were then subjected to triplex PCR to determine their phylogenetic groups. Once the class of each isolate was determined, it was found that none of the isolates fit into class A. This result stands to reason, as most strains that fall into class A are commensal strains and would therefore not be expected to be found in the freshwater Blue River environ (). Therefore, the laboratory strains, excluding class B1 ECOR 26 which was not available, along with isolates from groups B1, B2 and D were chosen for persistence experimentation in the nanopure water system. The following designations were made to simplify the naming process of the many strains from a system based on the location where the isolates were collected to one based on their phylogenetic classes once determined.

Isolate Number	Strain Designation
6732-2	B1-1
6728-1	B1-2

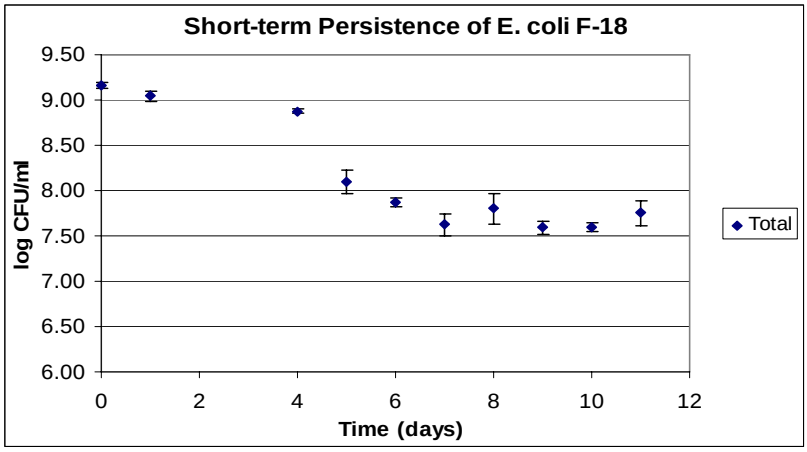
6725-3	B1-3
6731-2	B2-1
6731-3	B2-2
6726-1	B2-3
6730-3	B2-4
F-18	B2-5
EDL933	D-1
6728-5	D-2
6724-2	D-3
6731-4	D-4

Table 1. Table showing new strain designations based on phylogenetic class rather than isolate location from the river system. Isolates from the same group were chosen from different locations when possible to lower the probability of the strains being identical, furthering the diversity of the study.

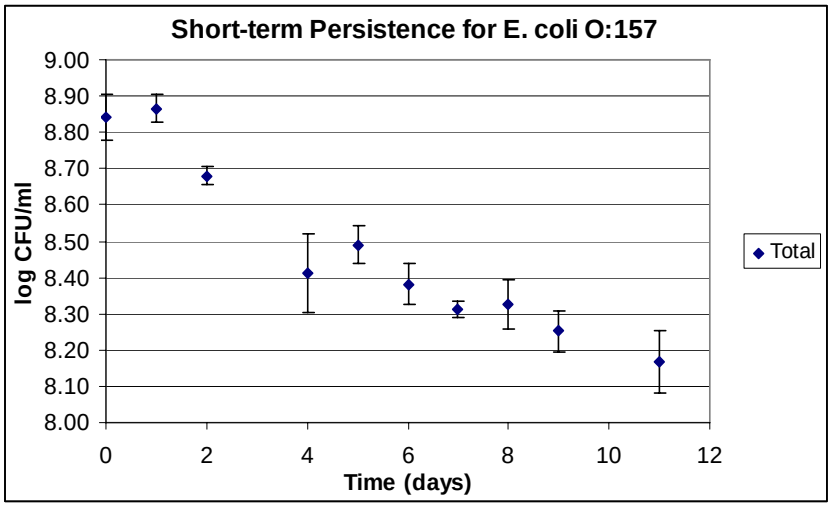
Short-term *E. coli* persistence analysis. The river isolates had not yet been classified when the start of the short-term persistence experimentation began; therefore, only the three laboratory strains, *E. coli* MG1655 (A), F-18 (B2) and EDL933 (D) were available for persistence studies. This particular study was designed to examine the change in bacterial numbers on a daily basis when subjected to starvation conditions while cultured in nanopure water. The results are to be used to determine the short term, approximately 2 week period, effects that these conditions have on the viable bacterial numbers of *E. coli* strains from the different phylogenetic classes.



Graph A.



Graph B.



Graph C.

Figure 3. Plots representing the trends of persistence over an eleven day period under starvation conditions for differing phylogenetic classes of *E. coli.* (A) Results for class A *E. coli* laboratory strain K-12. (B) Results for class B2 *E. coli* laboratory strain F-18. (C) Results for class D *E. coli* laboratory strain O:157.

The results for both the K-12 and F-18 strains bare a striking resemblance to one another. Both of these *E. coli* strains seem to follow a fairly linear decline over approximately one week of being subjected to the nutrient limited environment and then level off afterwards until the end of the eleven day period. Additionally, these two strains both drop approximately one and one-half log units during the course of experimentation, which is a noteworthy drop from the original concentration of cells (~16 and 17%, respectively). *E. coli* EDL933 on the other hand, follows the same linear declining trend, but occurs throughout the experiment. However, the decline is not nearly as significant as with the other two strains, resulting in less than a one log unit decline in viable cellular numbers (~8%). These results show that the class A and B2 *E. coli* laboratory strains act very similarly under starvation conditions for at least the first two week period, and that the class D EDL933 lab strain does not show as nearly as marked a decline. This seems to suggest that the K-12 and F-18 strains have similar systems to deal with the stress of the starvation conditions or at least in the short term. *E. coli* O:157 is known as a pathogen, and its virulence may play a key role in its ability to more suitably survive the severely nutrient limited environ. This may involve this pathogenic strain having a more highly modified system for stress response, as it has adapted to all manner of stresses while within a host.

Long-term *E. coli* persistence analysis. This second part to the experimentation was designed to examine how a longer duration under starvation conditions would effect the decline of *E. coli* cellular concentrations. Additionally, it was intended to expose whether or not trends exist between strains of the same phylogenetic group, as to their survival patterns in a similar environment. It could also show possible variances or similarities between the survival patterns within both the natural isolates and their laboratory strain counterparts, or between the two groups. It should be noted that the *E. coli* K-12 group A batch culture did not grow as planned and was therefore left out of experimentation; therefore, no representative for the class A phylogenetic group was used.

From the onset of experimentation red and white colonies were noticed on some plates, which were both determined to be *E. coli*. While this result could help to explain the observed results, the focus of this study remains the survival of *E. coli* strains in nanopure water. Results are therefore presented according to each individual strain's change in total viable numbers over time, followed by a discussion of any noticeable trends that may exist within the groups discussed above. To determine whether a noticeable declining trend is occurring over the course of experimentation, a borderline value for the slope will be arbitrarily set at -0.015 which would correspond to a drop of approximately 0.093 log units per week or a 0.70 total log drop over the duration. Any strain that has a linear trend line for the total colonies with a slope greater than or equal to this cutoff value will be deemed to have sustained relatively consistent numbers of colony forming units over the course of testing. Any strain with a slope less than this level will be considered to have undergone a perceptible decline of total viable *E. coli* cells over the duration of the trial.

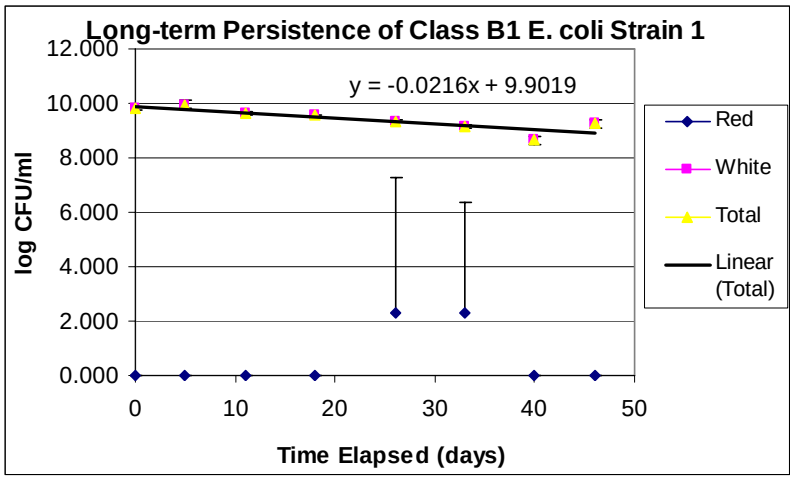


Figure 4. Plot representing the survival over a forty six day period for class B1 *E. coli* strain 6732-2, a river isolate.

Figure 4 seems to show a fairly consistent linear decline over the course of the experiment, and this trend can be deemed to be significant. The slope would correspond to a drop of nearly 1 log unit over the forty six day period. This would mean that over the course of the strain's incubation in water for the allotted time, the total viable cell count would decrease by a factor of 10 overall.

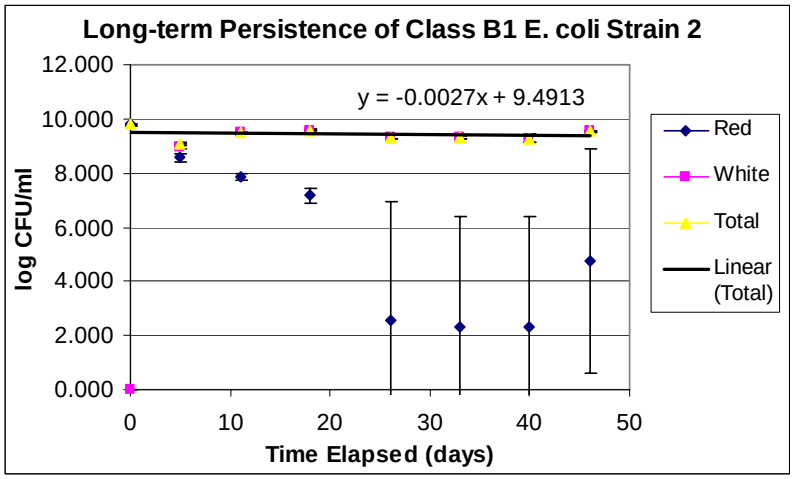


Figure 5. Plot representing the survival over a forty six day period for class B1 *E. coli* strain 6728-1, a river isolate.

Figure 5 seems to show that this strain is able to persist at relatively consistent numbers even over the long-term duration of this experiment. The trend line would also indicate that the viable numbers of the strain remains quite steady, as it would only correspond to a total drop of approx. 0.1 log units over the entire incubation period. This drop can be deemed insignificant, with the strain able to survive the stressful conditions quite well.

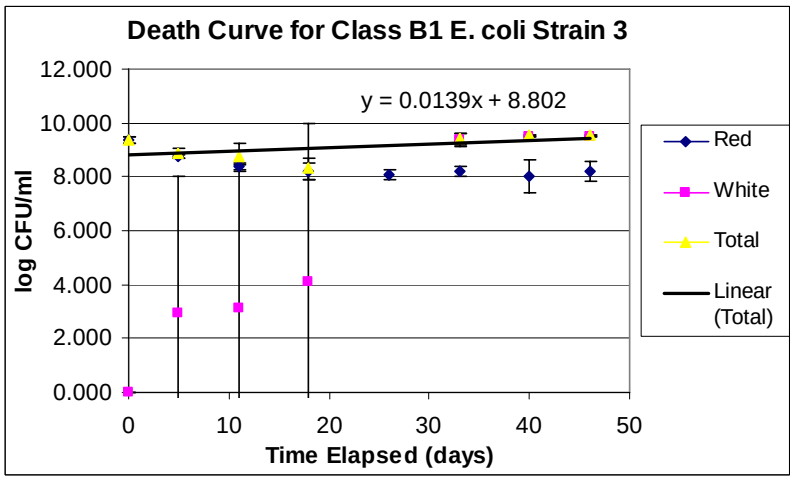


Figure 6. Plot representing the survival over a forty six day period for class B1 *E. coli* strain 6725-3, a river isolate.

The results for the strain illustrated in Figure 6 show no declining trend, and if anything there seems to be in increase in the total cellular concentrations during the testing. As this result would imply that the cells are no longer in a stationary or death phase but are going through replication and growth, the increase can therefore be ignored. Therefore, the cellular numbers

can be considered to remain at a consistent level over the course of the experiment, with the strain showing a reliable system for survival under the starvation-like conditions.

Class B1 trend analysis. This particular class does not seem to show any noticeable trends that exist between all of the strains, as one strain shows a marked decline while two strains are deemed to retain relatively consistent numbers over the course of experimentation. Moreover, these two better surviving isolates do not seem to follow similar growth trends overall, as strain 6728-1 seems to remain quite level over the duration, while strain 6725-3 appears to drop in numbers and then rebound to viable concentrations near the original levels. Therefore, it can be judged that the strains of this particular phylogenetic class of *E. coli* do not show similar trends in survival, and the individual strains most likely use a stress response system that involve differing signal cascades of gene repression and expression when exposed to stresses such as starvation.

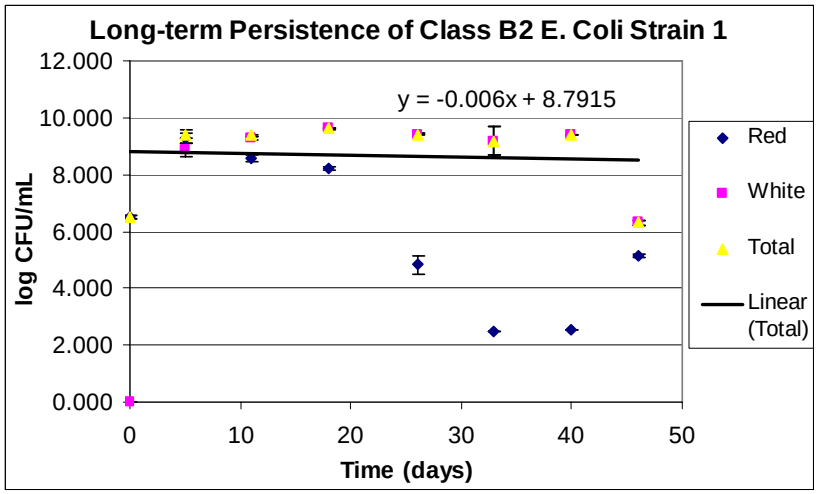


Figure 7. Plot representing the survival over a forty six day period for class B2 *E. coli* strain 6731-2, a river isolate.

Figure 7 seems to illustrate that strain 6731-2 remains at a more or less constant number during the forty six day period, with a couple of outliers for the first and last days. The trend line would show that the decline is insignificant, describing an overall decrease of approximately 3% of the original concentration of bacterial cells. This would tend to show that this particular *E. coli* strain is able to persist in the environment reasonably well over a nearly seven week period in the de-ionized water.

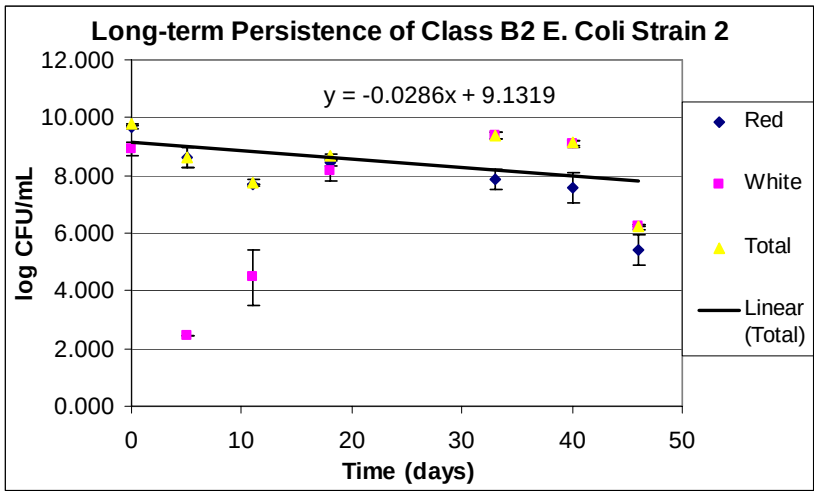


Figure 8. Plot representing the survival over a forty six day period for class B2 *E. coli* strain 6731-3, a river isolate.

The above plot seems to have fairly random values for total viable numbers over the course of experimentation, but a decreasing overall trend can be distinguished. The trend would represent an overall reduction of 1.3 log units over the forty six day period. This is a relatively marked decrease and can be judged as significant, as it would correspond to a 14% decrease in total cellular numbers during the course of the test.

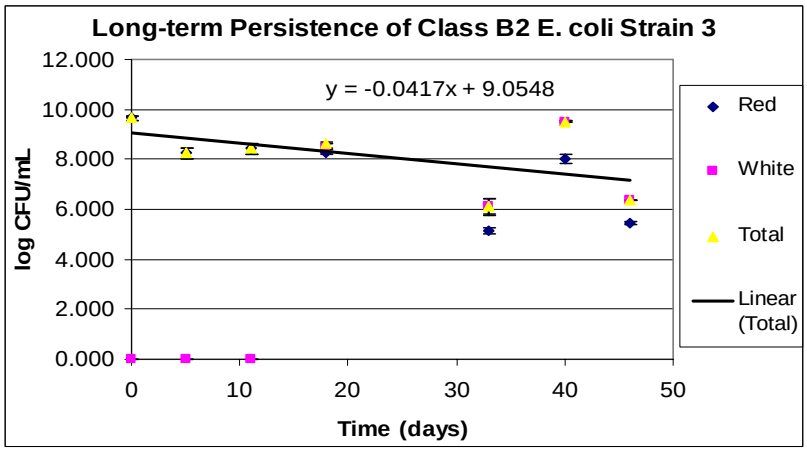


Figure 9. Plot representing the survival over a forty six day period for class B2 *E. coli* strain 6726-1, a river isolate

Once again, the plot seems to show a fairly consistent overall declining trend for the total number of colonies witnessed with time. This trend would equate to a nearly 2 log drop or an over 20% reduction from the original concentration of cells when incubated in water for this particular duration, which is quite significant.

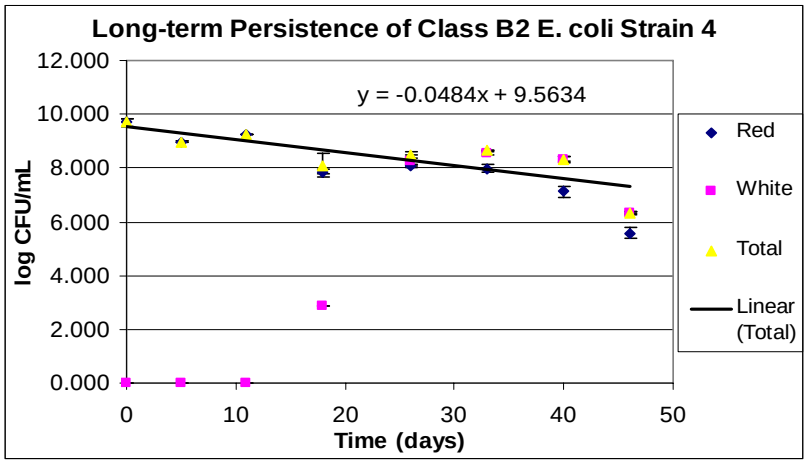


Figure 10. Plot representing the survival over a forty six day period for class B2 *E. coli* strain 6730-3, a river isolate

The above figure illustrates that this specific isolate follows a fairly consistent trend of decline with increased incubation time. The overall linear decline would correspond to a the cells reaching a final concentration nearly one quarter that of the original or a 2.2 log drop in the period of forty six days under the stressful conditions of incubation.

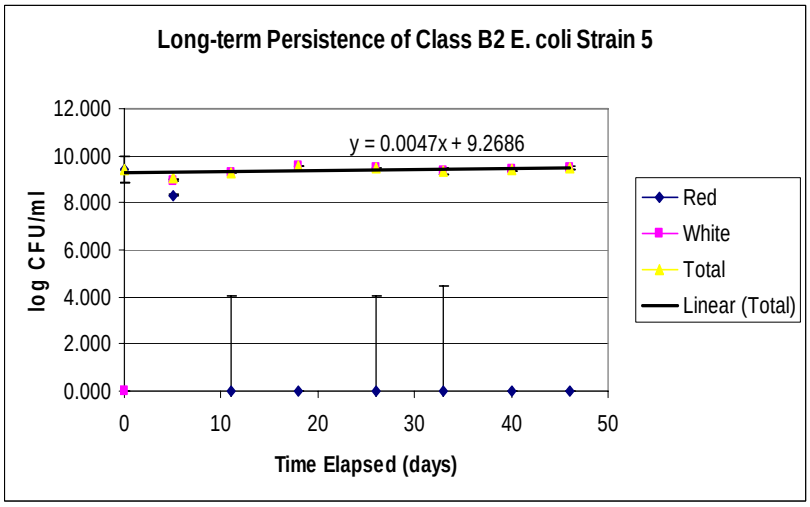


Figure 10. Plot representing the survival over a forty six day period for class B2 *E. coli* laboratory strain F-18.

The data points seem to remain at a reasonably constant level throughout the testing period. This is once again illustrated by the trend line, which would seem to show that the viable cellular number, if anything, increases over the course of time. Therefore, the F-18 strain can be considered to stay at consistent cellular concentrations over the duration of the experiment.

Class B2 trend analysis. Some trends are noticed within this particular *E. coli* phylogenetic group; however, they do not seem to be class-wide. River isolate strains 6731-3, 6726-1 and 6730-3 all have marked trends of linear decline over the trial time. However, the decline for the first of these strains is not nearly as significant as that of the other two. If the seemingly outlying day 40 data point for isolate 6726-1 were removed, the death curves for the latter two strains would be nearly identical over the duration of the experiment. This could

illustrate that these two isolates are possibly the same strain, which could be determined during 16S rRNA sequencing analysis, or possibly use similar stress-induced response systems for survival, which does not seem to be overly efficient when compared to other *E. coli* strains from their own and differing classes. Additionally, strain 6731-3 most likely uses a similar but modified system that enables the strain to survive more efficiently over the same period of time in the nanopure water environment. Finally, isolate 6731-2 and the laboratory F-18 strain have quite similar trends of persistence overall, excluding the first and last days time points for the river isolate which don't seem to fit the accompanying data, with a consistent level of viable cellular numbers throughout experimentation for both. This would signal, once again, that these strains are identical or have evolved remarkably similar overall gene regulation patterns for responding to starvation conditions to be able to not only persist at consistent levels for prolonged periods. Additionally, this could indicate that if the strains are indeed different, that the river isolate may be able to produce virulence factors like the type 1 fimbrae that F-18 type *E. coli* are known to produce, which are most likely induced under the general stress response.

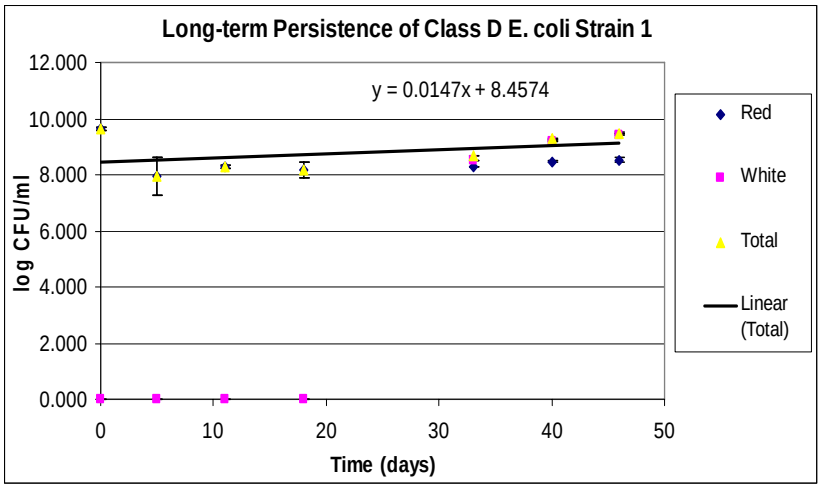


Figure 11. Plot representing the survival over a forty six day period for class D *E. coli* laboratory strain EDL933.

The above plot for pathogenic *E. coli* EDL933 seems to show a trend of initial decrease followed by a rebound to original viable concentrations. This trend would tend to show that the lab strain was able to persist in the environment, staying at a relatively consistent level with respect to the duration of the experiment as a whole. This ability to survive starvation-like conditions for a long period of time could be due to the pathogenic nature of the organism, which has acquired specific characteristics that make it more hardy while in stressful environments.

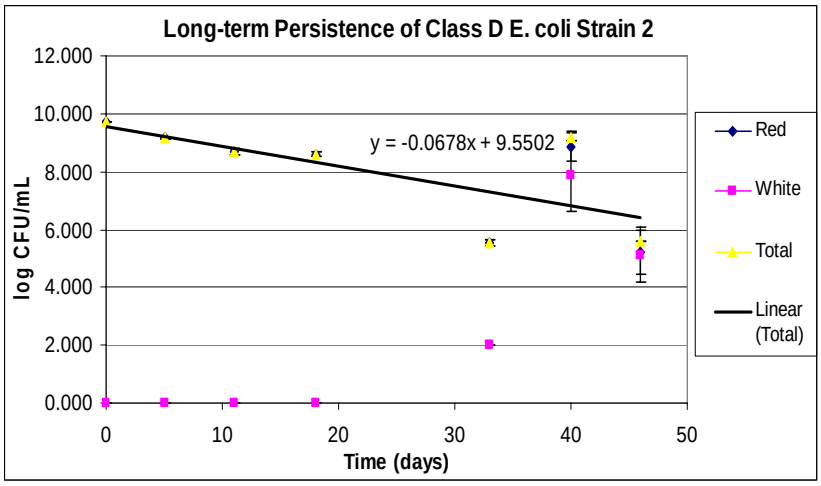


Figure 12. Plot representing the survival over a forty six day period for class D *E. coli* strain 6728-5, a river isolate.

Figure 12 seems to show that this particular strain follows a general decline in cellular numbers overall, remaining fairly linear over the first three weeks with some randomness noticed over the last few time points. Overall, the general trend would correspond to a decrease of over 3 full log units over the period of incubation in water.

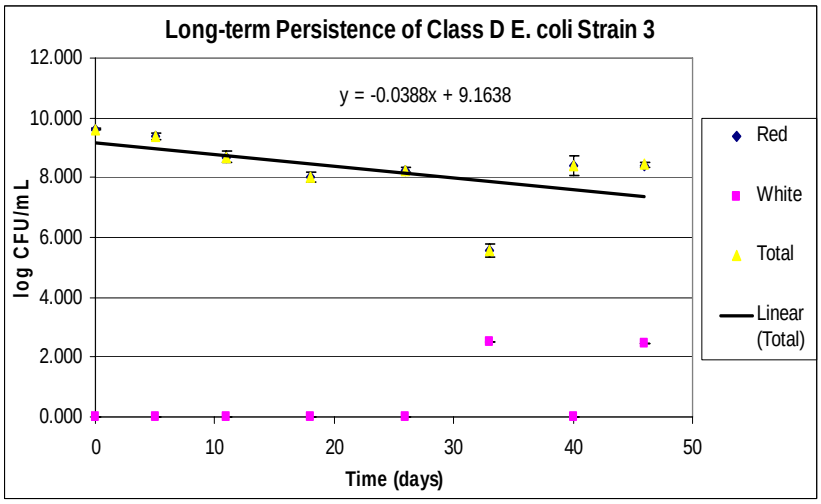


Figure 13. Plot representing the survival over a forty six day period for class D *E. coli* strain 6724-2, a river isolate.

This plot once more shows a tendency for decreasing numbers of colony number over time, with an initial fairly linear decline over the first four weeks and seemingly random data points thereafter. On the whole, the pattern of decline would reveal a nearly 2 log decrease, which is a quite significant decrease over the duration of the experiment.

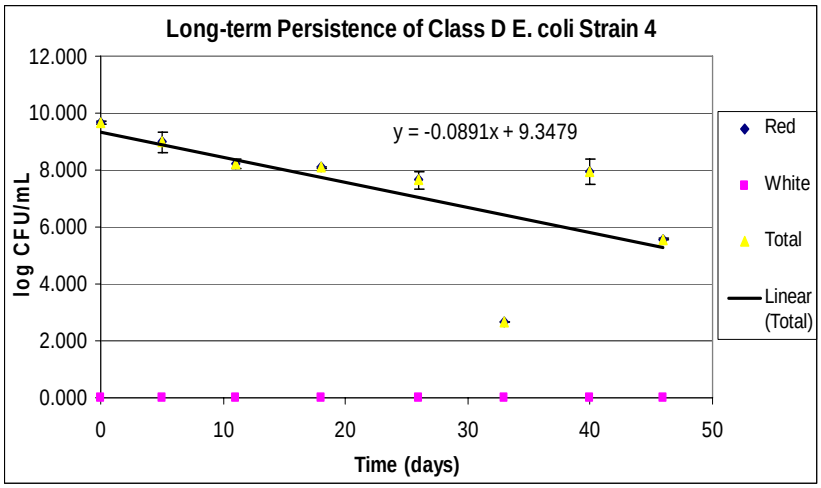


Figure 14. Plot representing the survival over a forty six day period for class D *E. coli* strain 6731-4, a river isolate.

In regards to total cellular concentrations, this particular strain seems to show a significant pattern of decline. This trend seems to follow a more linear pattern over the course of the first four weeks, with more variance in the time points taken afterwards. The slope would indicate an overwhelming 43% decrease in cellular numbers with an over 4 log total decrease during the course of the incubation, a very significant decrease.

Class D trend analysis. Here, at the surface it may appear as though patterns for survival may not be present for all of the river isolates; however, if the more random time points were eliminated for these strains, it could be seen that all of these natural isolates follow fairly the same trend for decreasing numbers over the course of the experiment. Each of these strains show a three to four week initial period of almost linear decrease that all closely mimic one another, including the rate of viable cellular decline. Therefore, it can be seen that these isolates may be of the same strain or have all obtained comparable RpoS global regulation methods for dealing with the stresses of surviving in a nanopure water system. The laboratory *E. coli* strain EDL933 ability to survive differs greatly from the other three phylogenetic group D isolates. This illustrates that the pathogenic EDL933 strain is quite well suited to survive prolonged exposure to an environment devoid of substantial nutrients, implying that a reliable system of stress response must be in place to regulate metabolism in favor of persistence rather than proliferation. This may be an indication that the three river isolate strains are not able to produce virulence factors, and therefore, contain overall less efficient mechanisms for dealing with their outside environment and the stresses that correspond with it. This would show that the pathogenic traits acquired by EDL933 over time have had a positive effect on the organism's ability to survive under highly stressful conditions such as starvation, and that the natural isolates are most likely not pathogenic themselves.

River Isolate and laboratory strain trend analysis. Based on the above figures, there does not seem to be any correlation that would relate the river isolate strains to one another, as the isolates' patterns of survival do not even seem similar within the same phylogenetic groups. When disregarding phylogenetic class, the results become even further skewed, as there seems to be no consistent pattern to relate an isolate's natural origins to its ability to persist and survive. This would tend to show that a strain's original environment is not key to its machinery directed towards survival, but there are more likely overriding factors, such as phylogenetic class,

virulence factors, pathogenicity or other acquired characteristics specific to the strain, which play a role in the stress-induced response mechanisms utilized by these various *E. coli* isolates. The only noticeable trend when looking at the river isolates as a group is that seven out of the ten isolates showed a noticeable decline in cellular numbers over the six and a half week period of the experiment. This could be attributable to the strains coming from a natural river system, resulting in these isolates being more prone to decline in total numbers as compared to the laboratory strains. However, this general decline would be expected to occur in most bacteria when exposed to stressful conditions for a prolonged period of time and would more likely point towards isolates 6731-2, 6725-3 and 6728-1, having somehow acquired more efficient RpoS gene control response mechanisms. While Figures 11 and 14 for laboratory strains, F-18 and EDL933, respectively, seem to show similar patterns for survival under the experimental conditions over the duration of the experiment, there seem to be subtle but noticeable differences which may show these strains' mechanism of survival may not be the same. For F-18, the total viable cellular numbers seem to stay quite consistent over the course of the entire experiment, while for pathogenic EDL933 strain, these numbers seem to decrease over the first approximately three weeks, where they seem to rebound back to total numbers equivalent to that of the start of experimentation from this time onward. Since both of the lab strains are able to remain at consistent numbers over the duration of the experiment, this may seem to show that these lab strains are more fit to survive overall than the collected river isolates. However, this ability to persist may be afforded to the lab strains due to a variety of other factors discussed above, especially the ability to elicit virulence factors, or may actually be due to the laboratory nature of the strains, which has possibly allowed them to make advantageous mutations over time to deal with the multiple stresses that they normally encounter within the lab setting. Therefore, no true determination can be made at this time in regards to the effects that the original environment of these strains play on their ability to survive under the starvation-like conditions of the experiment, as too many other variables could possibly confound the observed results.

Red and white colony observations. Within the first day of experimentation, designated Day 0, it was noticed that the aliquots plated on MacConkey agar for some of the strains revealed not only the expected red colonies indicative of the normally lac + *E. coli*, but also white colonies were observed, indicating, if the colonies still were *E. coli*, the inability of the colonies to utilize lactose as the carbon source. Since the short-term persistence experiment used LB agar for platings, this variance in colony phenotype was not noticed, as all colonies appeared to be the same pale yellow coloration of the agar itself. However, the only repercussion of this is that it is not known how the class A *E. coli* K-12 strain reacts in terms of possible repression under the starvation conditions, as the other two laboratory strains were involved in this second experiment as well. The results were compiled weekly over a nearly seven week period, taking care to note the numbers of red and white colonies that appeared over the course of the persistence study for each strain in triplicate.

Class B1 trend analysis. Figure 4 for isolate 6732-2 shows that the white colonies were not only observed on the initial plating, but were the only colonies witnessed for a majority of the duration of the experiment. Figures 5 and 6 show only red colonies initially, but these numbers declined throughout the remainder of the experiment while the number of white colonies rapidly increased up to the levels nearly the same as that of the red colonies on the original plates. Figure 5 for the river isolate 6728-1 shows that the number of white colonies surpassed that of the red after only one week after being subjected to the starvation conditions.

Additionally, the red colonies continued to decrease drastically, while the amount of white colonies quickly reached a maximum. Figure 6 for the 6725-3 strain shows a more gradual overtaking by the white colonies, with their numbers surpassing those of the red after approximately 3 weeks of incubation. In this case, the red colonies do not drop off as drastically as for the 6728-1 strains, with their number remaining at a relatively high level throughout the course of experimentation. Therefore, no trends are witnessed for any of the three experimental river isolates in regards to the change of colony coloration. This would once again seem to show that each of these isolates has its own unique mechanism of stress response.

Class B2 trend analysis. Figures 7, 8 and 9 for the isolate *E. coli* strains 6731-3, 6726-1 and 6730-3, respectively, of phylogenetic class B2 all follow similar trends, with the white colonies starting at a low initial level and eventually reaching a concentration similar to that of the red colonies originally. The red colonies seem to follow a general decline, with the white colonies overtaking them in numbers for all strains; however, the red colonies also seem to stay at a relatively high cellular number throughout the course of experimentation for these isolates. In the tubes containing these three strains the white colonies surpass red in number after approximately three to four weeks have passed since inoculation in the nanopure water. This seems to show that these three isolate B2 strains of *E. coli* are able to survive quite well under this particular form of stress, but their mechanisms of starvation response may not be quite as well suited to persist in an environment nearly devoid of nutrients as other strains of the same organism. Figures 10 and 11, displaying the results for 6731-2 and F-18 strains, seem to follow very similar trends for persistence and survival in the nanopure water system. Each shows a decline in the number of red colonies with a simultaneous rise in white colonies after approximately one week of experimentation has elapsed. Additionally, the total cellular numbers seem to remain quite constant over the entire scope of testing. The only true difference would be that the isolate strain does not show as marked a decline in red colonies as the F-18 lab strain. It seems that these two strains must rely on a slightly more efficient stress response mechanism than the other three isolates which seem to show an overall decline in cellular numbers over the scope of the experiment.

Class D trend analysis. Figures 13, 14 and 15 for the river isolate strains of phylogenetic class D reveal some interesting trends. First, for all of these strains, the white colonies never reach the same numbers as the red colonies, with no white colonies even being observed until after nearly five weeks of incubation for isolates 6728-5 and 6724-2 and none ever being witnessed for strain 6731-4. This would further illustrate that these isolate strains have quite similar mechanisms of stress response overall. Figure 12 shows the results for the *E. coli* O:157 lab strain show similar results over the first four weeks of experimentation, but then the number of white colonies reach the same levels as that of the red colonies and eventually outnumber the red over the last two or so weeks of incubation. This once again shows that the mechanism by which the lab strain and river isolates use differ and could help to provide a clue as to how the EDL933 strain is able to persist the starvation-like conditions for a prolonged period more efficiently

White colony analysis. Since the total colony results may help to explain specific persistence patterns or trends that are exclusive to certain phylogenetic groups, no definitive conclusions could be made until it was determined whether or not the white colonies were actually *E. coli* cells that had undergone physiological changes that may be advantageous to

survival. Also, finding that these white colonies are still *E. coli* would lend credence to the generalizations and trends noticed above pertaining to the total colony concentrations and short-term persistence findings. A first indicator that the white colonies had most likely undergone an internal modification is that the experimentation was re-started three separate times, stopping experimentation after approximately two weeks of incubation on the first two attempts, based on the thinking that the white colonies appearance was a sign of contamination. However, similar results were noticed for all three rounds of testing with additional care being focused on aseptic technique, signaling that the results were most likely legitimate and not the cause of another bacterial species. Another result indicative of a gene change occurring within the experimental tubes to produce the abnormal white colonies involves the plating of the control tubes on the final day of experimentation. These tubes had not been disturbed during the course of the nearly seven week period of testing except to open them for a very short period along with every other experimental time point to allow the exchange of gases with the environment and prevent the complete depletion of oxygen. When these cultures were plated, the results coincided with the experimental results very well, with total numbers as well as proportions of red and white colonies seeming to follow the same trends. While the actual plate count results are not provided for comparison, it was noted that the plate counts seemed to show that the control tubes seemed to have overall similar trends as the tubes used throughout experimentation. One final indicator involves the sectoring of colonies which seemed to be noticed on some plates during the course of experimentation. This result would seem to imply mutagenesis was occurring within the tubes to various colonies. Here, a mostly red colony would have a white portion within the borders of the colony itself, almost like a pie piece. In these sectored colonies there would be only slight disturbances or protuberances around the perimeter of the colony, which would seem to show a single colony forming instead of two separate colonies growing together. This would represent a mutation happening after the colony had been plated and was starting to grow on the MacConkey agar, with this mutation propagating outwards from this single initial mutant cell and leaving a region of different color within the colony. If the white colonies can be proven to be *E. coli* as well, these results would all provide further validation that the appearance of the white colonies was not the result of poor experimental technique or induced by the experimenters, but rather an occurrence that was happening within the *E. coli* cellular cultures.

Gram Stain and re-isolation results and analysis. The next logical step to determine whether or not the white colonies appearing on the MacConkey agar plates were actually still *E. coli* or not was to perform a standard Gram Stain. After the final time point numbers were logged, the red and white colonies from each strain were isolated from one another on separate MacConkey agar plates. Gram stains were performed on each of the red and white colonies for a single strain from each phylogenetic group. A normal compound microscope was used at a magnification ranging from 100 – 500x, and the equipment necessary to capture an image of the stain was not available. The results were rather inconclusive overall. The bacterial cells had shrunk greatly in size due to the stressful conditions under which they had become accustomed over the course of experimentation. Therefore, it was hard to distinguish the coloration of the cells even under the highest magnification. Also, the morphology of the cells was even hard to distinguish at times, as you could tell the cellular dimensions had become distorted due to the experimental conditions, due to the differing shapes and sizes of cells that had been collected from a single colony. The cells ranged from short bacilli to tiny cocci, with most cells being too small to visualize clearly. This was the case for both the red and white colonies for all strains that were stained and at best these results can be judged as inconclusive.

One interesting result did come out of the re-isolation platings, however. After the red and white colonies of each strain had been isolated on separate plates, the final day's time point plates were allowed to incubate in a freezer at -40°C. After approximately seven to ten days had elapsed, the plates were removed from the freezer and observed. All of the colonies were now red, illustrating a possible reversion of the white colonies back to their normal state when allowed to incubate and grow for an extended period of time in the more nutrient rich environment of the MacConkey agar plates. This would lead one to believe that the *E. coli* cultures had not gone through a permanent mutation, but instead stress-induced systems were involved in shutting off certain genes and operons, involved in various forms of metabolism to conserve energy to preserve their viability. In this situation, the lactose system had been altered in a way that this assay could detect due to the use of MacConkey agar, which has lactose as the main carbon substrate for metabolism. And when these starved cells are returned to the environment more suitable to growth, they go through a short lag and then return to a normal form of metabolism, utilizing all possible substrates within the media available for growth. Here, their stress response mechanisms are no longer repressing the production of various gene products, such as the lac operon, to conserve energy for prolonged survival but replicating all necessary genes and their products for growth to once again occur at an optimal level. This, along with the above observed results would indicate the cells are going through extreme measures to greatly repress systems necessary for the cells to undergo log phase growth in favor of genes that aid in stress response and stationary phase survival.

Triplex PCR analysis of red and white colonies. While the Gram Stain proved to be unhelpful in the determination of whether or not the white colonies were actually *E. coli*, subjecting these different colored colonies of a strain from each phylogenetic group to the triplex PCR protocol could prove that the white colonies banding patterns were not different from the original red colonies from the ChuA, YjaA and TspE4.C2 fragment production or lack thereof. The second round of triplex PCR exhibited the expected results, showing that the white colonies produced indistinguishable amplification profiles to that of the red colonies from the same plate. These results were observed by agarose gel electrophoresis; however, a picture was not able to be taken of the gel. This result helps to validate the use of the total colony counts as a means to determine the survival patterns of the individual strains and to make comparisons.

Conclusions

First, the results indicate that *E. coli* strains, both natural isolates as well as those from the lab, are able to survive quite well on the whole when incubated in nothing but nanopure water. Additionally, nearly one-half of the strains tested were able to remain at relatively consistent numbers over the course of experimentation. These findings agree well with other studies that show that *E. coli* is well fit to persist in a variety of environments, including those that have a variety of stresses (25, 11, 68).

This experiment also shows that there are no significant class-wide trends present within the phylogenetic groups of *E. coli*. While there may be similarities between some of the strains within the same class, there does not seem to be a noticeable pattern that could be used to classify how a strain will behave when introduced into starvation conditions based on phylogentic classification alone. This tends to agree with other studies that show that although differing laboratory and isolated strains of *E. coli* express and repress many common genes under starvation conditions, these strains often express these genes at varying levels (57). In

addition, other findings show that differing *E. coli* strains often have their own, unique stationary phase genes under regulation (46, 65), these variances would account for the differentiation in persistence patterns noticed in this study. However, these strains that behave similarly to stress may actually be identical stains, as a future 16S rRNA sequence analysis should be performed to elucidate whether or not this is the case. As far as a group comparison, it seems as though the laboratory strains are more capable of survival and persistence for a prolonged period in starvation conditions than their natural river isolate counterparts; however, this conclusion is only tentative, as there were only two laboratory strains available for experimentation which may have confounding variables discussed below.

While there may not be patterns that encompass entire phylogenetic groups of the species, there seem to be evident class trends when taking into account only the strains that went through a significant decline in viable numbers over the course of the experiment. The results show that, in terms of general decline, phylogenetic group D *E. coli* tend to be the least efficient at dealing with the stress of starvation, with an average decline of approximately 3 log units (cells/ml) over the duration of testing. On the other hand, class B1 proves to be the most resilient to decline, with only a 1 log drop over the forty six day period; while group B2 showed an average reduction in viable numbers of nearly 2 log units. It would be of interest to test strains of phylogenetic group A to see how they fit into this trend of general declination, especially if their overall decline is similar to that of their evolutionary sister class B1 *E. coli* strains (17).

Furthermore, the results suggest that there is no correlation between the appearance of Lac^- colonies and the ability of the strain to persist under the stressful conditions of incubation in nanopure water. This is evidenced by the variance between when the Lac- colonies would overtake the normal red *E. coli* colonies and whether or not the particular strain showed a noticeable decline in viable numbers. For example, strain 6730-3 showed a significant drop over the period but the white colonies did not become the majority until after four weeks had elapsed, while for strain 6732-2, which also showed a general decline, the white colonies were in the majority from the initial day of experimentation. This same inconsistency can be noticed for strains that remained at fairly level numbers over the course of testing. While the appearance of the white colonies may help to illustrate how and when the specific strains repress genes unnecessary during survival, such as the Lac operon, these results do not seem to lend much credence as to the overall persistence patterns of the strains tested. These findings show that the strains tested are most likely not going through stationary phase adaptive mutations, as in studies showing *E. coli* strains deficient in lactose metabolism obtaining a gain of function mutation when plated on lactose medium, but instead they are going through global repression controlled primarily by the RpoS system (63).

There does seem to be some evidence that could lead to the determination that strains able to express virulence factors are more able to deal with the stresses of starvation for prolonged periods of time. Both laboratory strains used, which are known to elicit these factors, are able to persist at consistent levels throughout the experimentation. This means that two out of the five strains capable of efficiently surviving the prolonged incubation in nanopure water are known to express specific virulence factors. Additionally, other studies show that virulence factors are often under the regulation of the RpoS system, which would indicate their importance in dealing with various stresses (26). Since three of the river isolates (6728-1, 6725-3, 6731-2) are able to remain at relatively consistent numbers, it is likely that there is some other factor responsible for strains being able to survive for prolonged periods other than the fact that they are laboratory strains, which these results could be misconstrued as showing. It has been found that all phylogenetic classes of *E. coli* are able to elicit virulence factors, with class B1 showing the highest frequency (17). This could show that each of these isolate strains that are able to

persist consistently over the trial may include genes encoding VFs, which would further show their importance in dealing with stressful environments. This proposed conclusion could be further bolstered by sequencing the genomes of these three river isolates or by performing PCR on them with specific primers to determine if genes of known virulence factors are present. Additionally, other strains that are known to express virulence factors could be subjected to experimentation to determine whether or not they are able to survive at consistent numbers for the prolonged period.

To lend additional credibility to the above conclusions, further experimentation should be performed, using various different natural isolates and laboratory strains, making sure to include strains from phylogenetic group A. This would allow more definitive conclusions to be drawn on how laboratory strain survival compares to the isolates' as well as the effects of virulence. Additionally, 16S rRNA sequencing should be performed on all isolates to ensure that identical strains are not being tested, preventing duplication of results. The most important next step would be to include microarray analysis of the strains during the course of experimentation. By using this procedure daily or along with every time point, it could be illustrated how the actual genetic machinery of each specific strain is being affected during incubation. This could hopefully elucidate how the RpoS master regulator effects the regulation of various genes known to be under its control, whether expressed or repressed, including the time component. As the RpoS cascade is further unraveled, this could allow a genetic model of the starvation-induced response to be made that would show the negative and positive impact on numerous genes of the various strains as time elapses, which could have important repercussions on the understanding of how *E. coli* is able to respond to the variety of stresses that are always present in the ever-changing environment.

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Appendix D

Escherichia coli Strains of the Major Phylogenetic Groups Subjected to Simulated Starvation Conditions in Nanopure Water

Logan D'Souza

I. Abstract

The goal of this research study was to compare the survival rates of *Escherichia coli* phylogenetic groups in nanopure water. Isolates from the Blue River in Oklahoma and known laboratory strains were classified into one of the four major phylogenetic groups of *E. coli* using triplex PCR techniques. These strains were then placed in starvation conditions simulated in the laboratory by incubation in nanopure water for over six weeks. Such taxing environments are known to induce the master regulator of general stress response, RpoS, to cope with the problem. The cultures were sampled weekly by serial dilutions and plating to obtain viable cell counts and determine if there were any conserved features of the strains in relation to their respective group. While some strains were able to maintain their viable cell counts, the majority decreased over 1 log from the original amount. In addition, the presence of lac^- colonies was noted in most strains at varying time points. No significant correlation was observed between survival patterns and phylogenetic groups. Future studies should include 16s rRNA sequencing to confirm the strain identity and microarray analysis to determine which genes are preferentially expressed and repressed in response to starvation.

II. Introduction

Escherchia coli is a Gram-negative microorganism that is a habitual occupant of the intestines of most animals. Consequently, whenever animals excrete fecal matter, they are releasing some of the *E. coli* that is in their intestines into the outside environment. Other contributors to the coliform population include inadequate septic systems and sewage overflow (15). Thus *E. coli* has been found in various environments other than the gastrointestinal tract that include soil, water, and sediments (10). Such contamination of soil and water has been linked to human health risks (27), and coliform counts in many bodies of water around the world are well above acceptable levels (12). As a result, studies are underway to determine more specific causes of the contamination (12, 36). *E. coli* has long been the focus of many of these studies, as it is both the primary coliform in the human normal flora and the cause of many

different bacterial diseases, including diarrhea, urinary tract infections, septicemia, and neonatal meningitis (27, 34, 43).

As a common practice, *E. coli* is defined as having two classes of environments of considerable differences in nutrition and stress factors (15, 40). The primary environment is in the intestines of animals, and the more arduous secondary environment is in soil, water, and sediments (40). Upon physiological study, it was first suggested that *E. coli* responds to the transition of environments using a dual regulation system, in which some genes are preferentially expressed in the external environment (13, 40). However, only a few studies have examined the specific genetic structure of *E. coli* in the secondary environment with regard to its ability to survive such stressful conditions (35, 36, 39).

Recent studies have shown that the master regulator of the general stress response in both *E. coli* and many other enteric and related bacteria is the RpoS sigma (σ^S) subunit of RNA polymerase (21, 22, 26). Examples of other general stress regulators include σ^B in various Gram-positive bacteria and the sporulation initiation regulator Spo0A in *Bacillus subtilis* (38, 44). RpoS is induced during several different stress conditions, including stationary phase, oxidative stress, near-UV irradiation, heat shocks, hyperosmolarity, ethanol, and acidic pH, to name a few (17, 20, 28, 32, 33). When induced, RpoS can replace the latent sigma factor RpoD and stimulate transcription of more than seventy RpoS-dependent genes (16, 18, 21, 29). Though the functions of these genes are not yet completely understood, the vast majority of them are thought to confer resistance to stress (21, 23, 30). Other genes are thought to function in metabolism, morphological changes that make the cells significantly smaller for conservation of energy, apoptosis where a portion of cells is sacrificed to yield nutrients, and the expression of several different virulence factors (5, 20).

Scientists in the field expect that many more genes will be detected as RpoS-dependent in the future, and with more research it is hoped to find specific functions of such genes as well as those already identified (21). With the continued findings of more factors that contribute to the RpoS transcription, translation, and proteolysis, it seems that this is one of the most complex regulation systems yet uncovered. In addition, the large number of genes that RpoS induces can yield a plethora of phenotypes. This depends on other factors that may stimulate one gene over another or the processing of the gene product, including transcription factors and post-transcriptional modification.

Our goal has been to study the prevalence and persistence of such *E. coli* strains that eventually inhabit the secondary environment of fresh water systems in their journey between animal hosts. In conjunction with the Oklahoma Water Resource Board, Dr. Guy Sewell of East Central University collected water samples from various sites along the Blue River near Tishimingo, Oklahoma, and subsequently isolated several dozen coliforms using nitrocellulose filters. These isolates were then frozen and transferred to our lab at the Stephenson Research and Technology Center in Norman, Oklahoma.

Once the strains were collected, we attempted to categorize them into one the four main phylogenetic groups of *E. coli*: A, B1, B2, and D (23, 42). This classification was first accomplished by using laborious techniques of multilocus enzyme electrophoresis and ribotyping (2, 3, 4), and was later confirmed by comparing genetic markers (11, 23, 41). Statistical analysis shows that the most common *E. coli* strains that have been classified belong to groups A and B1, the most common of the human normal flora, while the most rare are those of group B2 (11). However, it has also been found that the group with the highest frequency of strains with virulence factors is also B2 (4, 9, 24, 37). In a mouse model study, eighty-two *E. coli* strains were isolated from human feces during infections, and it was shown that those from group B2 killed mice at the highest frequency (37). In many of these cases, pathogenicity was linked to

large blocks of genes commonly referred to as pathogenicity islands that code for virulence factors.

After searching for a protocol to achieve group categorization of our strains, we found a French study by Clermont, *et al.*, that demonstrated success and ease in such classifications. According to this study, the goal was accomplished using triplex PCR techniques with DNA forward and reverse primers based on two genes, *chuA* and *yjaA*, and an anonymous DNA fragment, TspE4.C2. *ChuA* is a gene functioning in heme transport in *E. coli* strain O157:H7 (**8**, 31, 45, 46), *yjaA* is a gene of unknown function found in the genome sequence of K12 (7), and DNA fragment TspE4.C2 was found in a library made by Bonacorsi, *et al.*, using fragments of pathogenic strain C5 (8). PCR products were then analyzed using gel electrophoresis, as group A is defined as *chuA*-, TspE4.C2-, group B1 as *chuA*-, TspE4.C2+, group B2 as *chuA*+, *yjaA*+, and group D as *chuA*+, *yjaA*-. The procedure is especially advantageous because it is a colony PCR technique, in which a plucked colony isolated on agar can be substituted for purified DNA. This eliminates the extra step of purifying DNA from the microorganism.

Once the strains were characterized according to groups, stream conditions were simulated in the lab in order to see if there were any conserved features of the strains in relation to their respective groups. After growing cultures to a standard optical density, cells were resuspended in nanopure water. The cultures were sampled weekly by serial dilutions and plate counts, and the viable cell count was compiled and can be seen in the Results section. Future studies will utilize more strains in order to view a more comprehensive analysis of each phylogenetic group. In addition, microarray analysis of genes expressed during the course of the experiment will be helpful in discovering specific modes of survival.

III. Materials and Methods

Naming.

In order to keep track of the dozens of strains handed over by Dr. Sewell, a naming system was adopted. The first four numbers correspond to a specific sampling site on the Blue River, and the last number is the number of the strain found there. For example, the strain number 6347-3 means that the strain was the third one isolated from site 6347.

Isolation.

Four lab strains from each of the phylogenetic group were provided by the Conway lab: K12, F18, ECOR26, and EDL933 of groups A, B1, B2, and D, respectively. Additional river strains were sampled from the collection made by Dr. Sewell. Bacteria were isolated by quadrant streaking on Luria-Burtani (LB) agar plates and incubated overnight at 37˚C. Difco MacConkey agar plates were later used to avoid contamination that was observed on LB.

Working and Reserve frozen stocks.

As a proper practice, both working and reserve frozen stock cultures of each of our isolates were prepared. This was done by the following protocol. First, an overnight culture was made by inoculating a tube of 5mL LB broth with the appropriate strain. The tube was then placed in the Barnstead/Labline MaxQ 5000 shaker and gently shaken at 37˚C and 250rpm overnight. The next morning, 900µL of the overnight culture was added to 900µL of filter-sterilized glycerol in a properly labeled cryo tube. This step was repeated to make the second stock.

PCR.

There was some initial difficulty in replicating the triplex PCR techniques from Clermont, *et al.*, but after tweaking the system, the following procedure was finalized. For a total of a 25µL reaction in a PCR tube, we added 2.5µL 10x reaction buffer, 1.0µL 50mM $MgCl_2$, 0.1µL Invitrogen Taq DNA polymerase, 2.0µL 10mM dNTPs, 0.2µL forward primer (of each primer), 0.2µL reverse primer (of each primer), 1 picked colony, and 18.2µL autoclaved nanopure H_2O. The specific primer sequences were as follows:
ChuA forward (5'-GACGAACCAACGGTCAGGAT-3'),
ChuA reverse (5'-TGCCGCCAGTACCAAAGACA-3'),
YjaA forward (5'-TGAAGTGTCAGGAGACGCTG-3'),
YjaA reverse (5'-ATGGAGAATGCGTTCCTCAAC-3'),
TspE4.C2 forward (5'-GAGTAATGTCGGGGCATTCA-3'),
TspE4.C2 reverse (5'-CGCGCCAACAAAGTATTACG-3'). The primers yield 279, 211, and 152bp fragments, respectively.

Using the thermal cycler GeneAmp PCR System 9700 made by Applied Biosystems, the following PCR cycle was used: DNA denaturation for 4 minutes at 94°C, 30 cycles of 5 seconds at 94°C and 10 seconds at 59°C, and a final extension step of 5 minutes at 72°C.

Choice of strains.

Although dozens of strains were collected by Dr. Sewell, only a few had been classified by the other half of our research group at the beginning of our experiment, with no strains found from group A. Based on this information, an attempt was made to use similar numbers of each classification group in our experiment while maintaining a manageable number of strains with which to perform the many dilutions. In all, thirteen strains were sampled including some lab strains, with one strain from group A, three strains from group B1, five strains from group B2, and four strains from group D.

In order to make our naming system more readable, a new naming system was adopted according to the following system: A (lab strain K12), B1-2 (6732-2), B1-3 (6728-1), B1-4 (6725-3), B2-1 (6731-2), B2-2 (6731-3), B2-3 (6726-1), B2-4 (6730-3), B2-5 (lab strain F18), D1 (lab strain EDL933), D2 (6728-5), D3 (6724-2), and D4 (6731-4).

Agarose Gel Electrophoresis.

PCR reactions were analyzed using agarose gel electrophoresis. 1% gels were made with 0.5g agarose and 50mL 1x TBE that were heated by microwave for approximately 1 minute. Once the solution cooled for roughly 3 minutes, 10mg/mL of stock solution Ethidium bromide was added and mixed (0.5µL/mL buffer). To each PCR product, 5µL tracking dye was added to the total 25µL solution, of which 12µL was loaded. As a marker, 12µL of the 100bp Invitrogen DNA ladder was loaded. Gels were run at 70mV using the BioRad Power Pac 300 voltage supply until the dye had progressed a little over half of the gel. Pictures of the gel were then taken using the UV Products Epi Chemi II Darkroom and the UVP Labworks Image Acquisition and Analysis Software.

Serial Dilutions and Plating.

First, an overnight culture was made by inoculating a tube of 5mL LB broth with the appropriate strain, and then gently shaken at 37˚C and 250rpm as before in the incubator. The next morning, 0.5mL of the overnight was transferred to a fresh flask of 50mL LB. The new culture was grown in the Incubator Shaker Series Innova44 made by New Brunswick Scientific

Co. at 37˚C and 250rpm until an approximate measured optical density of 0.8 in order to define a relatively standard starting concentration for each culture. The optical density was measured by the Beckman DU530 Life Science UV/Vis Spectrophotometer at 260nm. At the specified optical density, the 20mL cultures were pulled out of the incubator, and 20mL of each culture was pipetted into their own large, plastic screw cap tube. The tubes were then centrifuged by the Beckman Coulter Allegra 21R centrifuge at 10˚C and 12,000 rpm for three different steps, being re-suspended by 15mL of nanopure H_2O after every centrifugation. After the third re-suspension, the 20mL of cells in water were divided into four different screw cap tubes with 5mL each such that we could run our experiment in triplicate while having one unaltered control tube. Culture tubes were incubated at room temperature for the duration of the experiment. For each tube, serial dilutions and subsequent platings were performed. This was accomplished by vortexing the large screw cap tubes gently, and then removing 100µL from the tube and adding it to an Eppendorf tube containing 900µL of nanopure H_2O. The Eppendorf tube was then vortexed, and a similar dilution step was performed into another Eppendorf tube with 900µL of nanopure H_2O until dilutions were obtained from 10^{-1} to 10^{-7}. The 10^{-5} through 10^{-7} Eppendorf tubes were then plated onto MacConkey agar plates by transferring 100µL to the plates and spreading aseptically with a glass hockey stick. Results were then entered into spreadsheet format for all platings of every strain of each timepoint. While the protocol was at first carried out with daily samplings over two weeks, these results were inconclusive and led us to conduct a more long-term experiment with weekly samplings over a total of 46 days. We used MacConkey agar for the platings because of its known selection for lactose-fermenting, Gram-negative microorganisms such as *E. coli*. This is done by the media's crystal violet that inhibits Gram-positive bacterial growth and its neutral red dye, a pH indicator that stains microorganisms that ferment the media's lactose into lactic acid. Though LB was initially used for plating, observed contamination by colonies displaying dissimilar morphology than typical *E. coli*.

Check for Contamination.

In order to make sure that the white lac^- colonies were indeed *E. coli*, both red and white colonies were isolated from each strain and quadrant streaked on MacConkey agar plates to be Gram stained the following day. In conjunction with the Gram stains, triplex PCR and subsequent agarose gel electrophoresis as stated above were also used to definitively prove that the white colonies were indeed *E. coli*. In addition, serial dilutions and platings were performed from the one unaltered control tube of each strain in order to see if the control culture also displayed the white colonies.

IV. Results and Discussion

Triplex PCR.

Triplex PCR was first tested on laboratory strains of known phylogenetic class: K12, F18, ECOR26, and EDL933 of groups A, B1, B2, and D, respectively. This was done to make sure that the procedure was accurate. Gels were obtained that resemble Figure 1 shown below, and the procedure was proven useful for our study.

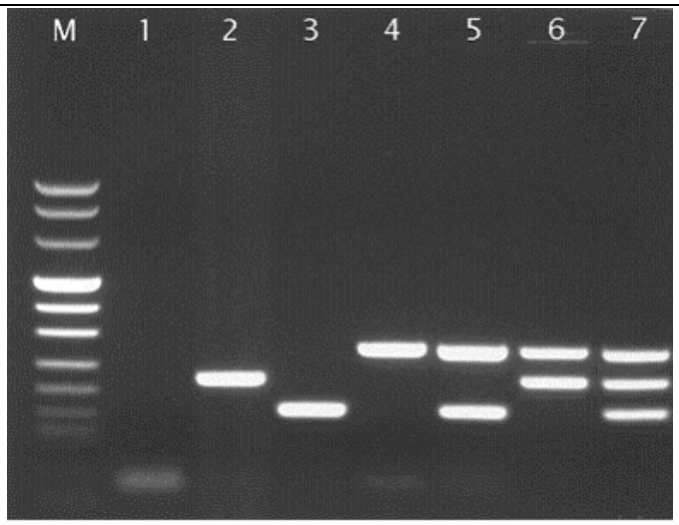


Figure 1

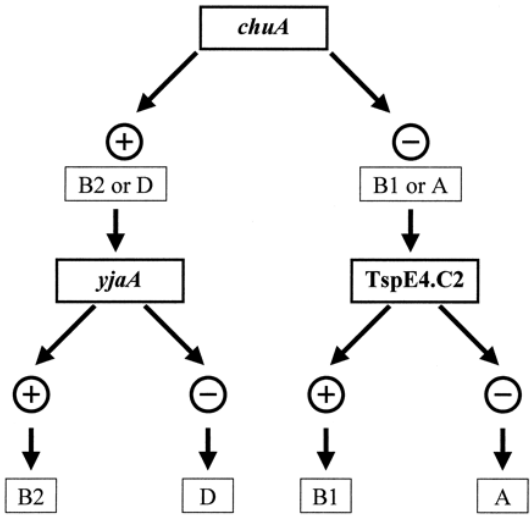


Figure 2

Figure 1 is an example of a triplex PCR gel used to determine phylogenetic groups in *E. coli* using the stated primers.
Figure 2 is an easily readable key used to analyze the gel for classifying phylogenetic group.
(Clermont, *et al.*, 2000)

The primers for *chuA, yjaA,* and TspE4.C2 yield 279, 211, and 152bp fragments, respectively. The top band in Figure 1, lane 7, is the heaviest and thus corresponds to *chuA*, the middle band is the *yjaA* product, and the bottom band is the lightest and thus corresponds to TspE4.C2. Using the Figure 2 key to read the gel, the strains in lanes 1 and 2 must belong to group A, lane 3 to group B1, lanes 4 and 5 to group D, and lanes 6 and 7 to group B2 with lane M as the marker.

After testing the triplex PCR method on laboratory strains, the technique was used to classify all the river isolates obtained from Dr. Sewell. This was primarily done by the other half of our lab group.

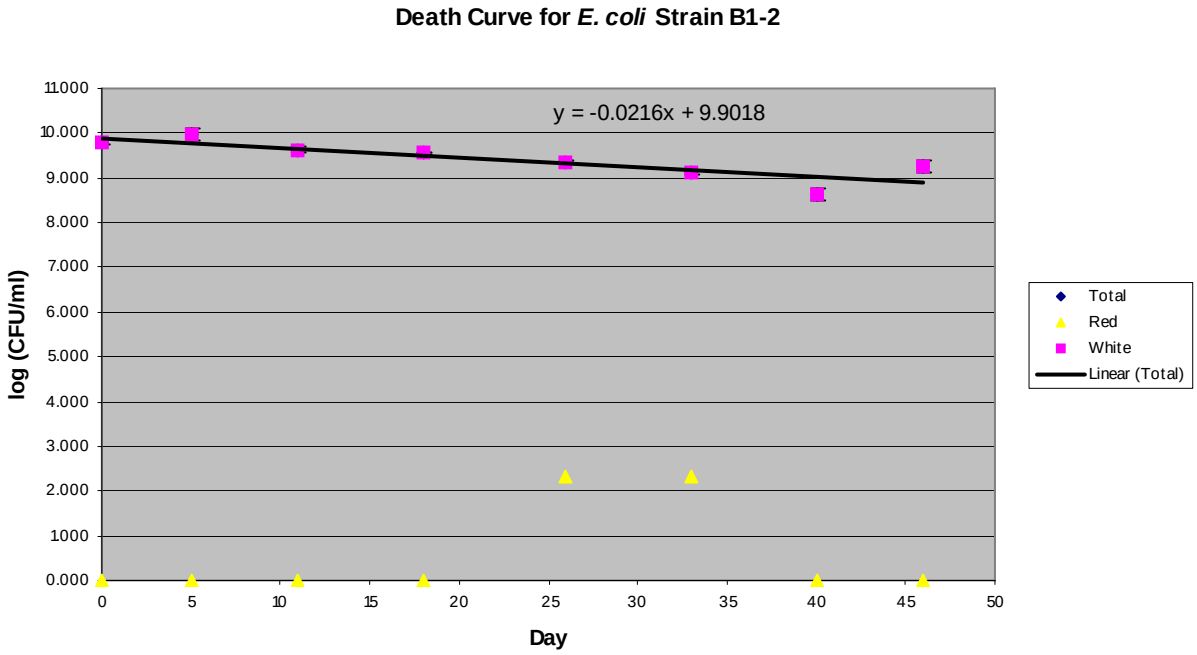
Persistence Study.

The study of long-term persistence in our simulated water system sought to correlate some similarity between phylogenetic groups and patterns of survival. In addition, we hoped that such a correlation might exist between lab strains versus those isolated from the river. It must be noted that lab strain in K12 of group A did not grow sufficiently and was deleted from the experiment. Consequently, there were no strains of group A sampled. While this was disappointing, if only one strain from group A were sampled, it would be inconclusive to use it as the sole representative of the entire group.

While the protocol was at first carried out with daily samplings over two weeks, these results did not show a significant decrease in viable cell counts and led us to conduct a more long-term experiment with weekly samplings over a total of 46 days. From the very first sampling, it was noted that for some strains, there were both red and white colonies plated. At first, we considered the results to be attributed to contamination. But upon repeating the experiment from the beginning with extra care for aseptic technique, highly similar results were noted in the exact same strains. In addition, subsequent triplex PCR and Gram staining techniques proved that the white colonies were indeed *E. coli*. Our initial daily plating did not account for such changes because we had only been plating on LB, and all the colonies appeared a yellowish-white. Only when we switched to MacConkey agar for the weekly plating did we observe the phenotypic change. While such a phenomenon of possible lac^- phenotypic change from the normal ability of *E. coli* to ferment lactose may play a role in survival, our study did not

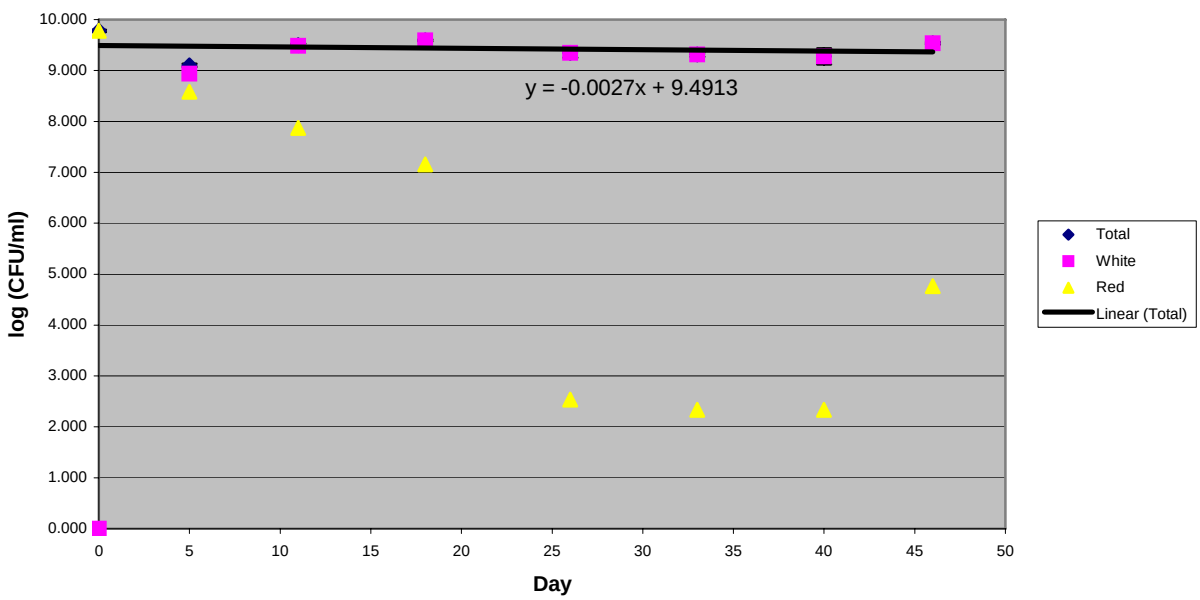
determine the reasons for this phenomenon. Thus our study focused on overall survival during the course of the experiment, though we noted the white colonies for discussion of patterns in future studies.

In order to facilitate a discussion of whether or not a strain survived well, an official boundary with regard to persistence must be defined. I propose that in our linear regression of log(CFU/ml) vs. time, a slope of –0.0215 or lower will correspond to a significant decline in persistence. Any slope higher than this value will be deemed a strain that has maintained itself well. I selected the slope value of –0.0215 because it equals roughly a 1 log or 10% decrease over the duration of our experiment.



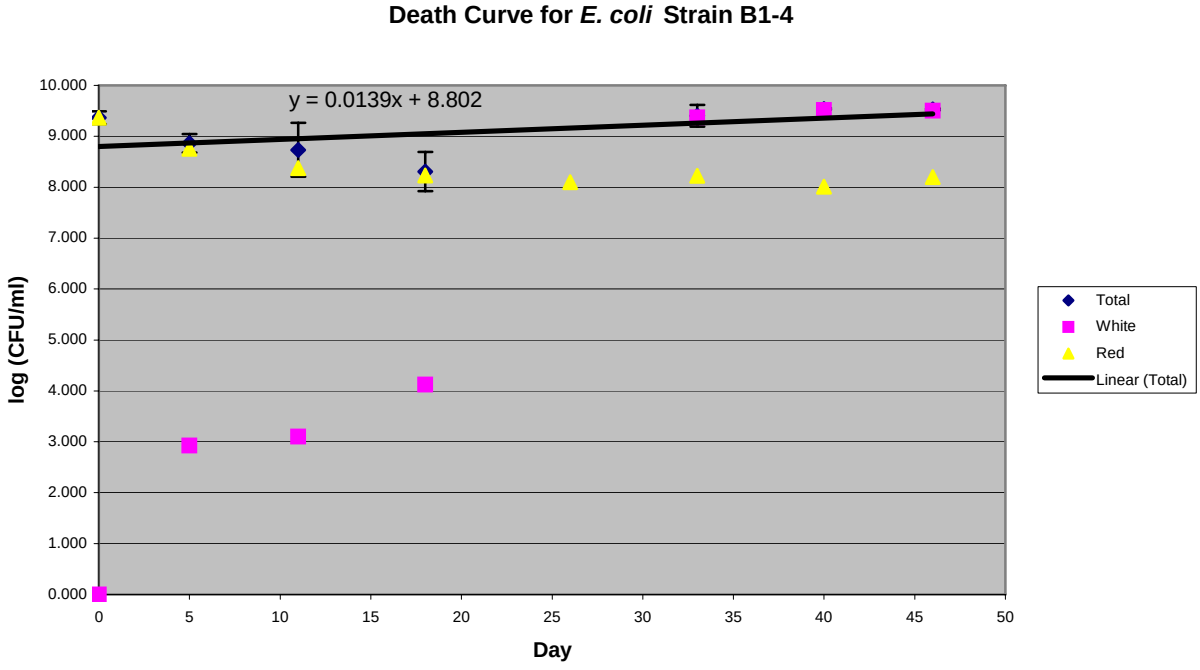
Graph 1: Survival of the Group B1 river isolate *E. coli* strain 6732-2 over 46 days in a simulated nanopure water system.

Graph 1 shows a linear regression slope of –0.0216 for B1-2 that is slightly greater than the experimental boundary of –0.0215. This corresponds with a steady decline in viable cells present by approximately 10% over the 46 day study. White colonies were noted from day 0, and red colonies were rarely noted through the course of the experiment.



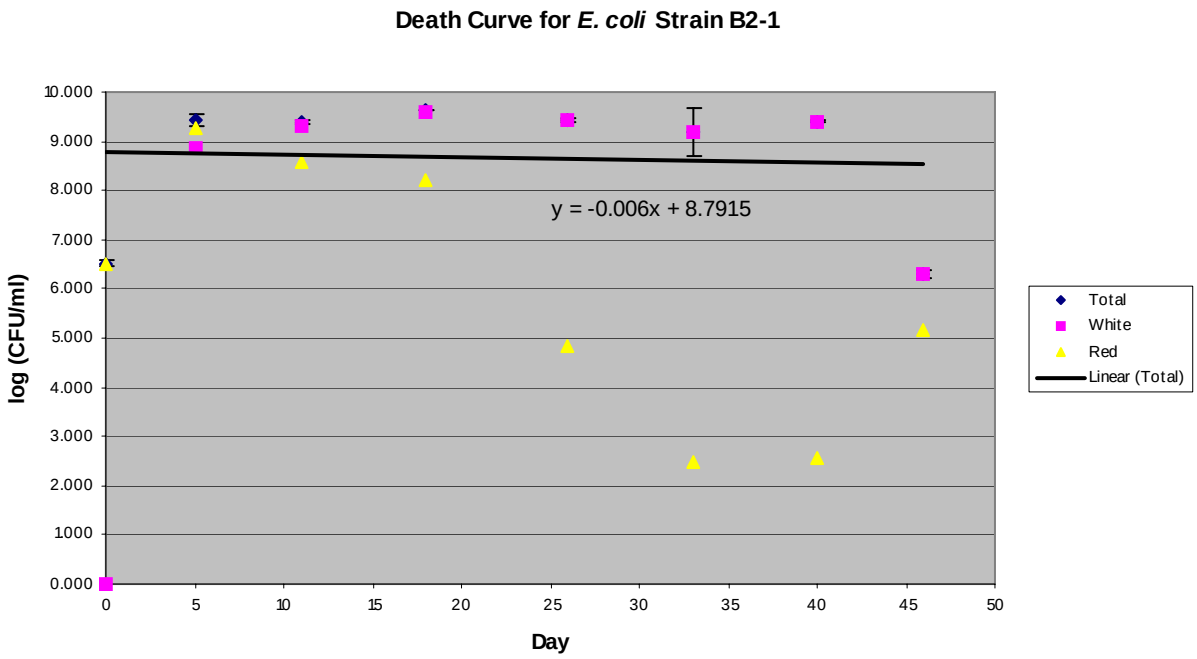
Graph 2: Survival of the Group B1 river isolate *E. coli* strain 6728-1 over 46 days in a simulated nanopure water system.

With a linear regression slope of approximately zero, B1-3 was able to maintain itself at a fairly constant level through the course of the experiment. The slope indicates only a roughly 0.1 log decline from day 0 to day 46. White colonies were noted in higher numbers from day 6 onward, while the number of red colonies steadily declined over time.



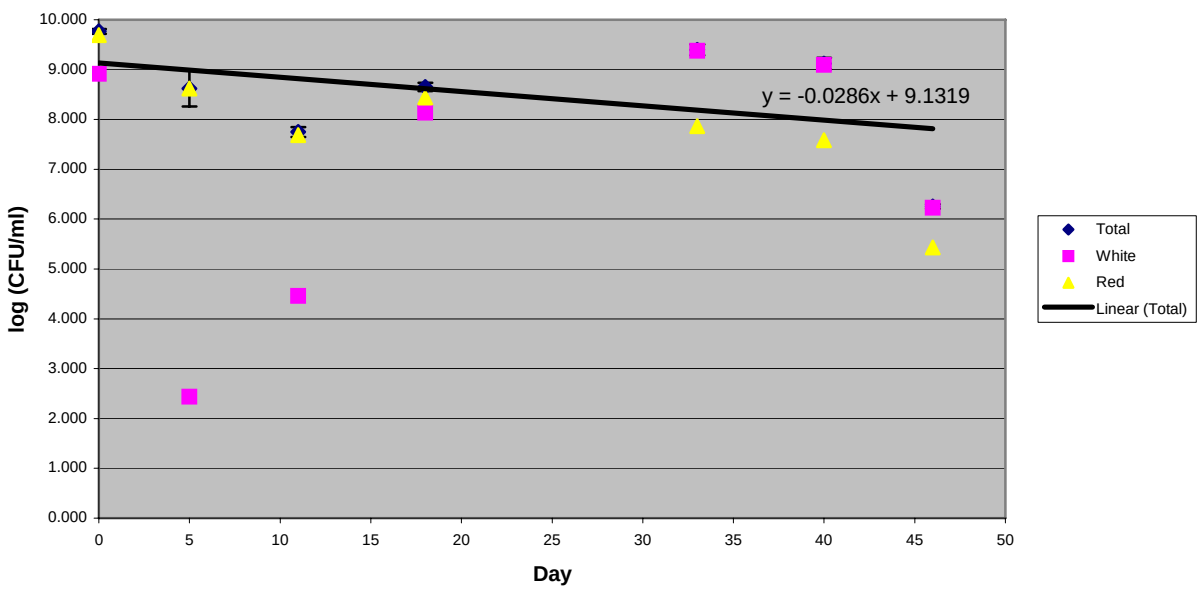
Graph 3: Survival of the Group B1 river isolate *E. coli* strain 6725-3 over 46 days in a simulated nanopure water system.

Graph 3 is indicative of only one of two strains sampled that actually increase its viable cell count during the course of the experiment with a positive linear regression slope. Clearly, B1-4 was able to maintain itself in the limiting environment. While numbers seemed to decrease in the first few weeks, a notable rebound occurred after white colonies were observed. White colonies were only noted in higher numbers halfway through the experiment, and red colonies were present to a high degree at all samplings.



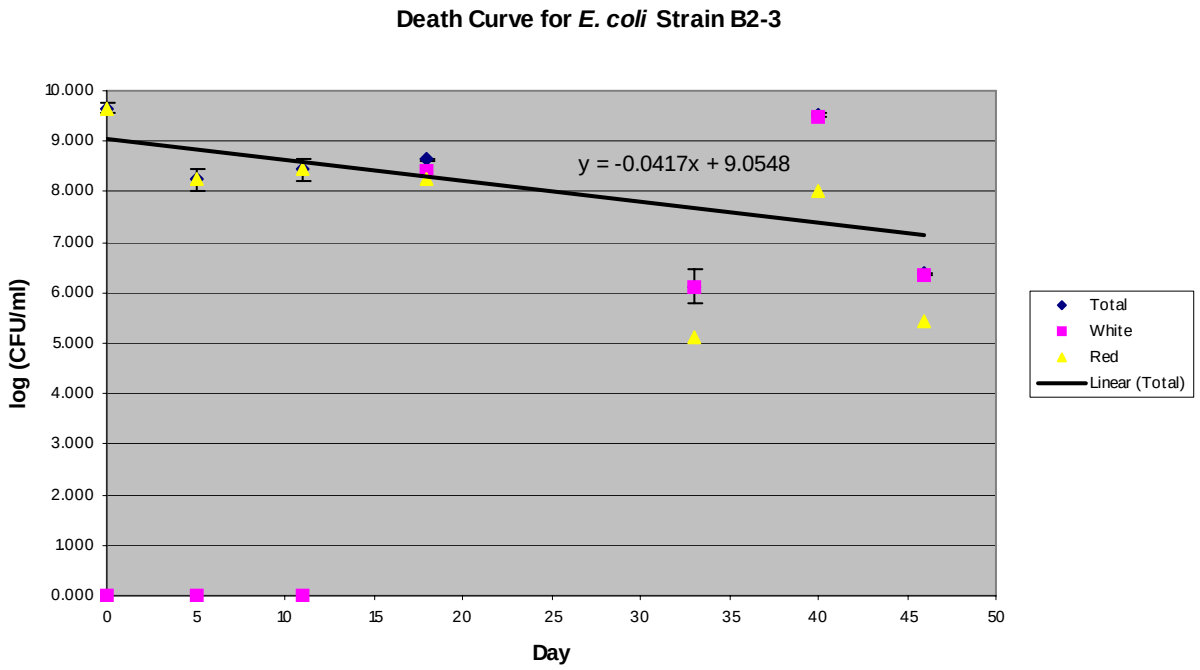
Graph 4: Survival of the Group B2 river isolate *E. coli* strain 6731-2 over 46 days in a simulated nanopure water system.

With a linear regression slope of approximately zero, B2-1 was able to maintain itself at a fairly constant level through the course of the experiment. Despite oddly low numbers on the first and last day of samplings, the overall slope indicates only a roughly 0.1 log decline from day 0 to day 46. White colonies were noted in higher numbers from day 6 onward, while the number of red colonies steadily declined over time.



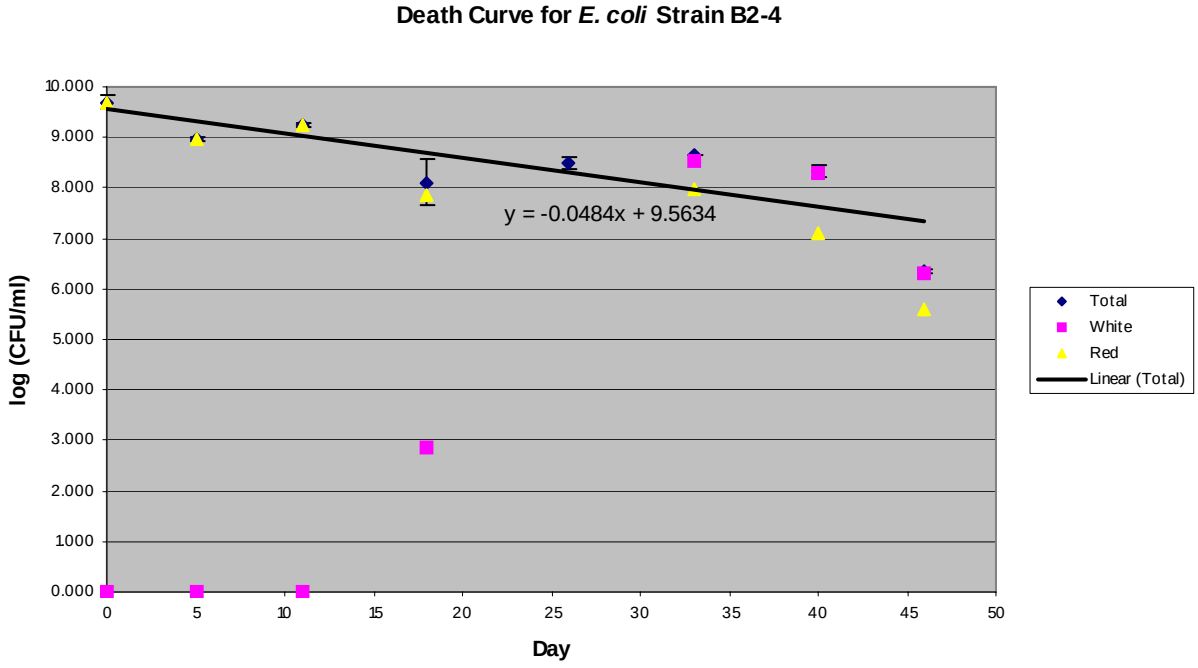
Graph 5: Survival of the Group B2 river isolate *E. coli* strain 6731-3 over 46 days in a simulated nanopure water system.

Though the viable counts were observed to both increase and decrease at different times of the experiment, graph 5 shows an overall linear regression slope of –0.0286 for B2-2 that is slightly greater than the experimental boundary of –0.0215. This corresponds with a consistent decline in viable cells present by over 10% during the 46 day study. White colonies were noted in significant numbers around day 18, and red colonies consistently high until the end of the experiment.



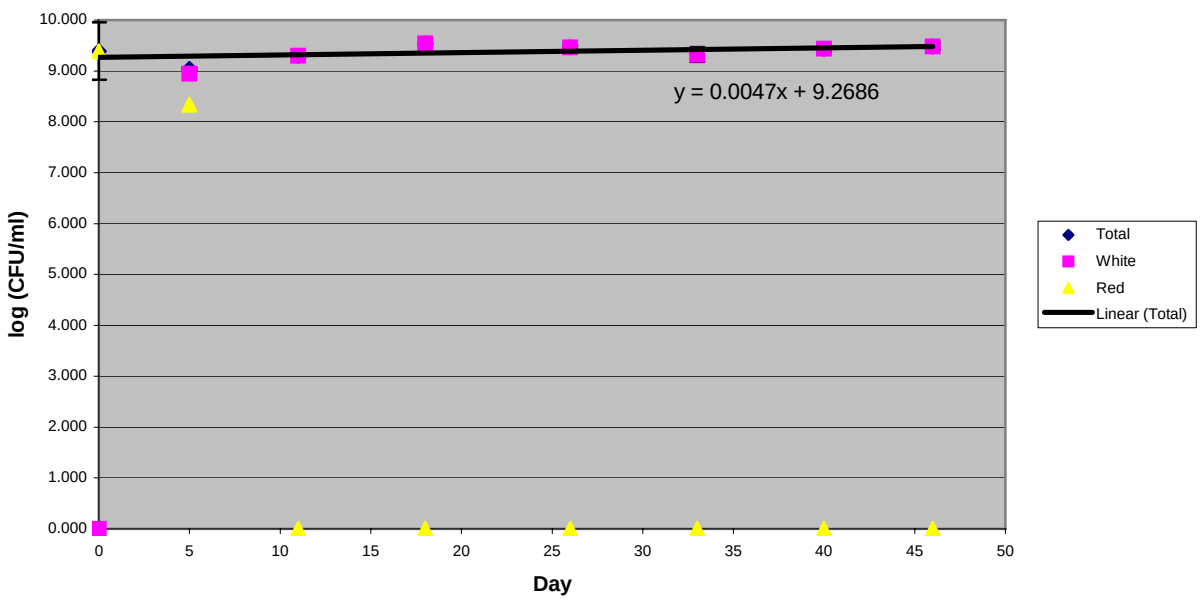
Graph 6: Survival of the Group B2 river isolate *E. coli* strain 6726-1 over 46 days in a simulated nanopure water system.

Though the viable counts were both up and down towards the end of the experiment, graph 6 shows an overall linear regression slope of almost double the experimental boundary of –0.0215. This corresponds with a relatively significant decline in viable cells present by approximately 2 logs over the 46 day study. White colonies were noted in high numbers around day 18, and red colonies were noted in steadily declining numbers through the course of the experiment.



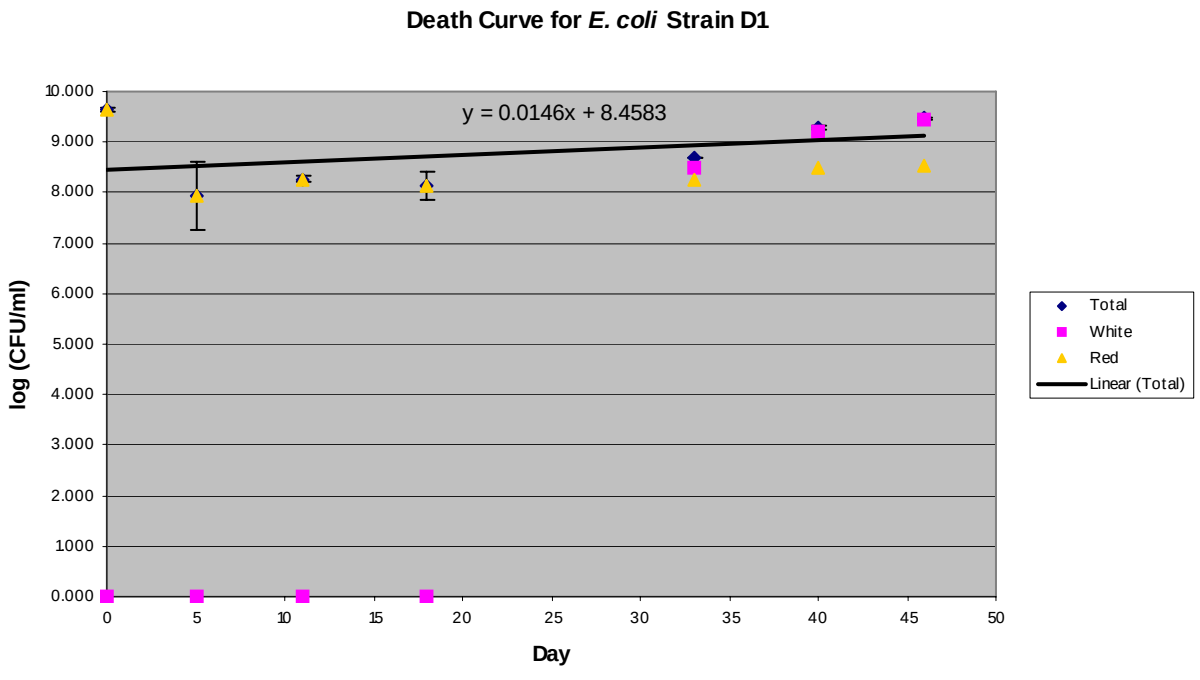
Graph 7: Survival of the Group B2 river isolate *E. coli* strain 6730-3 over 46 days in a simulated nanopure water system.

Graph 7 shows a linear regression slope of more than double the experimental boundary of –0.0215. This corresponds with a relatively significant decline in viable cells present by over 2 logs during the 46 day study. White colonies were noted from in high numbers around day 18, and red colonies were noted in steadily declining numbers through the course of the experiment.



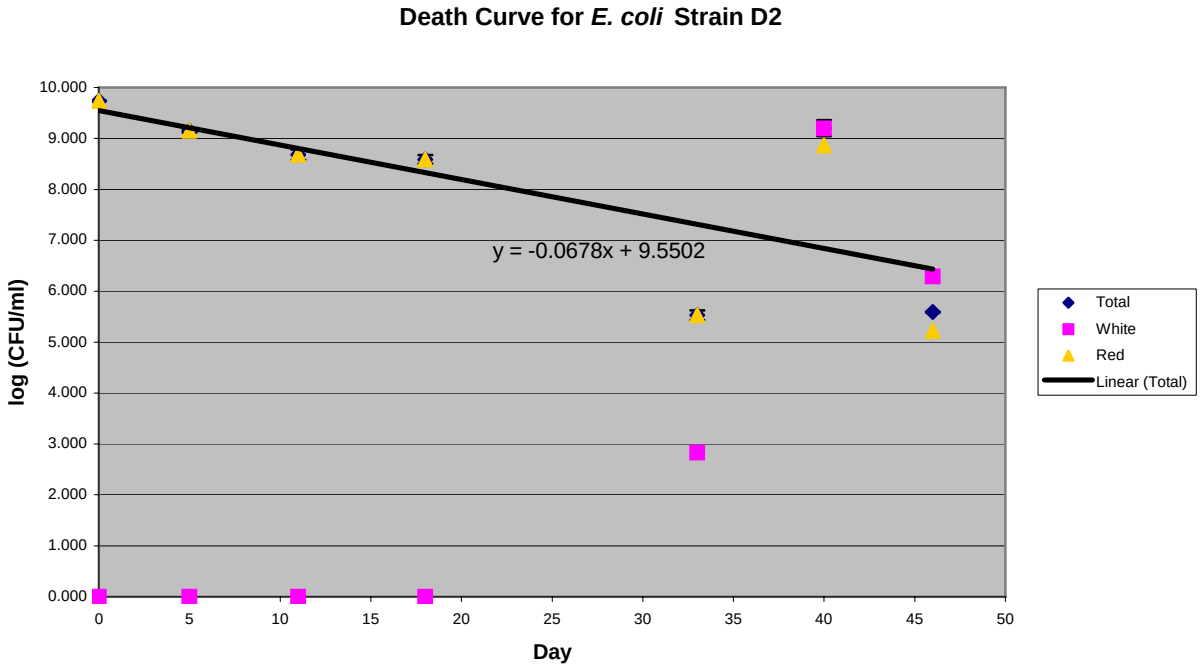
Graph 8: Survival of the Group B2 laboratory isolate *E. coli* strain F18 over 46 days in a simulated nanopure water system.

Graph 8 is notably only one of two strains sampled that exhibits an increase in its viable cell count during the course of the experiment as displayed by a positive linear regression slope. Clearly, B2-5 was able to maintain itself well during the course of the experiment. White colonies were noted in higher numbers from day 5 onward, and red colonies were nonexistent after day 5.



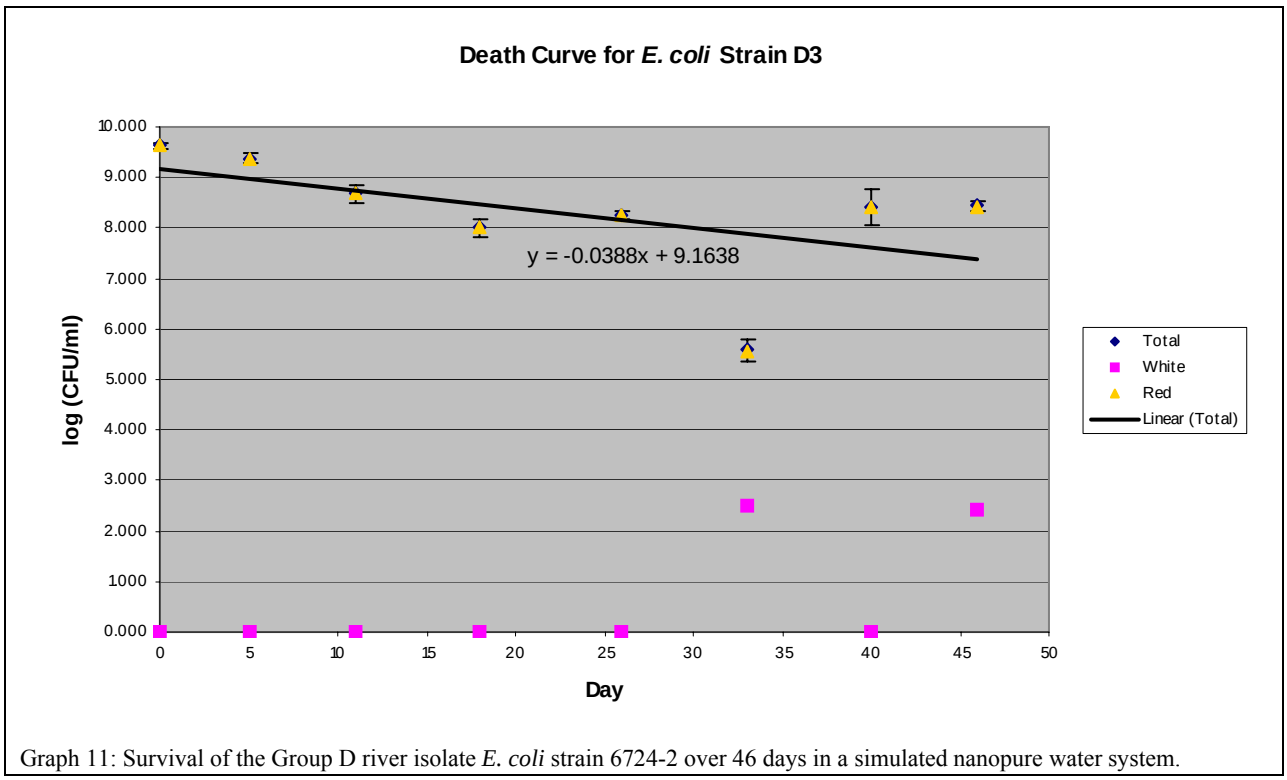
Graph 9: Survival of the Group D laboratory isolate *E. coli* strain EDL933 over 46 days in a simulated nanopure water system.

Though viable counts showed some outliers during the course of the experiment, graph 9 shows an overall linear regression slope of well above the experimental boundary of –0.0215. This corresponds with a relatively high significant maintenance in viable cells present during the 46 day study. White colonies were noted in high numbers only towards the end of the study, and red colonies were noted throughout the study.



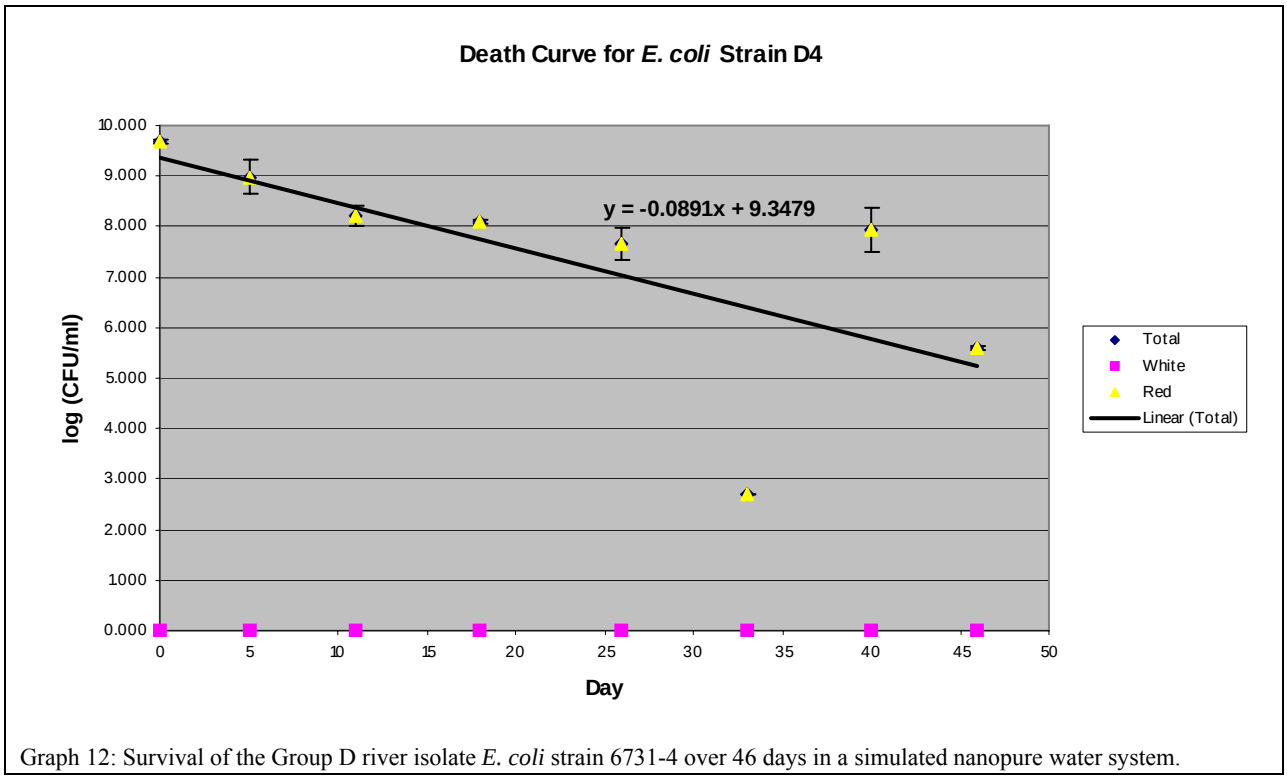
Graph 10: Survival of the Group D river isolate *E. coli* strain 6728-5 over 46 days in a simulated nanopure water system.

Noted in graph 10 is a linear regression slope that is well over triple the experimental boundary of –0.0215. This value indicates a relatively high significant decline in viable cells present by over 3 logs during the 46 day study. Without the outlier of a high count on day 40, the slope would be even more negative. White colonies were noted in high numbers only towards the end of the study, and red colonies were noted throughout the study.



Graph 11: Survival of the Group D river isolate *E. coli* strain 6724-2 over 46 days in a simulated nanopure water system.

Evident in graph 11 is a linear regression slope that is well above the experimental boundary of –0.0215. This corresponds with a relatively significant decline in viable cells present by nearly 2 logs during the 46 day study. However, if we throw out the outlier from day 33, a slightly more positive slope would be obtained. White colonies were never noted in high numbers, and red colonies were noted throughout the study.



Graph 12: Survival of the Group D river isolate *E. coli* strain 6731-4 over 46 days in a simulated nanopure water system.

Graph 12 shows the most negative linear regression slope of all twelve strains sampled. The slope is over quadruple the experimental boundary of –0.0215. This corresponds with an extremely high significant decline in viable cells present by almost half the initial number during the 46 day study. However, if we throw out the outlier from day 33, a more positive slope would be observed. White colonies were never noted, and red colonies were noted in steadily declining numbers throughout the study.

Group Analysis.

The strains sampled from group B1 do not exhibit a clearly shared trend when subjected to stress in our nanopure water system. B1-2 declined steadily, while B1-3 and B1-4 were able to maintain overall cell counts through the course of the 46 day experiment. And even though B1-3 and B1-4 both survived well, cell counts show a different trend of persistence. B1-3 maintained steady cell counts at nearly every week, while B1-4 declined over the first few weeks only to recover consistently after day 26 until the end of the experiment. The three strains were also markedly different in expression of the supposed lac^- white colonies. B1-2 showed only white colonies throughout the study with the absence of red colonies, B1-3 plated a majority of white colonies after day 5 with a fairly consistent drastic reduction in red colonies, and B1-4 showed a steady increase in the number of white colonies with a steady decrease in the number of white colonies. Clearly, the three strains sampled were highly different in their mode of survival, implying dissimilar response mechanisms.

While there were no apparent shared features of survival in group B2 on the whole, some similarities between individual strains that were observed. Both B2-1 and B2-5 persisted quite well when compared to other strains, maintaining cell counts to a high degree during the experiment. In addition, both strains showed a majority of white colonies after day 5. While this may be indicative of a shared stress response mechanism that is relatively successful when compared to their counterparts, it is possible that the strains are actually identical. Further studies will be necessary to determine if the strains are different. Such research may include sequencing of each strain's 16s rRNA. In the other three strains sampled from group B2, a steady decline in viable cell counts was noted. However, B2-2 declined at a rate of approximately half that of B2-3 and B2-4. In this case, B2-3 and B2-4 showed highly similar cell counts through the course of the experiment, with a decline of approximately 2 logs during the 46 day experiment. In addition, white colonies only became the majority of the population around the halfway point of the experiment in both strains. Again, this could imply a similar stress response, or that the strains are the same. Further tests are necessary to make such a distinction.

Group D showed the most overall trend in survival, with three out of the four strains exhibiting a significant decline in cell counts at varying degrees during the course of the experiment. In addition, each strain showed a majority of red colonies until late in the experiment, when both D1 and D2 began to plate a majority of white colonies. D3 and D4, on the other hand, maintained the majority of red colonies throughout the 46-day period. As stated before, these similarities may indicate an analogous response to stress conditions. However, the rate at which the different strains declined was highly variable, and no two strains exhibited a highly similar regression. Only D1 was able to maintain its viable cell counts over six weeks of incubation.

Overall, there seems to be no apparent similarity within the river isolates. Three out of the ten of the river isolates (B1-3, B1-4, and B2-1) displayed the ability to maintain viable cell counts through the course of the experiment, but none accomplished this feat through a similar survival pattern of viable cell counts from week to week. And because only two laboratory

strains were sampled, far-reaching conclusions can only be speculated. Both lab strains, B2-5 (F18) and D1 (EDL933) were able to persist to a relatively high degree during the experiment, even though the tracking of their persistence was markedly different with B2-5 exhibiting a consistent maintenance, whereas D1 showing initial decline followed by recovery. Thus it is possible that the mechanism by which the two strains persist is very different. It can be hypothesized that the lab strains are better equipped to handle stress because they have not been isolated in a weakened state, as is the case with the river isolates. It is also possible that the known presence of virulence factors in these two strains may contribute to their persistence.

Check for Contamination.

In order to make sure that the white colonies were indeed *E. coli*, a number of tests were performed. First, Gram stains were conducted on fresh red and white colonies that had been isolated from the final day 46 plating. Unfortunately, the Gram stains were inconclusive due to the inability to clearly visualize the cells. The cells were far too small to discern the difference between Gram positive and Gram negative. This is likely a result of the stress response, as the cells are possibly trying to conserve energy. It was, however, of noted interest that when the MacConkey plates containing the white colony quadrant streaks were incubated in the freezer over a weekend, the white culture had reverted back to the normal red color of a lactose fermenting bacteria. This implies that the change in phenotype is more likely contributed to a short-term repression of genes rather than a permanent mutation. It is possible that some operons that are commonly turned on during exponential growth are repressed during the stress response in order to maximize survival. When the cells are placed from a starved environment into more pleasant conditions, a certain amount of time may be necessary before the cells can adapt and change their expression.

Because Gram stains were inconclusive, we decided to return to triplex PCR techniques that would prove the white colonies as identical to their red counterparts. This final round of triplex PCR definitively proved that the white colonies were indeed *E. coli*, as expected banding patterns were observed after agarose gel electrophoresis. In addition, samples from the unaltered control tubes of each strain were sampled by serial dilutions and subsequent plating in order to compare results with the working tubes. Again, similar results were observed with the growth of both red and white colonies. These results prove that we did indeed have *E. coli* rather than contamination.

V. Conclusions

The experiment shows that there is no significant correlation between phylogenetic group and persistence of *E. coli* when subjected to starvation conditions in nanopure water. In general terms, the experiment shows that group D is most likely to decline over time, with three out of the four strains sampled unable to persist well during the 46 day period. On the other hand, group B2 showed the best survival rates, with two out of the five strains sampled capable of persisting to a relatively high degree during the experiment. Future studies will have to focus on a greater number of strains in all phylogenetic groups in order to make more meaningful conclusions, including strains from group A that were not available at the time. Though there were some noted similarities in decline between different strains, it is possible that the strains are identical. Further tests are necessary to determine whether the strains are indeed unique, and

such research could include the sequencing of 16s rRNA. This type of definitive identification would also eliminate duplication of results that would skew statistical information. While lac$^-$ phenotypes were noted from the first day of the experiment, it is possible that the lac operon was merely repressed and not mutated. This was evident in the white colony isolate plates that turned red after a few days. Furthermore, no discernable pattern was observed between the formation of white colonies and persistence. It is interesting to note that the two laboratory strains sampled, B2-5 (F18) and D1 (EDL933), were two of the five strains that were able to persist to a relatively high degree during the experiment. While this may be merely circumstantial data due to a lack of representation of lab strains, it could be indicative of the river strains isolated in a weakened state when compared to the lab strains, or the presence of known virulence factors in these strains might confer resistance to stress conditions. If the latter is true, then the three persisting river strains may also combat starvation in a similar manner. This trait could be shown by searching for pathogenicity islands using genome sequencing and analysis or pathogenicity island-directed PCR techniques. In addition to the repetition of this study with more strains and the search for virulence factors in the future, microarray analysis of specific genes associated with RpoS during the course of the experiment would be helpful in uncovering the specific mode of RpoS function as the general stress master regulator.

At this time, other research regarding *E. coli* persistence in water systems is being conducted, though the specific goals of these studies vary from ours. Recently, it was observed that *E. coli* incurred widespread damage to cell structure with a large, empty space forming between the cell wall and membrane after just a day of incubation in water (14, 25). While the shrinkage of cell volume found in this study concurs with our observed sizes, the implied major damage conflicts with our findings of consistent viability lasting over a much more extended period of time. In other reviews, starvation over an extended period of time in a water environment displayed a general expulsion of used substrates like dissolved amino acids and carbohydrates in a transition from a culturable state to a viable but non-culturable (VBNC) state (1, 14). This finding of the VBNC state during starvation in water was strengthened by independent studies. In one analysis that lasted over sixty days, only 0.7-5% of the initial *E. coli* population could be detected using standard cultivation methods while another 17-49% was detected using cultivation-independent methods (6, 14). These studies exhibit yet another factor that may complicate the future study of survival in water systems.

VI. Acknowledgements

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Information Transfer Program

Activities for the efficient transfer and retrieval of information are an important part of the OWRRI program mandate. The Institute maintains a website on the Internet at URL http://environ.okstate.edu/owrri that provides information on the OWRRI and supported research. The site provides links to information on publications of the Institute, grant opportunities and deadlines, and any upcoming events. Abstracts of technical reports and other publications generated by OWRRI projects are updated regularly and are accessible on the website.

The OWRRI produces a quarterly newsletter entitled "The Aquahoman" to disseminate research results and provide information on upcoming events and grant competitions.

The OWRRI also sponsors a water research symposium in the fall of each year at which OWRRI sponsored projects are presented. In addition, to keep state water professionals apprised of our work, updates on current-year projects are presented at the OWRRIs Water Research Advisory Board, which consists of representatives from 15 state and federal water agencies, and non-government organizations. As a result of that meeting, three of the OWRRI presenters were invited to address the Oklahoma Water Resources Board about their research.

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 NCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	11	0	0	0	11
Masters	10	0	0	0	10
Ph.D.	5	0	0	0	5
Post-Doc.	0	0	0	0	0
Total	26	0	0	0	26

Notable Awards and Achievements

In 2005, OWRRI continued its emphasis on expanding its outreach efforts. This has taken several forms. The annual water conference was held in Tulsa and attended by over 100 researchers and agency personnel. This meeting provides a much-needed opportunity for professionals to learn about recent water research in the state.

In January, OWRRI assembled its first Water Research Advisory Board (WRAB). The WRAB brings together representatives of state and federal agencies and NGOs with an interest in water research to learn about the current OWRRI research, set priorities for the following years competition, and recommend proposals for funding in the ensuing year. This inaugural meeting was a significant success. Several attendees mentioned that not only did they benefit from hearing the presentations but also from the opportunity to discuss issues with the other water agencies in the state. This lead to two outcomes: (1) three of the OWRRI researchers were invited to present their work to the Oklahoma Water Resources Board (OWRB) and (2) the OWRRI, OWRB, and USGS made plans to hold quarterly water forums among water agencies to discuss common issues.

One of the 2004 projects which was extended through 2005 (final report included herewith), Springs in Time: Comparison of Present and Historical Flows deserves particular mention. This project analyzed groundwater levels in 429 wells throughout the state that had at least 20 years of uninterrupted records. Contrary to expectations, the researchers found that 58% of the records showed increasing water elevations. Only 25% showed decreasing water levels; nearly all of these are located in the panhandle. Increased precipitation since 1970 is sufficient to account for the increased groundwater levels. These findings are very interesting and warrant further investigation as they have significant implications for agriculture and attracting new industry to the state, especially in light of the predicted reduction in precipitation throughout the southwest for the coming decades.

Publications from Prior Projects

None