

ACKNOWLEDGEMENTS

First I would like to sincerely thank my advisor Dr. Charles Hagedorn for his guidance and support of my graduate studies here at Virginia Tech. He pushed me to become a critical thinker and to develop my skills as an environmental scientist. I would also like to thank the members of my committee, Dr. Tamim Younos and Dr. Carl Zipper for their support and for helping me to expand the scope of my thoughts and the limits of my knowledge.

I would also like to thank the folks at the Occoquan Monitoring Lab (Civil and Environmental Engineering Department, Virginia Tech), who have supported the project through sample collection, and background data collection. Without them, this project would not have run as smooth. I would especially like to thank Harry Post, and those that worked directly on this project, Mark, Doug, and Phil.

I would like to thank staff from the Metropolitan Washington Council of Governments, Muhktar Ibrahim and TJ Murphy. Without their support, this project would not have been possible.

Known sample collection could not have been completed without the help of folks from DCR and from the Metropolitan Washington Council of Governments. Their work was much appreciated in the initial stages of this project.

Dr. Bruce Wiggins from James Madison University was also very gracious of his time and talents as he aided me with statistical analysis of portions of my data. Communications with him allowed my project to take on a direction that expanded the limits of the current information and research available for antibiotic resistance analysis.

I would like to help all those who gave me assistance in the laboratory. Annie Chapman, and Laura Stromberg. I would also like to thank Alexandria Graves for showing me the ropes of graduate school life.

Finally, I would like to thank my family and friends for their continued support, and for many painstaking hours of critiquing my work and providing advice.

TABLE OF CONTENTS

		Page
ABSTRACT.....		ii
ACKNOWLEDGEMENTS.....		iv
LIST OF TABLES.....		viii
LIST OF FIGURES.....		xii
CHAPTER I	INTRODUCTION.....	1
	Study Rationale.....	1
	Objectives.....	5
	References.....	8
CHAPTER II	LITERATURE REVIEW.....	10
	Total Maximum Daily Load Studies.....	10
	Bacterial Source Tracking.....	12
	Summary.....	29
	References.....	30
CHAPTER III	ANTIBIOTIC RESISTANCE ANALYSIS: ISSUES.....	34
	RELATED TO LIBRARY SIZE, LIBRARY EPRESENTATIVENESS, AND APPROPRIATE STATISTICAL MODELING	
	Introduction.....	34
	Materials and Methods.....	38
	Results and Discussion.....	42
	Summary.....	48
	References.....	50
CHAPTER IV	DETERMINING SOURCES OF FECAL.....	51
	CONTAMINATION IN THE POTOMAC RIVER, THE ANACOSTIA RIVER, AND ROCK CREEK USING ANTIBIOTIC RESISTANCE ANALYSIS	
	Introduction.....	51
	Materials and Methods.....	54
	Results and Discussion.....	58
	Summary.....	89
	References.....	94
CHAPTER V	EXPANDING THE LIMITS OF ARA.....	96
	Introduction.....	96
	Results and Discussion.....	99
	Summary.....	111
	References.....	115
CHAPTER VI	SUMMARY AND CONCLUSIONS.....	116

APPENDIX A	Background Information	121
APPENDIX B	Bacterial SourceTracking Results by Station.....	131
VITA		144

LIST OF TABLES

Chapter	Title	Page
CHAPTER I	1. EPA Recommended Bacterial Standards.....	2
CHAPTER III	1. Antibiotic Stock Solution Preparations.....	40
	2. Antibiotic Stock Concentrations After Addition..... to TSA	41
	3. Potomac River known source library.....	43
	4. Anacostia River known source library.....	43
	5. Rock Creek known source library.....	44
	6. Potomac Library RCC's from Random..... Assignments of Isolate Profiles to Sources	46
	7. Anacostia Library RCC's from Random..... Assignments of Isolate Profiles to Sources	47
	8. Rock Creek Library RCC's from Random..... Assignments of Isolate Profiles to Sources	47
	9. Test Results for Library Representativeness.....	48
CHAPTER IV	1. Sampling Schedule for the Potomac River,..... the Anacostia River, and Rock Creek	56
	2. Antibiotic plate concentrations for analysis of..... unknown stream isolates	57
	3. Percent of sampling events violating water quality..... standards by station on the Potomac River	60
	4. Percent of sampling events violating water quality..... standards by station on the Anacostia River	61
	5. Percent of sampling events violating water quality..... standards by station on Rock Creek	62
	6. Temperature and pH Data for the Potomac River by..... Sampling Station	63

Chapter	Title	Page	
CHAPTER IV	7. Temperature and pH Data for the Anacostia River by..... Sampling Station	63	
	8. Temperature and pH Data for Rock Creek by..... Sampling Station	63	
	9. Minimum Source Contributions for Significance..... in the Potomac	66	
	10. Percent of Source Contributions Below Minimum..... Detection Limits in the Potomac	66	
	11. Sampling Site Descriptions for the Potomac River.....	67	
	12. Source Distributions at Each Sampling Station on the.... Potomac as a Ten-Month Average	67	
	13. Minimum Source Contributions for Significance..... in the Anacostia	74	
	14. Percent of Source Contributions Below Minimum..... Detection Limit in the Anacostia	74	
	15. Sampling Site Descriptions for the Anacostia River.....	75	
	16. Source Distributions at Each Sampling Station on the Anacostia as a Ten-Month Average	76	
	17. Minimum Source Contributions for Significance in Rock Creek	82	
	18. Percent of Source Contributions Below..... Minimum Detection Limit in Rock Creek	83	
	19. Sampling Site Descriptions for Rock Creek.....	83	
	20. Source Distributions at Each Sampling Station..... on Rock Creek as a Ten-Month Average	84	
	CHAPTER V	1. Source Distributions (%) with Unknown Categories..... for the Potomac River	100
		2. Source Distributions (%) with Unknown Categories..... for the Anacostia River	100

Chapter	Title	Page
CHAPTER V	3. Source Distributions (%) with Unknown Categories.....	100
	for Rock Creek	
	4. Potomac Library Comparison of RCC's from.....	104
	DA and LR Models	
	5. Anacostia Library Comparison of RCC's from.....	104
	DA and LR Models	
	6. Rock Creek Library Comparison of RCC's from.....	104
	DA and LR Models	
	7. Potomac Source Distributions with DA and LR.....	107
	8. Anacostia Source Distributions with DA and LR.....	108
	9. Rock Creek Source Distributions with DA and LR.....	108
	10. Elimination of Duplicate Patterns in the Anacostia.....	109
11. Elimination of Duplicate Patterns in the Potomac.....	109	
12. Elimination of Duplicate Patterns in Rock Creek.....	110	
APPENDIX A	1. Potomac River Library Source Classification.....	129
	Numbers	
	2. Anacostia River Library Source Classification.....	129
	Numbers	
	3. Rock Creek Library Source Classification.....	130
Numbers		
4. Percent of Isolates Below Detection Limits in.....	130	
Potomac River Library Determined by Expected		
Frequency of Misclassification and Minimum		
Detectable Percentage		
5. Percent of Isolates Below Detection Limits in.....	130	
Anacostia River Library Determined by Expected		
Frequency of Misclassification and Minimum		
Detectable Percentage		

Chapter	Title	Page
APPENDIX A	6. Percent of Isolates Below Detection Limits in Rock Creek Library Determined by Expected Frequency of Misclassification and Minimum Detectable Percentage	130
APPENDIX B	1. Significant Differences of Mean Source Distributions on the Potomac	131
	2. DA Significant Difference of Mean Source Distributions on the Potomac	133
	3. Significant Differences of Mean Source Distributions on the Anacostia River	134
	4. DA Significant Differences of Mean Source Distributions on the Anacostia River	138
	5. Significant Differences of Mean Source Distributions on Rock Creek	139
	6. DA Significant Differences of Mean Source Distributions on Rock Creek	143

LIST OF FIGURES

Chapter	Title	Page
CHAPTER IV	1. Ten Month Average of Source Contributions..... from all stations on the Potomac River	65
	2. Dry and Wet Season Source Contributions on..... the Potomac River	69
	3. Average Source Contributions on the Potomac..... in the Months Surrounding Storm Events	71
	4. Average Source Contributions on the Potomac..... in the Months Surrounding a Winter Storm Event	72
	5. Ten-Month Average of Source Contributions..... from all stations on the Anacostia River	73
	6. Dry and Wet Season Source Contributions on..... the Anacostia River	77
	7. Average Source Contributions on the Anacostia..... in the Months Surrounding Storm Events	79
	8. Average Source Contributions on the Anacostia..... in the Months Surrounding a Winter Storm Event	80
	9. Average of Source Contributions from all..... stations on the Rock Creek	81
	10. Dry and Wet Season Source Contributions..... on Rock Creek	85
	11. Average Source Contributions on Rock Creek..... in the Months Surrounding Storm Events	87
	12. Average Source Contributions on Rock Creek..... in the Months Surrounding a Winter Storm Event	88
CHAPTER V	1. Source Distributions on the Potomac Before..... and After Exclusion of Isolates with < 80% Similarity to Known Source Profiles	101

Chapter	Title	Page
CHAPTER V	2. Source Distributions on the Anacostia Before..... and After Exclusion of Isolates with < 80% Similarity to Known Source Profiles	101
	3. Source Distributions on Rock Creek Before..... and After Exclusion of Isolates with < 80% Similarity to Known Source Profiles	102
	4. Potomac Source Distributions with DA and LR.....	105
	5. Anacostia Source Distributions with DA and LR.....	105
	6. Rock Creek Source Distributions with DA and LR.....	106
	APPENDIX A	1. Sampling Locations for Rock Creek and..... the Potomac River
2. Sampling Locations for the Anacostia River.....		122
3. Monthly and Storm Event Enterococci Counts..... for the Potomac River		123
4. Monthly and Storm Even Fecal Coliform Counts..... For the Potomac River		123
5. Monthly and Storm Event Enterococci Counts..... for the Anacostia River		124
6. Monthly and Storm Even Fecal Coliform Counts..... for the Anacostia River		124
7. Monthly and Storm Event Enterococci Counts..... for Rock Creek		125
8. Monthly and Storm Even Fecal Coliform Counts..... For the Rock Creek		125
9. Relating Flow to Indicator Organism Densities.....		126
10. Precipitation Totals at Reagan National Airport.....		126
11. Fecal Coliform and Enterococci Densities in the..... Potomac as a Function of Precipitation Between Sampling Events		127

Chapter	Title	Page
APPENDIX A	12. Fecal Coliform and Enterococci Densities in the Anacostia as a Function of Precipitation Between Sampling Events.....	127
	13. Coliform and Enterococci Densities in Rock Creek as a Function of Precipitation Between Sampling Events.....	128
APPENDIX B	1. Dry and Wet Season Source Contributions for Site FP01.....	131
	2. Dry and Wet Season Source Contributions for Site FP02.....	131
	3. Dry and Wet Season Source Contributions for Site FP03.....	132
	4. Source Contributions at Sampling Site FP01 in Months Surrounding Storm Events.....	132
	5. Source Contributions at Sampling Site FP02 in Months Surrounding Storm Events.....	132
	6. Source Contributions at Sampling Site FP03 in Months Surrounding Storm Events.....	133
	7. Dry and Wet Season Source Contributions for Site FA01.....	134
	8. Dry and Wet Season Source Contributions for Site FA02.....	134
	9. Dry and Wet Season Source Contributions for Site FA03.....	135
	10. Dry and Wet Season Source Contributions for Site FA04.....	135
	11. Dry and Wet Season Source Contributions for Site FA05.....	135
	12. Dry and Wet Season Source Contributions for Site FA06.....	136

Chapter	Title	Page
APPENDIX B	13. Source Contributions at Sampling Site FA01 in Months Surrounding Storm Events	136
	14. Source Contributions at Sampling Site FA02 in Months Surrounding Storm Events	136
	15. Source Contributions at Sampling Site FA03 in Months Surrounding Storm Events	137
	16. Source Contributions at Sampling Site FA04 in Months Surrounding Storm Events	137
	17. Source Contributions at Sampling Site FA05 in Months Surrounding Storm Events	137
	18. Source Contributions at Sampling Site FA06 in Months Surrounding Storm Events	138
	19. Dry and Wet Season Source Contributions for Site FR01	139
	20. Dry and Wet Season Source Contributions for Site FR02	139
	21. Dry and Wet Season Source Contributions for Site FR04	140
	22. Dry and Wet Season Source Contributions for Site FR05	140
	23. Dry and Wet Season Source Contributions for Site FR06	141
	24. Dry and Wet Season Source Contributions for Site FR07	141
	25. Source Contributions at Sampling Site FR01 in Months Surrounding Storm Events	141
	26. Source Contributions at Sampling Site FR02 in Months Surrounding Storm Events	142

Chapter	Title	Page
APPENDIX B	27. Source Contributions at Sampling Site FR04 in Months Surrounding Storm Events	142
	28. Source Contributions at Sampling Site FR05 in Months Surrounding Storm Events	142
	29. Source Contributions at Sampling Site FR06 in Months Surrounding Storm Events	143
	30. Source Contributions at Sampling Site FR07 in Months Surrounding Storm Events	143