URBAN BAYOU BACTERIA
SOURCE IDENTIFICATION STUDY

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URBAN BAYOU BACTERIA SOURCE IDENTIFICATION STUDY

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Linda Broach and Bob McElyea, TNRCC Region 12

David Whiteside, Steve Lewis, and John May, City of Houston, Health and Human Services Department

1.0 INTRODUCTION

Indicator bacteria such as *Escherichia coli* (EC) or fecal coliform (FC) are used to assess the suitability of a water body for contact recreation. Concentrations of these indicator bacteria in Houston-area bayous and other streams in southeast Texas have been documented to be among the highest in the state. In response, the Texas Natural Resource Conservation Commission (TNRCC) initiated a Total Maximum Daily Load (TMDL) study on two representative streams, Buffalo and White Oak Bayous, to determine the reasons for the high levels and to identify means to reduce the bacterial concentrations.

This project was designed to gather bacterial source data to support the TMDL study now being performed under contract to the TNRCC by the University of Houston and PBS&J. In brief overview, the project was designed to collect split water samples in the two bayous in coordination with the ongoing routine monitoring of the City of Houston and the TNRCC regional office (Region 12) in Houston. In this way all the water quality parameters analyzed in normal monitoring would be available, and the effort could focus on the unique source identification challenges. The split samples collected were analyzed by Dr. Shelley Payne and Dr. Ana-Maria Valle at the University of Texas at Austin. These water samples were analyzed for specific types of genetic material using Polymerase Chain Reaction (PCR). The genetic material analyzed included that for coliform bacteria, EC, two Bacteroides species that are closely associated with human sources, and a pathogen that is normally not from human sources. Together, these tests help determine the source of the high levels of bacteria that are reported in routine monitoring. The results were coordinated with the TNRCC's TMDL effort as they were obtained.

The main challenge facing the TMDL project is to understand the reasons for the high bacteria levels and if possible, the sources of the bacteria. In particular, it is important to determine the extent to which the bacteria are of human origin and thus correctable through specific actions. As has been documented (PBS&J, 2000) natural streams tend to exhibit low bacterial levels in dry weather, but high concentrations during runoff events when bacteria from many sources are washed from watersheds and transported in runoff. Streams in urban areas have additional potential sources from leakage and overflow of sanitary sewer systems, which can have a dramatic effect particularly under dry weather conditions.

Buffalo and White Oak Bayous are both highly urbanized streams, and sanitary sewer inputs may be major candidate sources of bacteria in the bayou during dry weather. However, there is an additional complicating factor in that essentially all of the dry weather flow in the two streams is from treated human sewage. For source identification to be effective, not only must there be a distinction between human waste and natural runoff, but also between treated and untreated human waste.

2.0 SAMPLING PROGRAM

The sampling program was intended to take maximum advantage of existing data collection efforts in the area. In this way most of the project resources could be concentrated on the source identification objective. Routine physical, chemical and microbiological parameter measurements are normally made on the split samples by the data collection agencies.

Two agencies were involved in the effort, the TNRCC and the City of Houston Department of Health and Human Services (HHS). Linda Broach was the sampler for the TNRCC and the City of Houston had dedicated personnel for each bayou.

White Oak Bayou – David Whiteside

Buffalo Bayou – Steve Lewis

Supervisor – John May

All of the routine monitoring station sampling was from bridges using a bucket to collect the sample. All sampling procedures for HHS followed the Clean Rivers Program Quality Assurance Project Plan (QAPP), and the TNRCC sampling followed similar requirements of the Surface Water Quality Monitoring Procedures Manual (TNRCC, 1999).

The basic procedure involved a PBS&J sampler picking up sterilized bottles from the UT Laboratory at Austin in the afternoon and driving to Houston. Early the next morning they would meet the agency sampling crew and travel together to the routine sites. At each site the agency person would conduct their normal sample collection and probe measurements and provide 1 liter of their bucket sample to the PBS&J crew. This sample would be stored on ice.

The end of the agency collection trip was typically at mid-day to allow time for samples to be processed at the laboratory. The PBS&J sampler would then do additional collections, usually at one of the City of Houston Wastewater Treatment Plants (WWTPs), and then drive back to Austin. The iced samples would be dropped off at the UT laboratory cold room with a UT lab technician signing for sample receipt.

The additional samples were typically of raw sewage, treated wastewater prior to disinfection, and treated wastewater after dechlorination. In addition to the wastewater samples, towards the end of the project samples were collected from chamber studies being conducted for the TMDL project directly.

Figure 2-1 contains a number of pictures taken during the monitoring process on both Buffalo and White Oak bayous. Several pictures illustrate the bucket sample collection work. Some of the

pictures were taken on the June 13 sampling trip, approximately one week after Tropical Storm Allison caused major flooding in the area. Others illustrate the chambers used for bacterial rate studies that are a part of the TMDL effort.

Before any sampling began, a QAPP was completed and approved by all involved agencies. A copy of this QAPP is on file with the HGAC and the TNRCC as well as the project team..

FIGURE 2-1 SAMPLING PHOTOGRAPHS

White Oak Bayou at Heights

Buffalo Bayou at Westcott

Chamber experiments

Buffalo Bayou below Barker Reservoir

FIGURE 2-1 SAMPLING PHOTOGRAPHS (CONTINUED)

Buffalo Bayou at Main

Buffalo Bayou at Main

White Oak Bayou at Studewood

Buffalo Bayou at Shepherd

3.0 MONITORING RESULTS AND DISCUSSION

The project was originally planned for sampling to begin in the March or April of 2000 and continue for a year, allowing time to complete a year of data collection and producing the report by July, 2001. For a variety of reasons, the first monitoring trip did not take place until 14 November, 2000. Sampling continued on a monthly basis as originally planned until May 2001, when it shifted to a semi-monthly schedule for the remainder of the summer. The final collection was on August 15.

In keeping with the overall study design, the station selection was left to the monitoring agency with each sampling run. Some sampling trips involved both bayous while others focused on one or the other. Also, some trips involved collections from one or more tributaries to the bayous. Figure 3-1 shows the locations of the sampling stations. Each station is listed within the Houston-Galveston Area Council (H-GAC) QAPP.

As data were obtained a master data table was constructed. The final version of this table is presented as Table 3-1. The table includes the conventional data collected along with the source information from UT, data on the bayou flow at a mid-point gage on each bayou, and antecedent rain at two representative rain gages. Note that the conventional chemical data were not available for the June 13 sampling because of flooding at the City of Houston laboratory.

Figure 3-2 shows the locations of the stream and rain gages employed. Table 3-2 lists the complete daily rain data from each rain gage. As can be seen by examining the table of daily rainfall data, there was a substantial degree of consistency between reported rainfall amounts at nearby rain gages.

3.1 DISCUSSION OF CONVENTIONAL SAMPLING RESULTS

The first sampling was on 11/14/00 with TNRCC and included stations on both Buffalo and White Oak bayous. After that sampling was with HHS and altered from one bayou to the next in the following months.

Buffalo Bayou Data

Data on Buffalo Bayou were collected on 11/14/00, 12/13/00, 2/21/01, 4/18/01, 6/13/01, 7/18/01, and 8/15/01. Briefly –

11/14 Rain occurred on 11/12 and flows were still fairly high on the 14th. Bacteria concentrations were still elevated at most Buffalo Bayou stations.

12/13 There was high bacteria levels and rain on day of collection.

FIGURE 3-1
LOCATION OF SAMPLING STATIONS

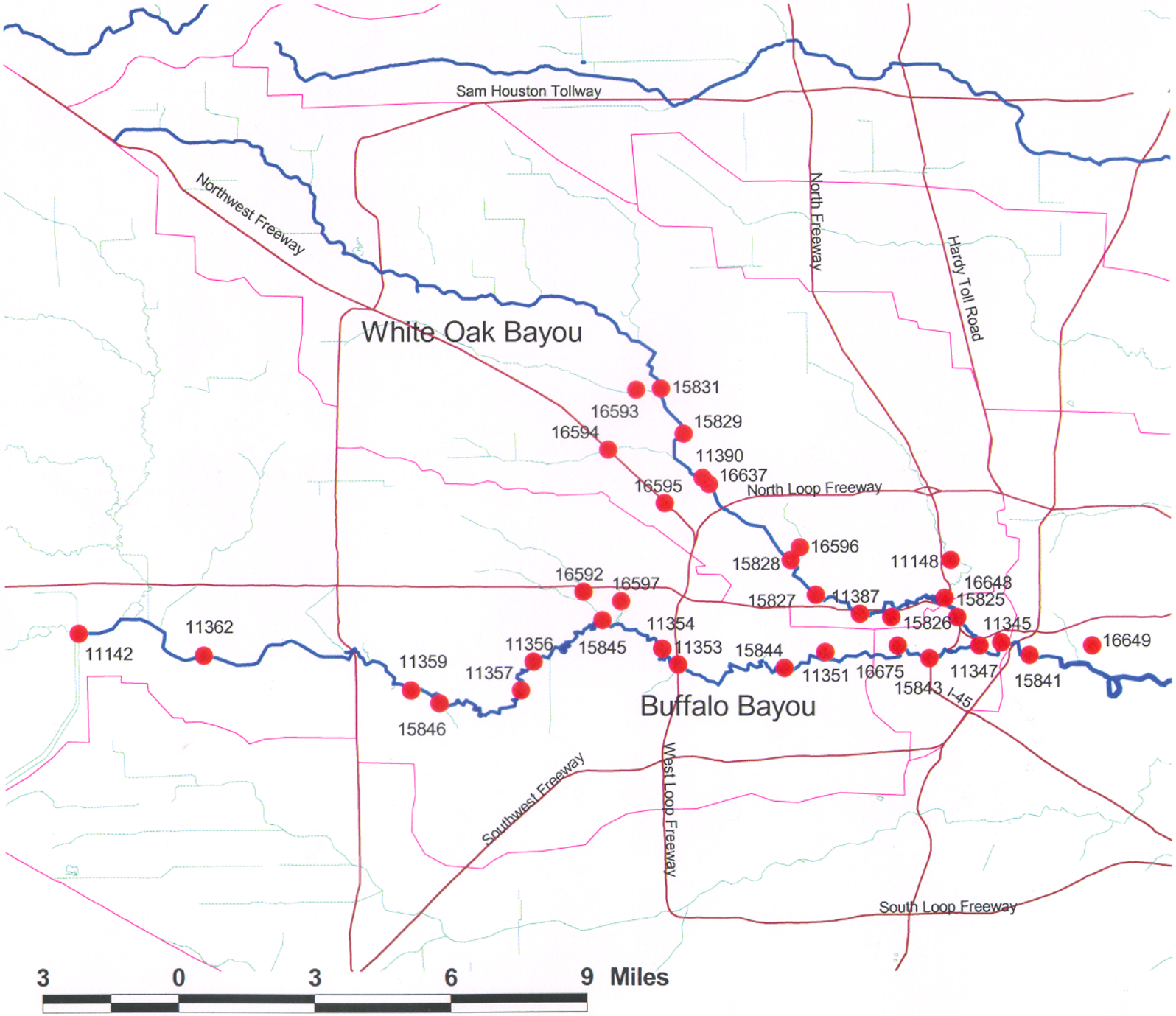


FIGURE 3-2
LOCATION OF RAIN AND FLOW GAGES

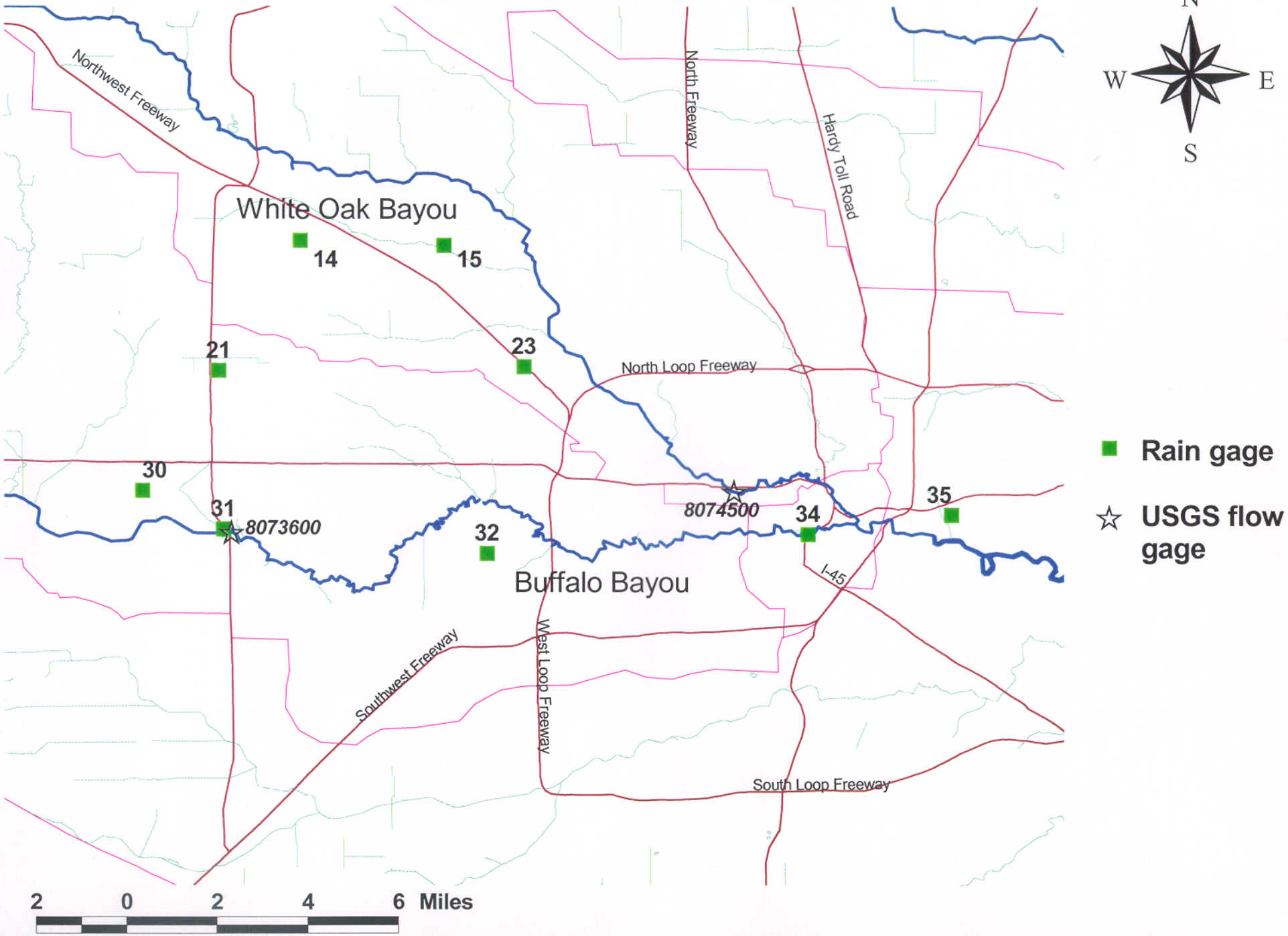


TABLE 3-1
PROJECT MASTER DATA TABLE

November 14, 2000 Station	Station Number	E. coli cfu/dL	FC cfu/dL	uidA lacZ	uid 1&3	uid 1&2	Bact. dist.	Bact. theta	Cam	TSS mg/L	VSS mg/L	NH_3-N mg/L	$NO_{2,3}$-N mg/L	TKN mg/L	PO_4-P mg/L	TP mg/L	TOC mg/L	TDS mg/L
Buffalo Bayou Tidal @ McKee Street	11345	1,968	21,000							140	18	0.13	1.05	1.50	0.31	*	7	207
Buffalo Bayou @ Woodway	11354	24,192	21,000							122	17	0.13	1.07	1.54	0.34	0.58	8	209
Buffalo Bayou @ Dairy Ashford	11362	17,329	17,100							103	13	0.11	0.96	1.40	0.32	0.52	8	197
White Oak @ Heights	11387	3,873	5,000			Pos.				44	7	0.20	2.13	1.20	0.46	0.56	6	287
White Oak @ Heights - Dup.	11387	3,088	3,400							47	8	0.20	2.13	1.22	0.45	0.56	6	314
White Oak @ Hogan	15825	3,609	1,640			Pos.				25	5	0.22	1.8	1.22	0.38	*	6	306
White Oak @ TC Jester	15828	5,475	2,250			Pos.				50	7	0.16	2.32	1.30	0.44	0.55	6	328
West District Raw Sewage				S. pos		Pos.	Pos.											
West District Effluent before Chlorination						Pos.												
West District Effluent after Dechlorination						Pos.												

December 13, 2000 Station	Station Number	E. coli cfu/dL	FC cfu/dL	uidA lacZ	uid 1&3	uid 1&2	Bact. dist.	Bact. theta	Cam	TSS mg/L	VSS mg/L	NH_3-N mg/L	Cond. µmhos/cm	pH SU	BOD mg/L	SO4 mg/L	Cl mg/L	TDS mg/L
Buffalo Bayou @ Loop 610	11353		43,000	S. pos		Pos.				115		0.24	308	7.8	9	14.7	34.8	232
Buffalo Bayou @Voss	11356		18,000	Pos.		Pos.	Pos.			180		0.38	520	7.7	5	25.3	71.9	364
Buffalo Bayou @ San Felipe	11357		10,000			Pos.				113		0.28	388	7.6	8	18.7	51.8	276
Buffalo Bayou @ Gessner	11359		35,000			Pos.				298		0.28	324	7.5	10	15.6	42.7	143
Buffalo Bayou @ Chimney Rock	15845		37,000	S. pos		Pos.	Pos.			99		0.19	414	7.6	8	19.4	48.1	281
Buffalo Bayou @ Briar Forest	15846		28,000	Pos.		Pos.				171		0.19	335	7.7	9	15.6	41.1	229
Neimans @ Memorial	16597		32,000			Pos.				59		0.19	112	7.8	10	6.4	4.7	114
Spring Branch @ Wirt	16592		39,000	Pos.		Pos.	Pos.			33		0.24	152	7.9	13	6.1	5.6	111

January 17, 2001 Station	Station Number	E. coli cfu/dL	FC cfu/dL	uidA lacZ	uid 1&3	uid 1&2	Bact. dist.	Bact. theta	Cam	TSS mg/L	VSS mg/L	NH_3-N mg/L	Cond. µmhos/cm	pH SU	BOD mg/L	SO4 mg/L	Cl mg/L	TDS mg/L
White Oak Bayou @ West 34th St	11390		10,000							47		0.095	297	7.52	<4	17.7	24.7	217
White Oak Bayou @ West 43rd St.	15829		5,700							49		0.107	300	7.53	<4	18	26.2	227
White Oak Bayou @ West Tidwell	15831		2,600							54		0.055	250	6.9	<4	17.2	20	222
White Oak Bayou @ West TC Jester	16637		16,000			Pos.				48		0.123	293	7.53	<4	17.5	24.4	223
Cole Creek @ Bolivia	16593		6,000			Pos.				31		0.093	316	7.16	<4	16.9	25.1	217
Brickhouse Gully @ US 290	16594		72,000							24		<0.05	322	7.54	<4	14.9	20.1	245
White Oak Trib @ US 290	16595		1,100							8		<.05	362	7.4	<4	17.7	17.4	220
Raw sewage				Pos.		Pos.	Pos.											
Pre-chlorination						Pos.												
Dechloronation																		

February 21, 2001 Station	Station Number	E. coli cfu/dL	FC cfu/dL	uidA lacZ	uid 1&3	uid 1&2	Bact. dist.	Bact. theta	Cam	TSS mg/L	VSS mg/L	NH_3-N mg/L	Cond. µmhos/cm	pH SU	BOD mg/L	SO4 mg/L	Cl mg/L	TDS mg/L
Buffalo Bayou @ McKee	11345		2,700							14		0.3	804	7.8	<4	37.7	94.9	473
Buffalo Bayou @ Main (& Commerce)	11347		2,300							20		0.22	827	7.8	5	45.4	97.8	495
Buffalo @ Shepherd	11351		25,000							17		0.3	793	7.8	4	31	93.7	447
Buffalo at Voss	11356																	
Buffalo Bayou @ South Jensen	15841		2,300				Pos.	Pos.		16		0.34	945	7.8	5	44.8	144.6	552
Buffalo @ Sabine	15843		21,000			Pos.				18		0.18	827	7.8	4	31.8	93.1	455
Buffalo Bayou @ Westcott	15844		32,000	Pos.		Pos.				14		0.26	768	7.7	6	31.6	95.6	442
Buffalo Bayou @ Westcott (QC)	15844		14,000							20		0.3	732	7.8	6	31.8	95.5	449
Buffalo Bayou @ Chimney Rock	15845			Pos.			Pos.	Pos.										
Buffalo Bayou @ Glenwood Cemetery	16675		590							<1		1.91	988	7.9	6	52.7	100.6	600
Buffalo Trib. @ Clinton (#1)	16649		240							3		1.5	704	7.1	<4	58.4	62.5	420
Buffalo Trib. at Clinton (#2, duplicate)	16649																	
Buffalo Trib. at Clinton (#3, blank)	16649																	
West District Raw Sewage				Pos.		Pos.	Pos.	Pos.										

Date	Flow (cfs) Buffalo @ West Belt 8073600	Flow (cfs) White Oak@ Heights 8074500	Rainfall (in) Buffalo RG32	Rainfall (in) White Oak RG23
11/9/00	619	44	0	0
11/10/00	963	39	0	0
11/11/00	109	36	0	0
11/12/00	392	285	1.48	1.39
11/13/00	1,070	506	0	0
11/14/00	**990**	**85**	**0**	**0.05**
12/8/00	112		0	0
12/9/00	92	38	0.01	0
12/10/00	76	38	0	0
12/11/00	74	38	0.03	0.02
12/12/00	60		0	0
12/13/00	**261**	**349**	**0.61**	**0.79**
1/12/01	1019	115	0	0
1/13/01	733	62	0.09	0.07
1/14/01	292	61	0.04	0.02
1/15/01	207	46	0.01	0.01
1/16/01	402	479	0.7	0.79
1/17/01	**411**	**402**	**0.24**	**0.32**
2/16/01	75	71	0.06	0.08
2/17/01	83	47	0	0
2/18/01	70	44	0	0
2/19/01	74	44	0	0
2/20/01	78	43	0	0
2/21/01	**73**	**43**	**0**	**0**

TABLE 3-1 (CONTINUED)
PROJECT MASTER DATA TABLE

3/21/2001 (started using extracted DNA) Station	Station Number	E. coli cfu/dL	FC cfu/dL	uidA lacZ	uid 1&3	uid 1&2	Bact. dist.	Bact. theta	Cam	TSS mg/L	VSS mg/L	NH_3-N mg/L	Cond. µmhos/cm	pH SU	BOD mg/L	SO4 mg/L	Cl mg/L	TDS mg/L
White Oak Bayou @ West 34th St	11390		670							8		0.253	816	8.16	<4	48.4	88.8	483
White Oak Bayou @ West 43rd St.	15829		170	Pos.		Pos.				10		0.329	827	8.08	<4	50.8	91.2	497
White Oak Bayou @ West Tidwell	15831		320							15		0.06	823	7.95	<4	32.2	93.7	491
White Oak Bayou @ West TC Jester	16637		4,100	Pos.		Pos.				7		0.178	817	8.34	<4	46.7	90.1	498
Cole Creek @ Bolivia	16593		730							12		0.063	513	7.9	<4	18.8	39	314
Brickhouse Gully @ US 290	16594		4,600	Pos.		Pos.	Pos.	Pos.		5		<.05	742	8.8	<4	20.9	63	451
White Oak Trib @ US 290	16595		<10							49		0.118	540	7.82	<4	23.9	43.4	332
West District Raw Sewage				Pos.			Pos.	Pos.										
Blank																		

April 18, 2001 Station	Station Number	E. coli cfu/dL	FC cfu/dL	uidA lacZ	uid 1&3	uid 1&2	Bact. dist.	Bact. theta	Cam	TSS mg/L	VSS mg/L	NH_3-N mg/L	Cond. µmhos/cm	pH SU	BOD mg/L	SO4 mg/L	Cl mg/L	TDS mg/L
Buffalo Bayou @ McKee	11345		3,200	Pos.		Pos.				29		0.27	319	7.4	<4	16.3	24.7	241
Buffalo Bayou @ Main (& Commerce)	11347		2,800			Pos.				34		0.52	288	7.3	<4	13.5	19.7	228
Buffalo @ Shepherd	11351		12,000	Pos.						76		0.3	359	7.4	<4	15.7	25.6	254
Buffalo Bayou @ South Jensen	15841		4,700							26		0.33	304	7.4	<4	15.3	22.6	227
Buffalo @ Sabine	15843		7,400	Pos.						21		0.3	344	7.4	<4	15.3	23.1	219
Buffalo Bayou @ Westcott	15844		6,600			Pos.				65		0.33	381	7.4	4	17.3	28.9	272
Buffalo Bayou @ Westcott (QC)	15844																	
Buffalo Bayou @ Glenwood Cemetery	16675		3,700			Pos.				5		<0.05	870	7.6	<4	47.1	73.7	548
Buffalo Trib. @ Clinton	16649		200,000	Pos.		Pos.	Pos.			6		1.46	741	7.2	6	67.2	72.4	486
Buffalo Trib. @ Clinton (Dup)	16649					Pos.												
West District Raw Sewage				Pos.		Pos.	Pos.	Pos.										

May 16, 2001 Station	Station Number	E. coli cfu/dL	FC cfu/dL	uidA lacZ	uid 1&3	uid 1&2	Bact. dist.	Bact. theta	Cam	TSS mg/L	VSS mg/L	NH_3-N mg/L	Cond. µmhos/cm	pH SU	BOD mg/L	SO4 mg/L	Cl mg/L	TDS mg/L
White Oak Bayou @ West 34th St	11390		2,200	Pos.		Pos.	Pos.	Pos.		12		1.29	744	8.19	<4	31.3	79.6	459
White Oak Bayou @ West 43rd St.	15829		2,100	Pos.		Pos.	Pos.	Pos.		23		1.38	750	7.97	<4	31.2	76.6	459
White Oak Bayou @ West Tidwell	15831		300			Pos.				34		0.062	643	7.68	<4	23.7	67.7	399
White Oak at West Tidwell (Dup)	15831																	
White Oak Bayou @ West TC Jester	16637		2,100	Pos.		Pos.	Pos.	Pos.		25		1.14	743	8.22	<4	31.1	80.5	461
Cole Creek @ Bolivia	16593		3,800	Pos.			Pos.			7		<0.05	567	7.94	<4	14	46.4	331
Brickhouse Gully @ US 290	16594		24,000			Pos.	Pos.			3		<0.05	708	8.87	<4	19	61.5	437
White Oak Trib @ US 290	16595		>200000	Pos.		Pos.	Pos.	Pos.		6		0.061	561	7.55	5	14.5	38.1	338
West District Raw Sewage				Pos.	Pos.	Pos.	Pos.											
West District Prechlorination				Pos.		Pos.	Pos.											
West District Dechlorinated																		

June 13, 2001 Station	Station Number	E. coli cfu/dL	FC cfu/dL	uidA lacZ	uid 1&3	uid 1&2	Bact. dist.	Bact. theta	Cam	TSS mg/L	VSS mg/L	NH_3-N mg/L	Cond. µmhos/cm	pH SU	BOD mg/L	SO4 mg/L	Cl mg/L	TDS mg/L
Buffalo Bayou @ McKee (No visible DNA)	11345		2,600															
Buffalo Bayou @ Main (& Commerce)	11347		2,400															
Buffalo @ Shepherd	11351		2,300															
Buffalo at Barker Dam (No visible DNA)	11364					Pos.												
Buffalo Bayou @ South Jensen (No visible DNA)	15841		2,100			Pos.												
Buffalo @ Sabine (No visible DNA)	15843		3,100															
Buffalo Bayou @ Glenwood Cemetery	16675		<99															
Buffalo Trib. @ Clinton	16649		77,000	Pos.		Pos.	Pos.											
Buffalo Trib. @ Clinton (Dup)	16649		55,000	Pos.		Pos.		Pos.										
West District Raw Sewage				Pos.		Pos.	Pos.	Pos.										
West District Prechlorination				Pos.		Pos.	Pos.											
West District dechlorination (No visible DNA)																		
Blank																		

Date	Flow (cfs) Buffalo @ West Belt 8073600	Flow (cfs) White Oak@ Heights 8074500	Rainfall (in) Buffalo RG32	Rainfall (in) White Oak RG23
3/16/01	1610	164	0	0
3/17/01	1610	89	0	0
3/18/01	599	67	0	0
3/19/01	735	62	0	0
3/20/01	636	55	0	0
3/21/01	**341**	**52**	**0**	**0**
4/13/01	85	75	0	0
4/14/01	80	72	0	0
4/15/01	78	69	0	0
4/16/01	471	465	1.15	0.98
4/17/01	236	370	0.02	0.05
4/18/01	**629**	**98**	**0**	**0**
5/11/01	1350	253	0.57	1.03
5/12/01	1019	227	0.04	0.07
5/13/01	585	319	0.01	0.05
5/14/01	470	139	0	0
5/15/01	200	75	0	0
5/16/01	**126**	**64**	**0**	**0**
6/8/01	489	1,780	3.79	4.81
6/9/01	2,680		4.81	7.78
6/10/01	427		0	0
6/11/01	268	354	0	0
6/12/01	1,350	179	0	0
6/13/01	**1,760**	**118**	**0**	**0**

TABLE 3-1 (CONTINUED)
PROJECT MASTER DATA TABLE

June 27, 2001 Station	Station Number	E. coli cfu/dL	FC cfu/dL	uidA lacZ	uid 1&3	uid 1&2	Bact. dist.	Bact. theta	Cam	TSS mg/L	VSS mg/L	NH_3-N mg/L	Cond. µmhos/cm	pH SU	BOD mg/L	SO4 mg/L	Cl mg/L	TDS mg/L
White Oak at Heights	11387					Pos.				12		.388	672	8.36	<4	32.7	6.66	404
White Oak at Crockett	15825		25,000			Pos.				20		.560	538	7.63	<4	28.2	45.9	350
White Oak at Crockett (Dup)	15825					Pos.												
White Oak at Studewood	15826		16,000	Pos.	Pos.	Pos.				6		.391	667	8.15	<4	32.2	65.7	425
White Oak at N. Durham	15827		22,000	Pos.	Pos.	Pos.				8		.468	687	8.53	<4	34.0	70.2	422
Little White Oak at Trimble	11148		49,000	Pos.		Pos.				14		.160	228	7.37	<4	12.9	10.5	160
White Oak Trib. at West 14th Street	16596		41,000			Pos.				3		.167	569	7.58	4	18.3	61.4	342
Little White at White Oak	16648		65,000	Pos.	Pos.	Pos.				14		.160	236	7.42	<4	13.6	11.2	161
Turkey Creek raw sewage				Pos.	Pos.	Pos.	Pos.	Pos.										
West District raw sewage				Pos.	Pos.	Pos.	Pos.											
W. District aeration basin (little visible DNA)																		
West District prechlorination				Pos.		Pos.												
Turkey Creek prechlorination						Pos.												
Turkey Creek chlorinated																		
West District dechlorination						Pos.												
Turkey Creek dechlorinated																		
Blank (No visible DNA)																		

July 18, 2001 Station	Station Number	E. coli cfu/dL	FC cfu/dL	uidA lacZ	uid 1&3	uid 1&2	Bact. dist.	Bact. theta	Cam	TSS mg/L	VSS mg/L	NH_3-N mg/L	Cond. µmhos/cm	pH SU	BOD mg/L	SO4 mg/L	Cl mg/L	TDS mg/L
Buffalo at Barker Dam (No visible DNA)	11142																	
Buffalo Bayou @ McKee (No visible DNA)	11345		4,100							19		.118	568		<4	24.4	64.3	320
Buffalo Bayou @ Main (& Commerce)	11347		2,400	Pos.		Pos.				51		.128	580		<4	23.3	69.4	326
Buffalo @ Shepherd	11351		6,600							241		.080	395		<4	16.8	40.9	283
Buffalo Bayou @ South Jensen (No visible DNA)	15841		7,400			Pos.				23		.113	412		<4	22.0	44.1	224
Buffalo @ Jensen	15841									22		.107	408		<4	19.1	41.5	236
Buffalo @ Sabine (No visible DNA)	15843		2,900							48		.095	641		<4	26.0	77.3	384
Buffalo Bayou @ Glenwood Cemetery	16675		3,900			Pos.				5		.241	630		<4	58.3	38.3	370
Buffalo Trib. @ Clinton	16649		200,000	Pos.		Pos.				16		.210	586		5	44.0	49.3	320
Buffalo Trib. @ Clinton (Dup)	16649																	
Turkey Creek raw sewage				Pos.		Pos.	Pos.											
West District raw sewage				Pos.		Pos.	Pos.											
Turkey Creek prechlorination						Pos.												
West District prechlorination				Pos.		Pos.												
Turkey Creek dechlorinated																		
West District dechlorination (No visible DNA)																		

July 31, 2001 Station	Station Number	E. coli cfu/dL	FC cfu/dL	uidA lacZ	uid 1&3	uid 1&2	Bact. dist.	Bact. theta	Cam	TSS mg/L	VSS mg/L	NH_3-N mg/L	Cond. µmhos/cm	pH SU	BOD mg/L	SO4 mg/L	Cl mg/L	TDS mg/L
White Oak Bayou at Heights	11387		3,500			Pos.				17		.309	718	8.24	<4	31.6	81.6	440
White Oak at Crockett	15825		2,000							15		.777	722	7.66	<4	32.4	77.3	412
White Oak at Studewood	15826		5,400			Pos.				8		.331	722	8.02	<4	31.2	80.6	431
Little White Oak at Trimble	11148		240,000							8		1.76	436	7.47	5	17.0	24.6	254
White Oak Trib at North Durham	15827		3,700							12		.326	730	8.35	<4	32.6	85.5	455
White Oak Trib at West 14 St.	16596		12,000			Pos.				2		.073	1230	7.79	<4	32.6	164	720
Little White Oak @ White Oak	16648		2,800			Pos.				7		.412	411	7.55	<4	20.3	23.1	242
Little White Oak at Cottage					Pos.	Pos.	Pos.											
West Dist. Raw Sewage						Pos.												
Buffalo, Clear, covered (No visible DNA) 133		667																
Buffalo, Clear, covered (No visible DNA) 134		5,231				Pos.												
Buffalo, Clear, uncovered (No visible DNA) 122		835																
Buffalo, Clear, uncovered (No visible DNA) 123		893																
Buffalo, Dark, covered (No visible DNA) 132		644																
Buffalo, Dark, covered (No visible DNA) 135		445																
Blank (No visible DNA) 136																		

Date	Flow (cfs) Buffalo @ West Belt 8073600	Flow (cfs) White Oak@ Heights 8074500	Rainfall (in) Buffalo RG32	Rainfall (in) White Oak RG23
6/22/01	568	277	0.35	0.49
6/23/01	920	69	0	0
6/24/01	361	49	0	0
6/25/01	101	49	0	0
6/26/01	84	170	0.02	0.94
6/27/01	**93**	**70**	**0**	**0**
7/13/01	63	56	0	0
7/14/01	63	57	0	0
7/15/01	61	60	0	0
7/16/01	88	226	0	0.14
7/17/01	170	214	0	0.35
7/18/01	**179**	**82**	**0.15**	**0**
7/26/01	138	423	1.37	0.98
7/27/01	170	378	0.26	0.4
7/28/01	52	161	0	0.01
7/29/01	43	74	0.17	0.12
7/30/01	356	61	0	0
7/31/01	**162**	**46**	**0**	**0**

TABLE 3-1 (CONCLUDED)
PROJECT MASTER DATA TABLE

August 15, 2001	Station	E. coli	FC	uidA	uid	uid	Bact.	Bact.	Cam	TSS	VSS	NH_3-N	Cond.	pH	BOD	SO4	Cl	TDS
Station	Number	cfu/dL	cfu/dL	lacZ	1&3	1&2	dist.	theta		mg/L	mg/L	mg/L	µmhos/cm	SU	mg/L	mg/L	mg/L	mg/L
Buffalo Bayou @ McKee (No visible DNA)	11345	3,400				Pos.				25		.45	714	7.9		34.3	90.9	447
Buffalo Bayou @ Main (& Commerce)	11347	5,500								48		.15	695	7.8		32.9	90.7	455
Buffalo @ Shepherd	11351	2,400								24		1.08	722	7.9		31.2	96.8	463
Buffalo @ Jensen	15841	1,900								25		.32	848	7.7		41.3	140.3	550
Buffalo @ Sabine (No visible DNA)	15843	4,600																
Buffalo Bayou @ Glenwood Cemetery	16675	>240000		Pos.		Pos.		Pos.		11		2.95	752	7.8		14.7	87.9	463
Buffalo Trib. @ Clinton	16649	52,000		Pos.		Pos.				2		1.22	592	7.1		48.1	50.3	377
Buffalo Trib. @ Clinton (Dup)	16649			Pos.		Pos.												
West Dist. Raw Sewage 156				Pos.		Pos.	Pos.											
West Dist. Prechlorination 144						Pos.	Pos.											
West District Dechlorination 145						Pos.												
West District Effluent 152		6,867		Pos.		Pos.												
Clear Chamber, BB @ Wilcrest		146																
Clear Chamber, WO @ 610		72																
Dark Chamber, BB @ Wilcrest 150		169																
Dark Chamber, WO @ 610 153		132		Pos.		Pos.												
Upstream Chamber, BB @ Wilcrest		146																

Date	Flow (cfs)		Rainfall (in)	
	Buffalo @ West Belt	White Oak@ Heights	Buffalo	White Oak
	8073600	8074500	RG32	RG23
8/10/01	84	52	0.05	0
8/11/01	69	47	0	0
8/12/01	60	46	0	0
8/13/01	59	41	0	0
8/14/01	58	44	0	0
8/15/01	**57**	**39**	**0**	**0**

Notes on PCR results:
Blank means non-detect.
Pos. = Band was seen at the right molecular weight for that organism.
Bejuida/lacZ= only lacZ was detected in this assay, so it gives a total coliform.
uid 1 & 3 = amplification of the E.coli gene uidA from the sample and/or extracted DNA less sensitive than expected, it may have to do with how many intact copies of gene are present during PCR.
uid 1 & 2 = semi-nested amplification of uid 1 & 3 for a more sensitive assay only if uid 1&3 products are present there will be a positive result.
Bacteroides distasonis = found mainly in humans and in rare occasions in pets that have very close relationships with their owners.
Bacteroides tethaiotaomicron = indicates general coliform present mainly human, but it can be animal related also.
Campylobacter = indicates animal run-off in the water.
Muddy or after rain waters that are turbid produce bad DNA extraction as DNA will bind to the sediments.

TABLE 3-2

RAINFALL DATA FOR BUFFALO AND WHITE OAK WATERSHEDS

Date	RG14 Upper WO	RG15 Upper WO	RG21 Upper BB	RG23 Middle WO	RG30 Upper BB	RG31 Upper BB	RG32 Middle BB	RG34 Confluence BB & WO	RG35 Below confluence
11/01/00	0.13	0.19	0.28	0.90	0.21	0.17	0.59	0.87	0.30
11/02/00	0.42	0.03	0.00	0.07	0.05	0.03	0.02	0.00	0.00
11/03/00	0.65	0.83	1.07	0.34	1.13	1.26	0.64	0.17	0.10
11/04/00	0.53	0.46	0.26	0.09	0.07	0.07	0.14	0.16	0.14
11/05/00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.02
11/06/00	1.97	2.40	1.81	1.65	1.68	2.46	1.52	1.41	1.11
11/07/00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00
11/08/00	0.13	0.12	0.04	0.16	0.03	0.06	0.14	0.20	0.12
11/09/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11/10/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11/11/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11/12/00	0.82	0.62	0.87	1.39	1.47	1.54	1.48	1.28	1.44
11/13/00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11/14/00	**0.00**	**0.00**	**0.00**	**0.05**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
11/15/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11/16/00	0.88	1.06	1.14	1.32	1.02	0.99	1.06	1.43	1.11
11/17/00	0.46	0.56	0.63	0.58	0.50	0.41	0.45	0.56	0.50
11/18/00	2.08	2.00	2.16	2.44	2.00	1.90	2.19	2.44	2.00
11/19/00	0.16	0.15	0.18	0.18	0.13	0.11	0.14	0.17	0.16
11/20/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11/21/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11/22/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11/23/00	0.14	0.35	0.08	0.20	0.07	0.00	0.08	0.24	0.33
11/24/00	0.71	0.89	0.83	1.04	1.00	0.00	0.98	1.12	1.11
11/25/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11/26/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11/27/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11/28/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11/29/00	0.01	0.02	0.01	0.01	0.02	0.00	0.01	0.06	0.00
11/30/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12/01/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12/02/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12/03/00	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.01
12/04/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
12/05/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12/06/00	0.34	0.35	0.29	0.38	0.23	0.25	0.17	0.22	0.35
12/07/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12/08/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12/09/00	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.00	0.01
12/10/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12/11/00	0.00	0.00	0.00	0.02	0.01	0.00	0.03	0.00	0.00
12/12/00	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00
12/13/00	**0.76**	**0.59**	**0.79**	**0.79**	**0.79**	**0.41**	**0.61**	**0.60**	**0.56**
12/14/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12/15/00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01

TABLE 3-2

RAINFALL DATA FOR BUFFALO AND WHITE OAK WATERSHEDS

Date	RG14 Upper WO	RG15 Upper WO	RG21 Upper BB	RG23 Middle WO	RG30 Upper BB	RG31 Upper BB	RG32 Middle BB	RG34 Confluence BB & WO	RG35 Below confluence
12/16/00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12/17/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12/18/00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.01
12/19/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12/20/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12/21/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12/22/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12/23/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12/24/00	0.85	0.89	1.08	1.04	1.05	0.77	0.60	0.55	0.51
12/25/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12/26/00	0.25	0.13	0.19	0.40	0.17	0.23	0.48	0.72	0.76
12/27/00	0.06	0.12	0.07	0.04	0.09	0.05	0.03	0.05	0.03
12/28/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12/29/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12/30/00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12/31/00	0.14	0.15	0.13	0.14	0.12	0.10	0.13	0.17	0.14
01/01/01	0.06	0.07	0.09	0.10	0.06	0.06	0.05	0.07	0.04
01/02/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
01/03/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
01/04/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
01/05/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
01/06/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
01/07/01	0.06	0.07	0.06	0.04	0.07	0.06	0.09	0.09	0.08
01/08/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
01/09/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
01/10/01	1.43	1.46	1.26	1.77	1.28	1.21	1.43	1.71	1.36
01/11/01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00
01/12/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
01/13/01	0.08	0.05	0.05	0.07	0.04	0.06	0.09	0.08	0.06
01/14/01	0.01	0.01	0.01	0.02	0.01	0.00	0.04	0.15	0.05
01/15/01	0.00	0.00	0.00	0.01	0.02	0.02	0.01	0.00	0.00
01/16/01	0.74	0.86	0.68	0.79	0.72	0.59	0.70	0.87	0.86
01/17/01	**0.27**	**0.28**	**0.25**	**0.32**	**0.21**	**0.19**	**0.24**	**0.24**	**0.18**
01/18/01	0.52	0.36	0.53	0.41	0.51	0.35	0.38	0.57	0.59
01/19/01	0.05	0.06	0.05	0.11	0.05	0.06	0.08	0.10	0.09
01/20/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
01/21/01	0.02	0.07	0.01	0.03	0.01	0.01	0.03	0.06	0.06
01/22/01	0.00	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.00
01/23/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
01/24/01	0.10	0.11	0.17	0.15	0.17	0.14	0.10	0.10	0.10
01/25/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
01/26/01	0.24	0.01	0.05	0.00	0.00	0.00	0.00	0.00	0.04
01/27/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
01/28/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
01/29/01	0.74	0.48	0.61	0.66	0.60	0.51	0.53	0.48	0.45

TABLE 3-2

RAINFALL DATA FOR BUFFALO AND WHITE OAK WATERSHEDS

Date	RG14 Upper WO	RG15 Upper WO	RG21 Upper BB	RG23 Middle WO	RG30 Upper BB	RG31 Upper BB	RG32 Middle BB	RG34 Confluence BB & WO	RG35 Below confluence
01/30/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
01/31/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
02/01/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
02/02/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
02/03/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
02/04/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
02/05/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
02/06/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
02/07/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
02/08/01	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
02/09/01	0.15	0.10	0.18	0.19	0.19	0.11	0.18	0.11	0.14
02/10/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
02/11/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
02/12/01	0.02	0.00	0.03	0.00	0.00	0.00	0.00	0.01	0.02
02/13/01	0.01	0.02	0.00	0.00	0.00	0.01	0.00	0.01	0.02
02/14/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
02/15/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
02/16/01	0.07	0.12	0.14	0.08	0.10	0.09	0.06	0.03	0.01
02/17/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
02/18/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
02/19/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
02/20/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
02/21/01	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
02/22/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
02/23/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
02/24/01	0.10	0.04	0.03	0.07	0.04	0.03	0.14	0.01	0.07
02/25/01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
02/26/01	0.00	0.03	0.00	0.01	0.00	0.00	0.02	0.00	0.00
02/27/01	0.02	0.03	0.00	0.02	0.00	0.00	0.00	0.01	0.02
02/28/01	0.03	0.06	0.07	0.04	0.08	0.01	0.04	0.01	0.00
03/01/01	0.10	0.07	0.07	0.33	0.08	0.06	0.33	0.25	0.30
03/02/01	1.65	1.29	1.49	1.16	1.50	1.10	0.88	0.90	0.76
03/03/01	0.26	0.28	0.24	0.31	0.23	0.23	0.30	0.38	0.39
03/04/01	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
03/05/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
03/06/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
03/07/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
03/08/01	0.77	0.55	0.43	0.53	0.58	0.58	0.49	0.72	0.77
03/09/01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
03/10/01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
03/11/01	0.16	0.18	0.21	0.50	0.32	0.41	0.34	0.16	0.10
03/12/01	0.16	0.17	0.23	0.38	0.29	0.17	0.30	0.12	0.18
03/13/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
03/14/01	1.47	1.53	1.51	1.73	1.50	1.46	1.45	0.99	0.70
03/15/01	0.01	0.00	0.01	0.00	0.01	0.01	0.00	0.00	0.01

TABLE 3-2

RAINFALL DATA FOR BUFFALO AND WHITE OAK WATERSHEDS

Date	RG14 Upper WO	RG15 Upper WO	RG21 Upper BB	RG23 Middle WO	RG30 Upper BB	RG31 Upper BB	RG32 Middle BB	RG34 Confluence BB & WO	RG35 Below confluence
03/16/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
03/17/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
03/18/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
03/19/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
03/20/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
03/21/01	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
03/22/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
03/23/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
03/24/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
03/25/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
03/26/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
03/27/01	3.47	3.43	3.29	4.31	3.55	2.88	2.78	3.05	2.77
03/28/01	0.70	0.86	0.83	2.47	1.32	1.87	2.61	1.52	1.50
03/29/01	0.13	0.08	0.09	0.04	0.07	0.05	0.03	0.04	0.04
03/30/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
03/31/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/01/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/02/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/03/01	0.01	0.00	0.01	0.02	0.01	0.00	0.01	0.01	0.00
04/04/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/05/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/06/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/07/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/08/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/09/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/10/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/11/01	0.20	0.03	0.10	0.03	0.08	0.00	0.03	0.07	0.10
04/12/01	0.01	0.00	0.02	0.00	0.00	0.01	0.01	0.02	0.00
04/13/01	0.02	0.01	0.02	0.00	0.00	0.01	0.00	0.00	0.00
04/14/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/15/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/16/01	1.75	0.70	2.27	0.98	0.78	1.12	1.15	0.46	0.16
04/17/01	0.11	0.13	0.05	0.05	0.01	0.01	0.02	0.05	0.00
04/18/01	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
04/19/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/20/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/21/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/22/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/23/01	0.03	0.01	0.01	0.18	0.15	0.00	0.00	0.03	0.18
04/24/01	0.04	0.01	0.04	0.04	0.06	0.15	0.02	0.09	0.06
04/25/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/26/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/27/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/28/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04/29/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

TABLE 3-2

RAINFALL DATA FOR BUFFALO AND WHITE OAK WATERSHEDS

Date	RG14 Upper WO	RG15 Upper WO	RG21 Upper BB	RG23 Middle WO	RG30 Upper BB	RG31 Upper BB	RG32 Middle BB	RG34 Confluence BB & WO	RG35 Below confluence
04/30/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
05/01/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
05/02/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
05/03/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
05/04/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
05/05/01	0.80	0.71	1.00	0.67	1.26	1.28	0.42	0.37	0.25
05/06/01	1.23	0.26	1.02	0.00	0.62	0.02	0.00	0.00	0.00
05/07/01	3.76	1.71	1.93	1.31	1.81	1.45	1.36	1.02	0.95
05/08/01	0.01	0.02	0.00	0.01	0.01	0.01	0.01	0.00	0.00
05/09/01	0.00	0.00	0.11	0.03	0.02	0.39	1.13	0.00	0.00
05/10/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
05/11/01	0.32	0.18	0.12	1.03	0.00	0.00	0.57	0.20	0.72
05/12/01	0.03	0.04	0.10	0.07	0.05	0.01	0.04	0.06	0.08
05/13/01	0.01	0.02	0.02	0.05	0.04	0.05	0.01	0.00	0.00
05/14/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
05/15/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
05/16/01	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
05/17/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
05/18/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
05/19/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
05/20/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
05/21/01	0.24	0.04	0.00	0.01	0.00	0.00	0.01	0.03	0.00
05/22/01	0.02	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00
05/23/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
05/24/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
05/25/01	0.04	0.04	0.02	0.04	0.03	0.01	0.02	0.03	0.04
05/26/01	1.01	0.93	0.94	1.56	1.27	0.60	0.84	1.51	0.67
05/27/01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
05/28/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
05/29/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
05/30/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
05/31/01	0.04	0.21	0.14	0.01	0.02	0.00	0.02	0.11	0.31
06/01/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01
06/02/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
06/03/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
06/04/01	0.00	0.28	0.02	0.02	0.02	0.00	0.20	0.12	0.09
06/05/01	1.83	2.74	2.14	4.93	1.43	0.00	3.45	6.44	8.10
06/06/01	0.07	0.04	0.08	0.01	0.09	0.00	0.00	0.03	0.02
06/07/01	0.20	0.20	0.24	0.67	0.13	0.17	0.14	0.41	0.86
06/08/01	3.52	5.05	2.58	4.81	2.38	2.01	3.79	2.91	3.59
06/09/01	4.91	7.25	3.02	7.78	1.95	1.51	4.81	10.86	8.68
06/10/01	0.19	0.06	0.04	0.00	0.03	0.00	0.00	0.06	0.01
06/11/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
06/12/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
06/13/01	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

TABLE 3-2

RAINFALL DATA FOR BUFFALO AND WHITE OAK WATERSHEDS

Date	RG14 Upper WO	RG15 Upper WO	RG21 Upper BB	RG23 Middle WO	RG30 Upper BB	RG31 Upper BB	RG32 Middle BB	RG34 Confluence BB & WO	RG35 Below confluence
06/14/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
06/15/01	0.41	0.48	0.33	0.18	0.46	0.32	0.19	0.24	0.09
06/16/01	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00
06/17/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
06/18/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
06/19/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
06/20/01	0.00	0.09	0.29	0.01	0.00	0.00	0.06	0.00	0.00
06/21/01	0.01	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.07
06/22/01	0.48	0.44	0.13	0.49	0.29	0.17	0.35	0.26	0.18
06/23/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
06/24/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
06/25/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
06/26/01	0.00	0.00	0.00	0.94	0.00	0.00	0.02	0.16	0.09
06/27/01	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
06/28/01	0.13	0.00	0.02	1.27	0.15	0.68	0.71	0.10	0.11
06/29/01	0.32	0.14	0.26	0.15	0.40	0.45	1.30	0.50	2.42
06/30/01	0.05	0.01	0.03	0.00	0.03	0.01	0.00	0.00	0.00
07/01/01	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.27	0.07
07/02/01	0.04	0.06	0.58	1.18	1.17	0.18	0.21	0.15	0.16
07/03/01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.29
07/04/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
07/05/01	0.36	0.63	0.12	0.07	0.00	0.00	0.00	0.49	0.02
07/06/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
07/07/01	0.04	0.00	0.22	0.00	0.22	0.12	0.96	0.00	0.00
07/08/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
07/09/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
07/10/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
07/11/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.41	0.00
07/12/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
07/13/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
07/14/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
07/15/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
07/16/01	0.05	0.44	0.02	0.14	0.58	0.00	0.00	0.44	0.00
07/17/01	0.00	0.00	0.01	0.35	0.31	0.02	0.00	0.34	0.26
07/18/01	**0.00**	**0.00**	**0.01**	**0.00**	**0.00**	**0.01**	**0.15**	**0.00**	**0.00**
07/19/01	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00
07/20/01	0.22	0.06	0.10	0.23	0.00	0.00	0.00	0.00	0.02
07/21/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
07/22/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
07/23/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
07/24/01	0.00	0.00	0.20	0.00	0.08	0.00	0.23	0.00	0.00
07/25/01	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00
07/26/01	0.01	0.21	0.05	0.98	0.02	0.84	1.37	0.04	0.45
07/27/01	0.44	0.13	0.13	0.40	0.12	0.11	0.26	0.75	1.42
07/28/01	0.02	0.14	0.03	0.01	0.01	0.00	0.00	0.00	0.06

TABLE 3-2

RAINFALL DATA FOR BUFFALO AND WHITE OAK WATERSHEDS

Date	RG14 Upper WO	RG15 Upper WO	RG21 Upper BB	RG23 Middle WO	RG30 Upper BB	RG31 Upper BB	RG32 Middle BB	RG34 Confluence BB & WO	RG35 Below confluence
07/29/01	0.02	0.02	0.01	0.12	0.00	0.02	0.17	0.17	0.00
07/30/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
07/31/01	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
08/01/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
08/02/01	0.00	0.00	0.00	0.04	0.03	0.00	0.17	0.51	0.54
08/03/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
08/04/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
08/05/01	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.04
08/06/01	2.68	0.84	1.38	0.00	1.28	0.65	0.19	0.00	0.00
08/07/01	0.14	0.11	0.03	0.13	0.00	0.02	0.63	0.28	0.00
08/08/01	0.20	0.13	0.04	0.00	0.00	0.00	0.00	0.35	0.00
08/09/01	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
08/10/01	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00
08/11/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
08/12/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
08/13/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
08/14/01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00
08/15/01	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

2/21 Bacteria were moderate in downstream stations but higher in upstream stations. There was no rain for five days and low flows.

4/18 Bacteria were moderate except for a downstream tributary station that was high. There was rain on 4/16 and fairly high flows remained on the 18th.

6/13 Sampling was preceded by Allison a week earlier and the bayou flows were moderately high with the water being in a muddy brown color. Bacteria levels were only moderate on the main stem, but high in one tributary. Conventional parameters were not available due to laboratory flooding.

7/18 Moderate flows and bacteria concentrations were observed. There was a small rain event on the day of sampling. High bacteria concentrations were seen at a tributary near Clinton.

8/15 A dry day with bayou flows at low levels. The EC bacteria concentrations were at moderate levels. There was one high concentration value on the main stem but not the tributaries.

White Oak Bayou

11/14 There was rain two days earlier but flows had declined and there were low bacteria levels.

1/17 There were fairly high bacteria levels with rain on the day before and the day of sampling.

3/21 This day had fairly low bacteria levels with no rain and low flow.

5/16 There was a moderate rain five days earlier and bacteria concentrations were moderate except for two tributary stations that were high.

6/27 This day had very high bacteria levels probably associated with a one-inch rain on the day before sampling.

7/31 There were moderate bacteria concentrations and bayou flows on this day. Small rains occurred two days before sampling and still larger rains occurred earlier. High bacteria concentrations were found in one tributary.

An initial conclusion is that there appears to be a reasonably close relation between rain and flow and bacteria levels. A second observation is that the tributary stations such as Brickhouse Gully and the tributary near Clinton Dr. had more variability and higher levels than the main stem at some times but not others.

Figures 3-3 and 3-4 plot the FC bacteria data for the main stem of the two bayous with flow measured at central United States Geological Survey (USGS) gages. There does not seem to be a relationship between flow and bacteria level in Buffalo Bayou, probably due to the regulating effect of the upstream reservoirs. However, there appears to be a positive relationship between flow and bacteria level in White Oak Bayou that does not have upstream reservoirs.

3.2 ANALYSIS

The TNRCC sampling included analyses of both FC and EC bacteria while the HHS monitoring only reported FC results through July and EC results in August. Another difference between TNRCC and HHS sampling was that TNRCC analyzed most of the nutrients as well as volatile suspended solids, while the HHS analyses focused on chlorides, sulfates and BOD. While there are differences in the monitoring parameters, the essential points of indicator bacteria and measures of the degree to which stream flow was impacted by runoff are similar.

The ammonia-N concentrations in both bayous tended to be in the tenths of a milligram per liter (mg/L) range. Values of over 1 mg/L were observed when bayou flows were at the lowest levels. BOD concentrations over the HHS reporting limit of 4.0 mg/L were rare and appeared limited to times of high flow and/or colder temperatures.

Figures 3-5 and 3-6 present plots of TSS versus FC in Buffalo and White Oak bayous, respectively. While neither shows a strong correlation between bacteria and solids, it is interesting to note the differences between the two systems. Because White Oak is concrete lined for much of its length, most of the TSS data are less than 50 mg/L. Conversely, most of the Buffalo Bayou TSS data are greater than 50 mg/L. Also, the FC data in White Oak appear to have a much greater range than the Buffalo data. Comparing just main stem data, the average TSS concentration in Buffalo is 2.5 times higher than in White Oak. The geometric mean FC concentration in Buffalo Bayou is about 2 times higher than that of White Oak Bayou.

FIGURE 3-3
RELATIONSHIP BETWEEN FLOW AND FC IN BUFFALO BAYOU

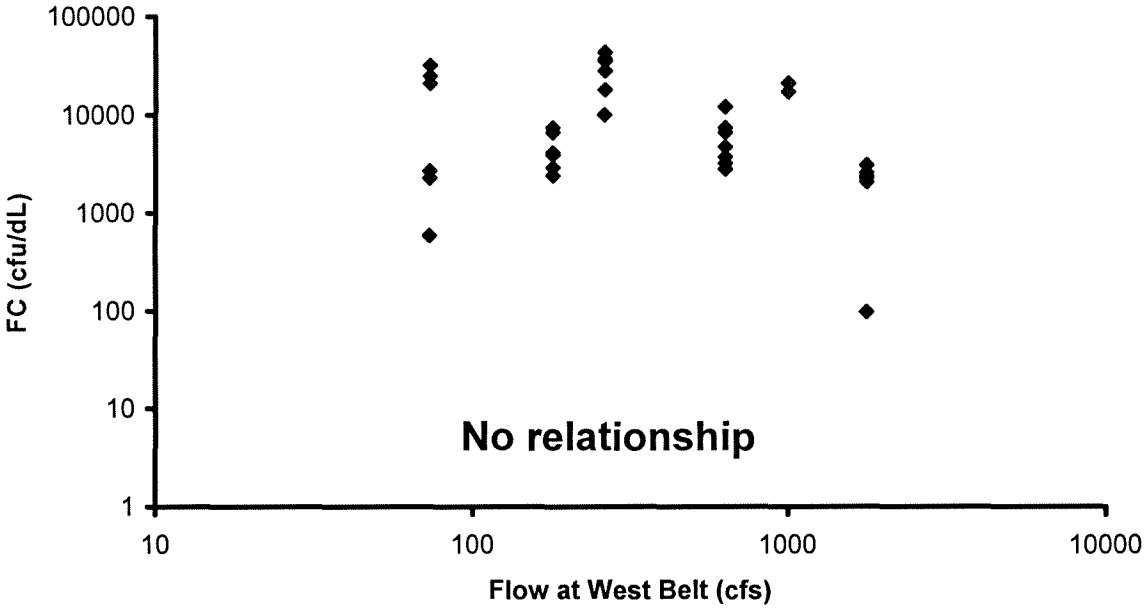


FIGURE 3-4
RELATIONSHIP BETWEEN FLOW AND FC IN WHITE OAK BAYOU

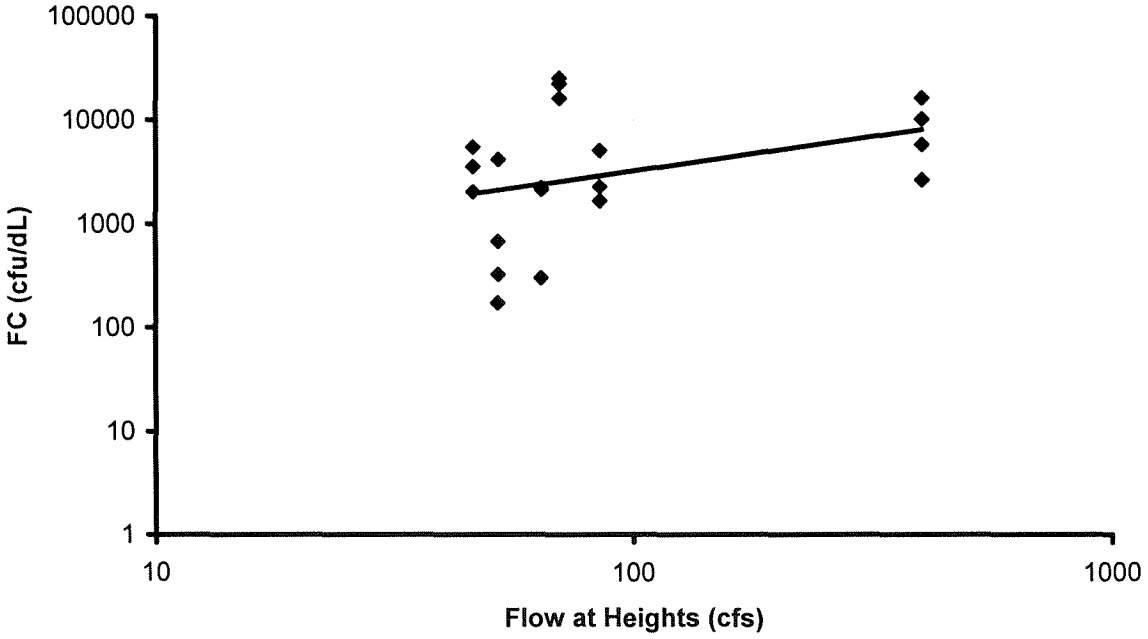


FIGURE 3-5
RELATIONSHIP BETWEEN TSS AND FC IN BUFFALO BAYOU

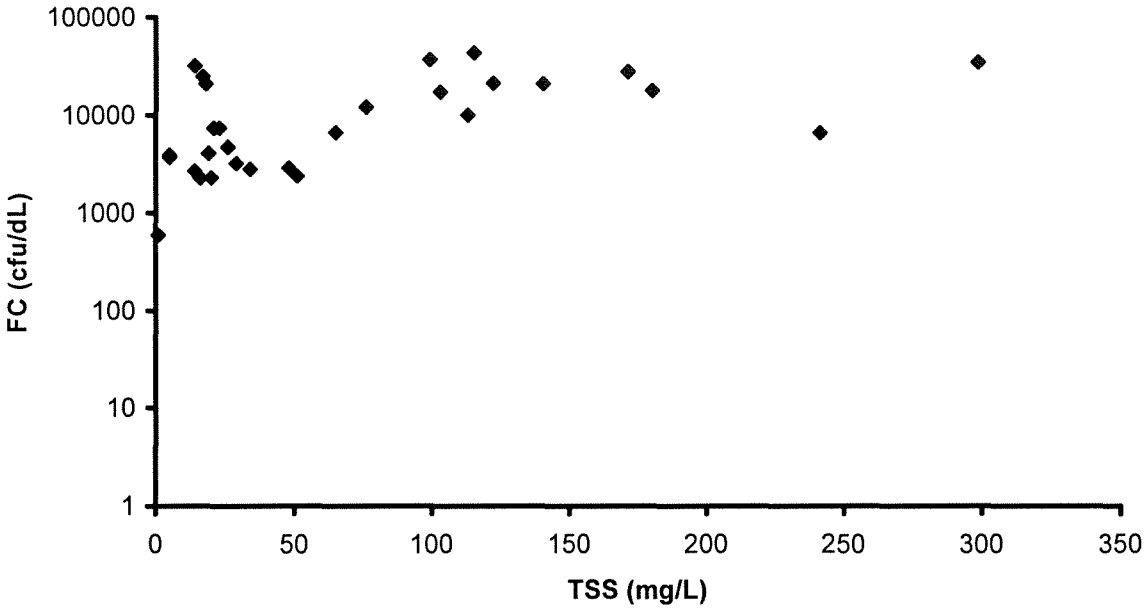
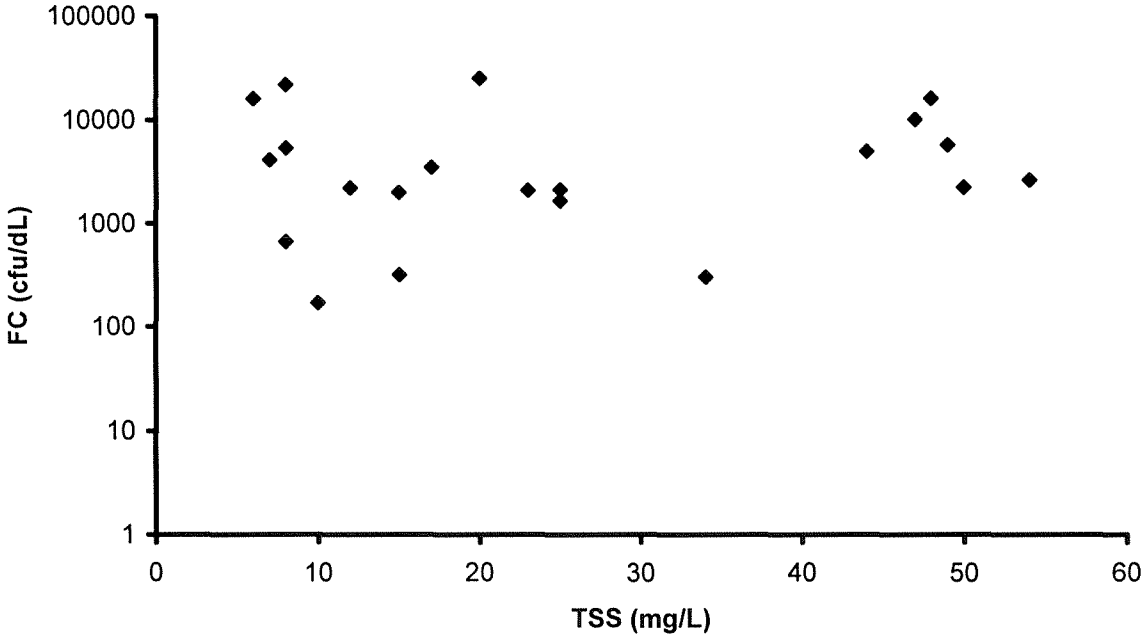


FIGURE 3-6
RELATIONSHIP BETWEEN TSS AND FC IN WHITE OAK BAYOU



4.0 ANALYSIS FOR HUMAN SOURCE IDENTIFICATION

The presence of specific bacterial DNAs was used as an indicator of the presence of particular types of bacteria in the water samples. Polymerase Chain Reaction (PCR) was used as a specific method for detecting DNA sequences. Two genes were targeted as indicators of coliform bacteria: lacZ, which is present in most coliforms and uidA, which is more specific for EC.

Samples were further analyzed for Bacteroides species. The presence of these bacteria indicates fecal contamination. Because these are strict anaerobes, they cannot be detected in surface waters or environmental samples by plating, but the DNA present in the cells can still be detected by PCR. Two Bacteroides species, B. distasonis (Bd) and B. thetaiotamicron (Bt) were chosen for detection, because one or both of these species are found in most humans but are much less commonly found in other animals (Kreader, 1995). However, Bd can occasionally be found in dogs and cats that are kept in close contact to humans. The presence of one or both of the Bacteroides species in samples that also have relatively high levels of fecal coliform is likely to indicate a human fecal source (Kreader, 1998). Kreader (1998) has also shown that Bd can be found in water for as long as a week from introduction, but only in cold temperature environment. Temperatures above 22 °C have been shown to increase the degradation and predation on these bacteria. Thus, most of these bacteria detected in Houston area would be expected to be the result of recent fecal contamination. Samples were also tested for the presence of Campylobacter jejuni. The presence of this pathogen represents a health hazard but its source is normally non-human.

Controls for each set of assays include samples of each of the bacterial strains, samples containing all the reagents except the DNA, and samples of wastewater pre- and post-treatment.

4.1 METHODS

Water samples (1 liter) were kept cold (4°) until processed. The samples were centrifuged as the first analytical step. The pellets produced by the centrifuge were lysed and extracted with the Qiagen QIAamp DNA mini kit using an adaptation of the tissue protocol. The DNA pellet was resuspended in 200 mL buffer and stored at -20°C. The DNA was electrophoresed in a 1.5% agarose gel to assess the amount and quality of DNA present in the sample.

A. Two sets of PCR reactions were performed to detect ECi DNA in the samples.

1. PCR was performed with uid1 and uid3 primers with the following conditions: 94°C/ 30s followed by 30 cycles of (94°C/ 1', 58°C/ 1', 72°C/ 1'), ending with 72°C/ 3'. PCR products were analyzed by electrophoresis; the presence of a 164 bp band indicated a positive result for the presence of uidA, which is present in EC, but absent in most other coliforms.

2. uidA/lacZ multiplex PCR: PCR was performed with primers BejuidA1, BejuidA2, lacZR and lacZL with the following conditions: 94°C/ 30s, 30X(94°C/ 1', 60°C/ 1', 72°C/ 1'), 72°C/ 2'. The presence of two bands indicated a positive result. If only a lacZ band was seen then the sample was considered a non-EC coliform.

B. Bacteroides distasonis (Bd) and Bacteroides thetaiotamicron (Bt) by PCR. PCR was performed with Bdist1 and Bdist2 primers or Btetha1 and Btetha2 primers as follows: 94°C/ 30s, 30X(94°C/ 1.5', 56°C/ 1.5', 72°C/ 1.5'), 72°C/ 3'. The presence of a band following gel electrophoresis indicated a positive result for the presence of Bacteroides for each primer pair.

C. Campylobacter jejuni PCR. PCR was performed with flagellin primers as follows: 94°C/ 30s, 30X(94°C/ 1', 42°C/ 1', 72°C/ 1'), 72°C/ 3'. The presence of a band indicated a positive result for the presence of Campylobacter jejuni for each primer pair.

4.2 RESULTS

For the first three sets of samples (11/14/00, 1/17/01, 2/21/01), the rates of detection of fecal coliforms by the PCR technique was lower than expected. Six of the 30 samples were positive for coliforms by multiplex PCR. Three of the 6 were also positive for Bacteroides. It was noted that the water had particulate material and there was interference with the PCR reactions. Therefore a DNA extraction step was added to the protocol to lyse the bacteria and free the DNA from possible interfering substances in the water samples. Using this protocol for the remaining sets of samples, the rate of detection of coliforms by PCR was improved. Ultimately, 55% of the bayou samples tested were positive for the EC gene. The negative controls (chlorinated water samples) were consistently negative, and the positive controls (raw sewage) were positive for coliforms and both Bacteroides species. Campylobacter DNA was not detected in any of the samples tested.

Table 4-1 summarizes the results obtained from the monitoring effort. Data are grouped into three classes, wastewater, bayou monitoring and chamber studies directly for the TMDL project. The goal of the wastewater samples was to provide a benchmark using water of known characteristics and source. The goal of the bayou monitoring samples was to assess the capability of PCR testing to indicate the contribution of human sources. The chamber testing was performed to take advantage of the availability of data and to shed additional light on the source identification process. The following sections provide a discussion of the testing results.

TABLE 4-1

SUMMARY OF PCR TEST RESULTS

Type of sample	Total number	uidA lacZ (Total coliform)		uid 1&3		uid 1&2 (E. coli)		Bacteroides distasonis (Human sources)				Bacteroides thetaiotamicron (Human sources)				Campylobacter jejuni (Pathogen)	
		Num of Pos.	% Pos.	Num of Pos.	% Pos.	Num of Pos.	% Pos.	Num of Pos.	% Pos.	Among uid 1&2 Pos.		Num of Pos.	% Pos.	Among uid 1&2 Pos.		Num of Pos.	% Pos.
										Num of Pos.	% Pos.			Num of Pos.	% Pos.		
Wastewater samples																	
Raw sewage	13	12	92.3%	3	23.1%	12	92.3%	12	92.3%	11	91.7%	5	38.5%	4	33.3%	0	0.0%
Aeration basin	1	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	NA	0	0.0%	0	NA	0	0.0%
Prechlorination	9	4	44.4%	0	0.0%	9	100.0%	3	33.3%	3	33.3%	0	0.0%	0	0.0%	0	0.0%
Chlorinated wastewater	1	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	NA	0	0.0%	0	NA	0	0.0%
Dechlorinated effluent	9	0	0.0%	0	0.0%	3	33.3%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
TOTAL	33																
Bayou samples																	
Buffalo (main stem)	46	11	23.9%	0	0.0%	19	41.3%	4	8.7%	2	10.5%	3	6.5%	1	5.3%	0	0.0%
Buffalo (tributary)	6	4	66.7%	0	0.0%	5	83.3%	2	33.3%	2	40.0%	0	0.0%	0	0.0%	0	0.0%
White Oak (main stem)	22	7	31.8%	2	9.1%	16	72.7%	3	13.6%	3	18.8%	3	13.6%	3	18.8%	0	0.0%
White Oak (tributary)	18	6	33.3%	2	11.1%	11	61.1%	6	33.3%	5	45.5%	2	11.1%	2	18.2%	0	0.0%
TOTAL	92	28	30.4%	4	4.3%	51	55.4%	15	16.3%	12	23.5%	8	8.7%	6	11.8%	0	0.0%
Chamber samples																	
Buffalo Bayou	9	0	0.0%	0	0.0%	1	11.1%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
White Oak Bayou	2	1	50.0%	0	0.0%	1	50.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Effluent	1	1	100.0%	0	0.0%	1	100.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
TOTAL	12																
Blanks	5	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	NA	0	0.0%	0	NA	0	0.0%

SUMMARY OF BAYOU SAMPLE FC AND TSS DATA

Type of sample	FC		TSS	
	Num of samples	Geomean (cfu/dL)	Num of samples	Mean (mg/L)
Buffalo (main stem)	35	5,962	35	64.0
Buffalo (tributary)	5	29,839	5	17.2
White Oak (main stem)	21	2,929	22	24.4
White Oak (tributary)	17	9,531	17	14.0

SUMMARY OF CHAMBER SAMPLE EC DATA

Type of sample	EC	
	Num of samples	Geomean (cfu/dL)
Buffalo Bayou	9	518
White Oak Bayou	2	97
Effluent	1	6,867

4.2.1 Wastewater Analyses

Over the ten-month period 33 samples were collected from area wastewater treatment plants. The samples were obtained from two City of Houston plants (West District and Turkey Creek) at several locations within the plants, including the raw sewage, after treatment and settling but before chlorination, and after dechlorination. In addition, 5 field blanks were collected and analyzed. Because these wastewater samples were not on the route of the routine agency monitoring, there were no analyses for bacteria by culture methods or other water quality parameters.

Almost all of the raw wastewater samples turned out to be positive for the uidA gene that is indicative of "total EC" and the lacZ gene that is specific to the "total coliform" organism group. This is simplified to an approximation of a "total coliform".

The uid1&3 test was generally negative despite the presence of E. coli by plate counts and in agency monitoring data. This may reflect a poor sensitivity of this particular PCR reaction, or it may be the result of inhibition of this reaction by components in the sample. Therefore, a second set of primers, uid1&2 was tested, and PCR using this set was found to be a more sensitive measure of E. coli DNA. The number of uid1&2 positive results was equal to the lacZ test for raw wastewater, but markedly higher than lacZ for treated wastewater before disinfection (prechlorination). Because of that it will be used as the benchmark for what PCR testing is capable of identifying in water samples.

The next group of results on Table 4-1 is that for the two *Bacteroides* species that are associated with human sources. *Bacteroides distasonis* and *Bacteroides thetaiotamicron* (Bd and Bt) are usually found in human intestines, but much less frequently found in other animals. Both *Bacteroides* species are adapted to the high organic content environment of the intestines and are strict anaerobes that die quickly in the presence of dissolved oxygen. At that point they become substrate for microorganisms that are adapted to aerobic environments such as wastewater treatment plants or surface waters.

Both the lacZ (total coliform) and uid1&2 (EC) genetic tests were positive for essentially all of the raw sewage samples. Results for *Bacteroides* are presented for the number of positives overall and for the number of positives when uid1&2 (the EC gene) was detected. When the EC gene was detected, the Bd gene was found in 11 of 12 samples while the Bt gene was only found a third of the time.

Moving through the treatment process, samples obtained after treatment with activated sludge and settling, but before disinfection, were always positive for the EC marker, but positive less than half the time for the total coliform marker. This suggests a limitation in the sensitivity of the lacZ total coliform test. It should be noted that samples from this point in the treatment process have been subjected to at least 8 hours of treatment by a different mix of microorganisms than those that dominate in the intestinal tract of birds and mammals. In addition, there has been time for the microorganisms to settle leaving a sample with relatively little particulate matter. Despite this, samples were always positive for

the EC genetic marker. In addition, a third of the samples were still positive for the Bd genetic marker for a human source. While the wastewater was clearly dominated by human source, it is somewhat surprising that a third of the samples had the genetic marker surviving. The Bt marker test had no positive results in the treated wastewater before disinfection.

After chlorination and dechlorination, one third of the samples were still positive for the EC marker, but none were positive for the *Bacteroides* markers. This is encouraging in that it suggests that *Bacteroides* will not exist in bayou samples from wastewater effluent, even though the effluent is the great majority of the flow. However, it is also somewhat surprising because the only difference between the prechlorination and dechlorinated samples was chlorination. This is designed to kill microorganisms, without necessarily destroying genetic material, but indicates that DNA may become fragmented or otherwise unsuitable for magnification following chlorination.

4.2.2 Bayou Water Analyses

A total of 92 surface water samples were collected from Buffalo and White Oak bayous and tributaries over a ten-month period. These are grouped in Table 4-1 by main stem and tributary for each bayou. The table also lists the geometric means of the FC bacteria and the average TSS concentrations where available. Some of the agency bacteria observations were with the IDEXX EC test, and most were with the membrane filter fecal coliform test referred to as the FC test. The August 15 sampling had only EC data, which is not included in the bayou part of the summary table.

Overall, more than half of the surface water samples were positive for the EC genetic marker, while only 30% were positive for the total coliform marker. This is a similar pattern of relatively poor sensitivity of the lacZ total coliform test relative to the two step uid1&2 test.

The Bd test suggesting human source was positive overall 16% of the time, but somewhat more often for those samples that were positive for the EC genetic material. The Bt marker was detected about half as often as the Bd marker.

There does appear to be some relation between the frequency of positive Bd detections and the level of bacteria present. The tributary samples were detected as Bd positive much more often than the main stem and these tributary samples had markedly higher bacteria levels than the main stem stations on both bayous. For the tributary samples, over 40% were positive for Bd if the sample was also positive for uid1&2. Three Bd and two Bt positive results (20% and 25% respectively) were obtained when the uid1&2 test was not positive.

The main stem bayou samples tended to have low detections of the Bd and Bt markers. Only about 10% of the time on Buffalo Bayou was a human source indicated if an EC source was detectable. If the EC source was not detectable for reasons of test sensitivity, it is not reasonable to expect that the human source marker can be detected for the same reason. The percentage was 19% on White

Oak Bayou. Knowing that *Bacteroides* would not survive for too long under aerobic conditions, this suggests that the bulk of the high indicator bacteria readings that are consistently obtained on the main stem of the bayous, are not from untreated, fresh human sources.

4.2.3 Chamber Study Tests

The last two sampling periods were conducted at a time when TMDL studies of bacteria dynamics were being conducted in Buffalo Bayou. These are water samples isolated in plastic bags under ambient bayou conditions. The main focus of these tests is on the rate of change in concentration as a function of light, bacterial regrowth after wastewater discharge and resupply from sediment. Samples from these chambers were collected to help address the human source issue associated with the EC data from the chamber samples.

On July 31, 2001 the chambers were set up to analyze the effects of light level on the rate of bacteria die-off. Buffalo Bayou water from the Beltway 8 location had been placed in the plastic chambers on the previous afternoon (7/30). The EC level of that water was about 3,000-4,000 MPN/dL. On the next day water samples were collected from the chambers for DNA testing using sterilized beakers. Water samples were also collected from the chambers for EC analysis using the IDEXX method. The results listed in Table 3-1 show that after one day most of the chamber EC concentrations had dropped to the 400 to 900 MPN/dL range, but one chamber had increased to over 5,000 MPN/dL. The water in this chamber also showed positive for uid1&2, while none of the other chambers had positive DNA test results. The reason for the spike in EC concentration in that chamber is not known. Given that this chamber was the first one on the float, a possible explanation could be a wave bringing fresh bayou water into the chamber, or some other form of contamination.

On August 14, 2001 there were two types of chamber studies underway. One was a regrowth study using West District effluent and water samples collected from Buffalo Bayou at Wilcrest and Beltway 8, and the other was a light level study with water collected from Buffalo Bayou at Wilcrest and White Oak Bayou at IH-610. All the DNA test samples were collected on the second day (8/15) of the chamber operation.

The regrowth study chamber was different in that the West District effluent sample was unusually high when first collected, almost 20,000 cfu/dL. The reason for the high EC count in chlorinated and dechlorinated wastewater is unknown. The chamber that contained 100% effluent had dropped in concentration to just under 7,000 cfu/dL after one day. It was this water that was tested and found to be positive for both uid1&2 and lacZ (total coliform). It did not show positive for Bd or Bt. Together with earlier results, this indicates that EC DNA could survive the chlorination process but not Bd or Bt DNA.

The other samples were day old water originally collected from Buffalo Bayou at Wilcrest and White Oak Bayou at 610. The initial samples had been at or slightly less than 500 MPN/dL EC. After the second day the levels had dropped still further to less than 200 MPN/dL. While most showed no positive DNA results, the White Oak Bayou sample was positive for both lacZ and uid1&2. It had no positive Bd or Bt results.

There is no definitive explanation available for this result, but it is perhaps useful to note that the water in the chambers is relatively quiescent that allows particulate matter to settle more than is the case in the bayou itself. A reduced amount of suspended particulate matter can be expected to both reduce the level of bacteria in the water and also to reduce the interference problems encountered in the PCR tests. This could allow the DNA testing results to be more sensitive at the same time the overall level of bacteria is reduced. Overall, the chamber study results appear to be consistent with the bayou and effluent samples, except that the bacteria concentrations are somewhat lower and the detections less frequent. Combining the Buffalo and White Oak Bayou chamber data, there were 2 detections of uid1&2 out of 11 samples or 18%. This is a little less than half the 41% found on the Buffalo Bayou main stem samples, but the average bacteria concentration in the chambers was near 500 MPN/dL, one tenth that of the Buffalo Bayou samples.

4.3 CONCLUSIONS

The results indicate that PCR is a specific method for detecting bacteria in water; positive PCR reactions were not detected in samples that did not contain culturable bacteria. The Bacteroides PCR reactions appear to indicate human contamination; human sewage samples were consistently positive for these DNA sequences. Most of the bayou samples tested were negative for Bacteroides, indicating that the major source of coliforms in these samples was unlikely to be untreated, fresh human sources.

The major problem with these assays was insufficient sensitivity. A number of samples that were positive by culture were negative for coliforms by the PCR reaction. This appears to be a result of presence of substances in the water that interfere with the PCR reaction and poor recovery of DNA from the samples. The addition of a DNA extraction step improved the sensitivity of the reactions, but increased sensitivity is still needed. Future efforts will focus on improving the recovery of DNA from the samples prior to PCR analysis.

Other findings of the work include:

- DNA concentration should be used in combination with uid1/2 for a more sensitive assay of EC
- lacZ PCR test must be made more sensitive if it is to be used for total coliform detection

- DNA extraction is possible but is impacted by particulate matter in the water, so events that add particulate matter such as flooding, pose special problems.

Campylobacter, a pathogen generally associated with animal waste, was never detected. This suggests that animal waste may not be a major source in a system where bacteria levels are rarely less than 1000 cfu/dL even in dry weather. While birds undoubtedly provide fresh droppings in the bayous during dry weather, few other animals do. Accumulated animal waste may be one of the reasons why levels tend to be elevated when rain runoff becomes a significant part of bayou flow.

A factor may be that the bayou sediments contain and support elevated bacteria levels. This is a separate subject of the TMDL process and will be discussed in other publications.

5.0 DISCUSSION

The major finding of the study is that the indicators of fresh human sources of bacteria, Bd and Bt, were found relatively infrequently in bayou water samples. Of the 35 main stem bayou samples where the EC marker was detected, only 5 (14%) showed positive for Bd or Bt. This should not be taken as a definitive result because of the sensitivity problems discussed above, but it nevertheless suggests that sources other than untreated, fresh human waste may be significant in these systems.

There appears to be a relationship between suspended particulate matter, as represented in the TSS measurement, and detection of Bd. Buffalo Bayou samples had over twice the TSS concentration of White Oak Bayou samples, and twice the bacteria concentrations, yet the frequency of Bd detection on Buffalo was half that of White Oak Bayou samples. The tributary samples tended to have the highest bacteria levels and the lowest TSS concentrations, and their frequency of Bd detection if the EC gene was detected, was much higher than the main stem stations.

Another finding is that while some EC genetic material is capable of surviving the chlorination process, the human source indicators do not. As mentioned earlier, one third of the wastewater samples were still positive for the EC marker after chlorination and dechlorination, but none were positive for the *Bacteroides* markers.

It must be recognized that determining the presence of fresh human wastes in a background of treated human waste will always be a challenge. If the sensitivity is raised too far it may be possible to detect residual genetic material from Bacteroides after the treatment process. Accordingly, there will always be a challenge in a system such as the effluent dominated Houston-area bayous. While it can always be expected to be a challenge, it is a challenge that must ultimately be met in order to formulate effective policies for dealing with a system that exceeds water quality criteria for contact recreation by a substantial margin.

These data and previous studies suggest that the predominant source of coliforms and E. coli in area bayous is non-human. Ideally, there should be a method for determining the source of these bacteria. The available literature indicates there is not a high correlation between particular Bacteroides species and specific non-human animals. Thus, while the presence of Bacteroides distosonis or thetaiotamicron indicates human fecal contamination, the presence of other Bacteroides species cannot be used as a marker for specific animal sources. An alternative approach would be to take advantage of RFLP data analysis that has shown that E. coli isolates associated with certain animal species have distinct RFLP patterns. By incorporating a PCR reaction spanning one or more of these sites and adding an additional restriction enzyme digestion step, it may be possible to determine the likely source for E. coli DNA detected in water samples.

6.0 REFERENCES

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