An Investigation of the Potential for Acute Toxicity to the Crayfish, *Procambarus peninsulanus*, Caused by the Application of the Herbicide, Aquathol, at Wakulla Springs State Park

Study conducted March through May 2005

Report date: July 2005

Biology Section

Bureau of Laboratories

Division of Resource Assessment and Management

Introduction

Wakulla Springs State Park biologists contacted the Florida Department of Environmental Protection's (FDEP) Bureau of Laboratories asking for assistance in addressing their concerns about crayfish mortality observed after an application of the herbicide, Aquathol, at the park. The park has been applying the herbicide at the spring periodically to reduce the abundance of *Hydrilla verticillata* (Appendix 1). Based upon toxicity data provided by the manufacturer, the concentration of Aquathol being applied at the park was not believed to be within the acutely toxic range for crayfish; however park staff had observed some crayfish mortality within the Wakulla River following at least one application.

The objectives of the study were two fold. The first objective was to determine if the concentrations of Aquathol within the Wakulla River were capable of causing mortality to crayfish in *in-situ* test chambers, and the second objective was to determine if the decay of the *Hydrilla* mats caused the dissolved oxygen (DO) concentrations in the river to drop to levels that the crayfish could not tolerate.

A preliminary study was performed in order to determine the feasibility of using caged crayfish within the Wakulla River. Results of the preliminary study were favorable; therefore arrangements were made to deploy caged crayfish during the next herbicide application.

In addition to outlining the method development, this report documents the results of the preliminary study, a follow-up trial, and the April 27th, 2005 Aquathol application at Wakulla Springs State Park.

Conceptual Study Design

The study design selected for this project was based upon aquatic toxicity methods, with multiple downstream test sites being established within the Wakulla River and a control site being established in McBride Slough. Since the herbicide application was to be performed at the spring head, a control site could not be established within the Wakulla River. McBride Slough is a much smaller spring run, but the water has physical-chemical properties similar to Wakulla Spring water. Crayfish were collected from McBride Slough and held overnight in aerated coolers before being deployed *in-situ* in polypropylene mesh test chambers the day of the herbicide application. The herbicide application did not exceed 48 hours; however the crayfish remained deployed for 4 days in order to determine if any latent mortality occurred. Approximately 7 to 10 days post-herbicide application, a second set of crayfish were similarly deployed in order to determine if the decomposing vegetation caused the DO concentrations within the river to be depressed to the extent that could cause crayfish mortality. These crayfish were also deployed for 4 days.

Preliminary Study Methods

The preliminary study had three objectives:

1) to determine if there was any latent mortality associated with the collection of wild crayfish from a nearby stream, McBride's Slough,
2) to determine the level of mortality associated with holding the crayfish collected at McBride's Slough in Wakulla Spring water, and
3) to determine if the caged crayfish deployed for four days in the Wakulla Spring run would suffer any mortality in the absence of the Aquathol application.

With the 3 objectives in mind, approximately 40 crayfish, *Procambarus peninsulanus* (Appendix 1), were collected on 3/9/05 from McBride Slough using D-frame dipnet sweeps. Different habitats (e.g. aquatic vegetation, roots) in the stream were swept and any crayfish captured were removed from the dipnet and placed into a cooler with site water. All other organisms and detritus were returned to the stream. Water was collected from two sites, McBride Slough (Control Site) and Wakulla Springs (Test Site). Physical chemistry parameters were measured at each site at the time the water was collected (see Table 1). The water and crayfish were then transported to the FDEP Laboratory in Tallahassee and put into three separate coolers. Two coolers contained water from Wakulla Springs and one contained water from McBride Slough. All three coolers were continuously aerated with an air stone. Based on length the 40 crayfish were divided into three class sizes: small, 1-1.25"; medium, 1.5-2"; and large $\geq$2.25" (23 small, 14 medium and 3 large). Once measured the crayfish were transferred to the coolers containing water from Wakulla Springs and McBride Slough. All 3 sizes were represented in each cooler. One crayfish from each cooler was placed in an *in-situ* test chamber (see Figure 1) inside the cooler in order to observe any effects of the chamber itself on the crayfish**.** Each chamber is made of 3-mm polypropylene mesh measuring 7" long and 3" in diameter and contains a 3" length of 2" (outer diameter) PVC pipe which serves as shelter for the crayfish in order to reduce stress. The crayfish were kept in these coolers overnight. Care was taken to reduce stress on the crayfish.

The next morning (3/10/05), the caged crayfish in the coolers were observed and appeared to be in good condition. The crayfish were then transported to McBride Slough. At McBride Slough five (1 large, 2 medium and 2 small) crayfish were selected and placed into a bucket with water from McBride Slough (cooler water mixed with stream water) in order to acclimate the crayfish to any differences in the water chemistry. The five crayfish were then transported to the in-stream site and each was placed into a separate chamber with some plant material for food. The chambers were hooked together and anchored using Hester-Dendy blocks on each end (Figure 2). The chambers were then placed into the middle of the stream at approximately 1m deep. The deployment site was bordered by mats of aquatic vegetation (e.g. *Vallisneria americana*); however the middle of the stream was sandy with good flow. The sampling team then went to the Wakulla Springs site. The same methods were used to deploy the crayfish; however ten crayfish were placed (2 large, 4 medium and 4 small) into ten chambers (two sets of five), with one crayfish in each chamber. The deployment site was adjacent to the boat dock in an area with minimal *Hydrilla* mats and approximately 2 m deep. Physical chemistry parameters were measured at both sites (Table 1). The crayfish were retrieved from the two sites four days (96-hours) later on 3/14/05.

Preliminary Study Results and Discussion

In the preliminary study, all crayfish appeared healthy and active when the chambers were retrieved (Table 2). The size of the crayfish did not appear to affect the probability of survival. The plant material was gone except for a small amount that remained in a few of the chambers. The crayfish were counted and released in the stream. Physical chemistry parameters were measured at each site (Table 1).

Herbicide Application Trial Methods

Aborted Herbicide Application

The herbicide application was scheduled to occur on 4/11/05. However due to rainfall during the previous weekend, the river's water level and flow rose to a point such that the herbicide concentration could not be established with the on-hand supply. Therefore this aborted application was used as an additional trial for this study. For this follow-up trial (4/11/05-4/15/05), the same methods as described in the preliminary study were carried out with the addition of 2 new sites at Wakulla Springs and recording data sondes. The sites were added to establish a gradient downstream of the herbicide dispersal site at the spring boil; resulting in a total of 3 Test Sites in the Wakulla River and a Control Site at McBride Slough (see maps in Appendix 2). Test Site 1 was established approximately 200 meters downstream from the spring vent. Test Site 2 was approximately 300 meters down from Test Site 1, and Test Site 3 was approximately 640 meters down from Test Site 2. The Control Site at McBride Slough was approximately 200 meters downstream of the bridge at Highway 267. The crayfish chambers were deployed at depths of 1-3 m with the Control Site being the shallowest and Test Site 1 being the deepest. Each site had mats of aquatic vegetation in close proximity of the chambers, but the chambers were not smothered. One data sonde was suspended at approximately 1 m deep at each of the 4 sites. The sondes were used to collect dissolved oxygen (DO), pH, temperature, and conductivity measurements over the course of the 4-day deployment. Crayfish and plant material for the test chambers were collected from McBride Slough on 4/8/05.

Successful Herbicide Application

On 4/27/05, the herbicide, Aquathol, was dispersed at the main boil for 46 hours at a rate to achieve a concentration in the river of 1-2 mg/L of Aquathol. Water samples were collected (by Jess VanDyke, FDEP Division of State Lands) at 6 sites along the river (Appendix 2) for determining herbicide concentration in the river.

Sondes and crayfish were deployed at the 4 established sites following the same methods as previously described. The crayfish were deployed just prior to the initiation of the herbicide application and remained at the sites until 5/2/05. Crayfish and plant material for the test chambers were collected from McBride Slough on 4/25/05.

Post Application test: On 5/6/05, nine days after herbicide application, sondes and crayfish were deployed again in the same manner for four days. Crayfish and plant material for the test chambers were collected from McBride Slough on 5/5/05.

Herbicide Application Trial Results and Discussion

Aborted Herbicide Application

All 20 crayfish were retrieved and appeared to be healthy and active (Table 2). All 4 data sondes were retrieved and in good condition, however the calibration for conductivity did not verify for the data sonde from Test Site 1, and the calibration for DO did not verify for the sonde from Test Site 2 (Table 3). Therefore those data (Appendix 4) are estimated. The DO ranged from 3.0 to 2.0 mg/L at Test Site 1, 5.5 to 2.8 mg/L at Test Site 2, and 5.7 to 2.7 at Test Site 3 (Appendix 4).

Successful Herbicide Application

Herbicide concentrations ranged from 0.41 mg/L at the Highway 98 Bridge to 2.06 mg/L at the boat dock (Table 4). Nineteen of the 20 chambers were retrieved on 5/2/05. All 19 crayfish from all 4 sites survived and were released. One chamber had become detached from the anchor and was missing from Test Site 2. This chamber was recovered on 5/6/05 near Test Site 2 and the crayfish inside was alive and in good condition after 9 days. Observations made at the sites noted large mats of *Hydrilla* sloughing off and floating downstream. A large mass actually became entangled with some of the cabling at Test Site 2, which may have caused the one chamber to become detached.

The data sondes were recovered and the calibrations verified for all parameters for all 4 sondes (Table 5). However, no physical chemistry data were reported from Test Site 3 or the Control Site due to data download malfunctions back at the laboratory. The DO level at Test Site 1 consistently measured well below the Surface Water Quality Criterion (Appendix 5, >5.0 mg/L, 62-302.530(31) F.A.C.). The DO level at Test Site 2 was higher, but still measured below 5.0 mg/L at times.

Post Application test: From the final deployment, 20 chambers were retrieved on 5/10/05. Nineteen of the 20 crayfish survived and appeared in good condition. One crayfish recovered from Test Site 1 was dead, to yield a 6.7% mortality. The mortality could have been due to the very low DO levels measured at Test Site 1, or it could have been due to chance. Test Site 1 had the lowest DO measurements compared to Test Site 3 and the Control Site (Figure 3, Appendix 5). As in all trials, the DO levels increased from upstream to downstream. The data sonde calibrations all verified, except for the sonde from Test Site 2 (Table 6). The sonde from Test Site 2 also failed to download any physical chemistry data, so no measurements were recorded from this site for this portion of the study. The sonde and chambers were moved slightly from the original position to avoid fouling by the sloughing plant material, so this is not suspected to be the cause of the equipment failure.

Summary

The preliminary study established that the crayfish did not appear to be effected by handling, living in a chamber for several days, or the normal levels of dissolved oxygen at Wakulla Springs and McBride Slough; and the follow-up trial reinforced these findings. The methods used in this study did not detect any effect of the herbicide on the crayfish in Wakulla Springs. The decay of the plant material showed an effect on the DO levels at Test Site 1. The levels cycled during the first days of the herbicide application ranging from 4.3 to 2.2 mg/L. After the application, the DO concentration was steadily measured at barely 2.0 mg/L. These persistent low DO levels may have had some effect on the crayfish, but the low % mortality measured by these methods does not provide enough evidence to be certain.

Table 1. *Physical Chemistry Results for the Preliminary Study*

Preliminary Crayfish Study	McBride Slough	Wakulla Springs	McBride Slough	Wakulla Springs	McBride Slough	Wakulla Springs
Date Measured	**3/9/2005**	**3/9/2005**	**3/10/2005**	**3/10/2005**	**3/14/2005**	**3/14/2005**
Dissolved Oxygen (mg/L)	2.46	1.59	4.1	0.59	5.5	0.77
Temperature (°C)	18.24	20.49	18.1	20.8	21.2	20.8
pH (S.U.)	7.42	7.94	7.7	7.9	7.0	7.3
Conductivity (µmhos/cm)	347	317	345	318	343	310

Figure 1. ***Chamber***

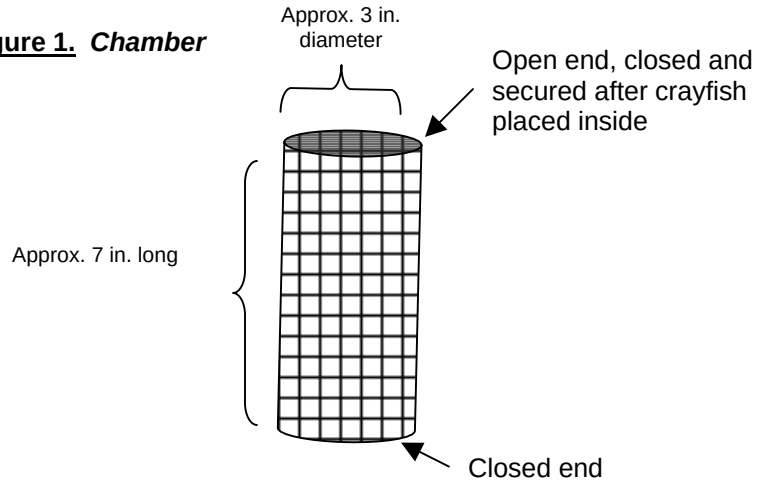


Figure 2. ***Chamber deployment arrangement***

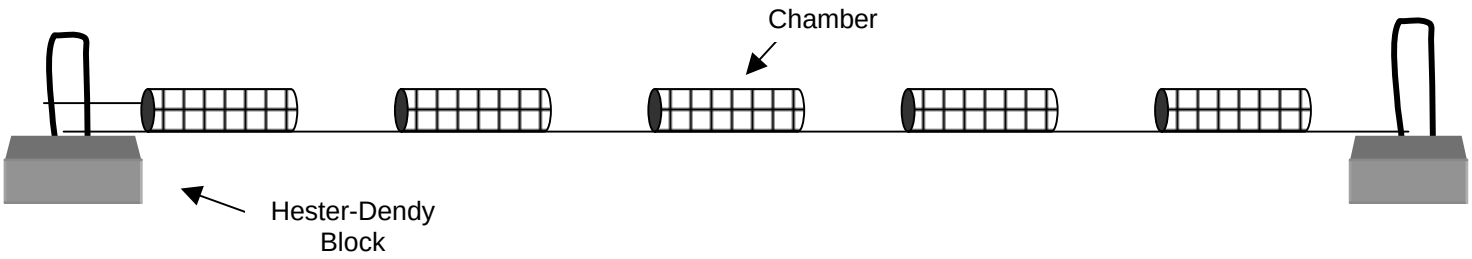


Table 2. Crayfish Mortality Results

Study Phase	Site location	Deployed	Retrieved	# Crayfish Deployed	# Crayfish Retrieved	% Mortality
Preliminary	Wakulla River	3/9/2005	3/14/2005	5	5	0%
Preliminary	McBride Slough	3/9/2005	3/14/2005	5	5	0%
Follow-up	Wakulla River	4/11/2005	4/15/2005	15	15	0%
Follow-up	McBride Slough	4/11/2005	4/15/2005	5	5	0%
Herbicide Applied	Wakulla River	4/27/2005	5/2/2005	15	15	0%*
Herbicide Applied	McBride Slough	4/27/2005	5/2/2005	5	5	0%
Post-application	Wakulla River	5/6/2005	5/10/2005	15	14	6.7%
Post-application	McBride Slough	5/6/2005	5/10/2005	5	5	0%

*One chamber was missing, but it was recovered on 5/6/05. Crayfish inside was still alive after 9 days of exposure.

Table 3. Data sonde calibration and verification results for the Follow-up Trial

Deployment Site	Sonde#	D.O mg/L		Temperature ºC		pH 7.0 S.U.		pH 10.0 S.U.		Conductivity 105 µmhos/cm	
		ICV 4/11/05	CCV 4/15/05	Actual 4/11/05	Actual 4/15/05	ICV 4/11/05	CCV 4/15/05	ICV 4/11/05	CCV 4/15/05	ICV 4/11/05	CCV 4/15/05
Control Site	**#118147**	8.5	8.8	23.9	22.1	7.0	7.1	10.0	*	105	103[1]
Test Site 1	**#118159**	8.6	8.4	22.8	24.3	7.0	7.2	10.0	*	105	86[1]
Test Site 2	**#111313**	8.5	7.5	23.5	23.3	7.0	7.0	10.0	*	105	101[1]
Test Site 3	**#118149**	8.4	8.6	23.7	24.1	7.0	7.2	10.0	*	105	102[1]

[1]Standard value = 99.2
ICV - Initial Calibration Verification
CCV - Continuing Calibration Verification
CCV failure
* Not necessary per DEP SOP FT1100

Table 4. Aquathol concentrations from samples collected on

Site Name	Sample 1 (mg/L)	Sample 2 (mg/L)	Average (mg/L)
Boat Dock	1.73	2.06	**1.90**
1st turn	1.25	0.97	**1.11**
Ways	1.34	1.48	**1.41**
Turnaround	0.96	0.8	**0.88**
365 Bridge	1.14	1.03	**1.09**
98 Bridge	0.44	0.41	**0.425**

Table 5. Data sonde calibration and verification results for the Herbicide Application Test

Deployment Site	Sonde#	D.O mg/L		Temperature ºC		pH 7.0 S.U.		pH 10.0 S.U.		Conductivity 99.2 µmhos/cm	
		ICV 4/27/05	CCV 5/2/05	Actual 4/27/05	Actual 5/2/05	ICV 4/27/05	CCV 5/2/05	ICV 4/27/05	CCV 5/2/05	ICV 4/27/05	CCV 5/2/05
Control Site	**#111313**	99.4%^	9.1	21.8	24.2	7.2	6.9	10.0	*	99	98
Test Site 1	**#118159**	99.7%^	8.6	22.3	23.3	6.9	7.0	10.1	*	99	100
Test Site 2	**#118147**	100%^	8.5	22.4	23.6	7.1	7.1	10.1	*	99	99
Test Site 3	**#118156**	100%^	8.7	21.7	23.3	7.1	7.1	10.2	*	99	98

^Recorded as %saturation
ICV - Initial Calibration Verification
CCV - Continuing Calibration Verification
CCV failure
* Not necessary per DEP SOP FT1100

<u>Figure 3.</u> Dissolved Oxygen measurements for the Herbicide Application phase of the study.

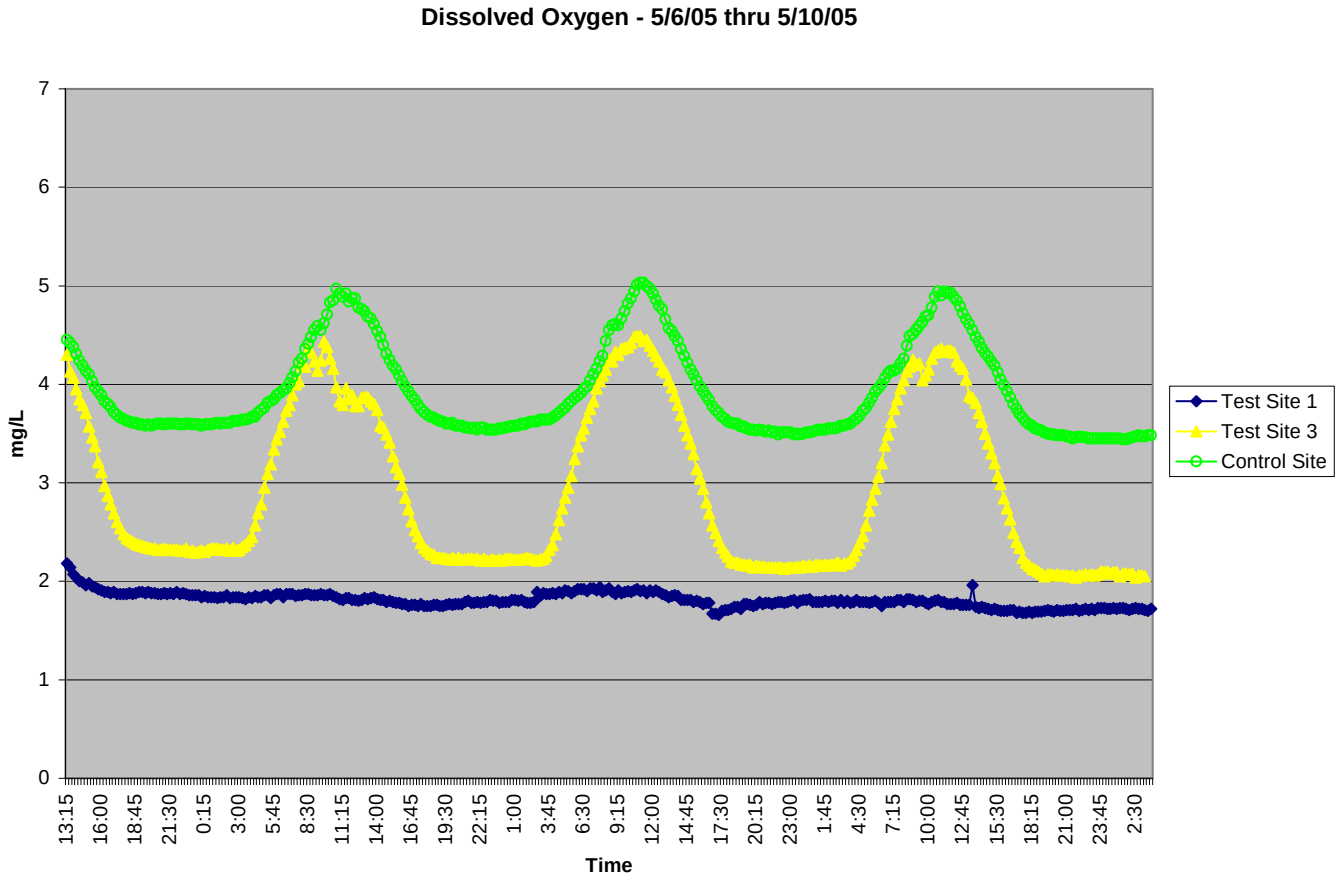


Table 6. Data sonde calibration and verification results for the Post-application Test

Deployment Site	Sonde#	D.O mg/L		Temperature ºC		pH 7.0 S.U.		pH 10.0 S.U.		Conductivity 94.7 µmhos/cm	
		ICV 5/6/05	CCV 5/10/05	Actual 5/6/05	Actual 5/10/05	ICV 5/6/05	CCV 5/10/05	ICV 5/6/05	CCV 5/10/05	ICV 5/6/05	CCV 5/10/05
Control Site	**#118156**	9.0	8.7	20.6	23.8	7.0	7.1	10.0	*	95	95[2]
Test Site 1	**#118147**	8.5	8.7	23.5	23.9	6.9	7.0	10.1	*	95	101[2]
Test Site 2	**Biology#3**	8.5	2.0	23.5	22.9	7.1	7.3	8.3	*	101[1]	97[2]
Test Site 3	**#118159**	8.5	8.8	23.7	23.6	7.0	7.0	10.0	*	95	97[2]

[1]Standard true value = 100.6

[2]Standard true value = 99.2

ICV - Initial Calibration Verification

CCV - Continuing Calibration Verification

CCV failure

* Not necessary per DEP SOP FT1100

Appendices

- Appendix 1. *Hydrilla verticillata* and *Procambarus peninsulanus*
- Appendix 2. Maps of sites
- Appendix 3. Graphs of data from sondes for Follow-up trial phase
- Appendix 4. Graphs of data from sondes for Herbicide Application phase
- Appendix 5. Graphs of data from sondes for Post-application phase
- Appendix 6. Sonde calibration and verification criteria

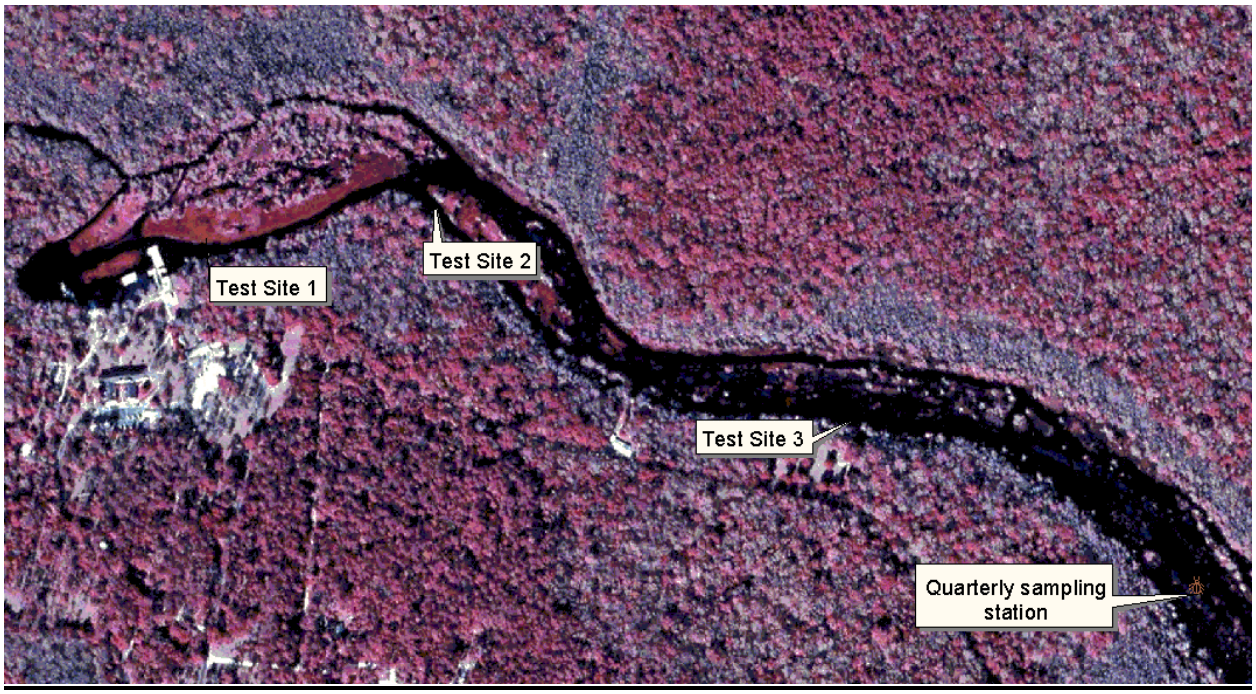
Appendix 1

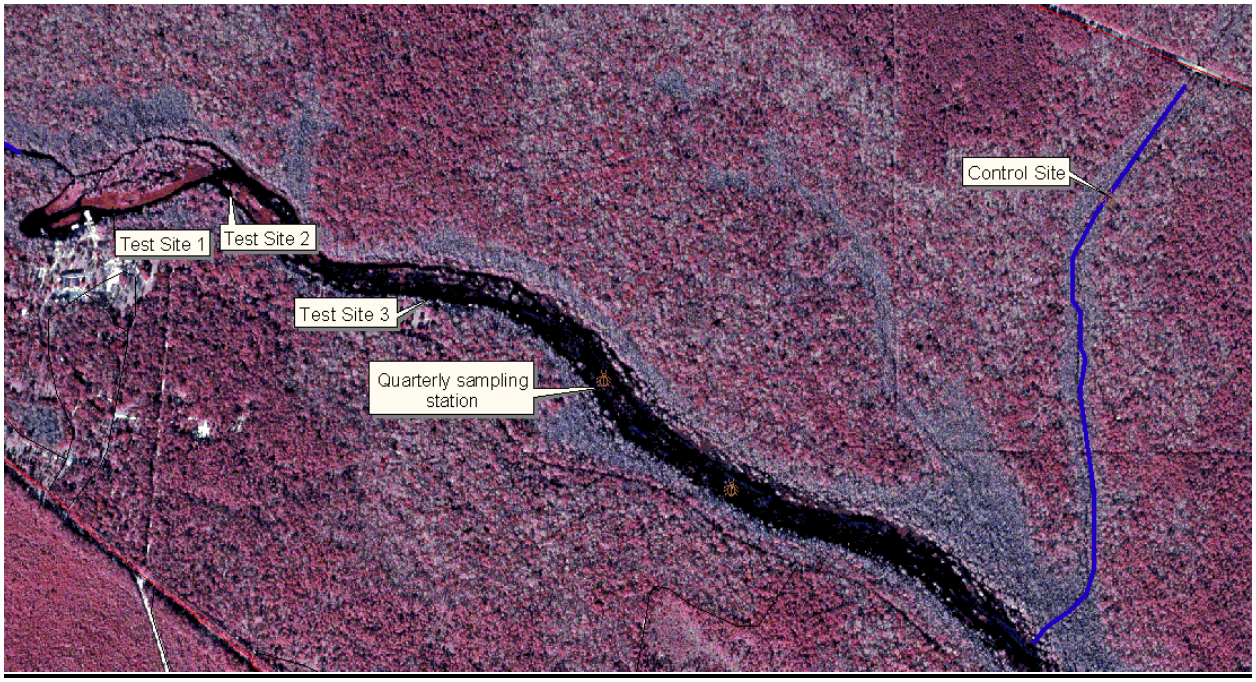
Hydrilla mats at Wakulla Springs on 5/2/05.


FL DEP REFERENCE
FL: Wakulla Co.
Location: M & Br. da Hwy
SR 267
Date: 8 Apr. 2005
Sample #
Coll: M. W. Heyn & J. Ray
Decapoda: Cambar. dea
Procambarus (S.)
paeninsulanus (Faxon)
det: M. W. Heyn. '05
INCHES
CENTIMETERS
Cat. No. 09-016

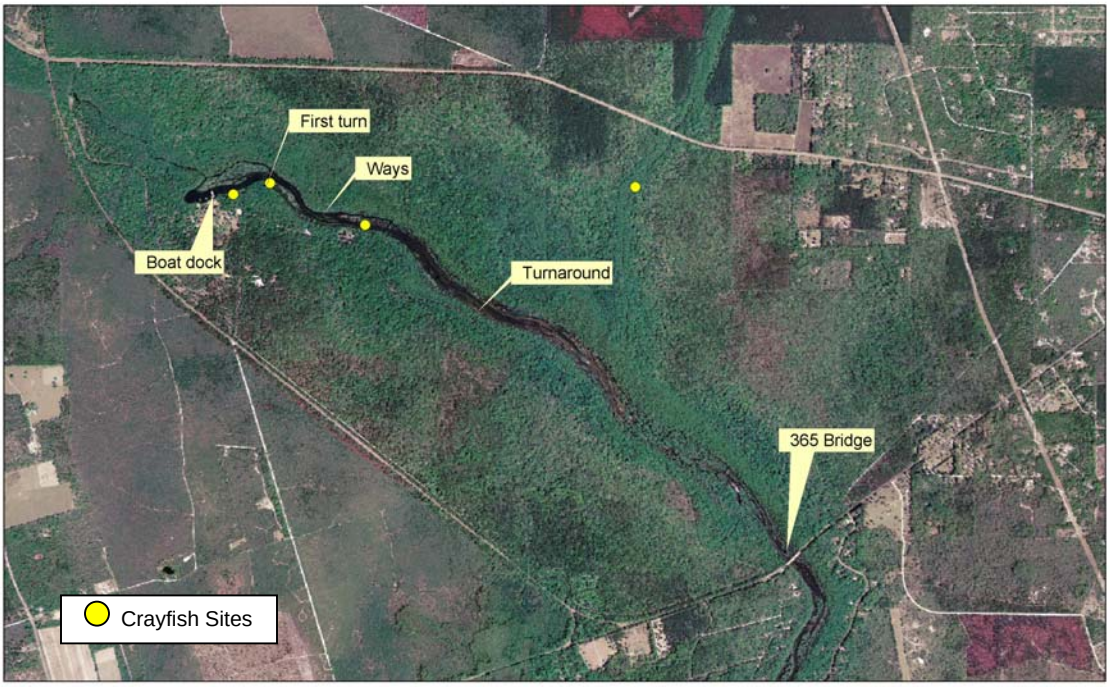
Appendix 2

Maps of Sites



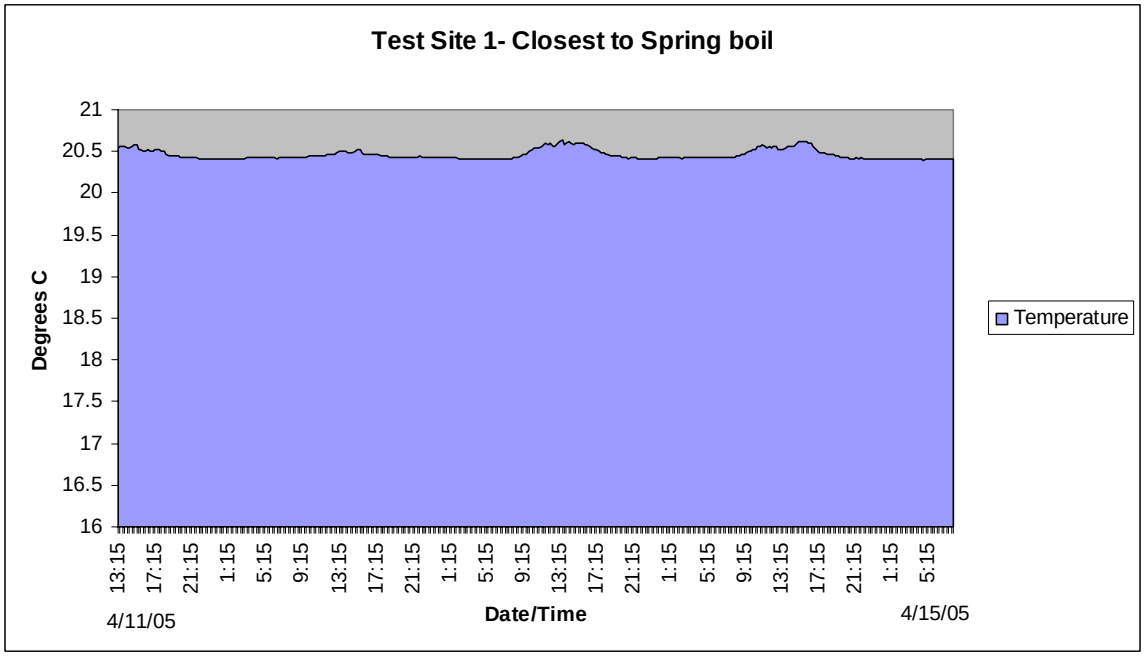


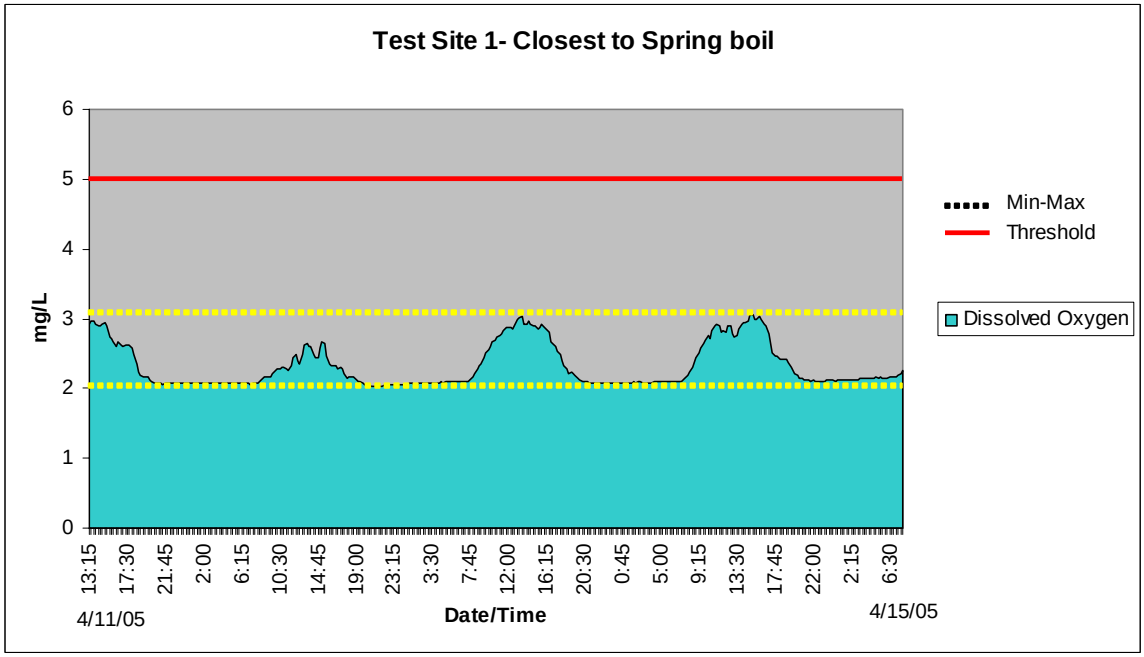
Map of Aquathol sample collection sites

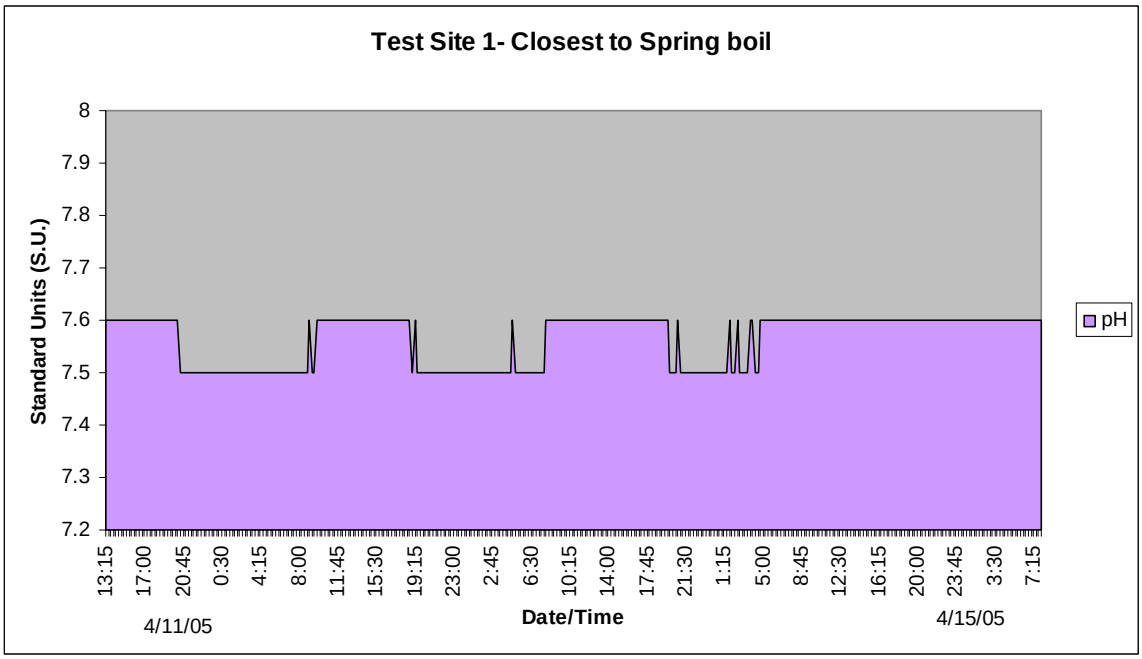


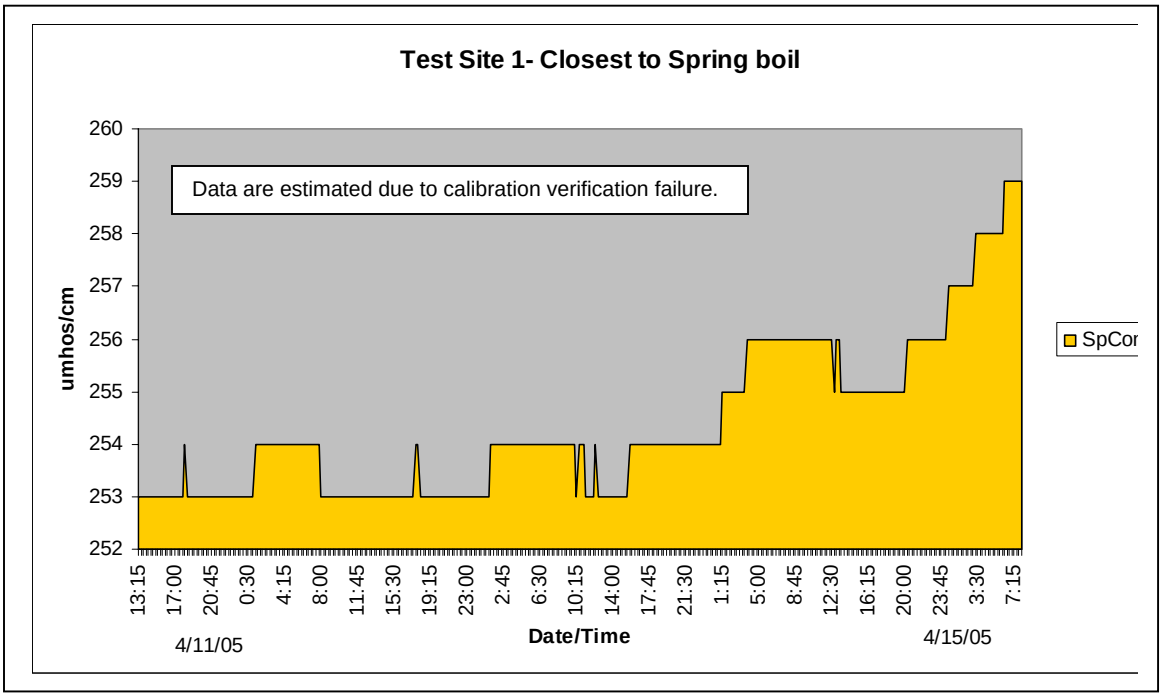
Appendix 3

Physical Chemistry Data from Test Sites 1, 2 & 3 and the Control Site 4/11/05 – 4/15/05






Test Site 1- Closest to Spring boil
Standard Units (S.U.)
8
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
13:15
17:00
20:45
0:30
4:15
8:00
11:45
15:30
19:15
23:00
2:45
6:30
10:15
14:00
17:45
21:30
1:15
5:00
8:45
12:30
16:15
20:00
23:45
3:30
7:15
4/11/05
Date/Time
4/15/05
pH


Test Site 1- Closest to Spring boil
Data are estimated due to calibration verification failure.
umhos/cm
260
259
258
257
256
255
254
253
252
13:15
17:00
20:45
0:30
4:15
8:00
11:45
15:30
19:15
23:00
2:45
6:30
10:15
14:00
17:45
21:30
1:15
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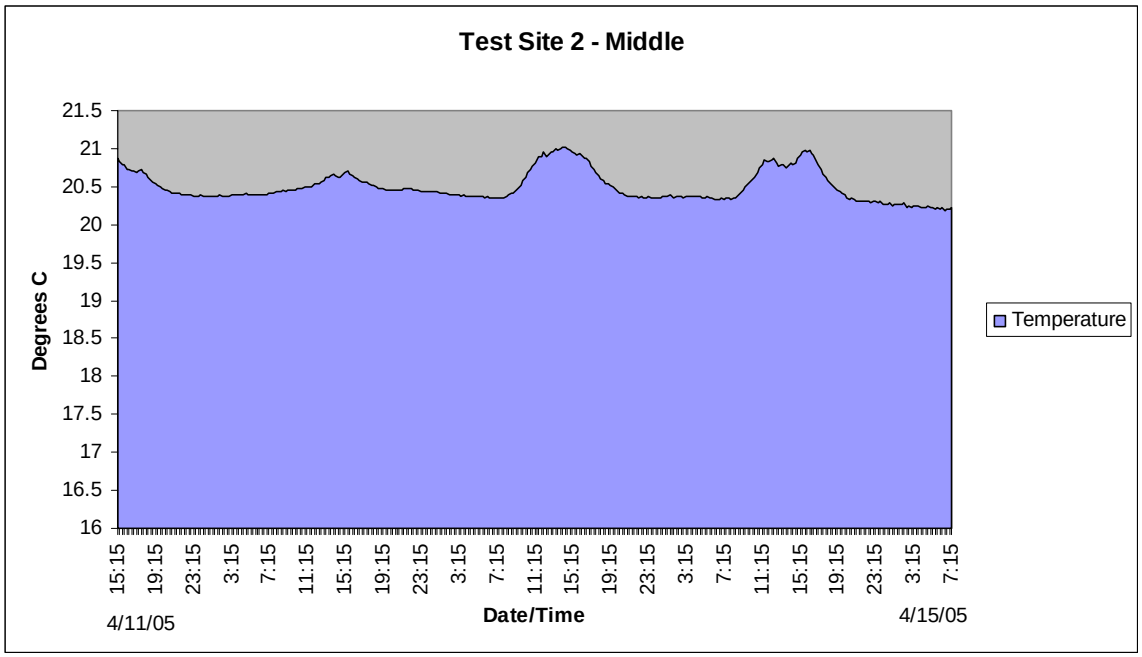
7:15
4/11/05
Date/Time
4/15/05
SpCor


Test Site 2 - Middle
Degrees C
21.5
21
20.5
20
19.5
19
18.5
18
17.5
17
16.5
16
15:15
19:15
23:15
3:15
7:15
11:15
15:15
19:15
23:15
3:15
7:15
11:15
15:15
19:15
23:15
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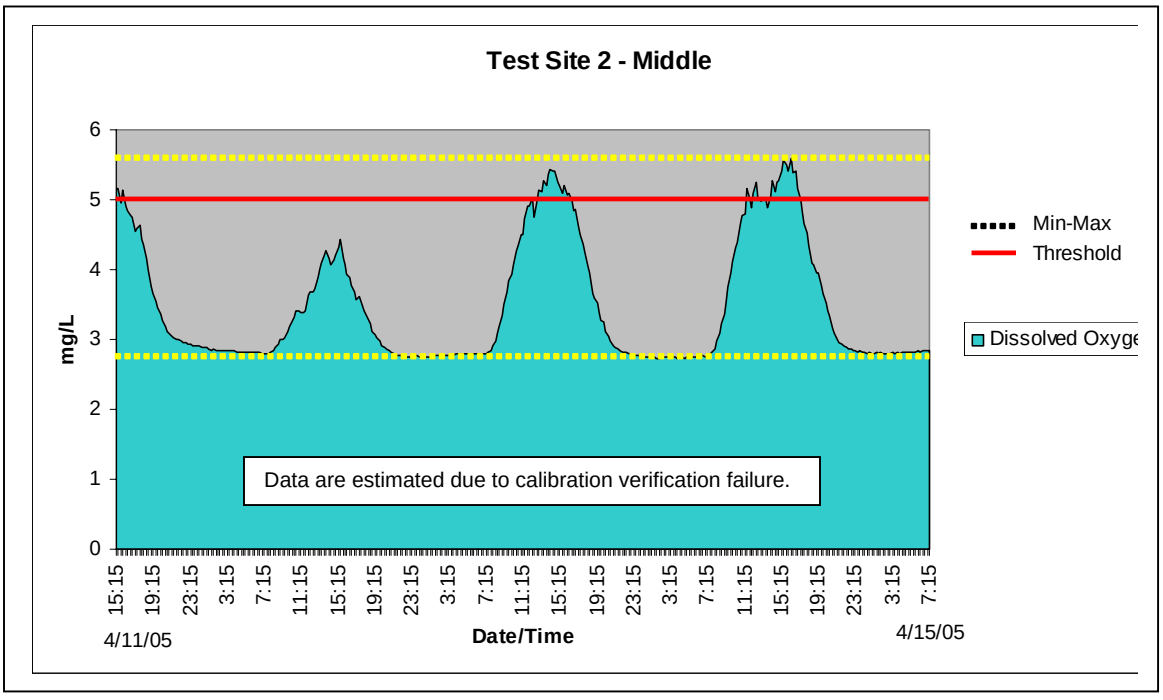
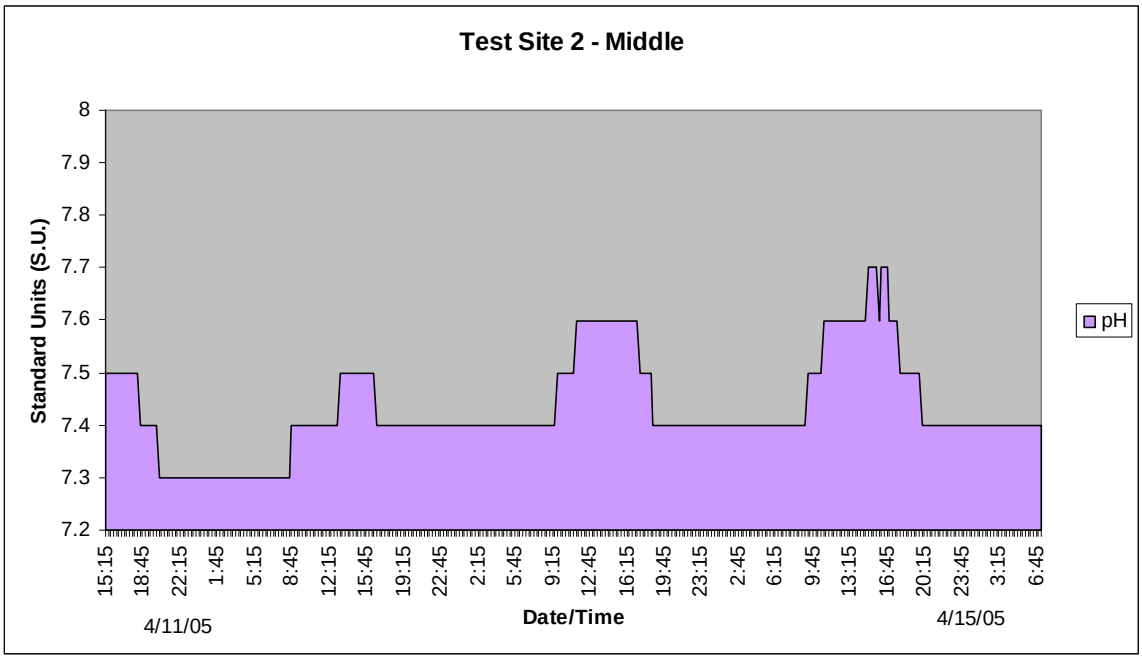
7:15
4/11/05
Date/Time
4/15/05
Temperature


Test Site 2 - Middle
mg/L
6
5
4
3
2
1
0
15:15
19:15
23:15
3:15
7:15
11:15
15:15
19:15
23:15
3:15
7:15
11:15
15:15
19:15
23:15
3:15
7:15
11:15
15:15
19:15
23:15
3:15
7:15
4/11/05
Date/Time
4/15/05
Min-Max
Threshold
Dissolved Oxyge
Data are estimated due to calibration verification failure.


Test Site 2 - Middle
Standard Units (S.U.)
8
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
15:15
18:45
22:15
1:45
5:15
8:45
12:15
15:45
19:15
22:45
2:15
5:45
9:15
12:45
16:15
19:45
23:15
2:45
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Date/Time
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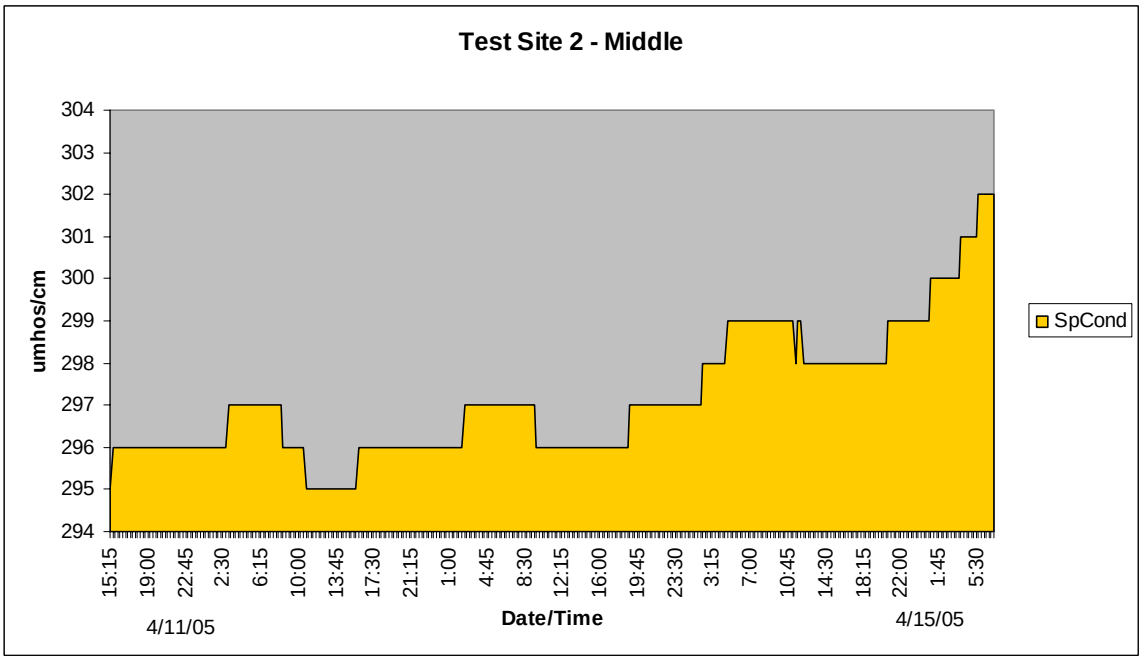
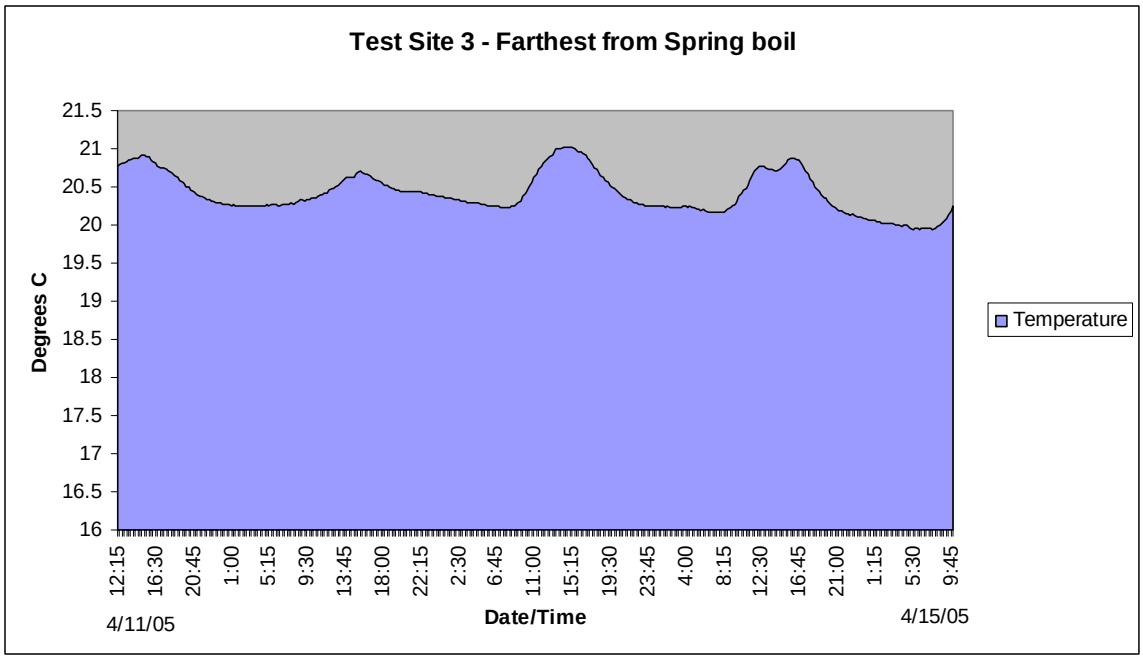
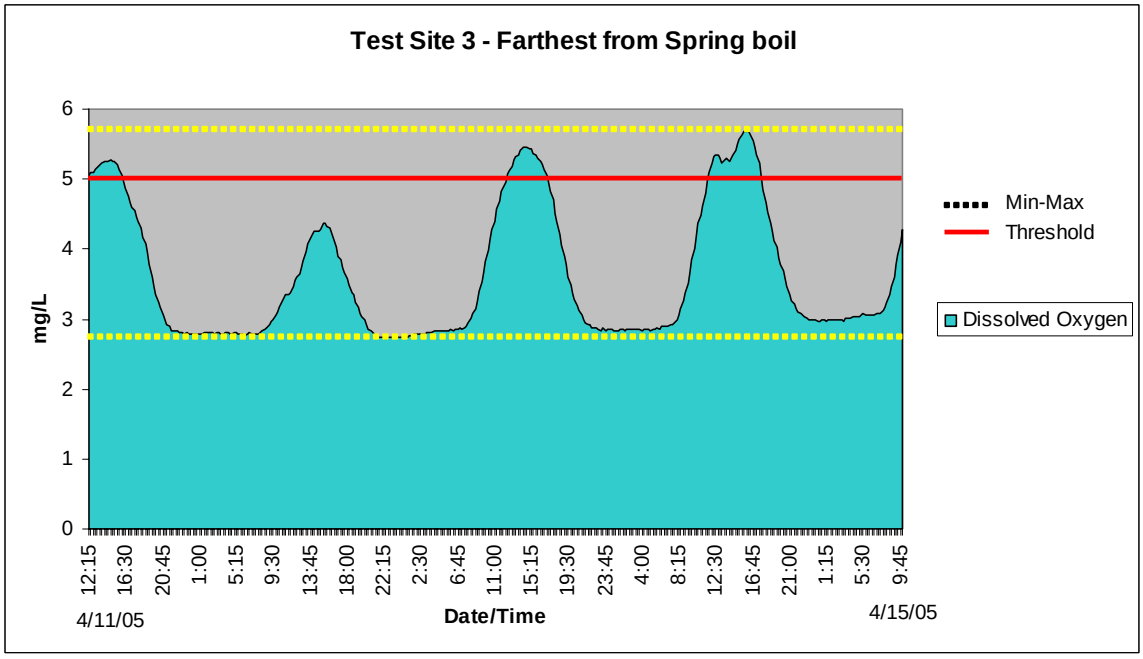
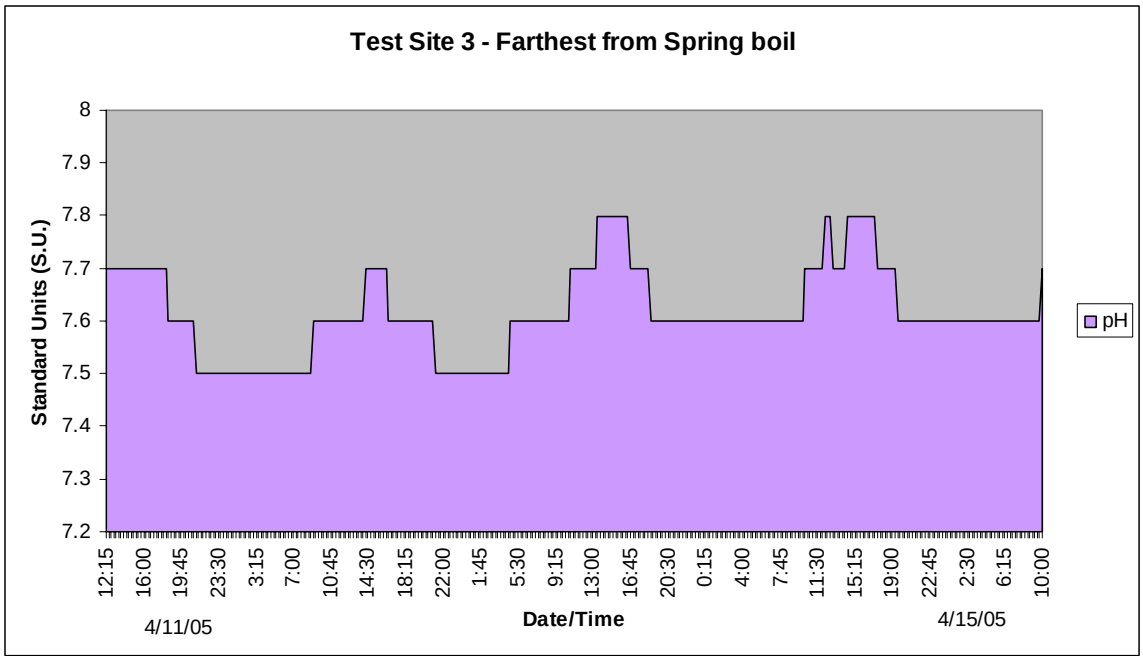
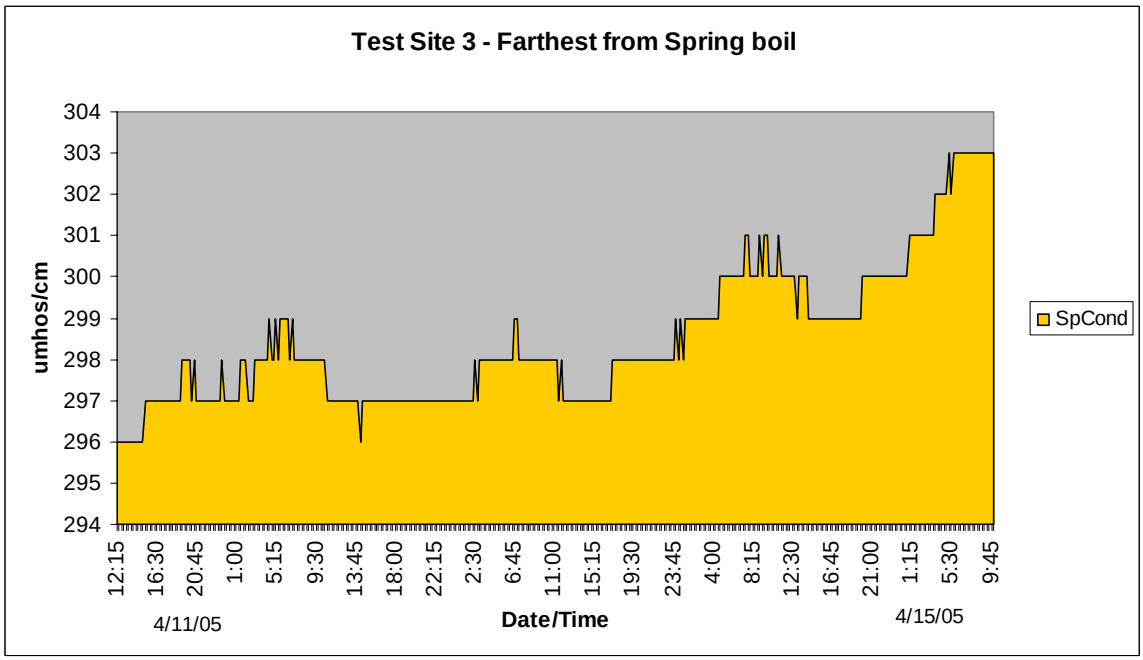
pH

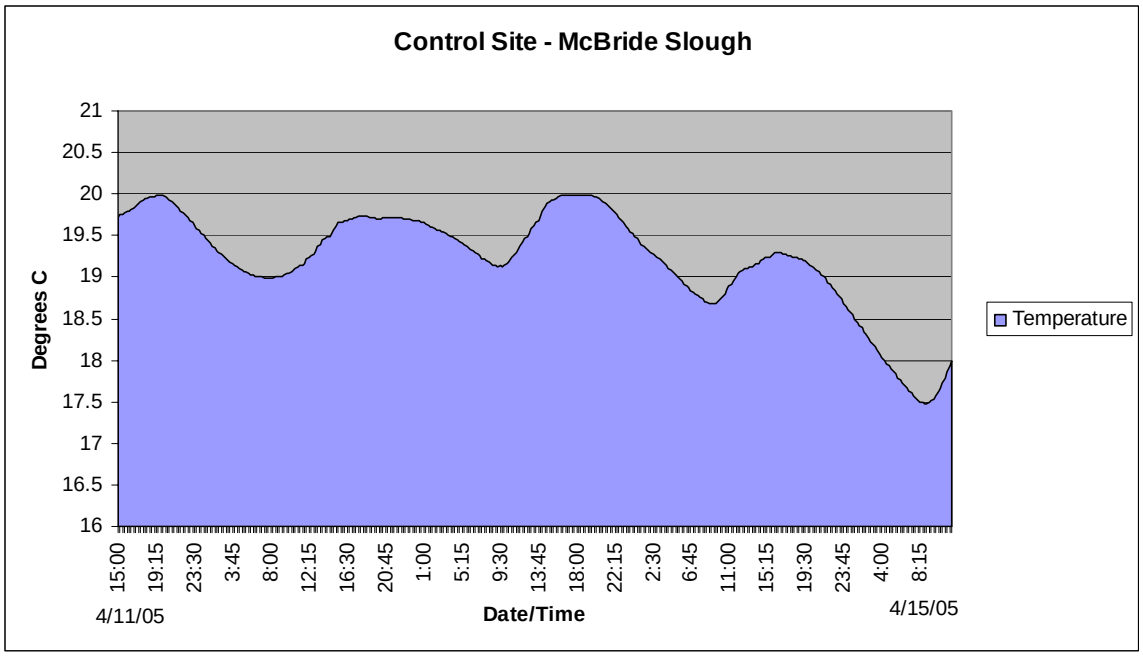

Test Site 2 - Middle
umhos/cm
304
303
302
301
300
299
298
297
296
295
294
15:15
19:00
22:45
2:30
6:15
10:00
13:45
17:30
21:15
1:00
4:45
8:30
12:15
16:00
19:45
23:30
3:15
7:00
10:45
14:30
18:15
22:00
1:45
5:30
4/11/05
Date/Time
4/15/05
SpCond

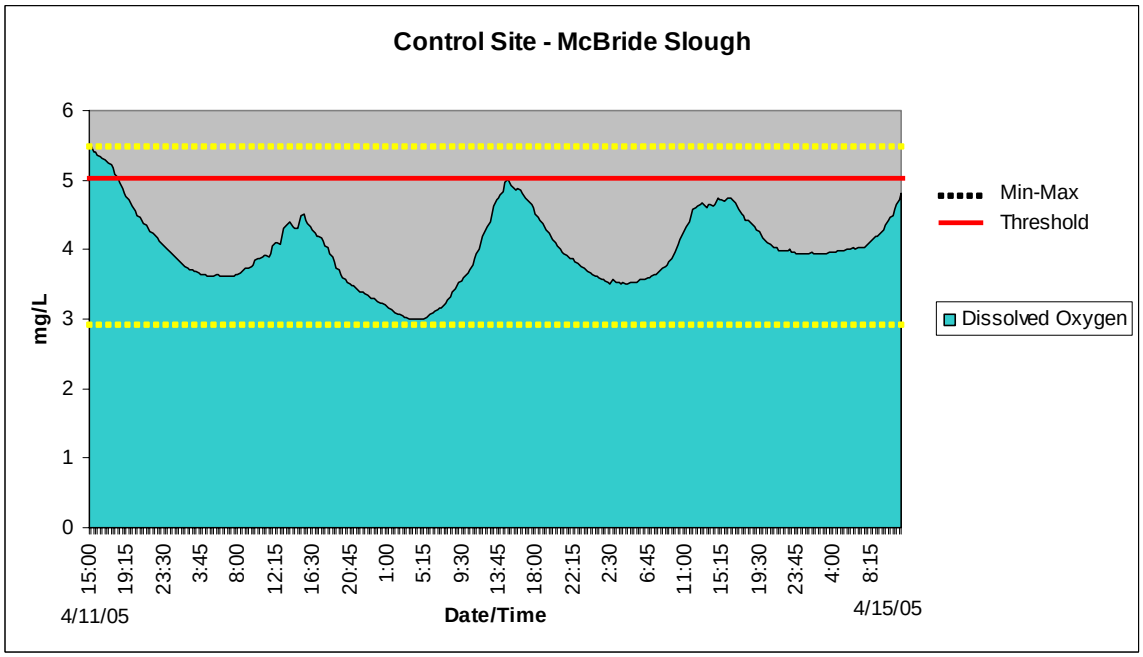

Test Site 3 - Farthest from Spring boil
Degrees C
21.5
21
20.5
20
19.5
19
18.5
18
17.5
17
16.5
16
12:15
16:30
20:45
1:00
5:15
9:30
13:45
18:00
22:15
2:30
6:45
11:00
15:15
19:30
23:45
4:00
8:15
12:30
16:45
21:00
1:15
5:30
9:45
4/11/05
Date/Time
4/15/05
Temperature

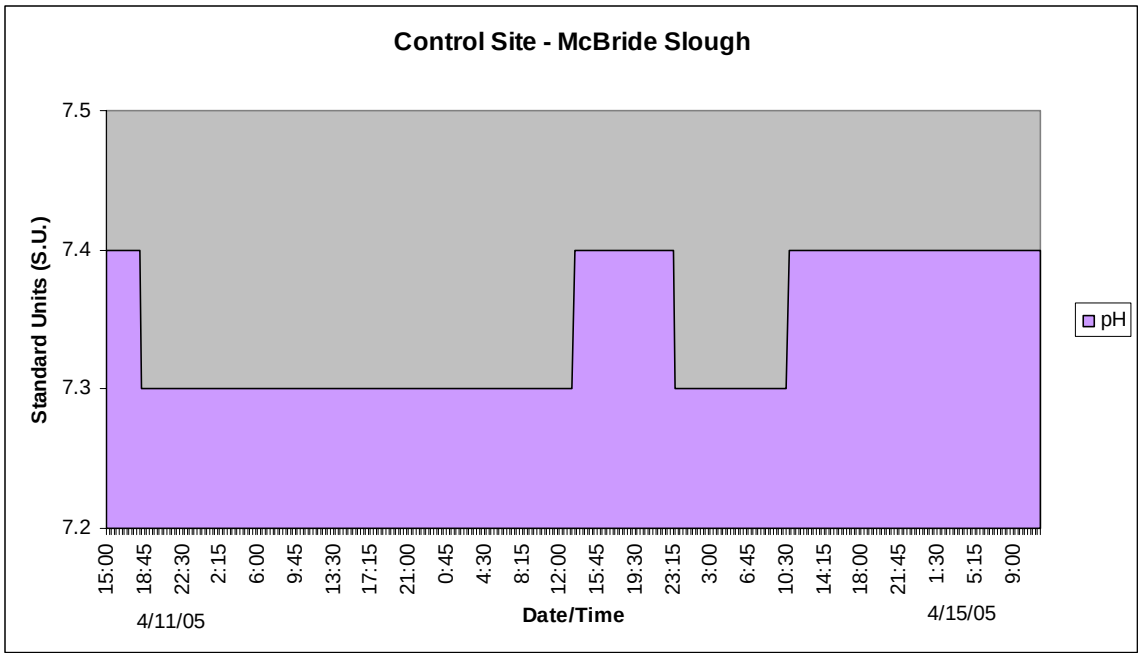

Test Site 3 - Farthest from Spring boil
mg/L
6
5
4
3
2
1
0
12:15
16:30
20:45
1:00
5:15
9:30
13:45
18:00
22:15
2:30
6:45
11:00
15:15
19:30
23:45
4:00
8:15
12:30
16:45
21:00
1:15
5:30
9:45
4/11/05
Date/Time
4/15/05
Min-Max
Threshold
Dissolved Oxygen

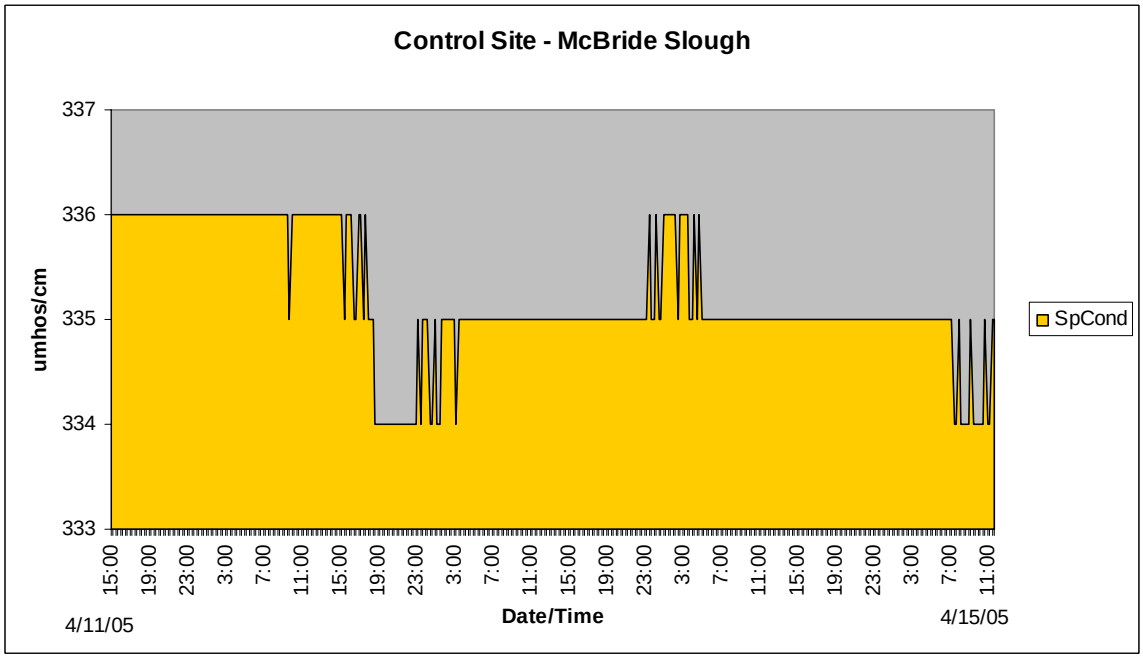

Test Site 3 - Farthest from Spring boil
Standard Units (S.U.)
8
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
12:15
16:00
19:45
23:30
3:15
7:00
10:45
14:30
18:15
22:00
1:45
5:30
9:15
13:00
16:45
20:30
0:15
4:00
7:45
11:30
15:15
19:00
22:45
2:30
6:15
10:00
4/11/05
Date/Time
4/15/05
pH


Test Site 3 - Farthest from Spring boil
umhos/cm
304
303
302
301
300
299
298
297
296
295
294
12:15
16:30
20:45
1:00
5:15
9:30
13:45
18:00
22:15
2:30
6:45
11:00
15:15
19:30
23:45
4:00
8:15
12:30
16:45
21:00
1:15
5:30
9:45
4/11/05
Date/Time
4/15/05
SpCond


Control Site - McBride Slough
Degrees C
21
20.5
20
19.5
19
18.5
18
17.5
17
16.5
16
15:00
19:15
23:30
3:45
8:00
12:15
16:30
20:45
1:00
5:15
9:30
13:45
18:00
22:15
2:30
6:45
11:00
15:15
19:30
23:45
4:00
8:15
4/11/05
Date/Time
4/15/05
Temperature

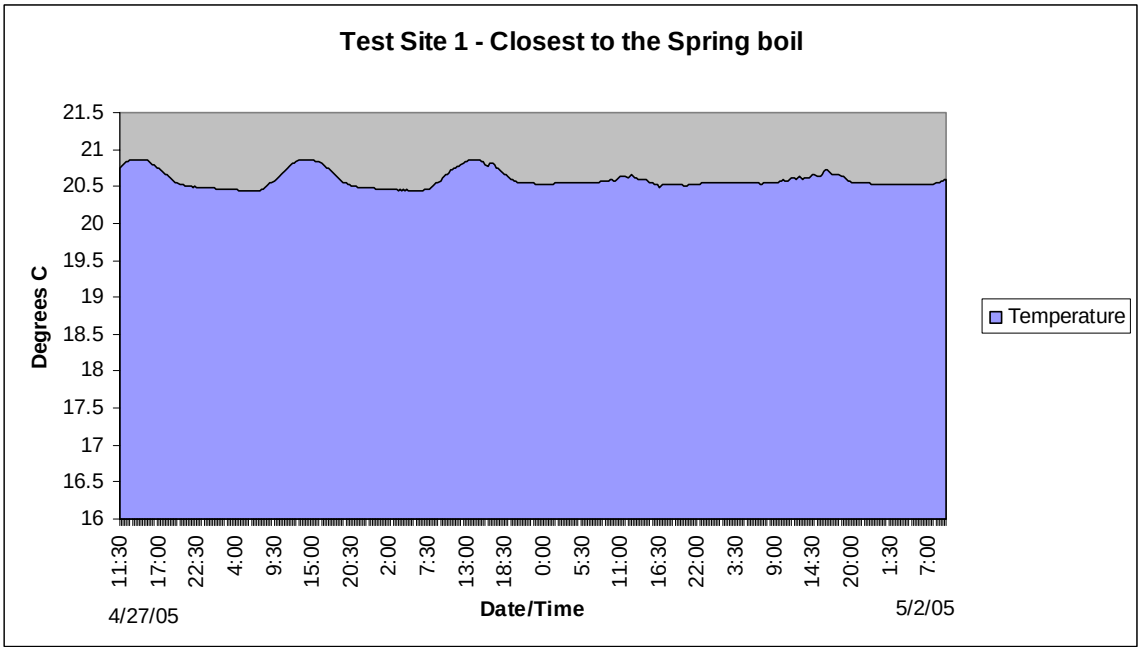

Control Site - McBride Slough
mg/L
6
5
4
3
2
1
0
15:00
19:15
23:30
3:45
8:00
12:15
16:30
20:45
1:00
5:15
9:30
13:45
18:00
22:15
2:30
6:45
11:00
15:15
19:30
23:45
4:00
8:15
4/11/05
Date/Time
4/15/05
Min-Max
Threshold
Dissolved Oxygen

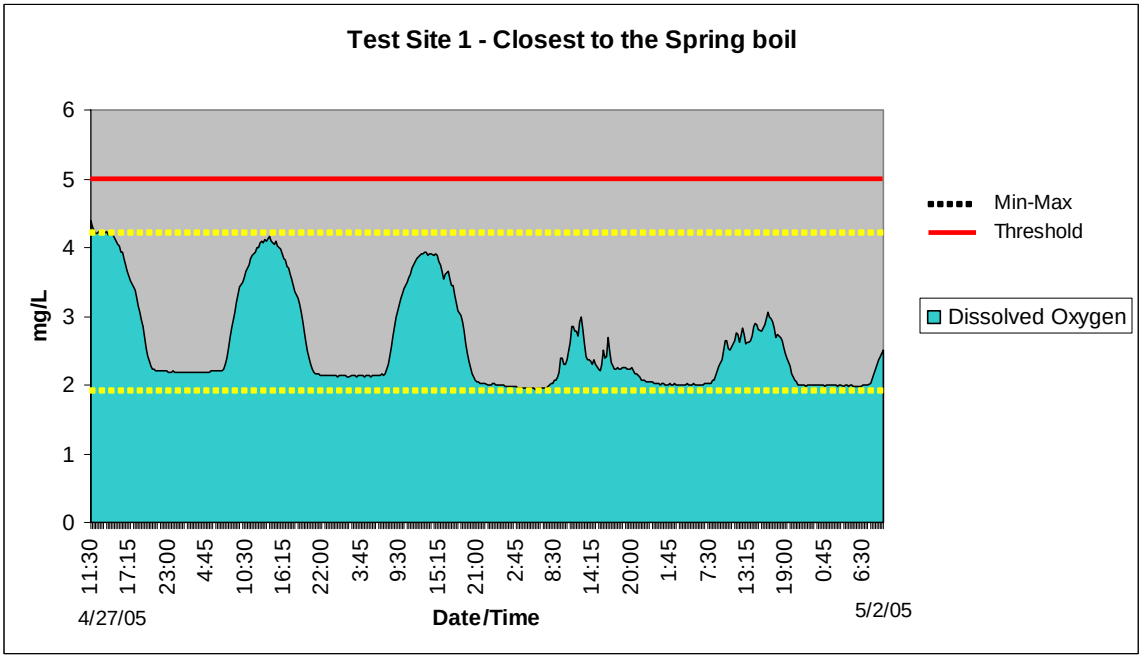

Control Site - McBride Slough
Standard Units (S.U.)
7.5
7.4
7.3
7.2
15:00
18:45
22:30
2:15
6:00
9:45
13:30
17:15
21:00
0:45
4:30
8:15
12:00
15:45
19:30
23:15
3:00
6:45
10:30
14:15
18:00
21:45
1:30
5:15
9:00
4/11/05
Date/Time
4/15/05
pH

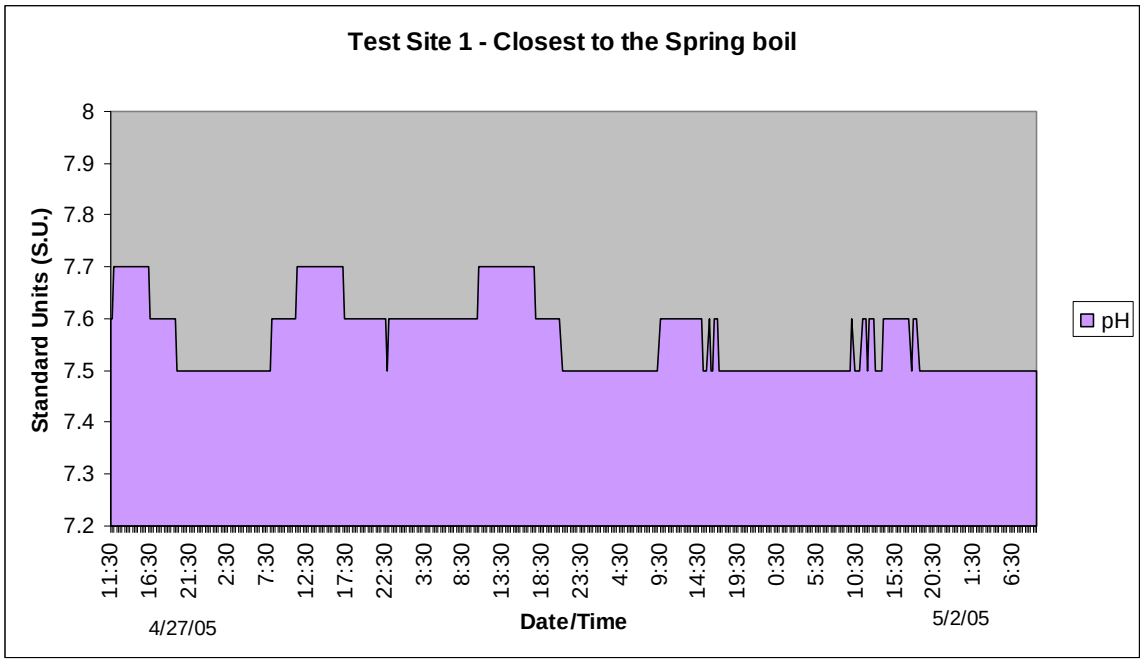

Control Site - McBride Slough
umhos/cm
337
336
335
334
333
15:00
19:00
23:00
3:00
7:00
11:00
15:00
19:00
23:00
3:00
7:00
11:00
15:00
19:00
23:00
3:00
7:00
11:00
15:00
19:00
23:00
3:00
7:00
11:00
4/11/05
Date/Time
4/15/05
SpCond

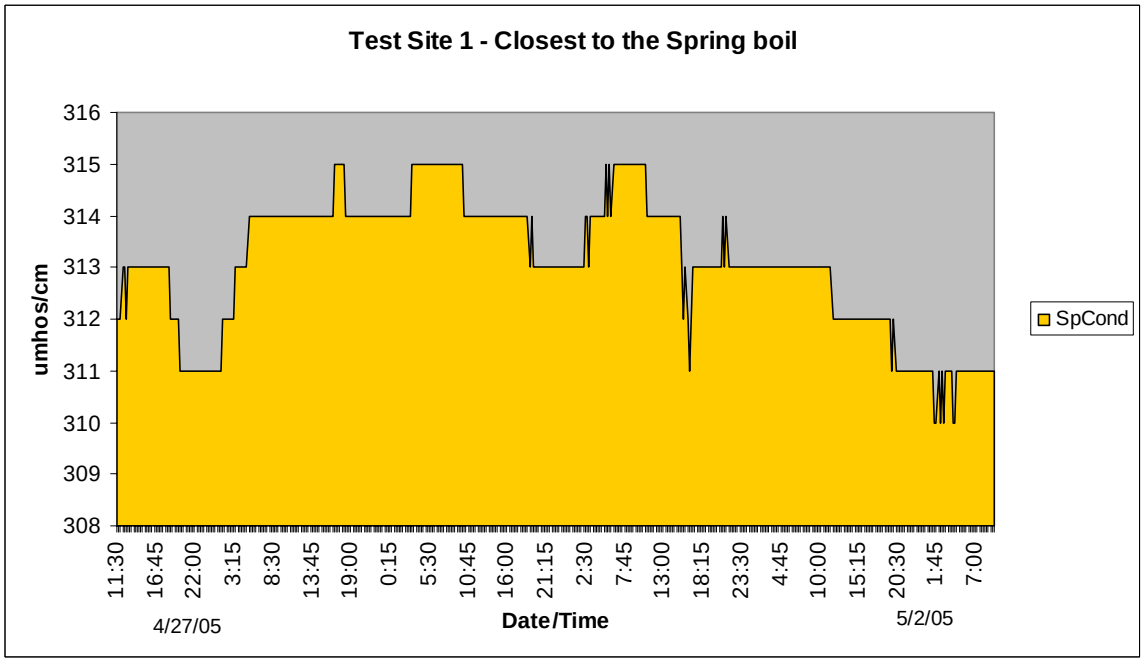
Appendix 4

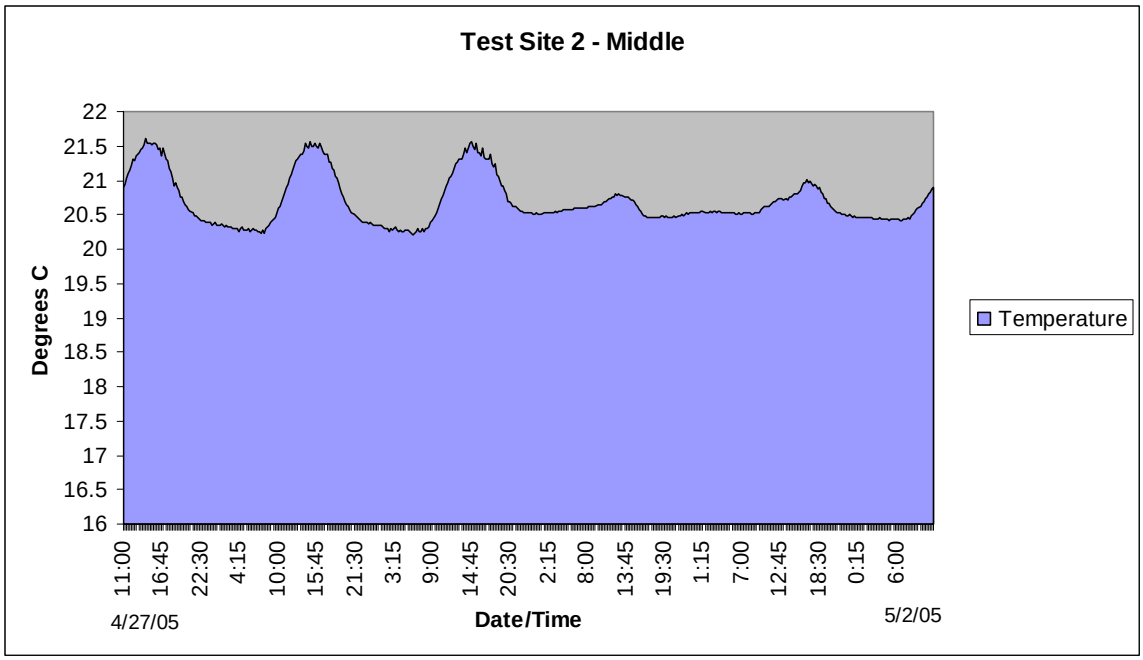
Physical Chemistry Data from Test Sites 1 & 2
4/27/05 – 5/2/05

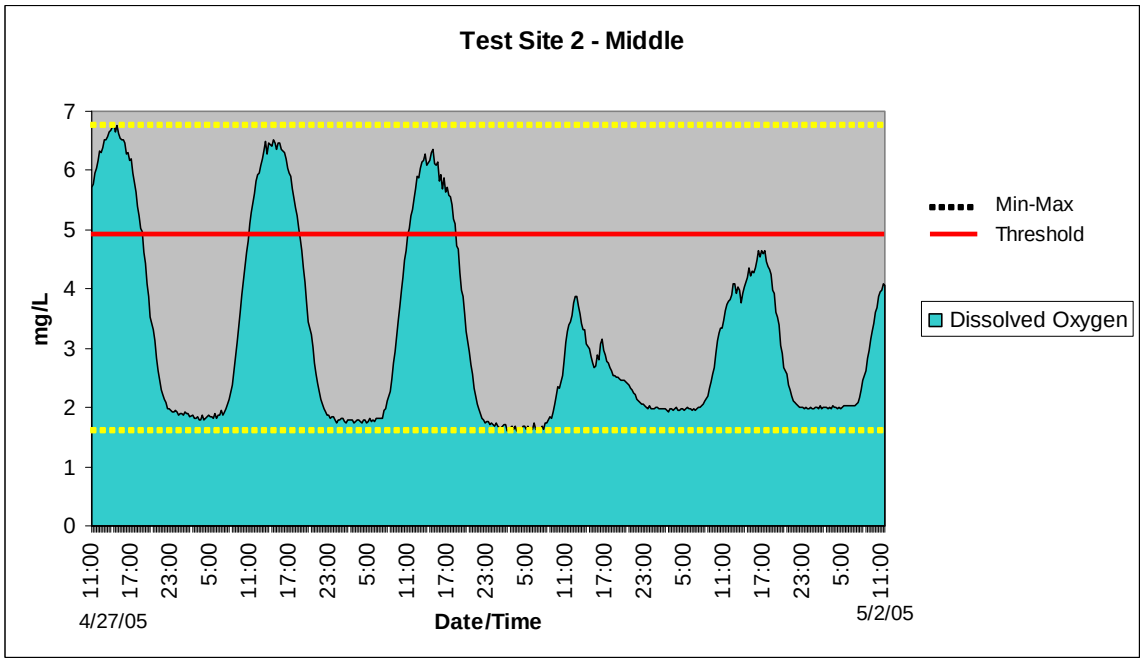


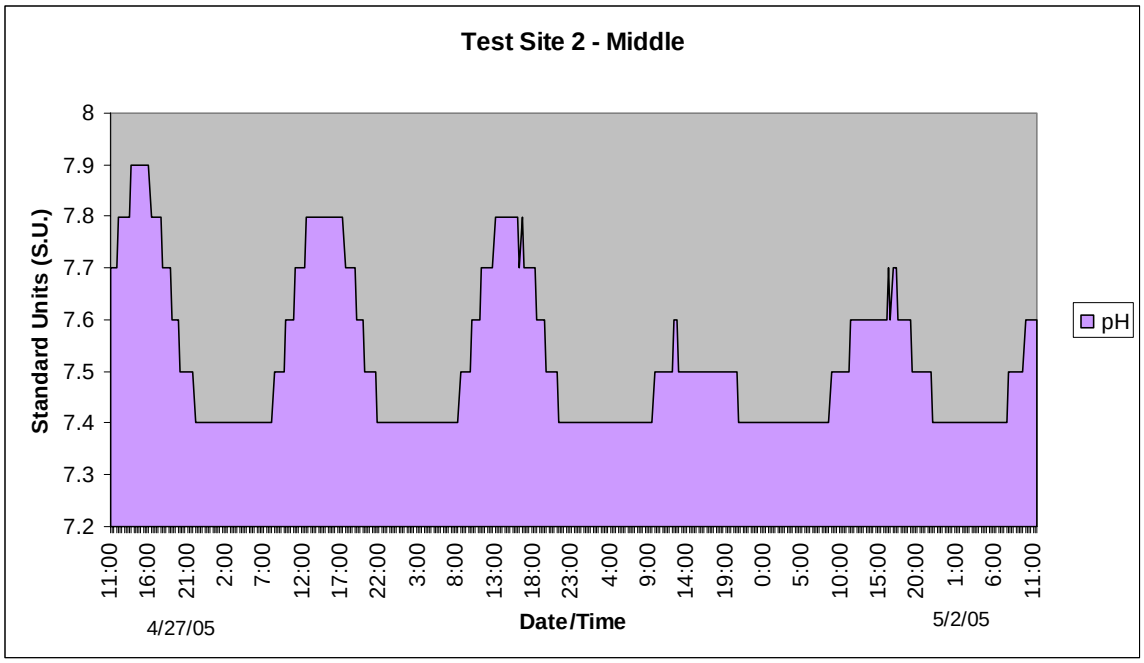


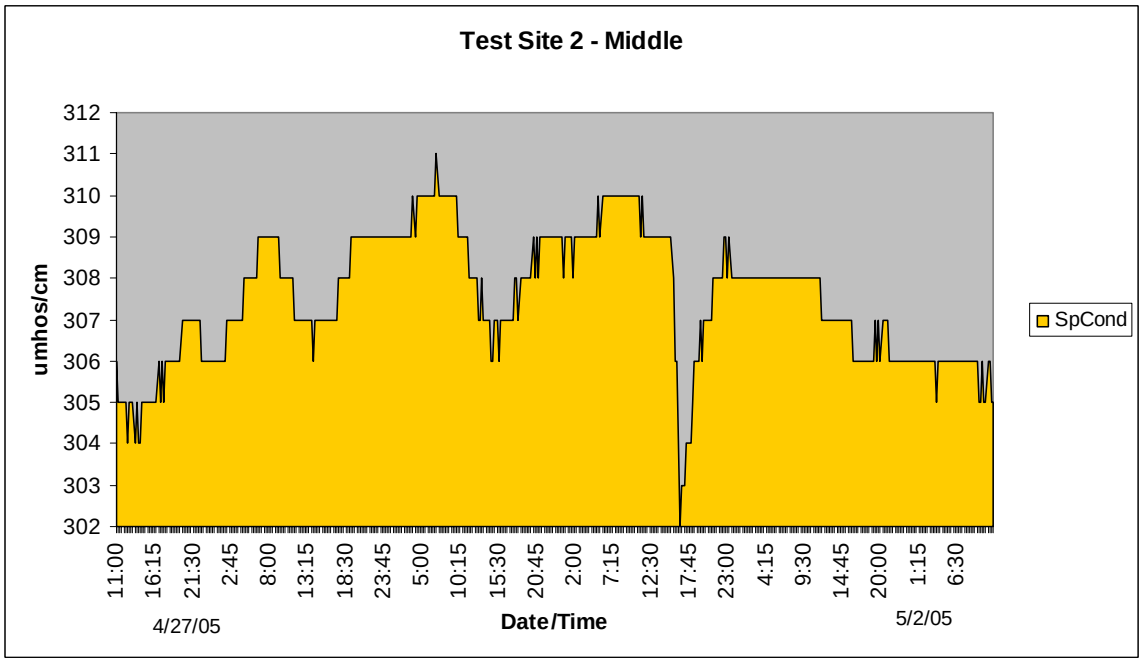

Test Site 1 - Closest to the Spring boil
Standard Units (S.U.)
8
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
11:30
16:30
21:30
2:30
7:30
12:30
17:30
22:30
3:30
8:30
13:30
18:30
23:30
4:30
9:30
14:30
19:30
0:30
5:30
10:30
15:30
20:30
1:30
6:30
4/27/05
Date/Time
5/2/05
pH


Test Site 1 - Closest to the Spring boil
umhos/cm
316
315
314
313
312
311
310
309
308
11:30
16:45
22:00
3:15
8:30
13:45
19:00
0:15
5:30
10:45
16:00
21:15
2:30
7:45
13:00
18:15
23:30
4:45
10:00
15:15
20:30
1:45
7:00
4/27/05
Date/Time
5/2/05
SpCond


Test Site 2 - Middle
Degrees C
22
21.5
21
20.5
20
19.5
19
18.5
18
17.5
17
16.5
16
11:00
16:45
22:30
4:15
10:00
15:45
21:30
3:15
9:00
14:45
20:30
2:15
8:00
13:45
19:30
1:15
7:00
12:45
18:30
0:15
6:00
4/27/05
Date/Time
5/2/05
Temperature

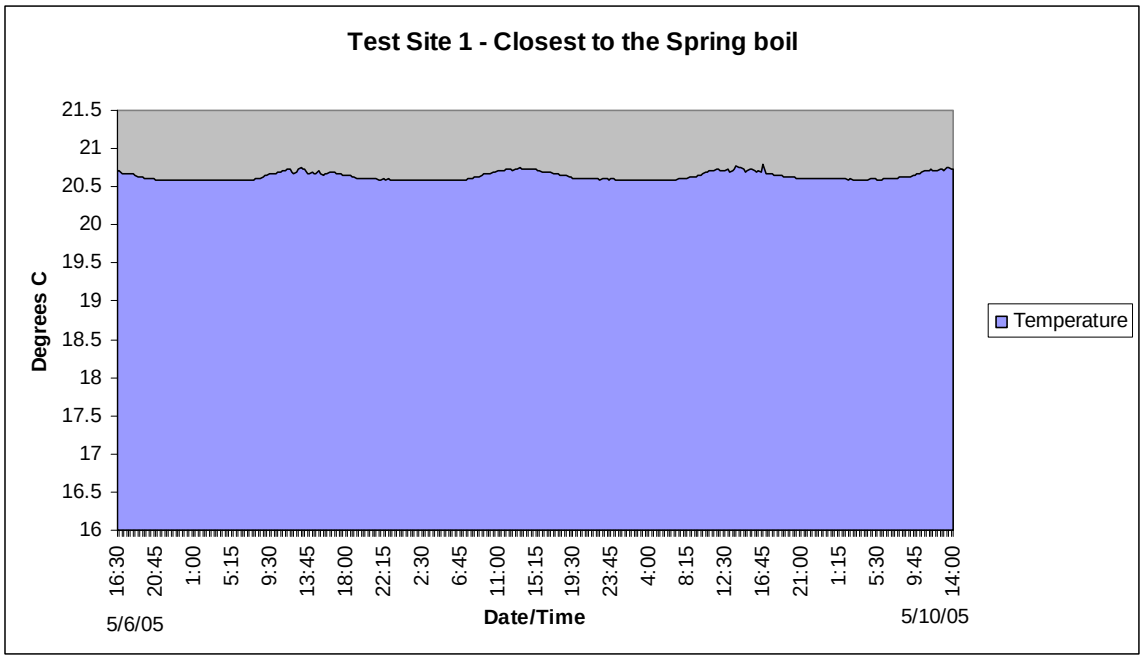

Test Site 2 - Middle
mg/L
7
6
5
4
3
2
1
0
11:00
17:00
23:00
5:00
11:00
17:00
23:00
5:00
11:00
17:00
23:00
5:00
11:00
17:00
23:00
5:00
11:00
17:00
23:00
5:00
11:00
4/27/05
Date/Time
5/2/05
Min-Max
Threshold
Dissolved Oxygen

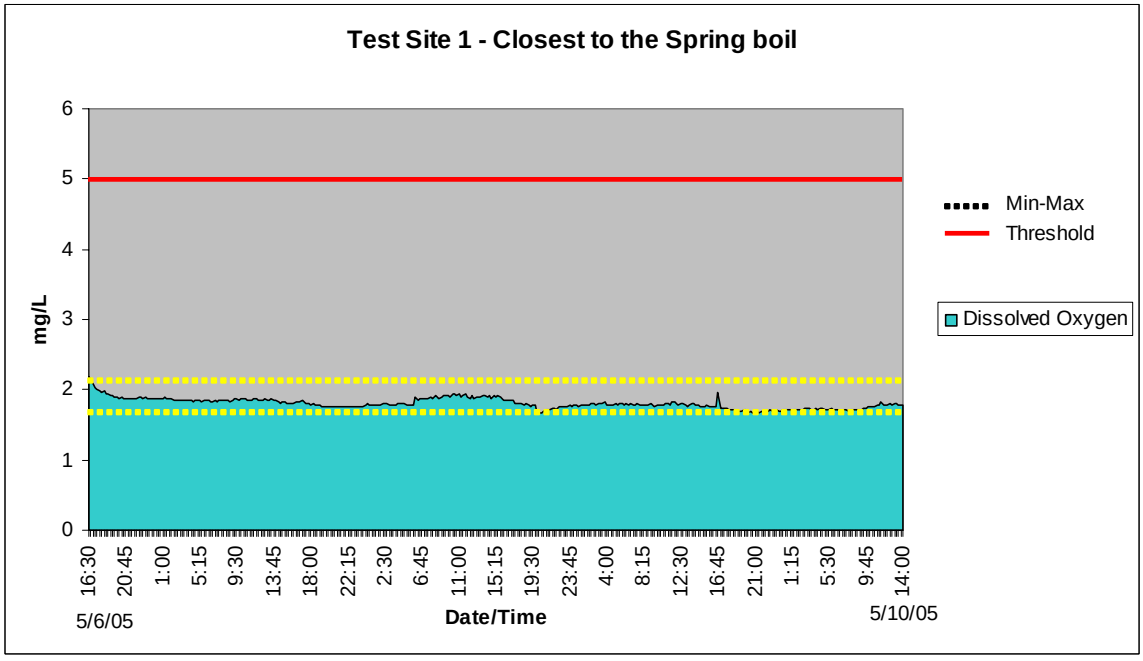

Test Site 2 - Middle
Standard Units (S.U.)
8
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
11:00
16:00
21:00
2:00
7:00
12:00
17:00
22:00
3:00
8:00
13:00
18:00
23:00
4:00
9:00
14:00
19:00
0:00
5:00
10:00
15:00
20:00
1:00
6:00
11:00
4/27/05
Date/Time
5/2/05
pH

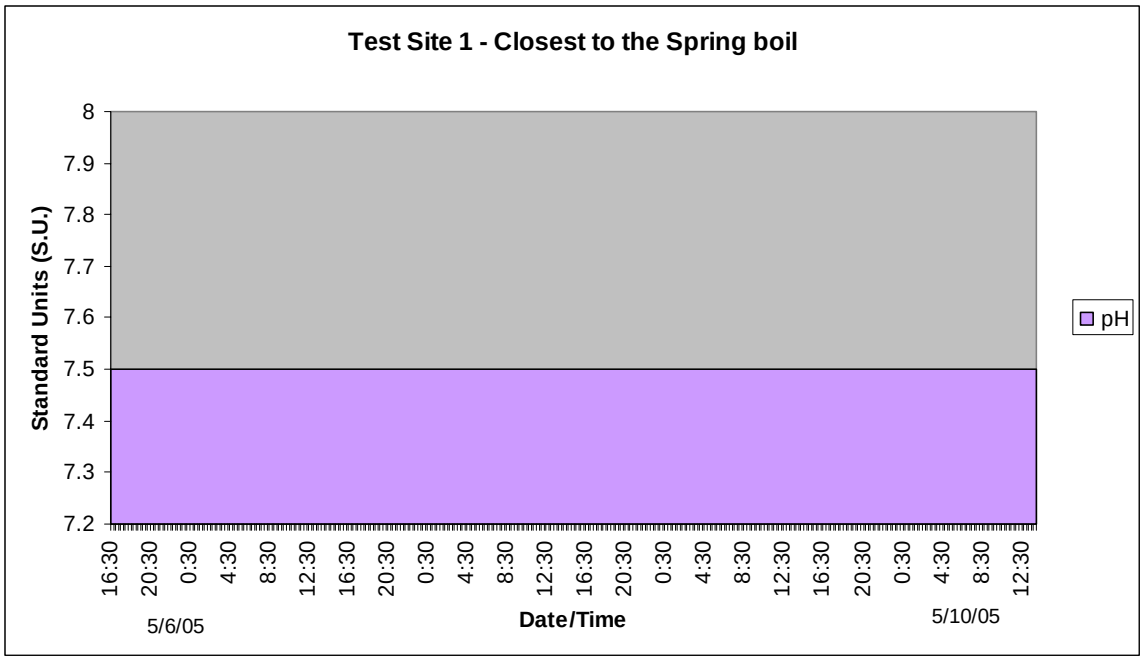

Test Site 2 - Middle
umhos/cm
312
311
310
309
308
307
306
305
304
303
302
11:00
16:15
21:30
2:45
8:00
13:15
18:30
23:45
5:00
10:15
15:30
20:45
2:00
7:15
12:30
17:45
23:00
4:15
9:30
14:45
20:00
1:15
6:30
4/27/05
Date/Time
5/2/05
SpCond

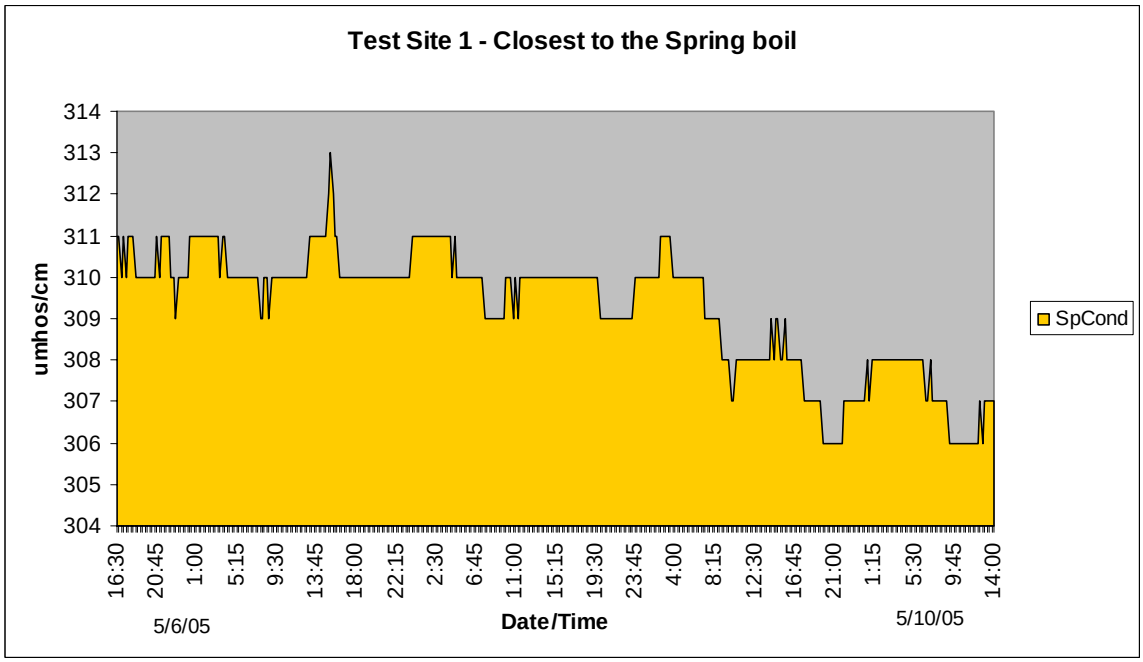
Appendix 5

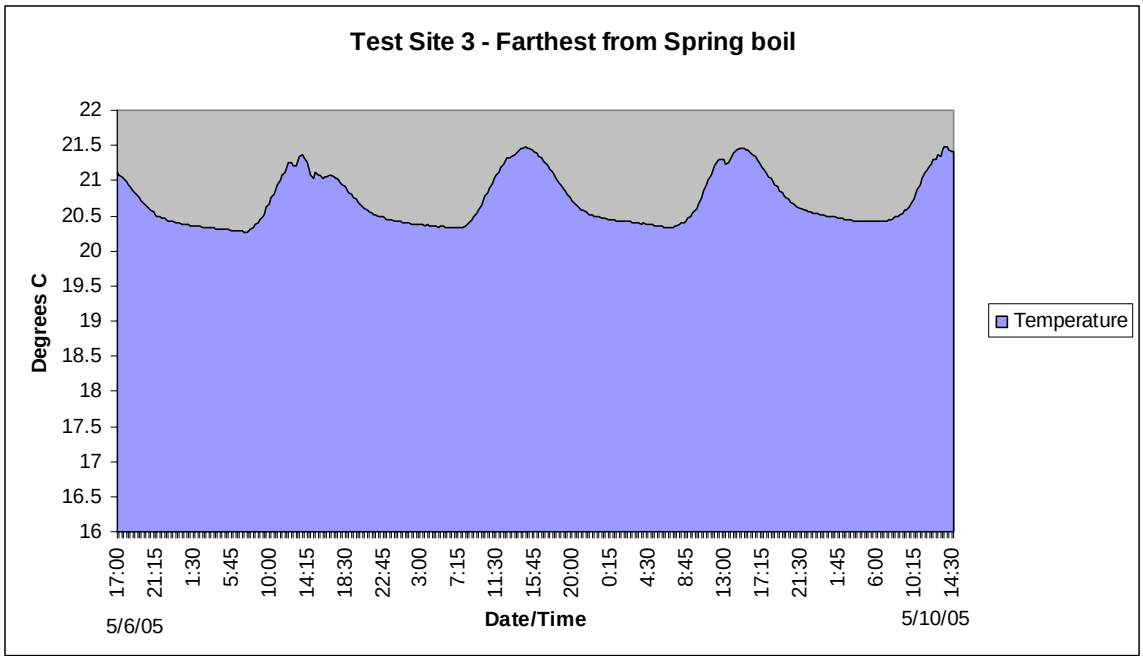
Physical Chemistry Data from Test Sites 1 & 3 and Control Site 5/6/05 – 5/10/05

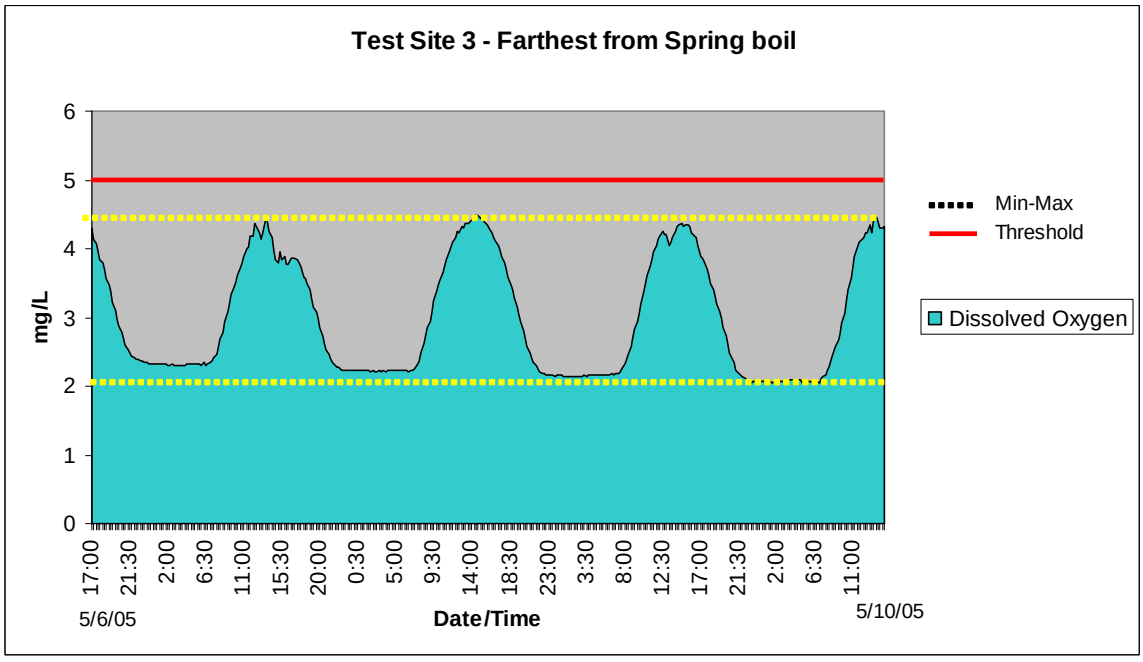


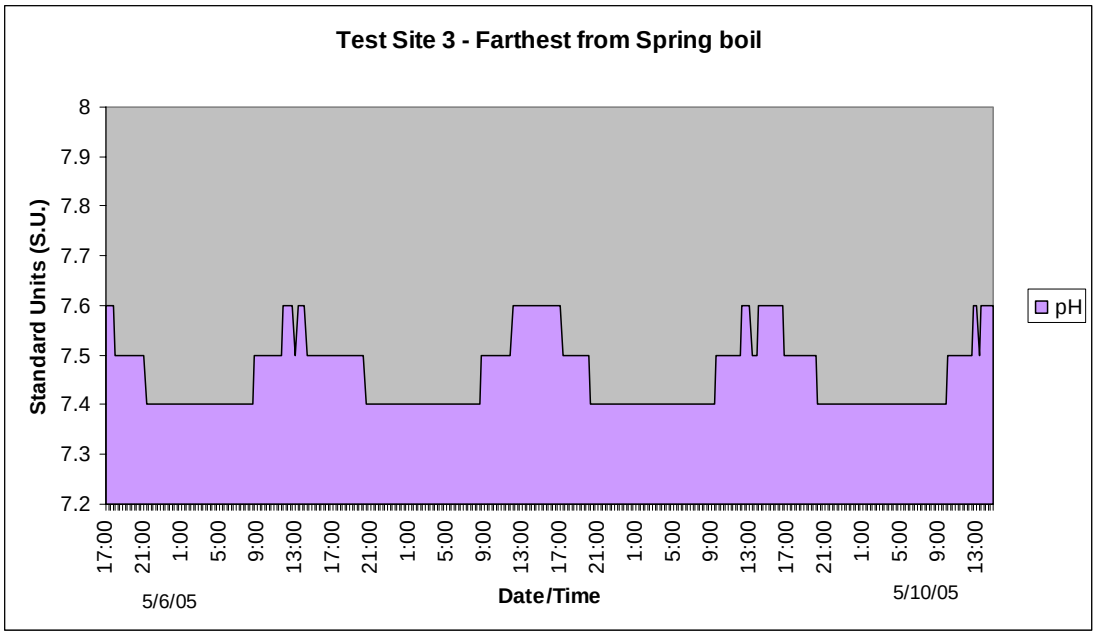


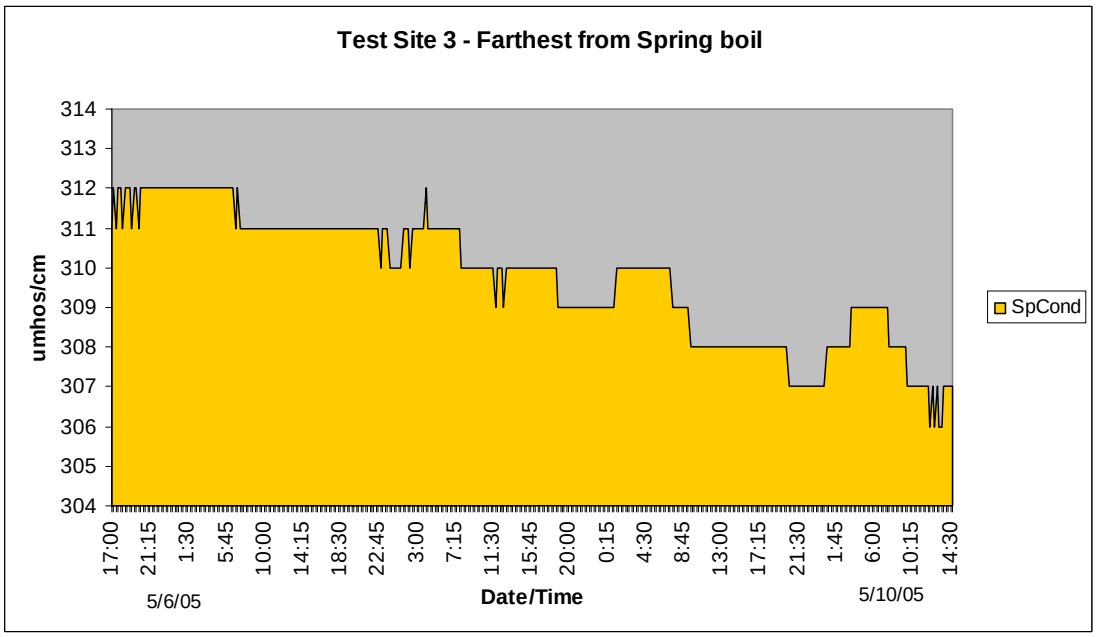

Test Site 1 - Closest to the Spring boil
Standard Units (S.U.)
8
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
16:30
20:30
0:30
4:30
8:30
12:30
16:30
20:30
0:30
4:30
8:30
12:30
16:30
20:30
0:30
4:30
8:30
12:30
5/6/05
Date/Time
5/10/05
pH

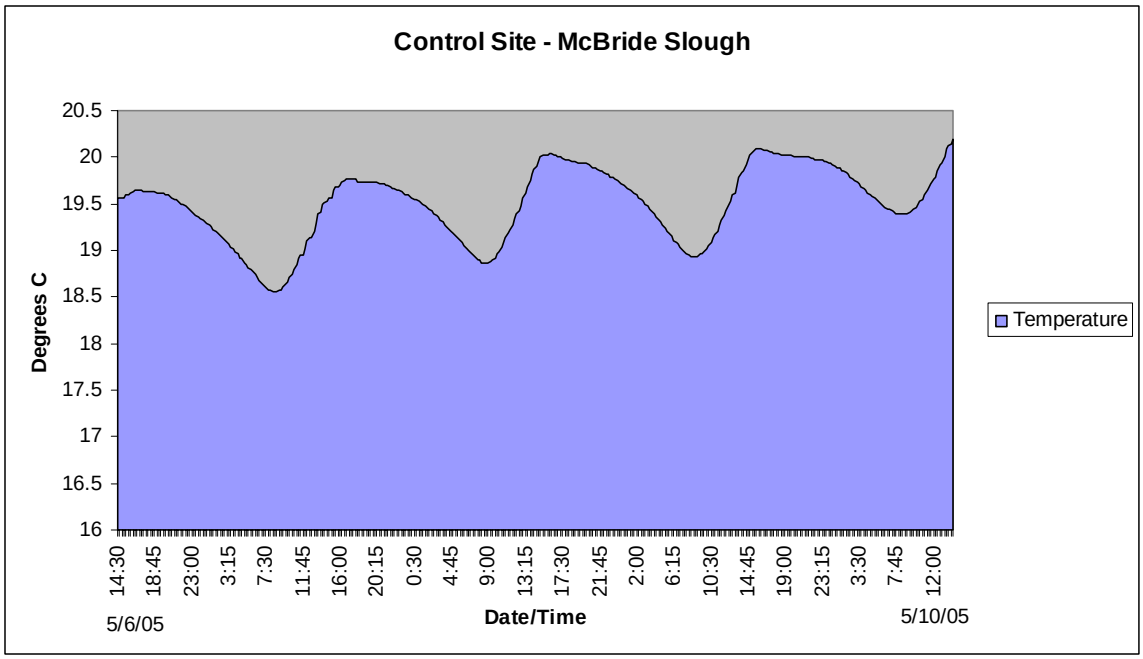

Test Site 1 - Closest to the Spring boil
umhos/cm
314
313
312
311
310
309
308
307
306
305
304
16:30
20:45
1:00
5:15
9:30
13:45
18:00
22:15
2:30
6:45
11:00
15:15
19:30
23:45
4:00
8:15
12:30
16:45
21:00
1:15
5:30
9:45
14:00
5/6/05
Date/Time
5/10/05
SpCond

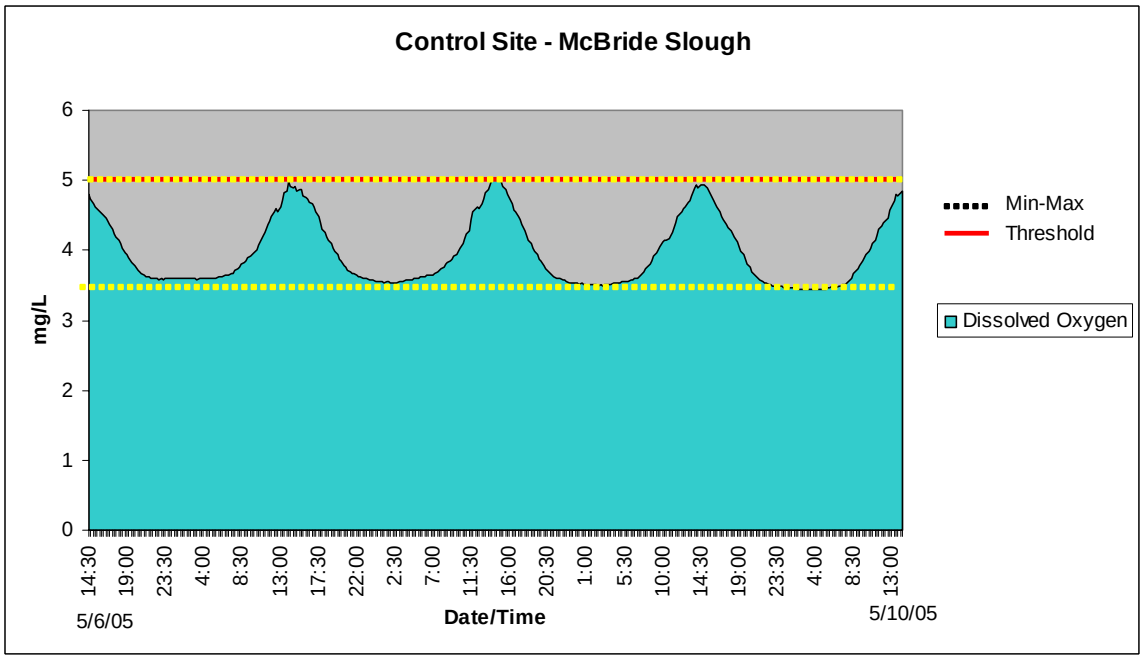

Test Site 3 - Farthest from Spring boil
Degrees C
22
21.5
21
20.5
20
19.5
19
18.5
18
17.5
17
16.5
16
17:00
21:15
1:30
5:45
10:00
14:15
18:30
22:45
3:00
7:15
11:30
15:45
20:00
0:15
4:30
8:45
13:00
17:15
21:30
1:45
6:00
10:15
14:30
5/6/05
Date/Time
5/10/05
Temperature

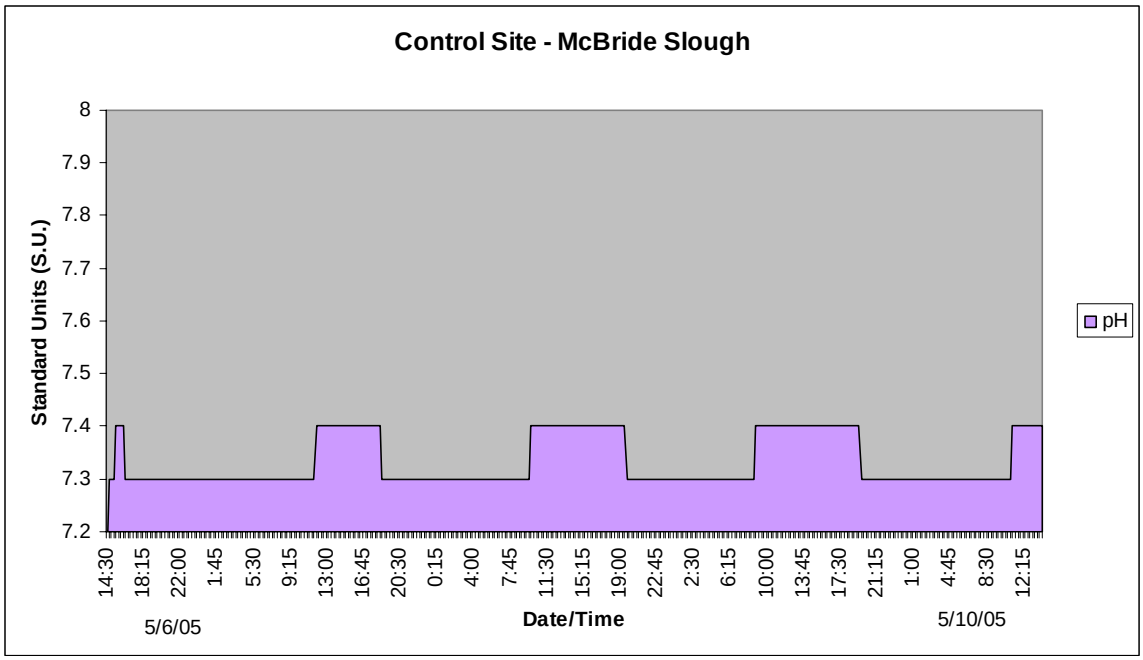

Test Site 3 - Farthest from Spring boil
mg/L
6
5
4
3
2
1
0
17:00
21:30
2:00
6:30
11:00
15:30
20:00
0:30
5:00
9:30
14:00
18:30
23:00
3:30
8:00
12:30
17:00
21:30
2:00
6:30
11:00
5/6/05
Date/Time
5/10/05
Min-Max
Threshold
Dissolved Oxygen

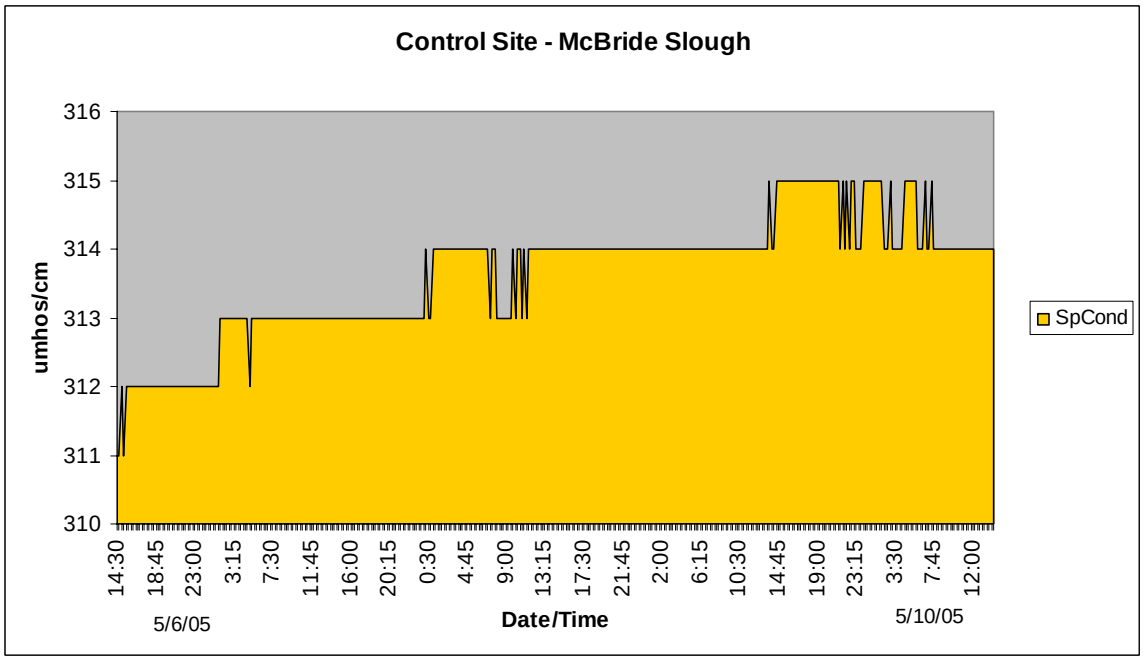

Test Site 3 - Farthest from Spring boil
Standard Units (S.U.)
8
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
17:00
21:00
1:00
5:00
9:00
13:00
17:00
21:00
1:00
5:00
9:00
13:00
17:00
21:00
1:00
5:00
9:00
13:00
17:00
21:00
1:00
5:00
9:00
13:00
5/6/05
Date/Time
5/10/05
pH


Test Site 3 - Farthest from Spring boil
umhos/cm
314
313
312
311
310
309
308
307
306
305
304
17:00
21:15
1:30
5:45
10:00
14:15
18:30
22:45
3:00
7:15
11:30
15:45
20:00
0:15
4:30
8:45
13:00
17:15
21:30
1:45
6:00
10:15
14:30
5/6/05
Date/Time
5/10/05
SpCond


Control Site - McBride Slough
Degrees C
20.5
20
19.5
19
18.5
18
17.5
17
16.5
16
14:30
18:45
23:00
3:15
7:30
11:45
16:00
20:15
0:30
4:45
9:00
13:15
17:30
21:45
2:00
6:15
10:30
14:45
19:00
23:15
3:30
7:45
12:00
5/6/05
Date/Time
5/10/05
Temperature


Control Site - McBride Slough
mg/L
6
5
4
3
2
1
0
14:30
19:00
23:30
4:00
8:30
13:00
17:30
22:00
2:30
7:00
11:30
16:00
20:30
1:00
5:30
10:00
14:30
19:00
23:30
4:00
8:30
13:00
5/6/05
Date/Time
5/10/05
Min-Max
Threshold
Dissolved Oxygen


Control Site - McBride Slough
Standard Units (S.U.)
8
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
14:30
18:15
22:00
1:45
5:30
9:15
13:00
16:45
20:30
0:15
4:00
7:45
11:30
15:15
19:00
22:45
2:30
6:15
10:00
13:45
17:30
21:15
1:00
4:45
8:30
12:15
5/6/05
Date/Time
5/10/05
pH


Control Site - McBride Slough
umhos/cm
316
315
314
313
312
311
310
14:30
18:45
23:00
3:15
7:30
11:45
16:00
20:15
0:30
4:45
9:00
13:15
17:30
21:45
2:00
6:15
10:30
14:45
19:00
23:15
3:30
7:45
12:00
5/6/05
Date/Time
5/10/05
SpCond

Appendix 6

Verification Criteria	
DO	± 0.3 mg/L from expected value*
Conductivity	± 5% from expected value
pH	± 0.2 S.U. from expected value

* See chart in FDEP SOP FT1500

Expected value = Known standard