Influence of the Waugh Street Bat Colony on Indicator Bacteria Levels in Buffalo Bayou

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Executive Summary

Bacteria and other pathogens are a growing concern in Texas waters due to the majority of water quality impairments on the 303(d) list are for indicator bacteria. One of the barriers to effective management of indicator bacteria levels and associated pathogens is successful source identification. Several potential sources of indicator bacteria exist within the urban watersheds. A recent study of Total Maximum Daily Loads for fecal pathogens in Buffalo Bayou and White Oak Bayou indicates a predominance of human sources for *E. coli* in Buffalo Bayou after rain events and a higher proportion of non-human sources in dry weather. One potential wildlife source of indicator bacteria, *E. coli*, in Buffalo Bayou is a large colony of Mexican or Brazilian Free Tailed Bat (*Tardarida brasiliensis*) that is located underneath the Waugh Street Bridge which overhangs Buffalo Bayou. The colony size is estimated to range between 250,000 – 300,000 bats. Mexican Free Tailed bats are also considered "guano" bats due to the large quantities of feces produced. Little information is known about the impacts of bat colonies on indicator enteric bacteria levels in adjacent waterbodies. Unpublished data collected by the City of Austin during 1999 failed to document any impacts on Town Lake and the Colorado River associated with the large bat colony. However, the State of California found that elevated fecal coliform levels associated with high densities of bats.

During 2008 and 2009 we conducted a comprehensive study of the relationship of Mexican free-tailed bats at the Waugh Street bridge and *E. coli* concentrations in Buffalo Bayou. The primary objective of our study was to determine the influence of the Waugh Street Bridge bat colony on indicator bacteria levels within Buffalo Bayou. We reviewed historical water quality data, collected new targeted ambient water quality data upstream and below the bat colony, and used both cultivation-based and molecular methods to identify and enumerate indicator bacteria in the guano (cells/gram feces) and in ambient water.

Multiple lines of evidence suggest that the Waugh Street bat colony is a significant source of *E. coli* bacteria in Buffalo Bayou. Historical routine monitoring data document long term trends in elevated *E. coli* levels downstream of the Waugh Street bat colony. However, new data collected during our study documented similar *E. coli* levels both upstream and downstream of the bat colony. Our experimental design did not, however, include sites as far down as the historical data which included sites located 8000 feet downstream of the bat colony. We hypothesized that *E. coli* deposited in fecal pellets from the bat colony may not have had sufficient time to reach elevated densities. In addition, other bat colonies have been recently observed upstream of the Waugh Street colony and may be confounding our observations. *E. coli* contributions by the bat colony exhibited diel and seasonal patterns consistent with the life history of bats. Lowest levels in *E. coli* levels were usually seen in the early morning (night samples) while bats were out foraging. Furthermore, these diel differences were smaller or absent during the winter months when bats are usually dormant or

have emigrated to warmer climates. Estimates of the bat colony population size, literature based per capita fecal production rates and estimates of *E. coli* densities per gram of feces and fecal pellet were used to to generate estimates of fecal and *E. coli* loading into Buffalo Bayou. We estimated that the colony likely produces between 524 and 628 million *E. coli* colony forming units per day.

Genetic analyses and related tests provide the strongest evidence that the Waugh Street Mexican free-tailed bat colony and other nesting sites in the watershed are a significant source of *E. coli*. The HFERP analyses determined that 15 to >50% of *E. coli* isolates at the Waugh Avenue Bridge and at downstream sampling locations had DNA fingerprints that clustered with the those from the bat guano indicating a strong likelihood of bats as a source of *E. coli* in Buffalo Bayou. While less conclusive, the sugar utilization data produced similar trends. During the study period we also found high numbers of bats living in bridges upstream from the Waugh Avenue Bridge bat colony that could explain the similarity of upstream sugar utilization to that of the Waugh Avenue Bridge *E. coli* isolates. The combination of the two methods indicates that the bat colony guano is a source of *E. coli*, at least to 1000 ft below the Waugh Avenue Bridge.

In summary, it appears that the Waugh Street colony represents a significant source of *E. coli* bacteria within Buffalo Bayou. In addition, bats are contributing to the *E. coli* loads in Buffalo Bayou at other locations. These large bat populations represent a potentially significant but poorly understood source of nutrients and *E. coli* bacteria in our urban watersheds. Additional research is critically needed to characterize the interaction of bat population dynamics, seasonal and diel movement, fecal and associated nutrient and bacteria loading, and resulting instream indicator bacteria concentrations.

Introduction

Bacteria and other pathogens are a growing concern in Texas waters due to the majority of water quality impairments on the 303(d) list are for bacteria. Nearly 50% of the designated streams in the Houston Galveston area are impaired due to elevated levels of indicator bacteria (TCEQ 2007). Several of these streams routinely exhibit indicator bacteria levels that are more than ten times the contact recreation standard in some urban watersheds. Indicator bacteria include fecal coliform, *Escherichia coli* (*E. coli*) and Enterococci groups. Although they are not generally harmful themselves, they indicate the possible presence of pathogenic (disease-causing) bacteria, viruses, and protozoans that also live in human and animal digestive systems. Therefore, their presence in streams suggests that pathogenic microorganisms might also be present and elevated health risks might be associated with contact recreation (e.g. swimming) and eating shellfish might be a health risk. The management of elevated indicator bacteria includes point source controls such as disinfection of wastewater and reduction of non-point sources such as repair of sanitary sewer collection systems, and implementation of urban and agricultural best management practices.

One of the barriers to effective management of indicator bacteria levels and associated pathogens is successful source identification. Several potential sources of indicator bacteria exist within the Buffalo Bayou watershed including contaminated runoff and storm water, malfunctioning wastewater collection systems, improperly functioning wastewater plants, wildlife and domesticated animals. The final report to the TCEQ of Total Maximum Daily Loads for fecal pathogens in Buffalo Bayou and White Oak Bayou indicates a predominance of human sources for *E. coli* in Buffalo Bayou after rain events and a higher proportion of non-human sources in dry weather (Rifai 2006; Petersen et al. 2005). Determining the relative contributions from each source is a first critical step in determining the most effective management strategy.

One potential wildlife source of indicator bacteria, *E. coli*, is a large colony of Mexican or Brazilian Free Tailed Bat (*Tardarida brasiliensis*) that is located underneath the Waugh Street Bridge which overhangs Buffalo Bayou. Bridges provide ideal habitat for many species of bats by providing refugia from predators, weather, and elevated temperatures during many months of the year that help conserve body temperature (Keeley and Tuttle 1999). This colony has been identified as a potential source of indicator bacteria (Fig. 1). The colony size is estimated to range between 250,000 – 300,000 bats and research is currently underway to provide better estimates (Buffalo Bayou Partnership web page: http:\\Waugh Bridge Bat Colony.mhtv and http://www.buffalobayou.org/WaughBatColony.htm). The potential impact of this large concentration of bats on indicator bacteria levels in Buffalo Bayou is unknown but could be substantial. Mexican Free Tailed bats are also considered “guano” bats due to the large quantities of feces produced (Schmidly 1991). In

the past large amounts of the guano was mined near cave roosting sites for agricultural use. Since Buffalo Bayou continues to exhibit elevated bacteria levels in violation of state water standards, it is imperative that water resource managers determine whether this bat colony is contributing significantly to overall loading of bacteria.

Little information is known about the impacts of bat colonies on indicator enteric bacteria levels in adjacent waterbodies. Unpublished data collected by the City of Austin during 1999 failed to document any impacts on Town Lake and the Colorado River associated with the large Mexican Free Tailed bat colony found underneath the Commerce Street Bridge in Austin (Pers comm. Herrington, Chris Herrington City of Austin chris.herrington@ci.austin.tx.us). The City of Austin and Lower Colorado River collected samples from a boat in the middle of the channel during non-storm flow conditions at surface and bottom depths. Based on the results of their sampling, they concluded that the bat colony did not significantly contribute to nutrient or bacteria levels in Town Lake. They sampled at six upstream locations and two locations (100 and 1000 feet) downstream of the colony. Four of the upstream locations and the 1000 ft downstream site represented routine monitoring stations where historical data has been collected. The City of Austin has collected extensive water quality data (more than 1500 sample events), including nutrients and bacteria, for sites upstream and downstream of the Congress Street bat colony since 1975. Based on this data, they have not observed any negative impact from the bats on Town Lake water quality. Town Lake is currently fully supporting contact recreation during non-storm flow conditions according to TCEQ assessment procedures (Pers comm. Herrington, Chris Herrington City of Austin chris.herrington@ci.austin.tx.us). Bacteria concentrations typically peaked at the 1st Street site, which is located upstream of the bat colony. The State of California found that elevated fecal coliform levels may be caused in part by high densities of bats and other organisms that inhabit an enclosed tunnel section of San Luis Obispo creek during studies conducted in support of a TMDL (CRWQCB 2004).

A recent report released by the Bat Conservation International, reported that no structural damage, aquatic pollution, or disease transmission to humans has been associated with even the largest bat colonies living in Texas bridges and culverts (Keeley and Tuttle 2000). Recent estimates of the bats at the Commerce Street Bridge range up to 1.5 million per year (Tuttle 2003). This is in contrast to the previously estimated 300,000 bats found at Waugh Street in Houston. Although several large roosting areas or colonies of Mexican Free Tailed bats are located in Texas, the only documented large colony in Houston is the Waugh Street Bridge. However, smaller roosting areas are commonly found in buildings and can be expected in the vicinity of Buffalo Bayou. The occupation of buildings by roosting bats is a state-wide phenomenon with the exception of East Texas. Every town in the Mexican Free Tailed bat's range is likely to have at least 15 roosts per 5,000 human inhabitants (Davis et al. 1962). Most roosts in buildings house less than 100 bats at a time. Season fluctuations occur in the density of

bats. The abundance of the bats follows an annual pattern where highest numbers of bats occurring during spring through fall. Most of these bats migrate to Mexico (early December through February) to overwinter (Schmidly 2004). A few though overwinter in buildings in southwest Texas. The eastern sub-species (*T.b. cyanocepha*) found in southeast Texas does not migrate and will overwinter (Schmidly 1991). However, they often enter a state of torpor and hibernate during the winter months (Tuttle 2003).

In addition to seasonal trends bats exhibit diel patterns in activity. Mexican free-tailed bats typically emerge from their roost areas shortly before dark to begin their nightly foraging. They can forage up to 50 miles and normally return back to their roost before sunrise (Tuttle 1994).

The primary objective of our study was to determine the influence of the Waugh Street Bridge bat colony on indicator bacteria levels within Buffalo Bayou. We proposed to accomplish the first objective by conducting a critical review of existing site data and pertinent published literature and conducting a focused field study on the ambient levels of indicator bacteria above and below the bat colony. A secondary objective will be to determine the percentage contribution of indicator bacteria attributable to bats. We will use a combination of cultivation-based and molecular methods to identify and enumerate indicator bacteria in the guano (cells/gram feces) and in ambient water in Buffalo Bayou.

Methods

Historical Data Review

We reviewed pertinent published summary data and electronically available data housed in agency databases, technical reports, and refereed literature dealing with the Buffalo Bayou watershed. Some of these reports and articles have already been mentioned and were discussed in the introduction section of this report. The primary water quality data sources that were reviewed and analyzed include the HGAC-CRP and TCEQ SWQMIS online database (HGAC 2010; TCEQ 2010). Our review focused on historical data sets and studies that included information on *E. coli* bacteria and associated variables that influence their survival within Buffalo Bayou. In addition, since we were mainly interested in quantifying the influence of the Waugh Street bat colony on indicator bacteria levels we largely confined our search to monitoring sites near the bat colony. We defined these sites as being within one mile both upstream and downstream of the Waugh Street Bridge. If we move beyond these boundaries other factors including significant tributary flows, storm sewer outfalls, and wastewater facilities might confound any signal attributable to the bat colony.

Historically fecal coliform bacteria were monitored to determine compliance with state water quality standards. Starting in the late 1990's the State of Texas promulgated new water quality standards that include *E. coli* and Enterococcus bacteria (TCEQ 2008a). Since then both of these indicators have been

monitored. Although the official regulatory water quality standard for the Buffalo Bayou tidal segment is Enterococcus bacteria (preferred EPA indicator for marine waters), we chose to evaluate *E. coli* levels and genetic composition. There were several reasons for this decision. The official state water quality standard indicator bacteria used to evaluate the upstream adjacent non-tidal segment of Buffalo Bayou (segment 1014) is *E. coli*, which is the EPA preferred freshwater indicator. Since the bat colony at the Waugh Street Bridge is located near the upper portion of the tidal segment and is influenced by water flowing in from the upstream non-tidal segments it is reasonable to use *E. coli* as an indicator. Finally although Buffalo Bayou segment 1013 is described as a tidal water body, the salinity and specific conductance show that it is freshwater (TCEQ 2008a). While there are tidal fluctuations at the United States Geological Survey (USGS) gauge at Shepherd, the salinity and specific conductance data do not support the continued use of the criteria for a tidal water body. Therefore, *Escherichia coli* (*E. coli*) were used as the indicator bacteria for all of the segments. Other variables evaluated included stream stage level (surrogate for flow), season, and water temperature. These variables are known to affect the survival and ambient levels of indicator bacteria.

We contacted the TPWD and Bat Conservation International (BCI) to obtain information and data on the history and population trends of the Waugh Street colony and other potential sites. Originally we planned to attempt to estimate current bat roosting population density at the bridge using techniques outlined in Kunz (1990). Depending on the available data we hoped to merge this data with past water quality data to determine if bacteria levels have increased as the colony has grown. However, long-term past quantitative estimates of population density do not exist for this colony. Therefore due to the inability to compare current populations to historical levels and logistical issues we were not able to conduct population surveys.

Surface Water Indicator Bacteria and Water Quality Data Collection

During 2008 and 2009 an intensive survey of ambient bacteria levels was conducted to determine if the Waugh Street bat colony is affecting bacteria levels in Buffalo Bayou. Our primary approach included upstream and downstream sampling in the vicinity of the colony. Our null hypothesis was that the upstream indicator bacteria are not statistically significantly lower than downstream levels. In order to test this hypothesis we conducted diel and seasonal sampling in the vicinity of the bat colony. Field sampling included sampling upstream and downstream of the colony (Table 1 and Fig. 1 and 2). Sampling was conducted quarterly starting in the summer 2008 through spring 2009 (Table 1). Additional source identification samples (water and fecal material) were collected in November 2009. The actual sample dates were 8/14-15/08, 9/25-26/08, 11/17-18/08, 2/5-5/09, 2/4/2-3/09, 4/2-3/09, 11/5/09, and 11/17/09.

Table 1. Ambient field monitoring sites and frequency.

Site	Location	Seasons
Site 1.	Upstream (100 ft)	* Summer (Aug and Sept 08), Fall (Nov) 08, Winter (Feb) 09, Spring (April) 09 5 events total (ambient monitoring)
Site 2.	Immediately below bridge (bat colony at Waugh St.	Same (*) + fecal pellet sampling (April & Nov 09)
Site 3	Downstream 100 ft	Source ID (water): (Aug 08, April and Nov 09)
Site 3A	Downstream 200 ft.	Same (*) + Source ID (water): (Aug 08, April and Nov 09)
Site 4.	Downstream 1000 ft	Same (*) + Source ID (water): (Aug 08, April and Nov 09)

Influence of the Waugh Street Bat Colony on Indicator Bacteria Levels in Buffalo Bayou Site Map.

Figure 1. Location of ambient sampling sites. Site 2 is location of Waugh Street bat colony

We collected two diel bacteria samples during each sampling period. These water samples were collected in the late afternoon (dusk) of the first day and early morning (dawn) of the next day at each site. Source sampling occurred during evening of the dates noted. We hypothesized that late afternoon sampling should yield the highest bacteria loading due to the high densities of roosting bats. Early morning sampling should yield the lowest possible loading due to the absence of bats, which have been foraging all night.

Diel variations in wastewater treatment (WWTP) flows and loading of indicator bacteria into Buffalo Bayou are a potential confounding factor. Therefore we reviewed data from the TCEQ wastewater and EPA PCS (permit compliance system) GIS layers that are shared with the HGAC Clean Rivers Program (CRP) to insure there were not wastewater plants nearby. We also sampled early in the morning and late in the evening to reduce the influence of WWTP (wastewater) flows. During these periods WWTP flows are lower. However, the influence of point sources cannot be totally removed since at low flows Buffalo Bayou, like many urban streams, is often dominated by wastewater effluent contributions.

Since stream flow can also influence bacteria levels by either increasing concentrations due to contaminated runoff and by dilution of source water. Therefore to increase the likelihood of detecting actual impacts associated with the bat colony and to reduce flow induced variation we attempted to collect all samples during dry weather conditions. Dry weather conditions were defined as a period of at least 3 days of no significant precipitation (< 0.1 inches in 24 hour period) within the watershed. In all cases stream gage level was used to confirm this and/or allow us to evaluate this source of variability.

Water quality and bacteriological sampling will be conducted using standard methods described in the TCEQ Surface Water Quality Monitoring and Clean Rivers Program guidance manuals (TCEQ 2008b). At each site five (5) replicate indicator bacteria per sampling event were collected. Bacteria samples were collected mid-stream with the use of a telescoping sampler which will hold a sterilized sampling bottle or Whirlpak. Samples were placed on ice and returned to the laboratory for analysis of E. coli bacteria. We then incubated and analyzed these samples using the IDEXX Colilert® defined substrate method which has been approved for use in Texas by both the TCEQ and EPA and is described in TCEQ (2008b). In addition to collected samples, we also analyzed one field duplicate and one blank during each sampling period. In addition to bacteria sampling, we also measured additional water quality measurements in the field including surface temperature, pH, specific conductance, dissolved oxygen with a YSI multiparameter meters, transparency with a secchi disk and turbidity with a NTU (nephelometric turbidity unit) meter.

Fecal Pellet Collection – Bacteria Enumeration

Bat guano (fecal pellets) were collected at the Waugh Street Bridge by the EIH-UHCL samplers on 4/2/09 and 11/17/09 and transferred to TAMUG for genetic analysis. During 11/5/09 pellets were also collected by the UHCL lab for direct enumeration of *E. coli* using a more crude method of estimation which utilizes a process where fecal pellets are weighted on an analytical balance and then crushed and vigorously shaken in a known volume of sterile buffer and allowed to settle. After a short time the supernatant processed using the IDEXX Colilert® defined substrate method TCEQ (2008b). Estimated numbers of *E. coli* MPN/100 ml are then converted back to the original weight of fecal material introduced and converted to MPN/g of feces. Again, this approach provides a very crude estimate because factors such as adherence to solid material and loss of this material during the settling stage may introduce a negative bias. In addition, the age of the fecal material collected will affect the amount of bacteria recovered.

Statistical Analysis – Indicator Bacteria

Bacteriological data was statistically analyzed to evaluate differences in bacteria levels between stations and diel periods. Three factors will be evaluated including station effects (which incorporate the bat colony influence), diel effects, and seasonal influence. A two-way Analysis of variance will be used to test the null hypothesis of no significant differences between sites and seasons and interactions after appropriate transformation of the independent variable (e.g log transformation of bacteria levels) to meet the assumptions of equal variance and normal distributions. If the null hypothesis is rejected, a multiple comparison test will be conducted to identify which site or season is significant. If significant interactions between factors are detected, mean plots will be generated to graphically evaluate these interactions. Elevated levels of bacteria downstream of the bat colony will provide sufficient evidence to conclude that the bats are having a significant effect on water quality in Buffalo Bayou. If downstream diel levels are greater in afternoon versus morning hours this will provide additional evidence in support of the hypothesis that bats are affecting water quality. Finally, there should also be a strong seasonal effect due to the absence of bats during winter months.

Graphical and correlation analyses were conducted on flow, temperature, stream level, and turbidity to determine their potential influence of these factors on observed bacteria levels. A principal components analysis was also conducted to evaluate the relative influence and relationship of these variables including location (as measured by a proxy distance variable), diel period, seasonality (as measured by the proxy variable water temperature) and measured ambient concentrations of *E. coli.*

Microbial Source Identification

Microbial source identification sampling efforts occurred during dry weather conditions and in most cases paralleled sample collection efforts for the

previously described IDEXX Colilert® and water quality analyses. Sampling occurred in August 2008, April 2009 and November 2009. Water samples from Buffalo Bayou were collected directly under the Waugh Avenue Bridge, 100, 200, and 1000 feet downstream on 8/14/08, 4/2/09, and 11/17/09. Bat guano (fecal pellets) was also collected by the EIH-UHCL samplers on 4/2/09 and 11/17/09 and transferred to TAMUG. Water samples were collected in sterilized cubitainers and stored on ice (4ºC) then transported back to the EIH or TAMUG within 4 hr. At TAMUG, the IDEXX Colilert method was used to determine the MPN of *E. coli*, followed by removal of the IDEXX growth media from 'positive' IDEXX trays for isolation of E. coli using selective media.

For source identification the Texas A&M at Galveston Microbiology Laboratory used a two pronged approach to determine the percentage of guano-like *E. coli* bacterial isolates in Buffalo Bayou water collected directly under the Waugh Avenue Bridge, 100, 200, and 1000 feet downstream. We conducted fingerprinting of whole genomic DNA (HFERP) and biotype analysis using different sugars in minimal agar.

We also used fluorescence in situ hybridization (FISH; Amann et al. 1995) with DNA probes specific for *E. coli* and Enterococcus spp. to quantify these indicator bacteria in guano and to estimate their contribution into the bayou as a cell number per gram feces. With this method, intact bacteria could be visualized and enumerated with epifluorescence microscopy.

Summary of Source Identification Methods

1. Isolates were confirmed to be *E. coli* by rapid fingerprinting analysis of 16S rRNA genes. HFERP fingerprinting was conducted with polymerase chain reaction (PCR) using BOX A1R primers labeled with 6-FAM (carboxyfluorescein) according to Johnson et al. (2004). The primers randomly amplify target sequences in the genomic DNA to produce the fingerprints. The PCR products were separated on 1% agarose gels, documented digitally, and then analyzed for similarity with Bionumerics software using four different cluster similarity analyses and correlation algorithms. This fingerprinting method was employed by a concurrent study funded by the TCEQ in Buffalo Bayou and White Oak Bayou for source identification and the bat data can be better interpreted in the context of this larger data set. However, increasing numbers of studies indicate that source tracking or identification with DNA fingerprinting of *E. coli* is unreliable because of the lack of strain specificity among hosts (Gordon and Cowling 2003; Hassan et al. 2006; Stoekel and Harwood 2007). Therefore, we also employed these DNA fingerprinting methods for *Enterococcus* isolated from the water samples provided. DNA fingerprints of *E. coli* and Enterococcus isolated from the bat guano were compared with those from bacteria isolated from the bayou.

2. Biotype analysis of isolated cultures of *E. coli* was conducted using different sugars in minimal agar. Souza et al. (1999) demonstrated significant heterogeneity in sugar utilization of *E. coli* based upon host diet. Since the Mexican free-tailed bats are predominantly insectivores, we expect to see this

diet reflected in the sugar utilization of *E. coli* isolated from the bat guano and the bayou samples versus that of human and other non-human sources. For biotyping by sugar utilization, agar plates containing only one of the following sugars: dulcitol, raffinose, rhamnose, salicin, sucrose, mannitol, arabinose, adonitol, or moltose were inoculated with each isolate and observed for presence or absence of colony formation (Souza et al. 1999). The presence/absence data were converted to binary format (i.e. 1, 0) and analyzed with the Bionumerics software using the Dice Coefficient/UPGMA cluster analysis.

Finally the UHCL-EIH team surveyed the literature and contacted TPWD and BCI for information regarding total amounts of guano deposited into the bayou from the Waugh Street Bridge colony. Unfortunately we were not able to locate any reliable published estimates for the Waugh Street colony. However, we did find estimates in the literature of fecal production by roosting Mexican free-tailed bats which inhabited a highway overpass near Belton, Texas (Sgro and Wilkins 2003). They conducted laboratory studies on captured wild bats and produced 12 hour estimates of fecal production. This 12 hour estimate approximates the normal roosting period when bats produce the majority of feces. They estimated that a single bat on average produces 0.0646 grams of feces, with a standard deviation of 0.0985 grams (Sgro and Wildkins 2003). They also found through their mark recapture study that this species of bat shows a high level (70% of population) of site fidelity, ranging between 1 week to 4 months. Using the previous estimates of 250,000 – 300,000 bats present at the Waugh Street bridge and the per capita production rate of 0.0985 grams of feces per day, we can estimate that during the period when bats are present and/or active the production of between 24.6 to 29.5 kg (54.2 to 65 lbs) of feces per day is possible (Buffalo Bayou Partnership web page: http:\\Waugh Bridge Bat Colony.mhtv and http://www.buffalobayou.org/WaughBatColony.htm).

Results

Historical Data

Based on review of electronic data sources we obtained recent ambient monitoring data from both TCEQ and local agencies through the Clean Rivers Program at the nearest monitoring site located upstream and downstream of the bat colony in Buffalo Bayou. These were located upstream at Buffalo Bayou at Shepard Drive (TCEQ station I.D. 11351) and downstream at the Sabine Street crossing (TCEQ station I.D. 15843). The Shepard Drive site is about 3,200 ft upstream whereas the Sabine Street site is located about 8,000 ft downstream. These sites are monitored by City of Houston Health and Human Services Department in cooperation with the HGAC under the Clean River Program. The period of record obtained extended from June 13, 2001 to January 22, 2008 for the Shepard Drive site and December 6, 2001 to July 24, 2006 at the Sabine Street site.

Bacteria levels at both sites were extremely variable during the period of record evaluated but appeared to have declined in recent years (Fig. 2). However, overall ambient *E. coli* levels do appear to increase downstream from Shepard Drive as the bayou flows past Waugh Street and ultimately to the Sabine Street site, an approximate distance of 11,200 feet (2.1 miles) (Fig. 3). This increase in bacteria levels is also highly statistically significant (Table 3). This data supports the hypothesis that the Waugh Street bat colony is contributing to historical increases in *E. coli* within Buffalo Bayou as measured at the downstream Sabine Street location.

Precipitation and Stream Level

We attempted to obtain flow and/or precipitation data from the nearest USGS gage (08074000) which are located at the upstream Shepard Drive site (USGS 2010). During the period of our study however no data were available for download including flow, stage or rainfall. However, both stream stage level and rainfall data were available at the co-located site at Shepard Drive (2240-W100) that is operated by Harris County Office of Emergency Management (HCOEM) (Harris County 2010). Based on precipitation and stream level data our samples were generally collected during dry weather events with the exception of the April 2009 (Table 3). Water levels however on this date were extremely low suggesting little impact on stream flow.

Upstream Point Source Loading

Based on watershed information obtained from the HGAC CRP program the nearest wastewater discharge points are over 5 and 9 miles upstream of the bat colony (HGAC 2010) (Figure 4). These include the Memorial Village Water Authority (Permit 10584-001) and UA HOLDINGS 1994-5 LP (Permit 12233-001) facilities that discharge into Buffalo Bayou. The average mean monthly flow varied between 1.79 and 1.93 MGD at the Memorial Village Water Authority, and 0.0013 and 0.0028 MGD at the UA Holdings facilities during the study period. Based on the reported flow it is highly unlikely that these remote facilities would significantly influence bacteria levels at the downstream study sites except during very low flow periods.

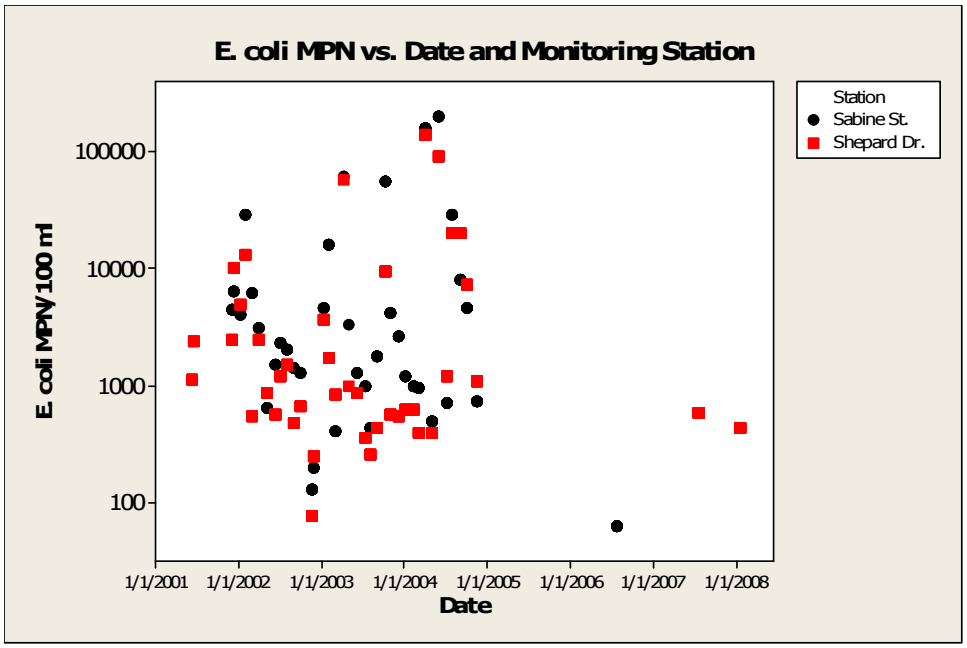

Figure 2. *E. coli* levels in ambient water measured at the upstream Shepard Drive (ID 11351) site and downstream Sabine Street site (ID 15843). Data from HGAC CRP database

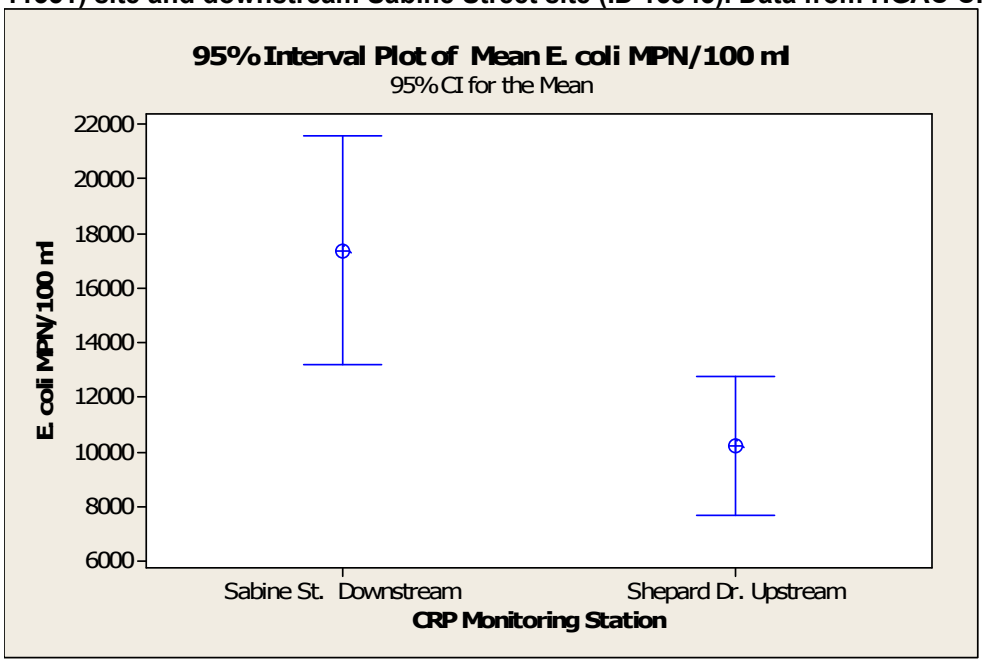

Figure 3. Confidence interval plot of mean *E. coli* levels at the upstream Shepard Drive (ID 11351) and downstream Sabine Street sites (ID 15843). Data from HGAC CRP database.

Table 2. One-way ANOVA: *E. coli* (MPN/100ml) versus Station

```
Source    DF           SS           MS      F      P
Station    1  11977279264  11977279264  8.55  0.004
Error    933  1.30623E+12   1400033902
Total    934  1.31821E+12

S = 37417   R-Sq = 0.91%   R-Sq(adj) = 0.80%

Level          N   Mean  StDev
Sabine St.   449  17371  44952
Shepard Dr.  486  10207  28753

             Individual 95% CIs For Mean Based on Pooled StDev
Level           +---------+---------+---------+---------
Sabine St.                            (---------*---------)
Shepard Dr.      (--------*---------)
                 +---------+---------+---------+---------
               7000     10500     14000     17500

Pooled StDev = 37417
```

Table 3. Water level and rainfall data recorded at the Harris County Office of Emergency Management Gage at Shepard Dr. Datum: top of bank 28 feet elevation.

Date/Time (* Central Standard)	Water Level (ft)	1 day rainfall (in)	3 day rainfall (in)
8/14/2008 (*14:19)	4.55	0.00	0.00
8/15/2008 (* 05:36)	4.19	0.00	0.00
9/25/2008 (* 14:26)	6.43	0.00	0.00
9/26/2008 (05:24)	4.9	0.00	0.00
11/17/2008 (14:49)	9.64	0.00	0.00
11/18/2008 (06:42)	9.66	0.00	0.00
2/5/2009 (15:28)	1.54	0.00	0.00
2/6/2009 (06:39)	1.63	0.00	0.00
4/2/2009 (15:26)	2.32	0.20	0.22
4/3/2009 (07:02)	1.51	0.16	0.20
11/5/09 (mid-day)	4.16	0.00	0.00
11/17/2009 (mid-day)	1.85	0.00	0.24

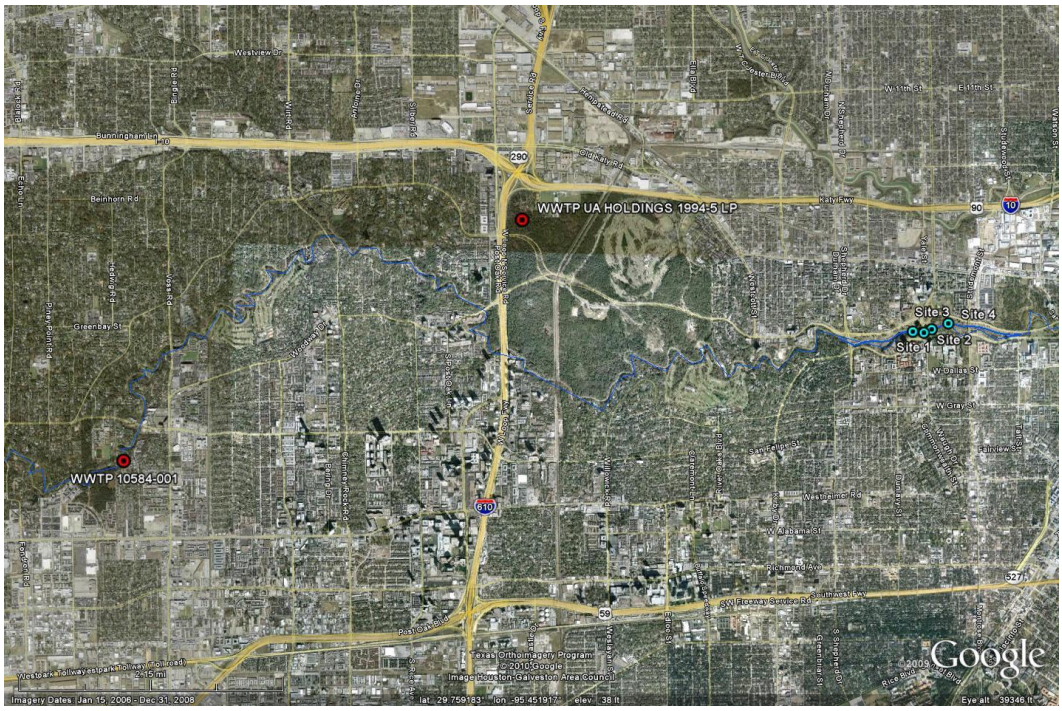

Figure 4. Location of wastewater discharge points (in red) upstream of the study site (in red) in Buffalo Bayou.

Bat Colonies

During a 2008-2009 a parallel study was conducted by Dr. Brinkmeyer which was funded by the TCEQ and entitled “Population Dynamics of *Escherichia coli* and *Enterococcus* spp. bacteria in Buffalo Bayou and White Oak Bayou”. During that study her research team observed additional large congregations of bats at various bridges upstream of Waugh Avenue including two large congregations at the intersection of South Mason Road, and downstream in downtown at the intersection of Smith and Franklin streets (Figure 5). There were highly visible layers of guano at each location.

Ambient Water Quality and E. coli levels

Data collected during our study documented elevated levels of *E. coli* in Buffalo Bayou (Fig. 6). The current freshwater primary contact recreation standard is a geometric mean criterion for E. coli is 126 per 100 ml. In addition, the single sample criterion for E. coli is 399 per 100 ml (TSL 2000). The bat colony is however located in the tidal section of Buffalo Bayou and the geometric mean criterion for Enterococci are 35 colonies per 100 ml. As discussed earlier, we used the *E. coli* bioindicator for evaluation. All collections at each site exceeded the *E. coli* criteria (Fig. 7). Overall patterns suggest extensive overlap between sites in terms of bacteria density (Fig. 8). Lowest concentrations of *E. coli* occurred in late fall through winter months when bats are either less active and/or have migrated to other locations.

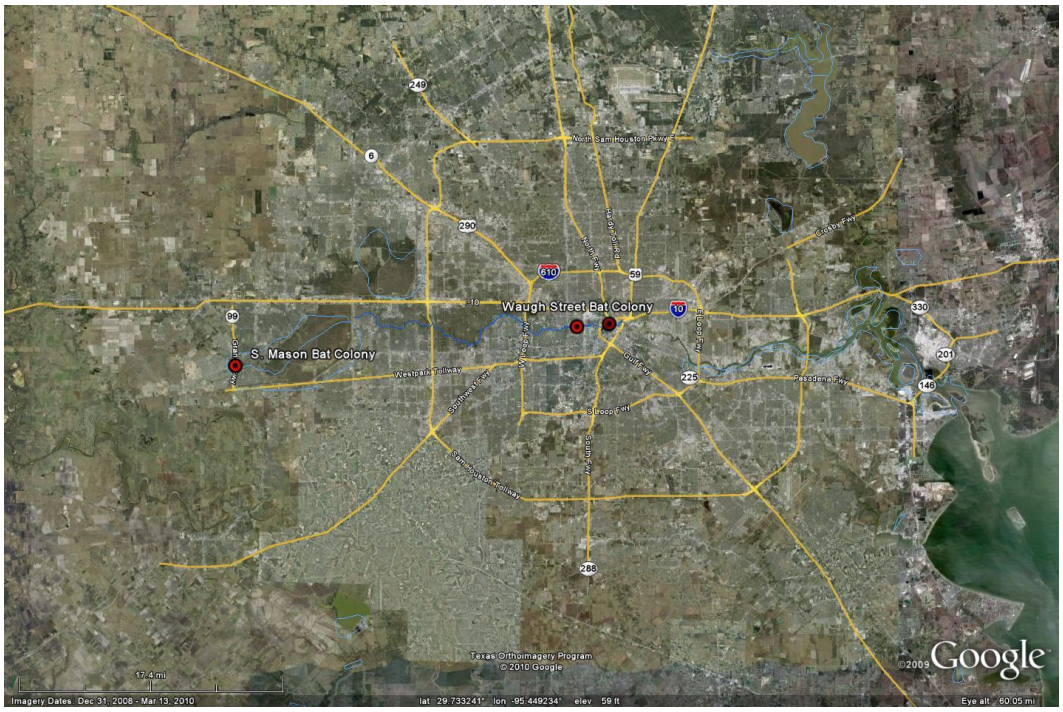

Figure 5. Location of additional bat "colonies" observed by Dr. Brinkmeyer's research team during 2008-2009 and the Waugh Street colony.

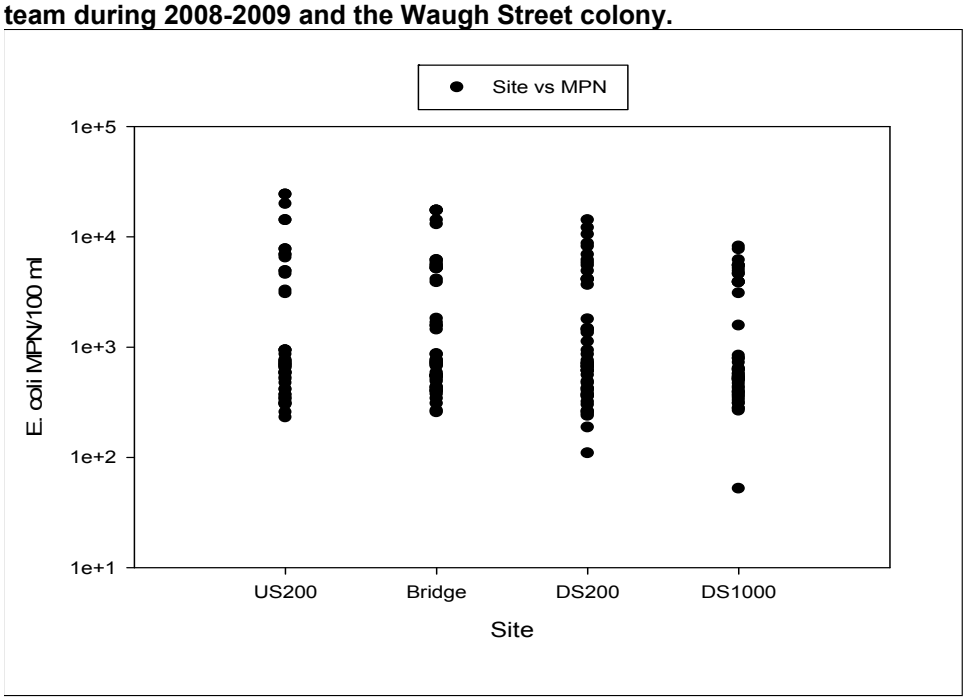

Figure 6. Overall trends in *E. coli* concentrations in ambient water upstream and downstream of the Waugh Street Bridge based on August, September, November 2008 and February and April 2009 monitoring. Log_{10} scale used in Y axis.

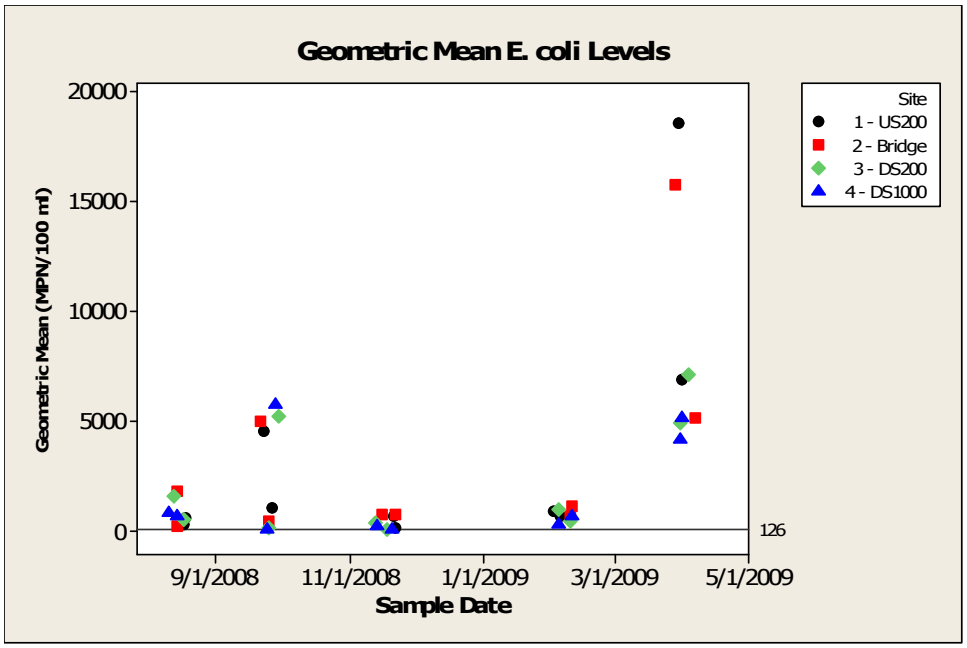

Figure 7. Geometric mean *E. coli* levels in ambient water upstream and downstream of the Waugh Street Bridge based on 2008 and 2009 monitoring. Horizontal line represents geometric mean criterion for *E. coli*.

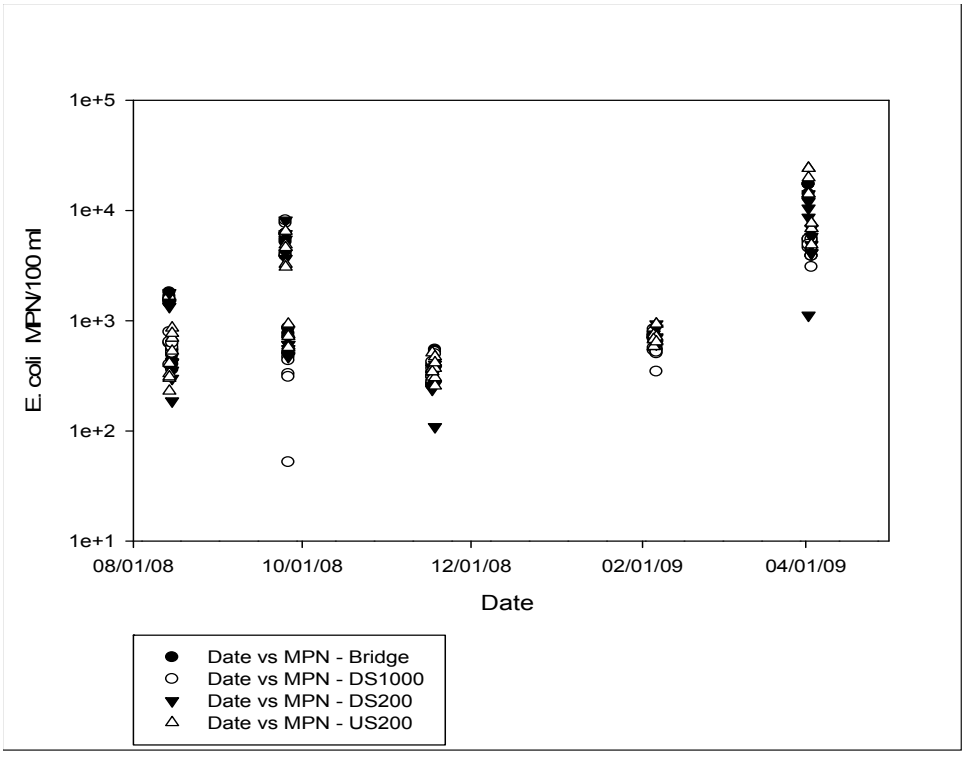

Figure 8. Temporal and spatial trends in *E. coli* concentrations in ambient water upstream and downstream of the Waugh Street Bridge based on August, September, November 2008 and February and April 2009 monitoring. Log_{10} scale used in Y axis.

E. coli levels were generally higher during “day” sampling events, that is, in late afternoon after an entire day of roosting (Figure 9). There was also a strong seasonal pattern in diel trends of bacteria levels. This overall diel trend was attenuated or absent during late fall and winter months when bats may have migrated away or are less active (Figures 10-12). This pattern was most obvious at the bridge and 200 feet downstream (Figure 12).

The two-way ANOVA that was performed yielded significant differences in log transformed *E. coli* levels between sites and sampling dates (Table 10). In addition, significant interactions were observed. Examination of interaction plots indicated that there were distinct seasonal differences in log transformed *E. coli* levels with highest levels occurring in summer and fall months (Figure 13). In addition, as previously observed with the raw data, highest log transformed *E. coli* levels usually occurred in daytime samples with the exception of fall and winter when little diel differences occurred. Log transformed and untransformed *E. coli* levels varied little between sites, but levels were generally higher at the upstream sites and immediately below the bridge (Figures 13 and 14). With the exception of April 2009, *E. coli* levels were generally lower upstream of the Waugh Street bat colony.

Environmental conditions were similar between all sites. Water temperature, specific conductance, pH, secchi disk transparency, turbidity, ammonia nitrogen and dissolved oxygen exhibited similar patterns between sites (Figs. 15-21). The distribution of ammonia nitrogen levels were however slightly higher at the bridge site which may reflect potential nitrogen loading from bat urea.

In order to evaluate the influence of various physical and water quality variables and *E. coli* levels we constructed scatter plots to visually examine the data (Figs. 22-25). In addition, linear correlation analysis was conducted (Table 5). Highest turbidity levels generally occurred at higher stream stage (Fig. 22). This probably reflects mobilization of stream sediments. Highest *E. coli* levels in turn were encountered at intermediate turbidity and water temperature (Figs. 23 and 24). At highest stream stage levels, associated flows are likely diluting bacteria levels (Fig. 25). This pattern has been observed in numerous studies of bacteria levels in streams. *E. coli* levels were significantly positively correlated with 1 and 3 day cumulative rainfall amounts (Table 5). In addition *E. coli* density was negatively correlated with stream level and consequently stream flow, although this was not significant at the alpha = 0.05 level. These data and correlations suggest that precipitation and stream flow were the primary variables measured that influence *E. coli* levels.

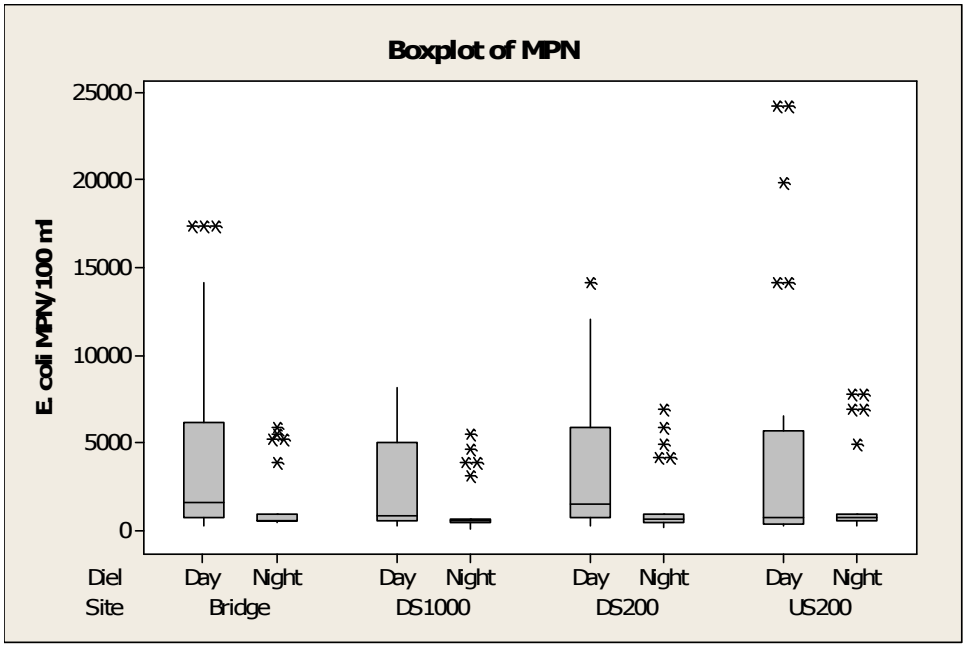

Figure 9. Interaction boxplot of site and diel factors for all sites combined.

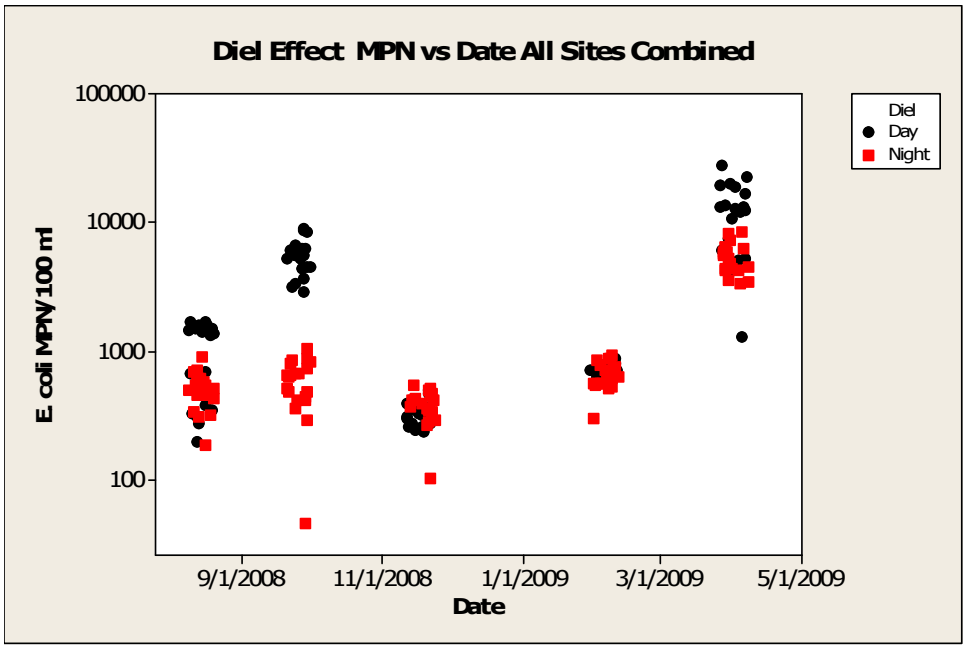

Figure 10. Interaction plot of date and diel factors for all sites combined. Log10 scale used in Y axis.

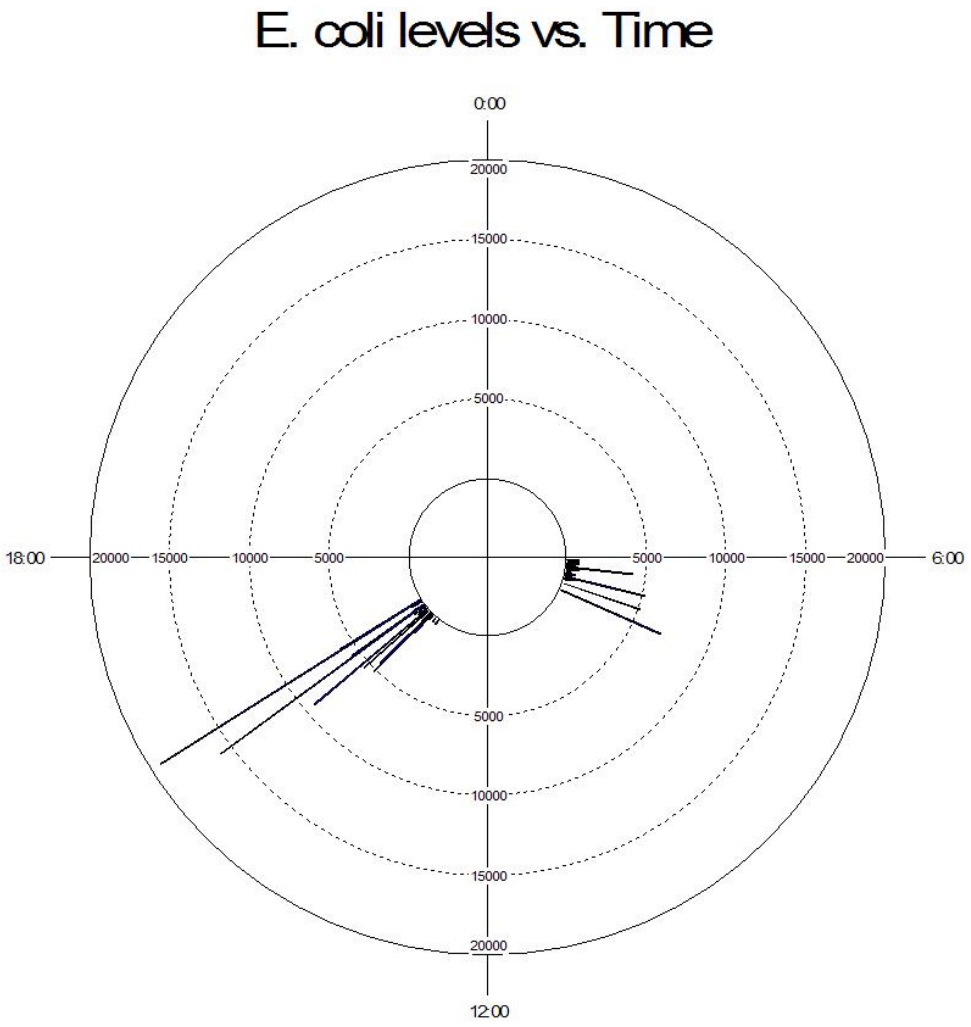

Figure 11. Average E. coli levels by time of collection for all sites. Perimeter scale = 24 hour time; vertical and horizontal scale is numbers of *E. coli* MPN/100 ml

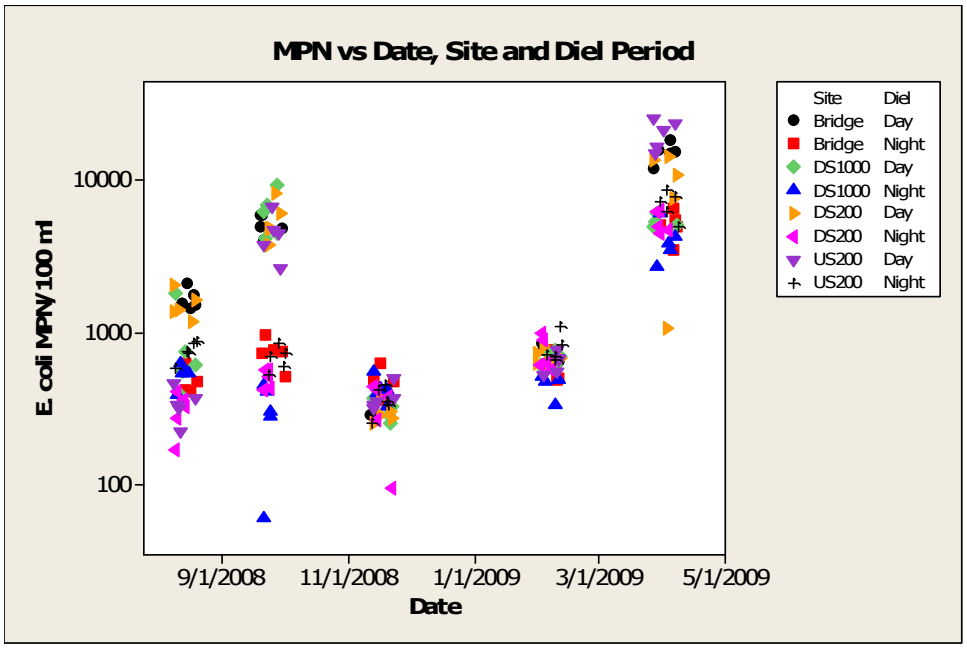

Figure 12. Plot of date, site and diel factors. Note log_{10} scale on Y-axis. *Note, replicate values for August 2008 are not true field replicates, but instead laboratory replicates or splits.

Table 4. Two-way ANOVA: Log_{10} transformed E. coli versus Date, Site

Source	DF	SS	MS	F	P
Date	9	53.8810	5.98678	327.15	0.000
Site	3	0.5639	0.18797	10.27	0.000
Interaction	27	3.6082	0.13364	7.30	0.000
Error	160	2.9280	0.01830		
Total	199	60.9812			

S = 0.1353 R-Sq = 95.20% R-Sq(adj) = 94.03%

*Note, replicate values for August 2008 are not true field replicates, but instead laboratory replicates or splits.

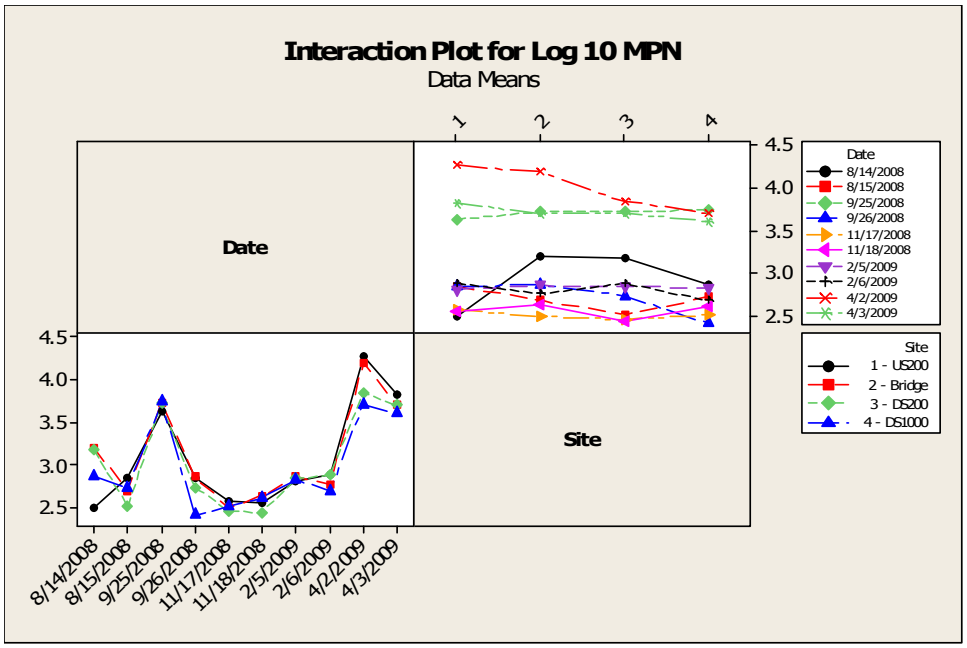

Figure 13. Interaction plot of average log_{10} transformed *E. coli* levels by date and site. For paired dates the first and second date represents day and night samples respectively. *Note, replicate values for August 2008 are not true field replicates, but instead laboratory replicates or splits.

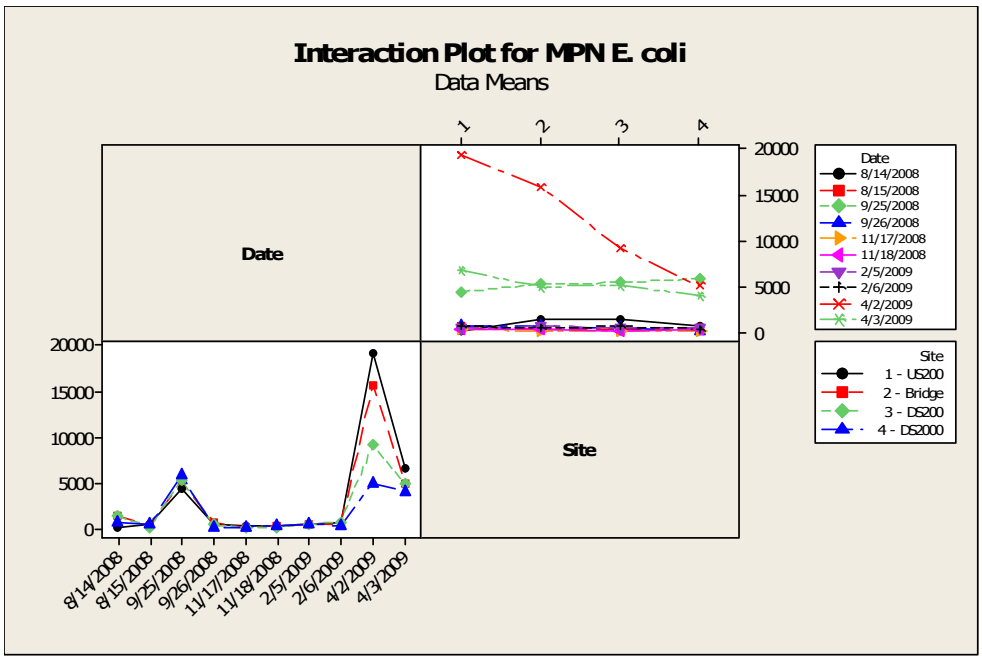

Figure 14. Interaction plot of average *E. coli* levels by date and site. For paired dates the first and second date represents day and night samples respectively. *Note, replicate values for August 2008 are not true field replicates, but instead laboratory replicates or splits.

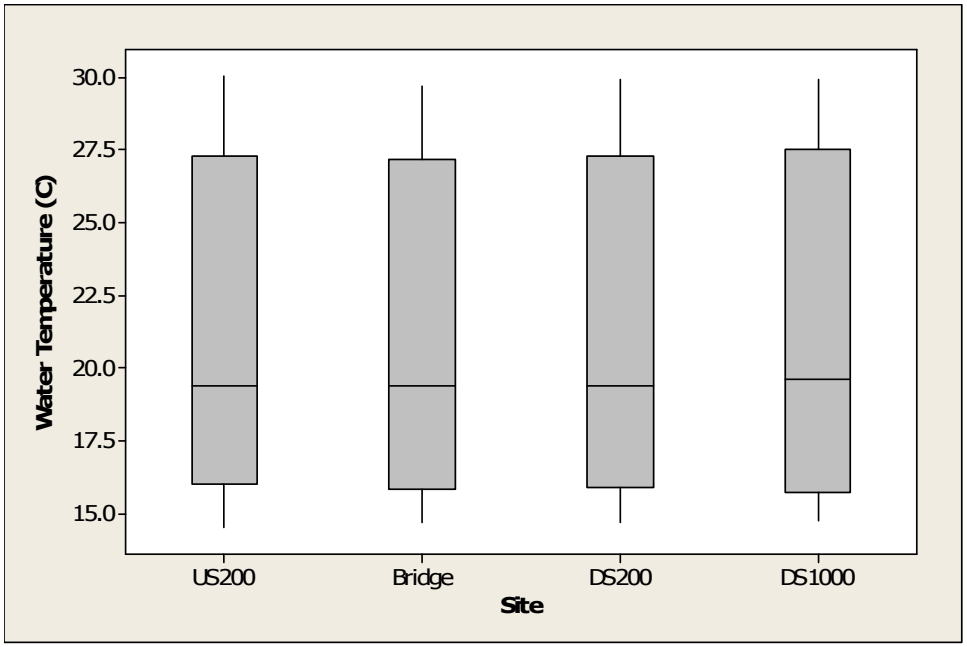

Figure 15. Range of water temperature (C) conditions at each site during study period.

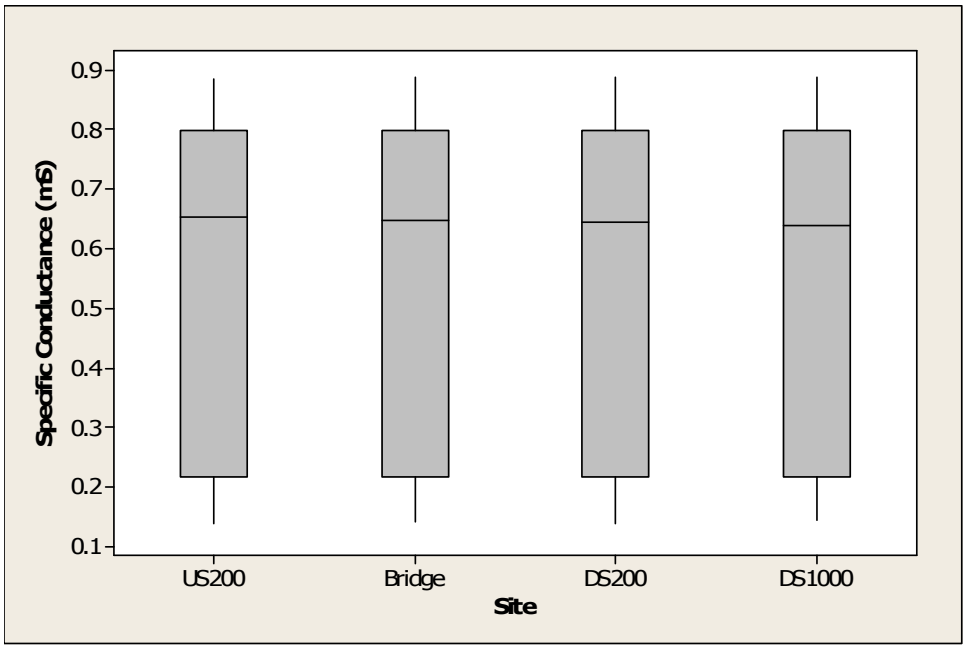

Figure 16. Range of specific conductance (mS) levels at each site during study period.

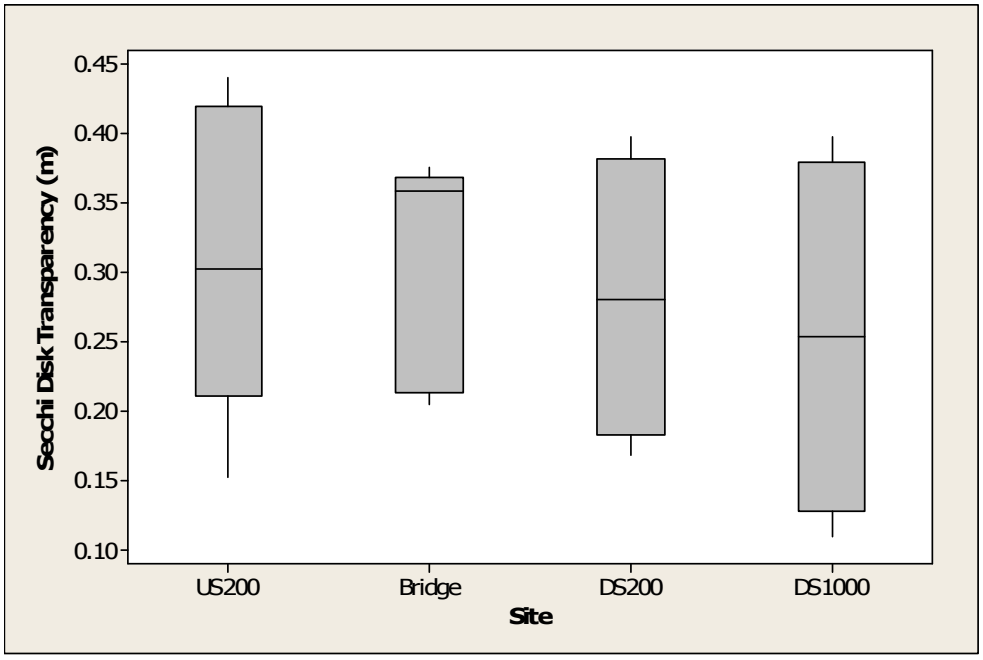

Figure 17. Range of secchi disk (m) levels at each site during study period.

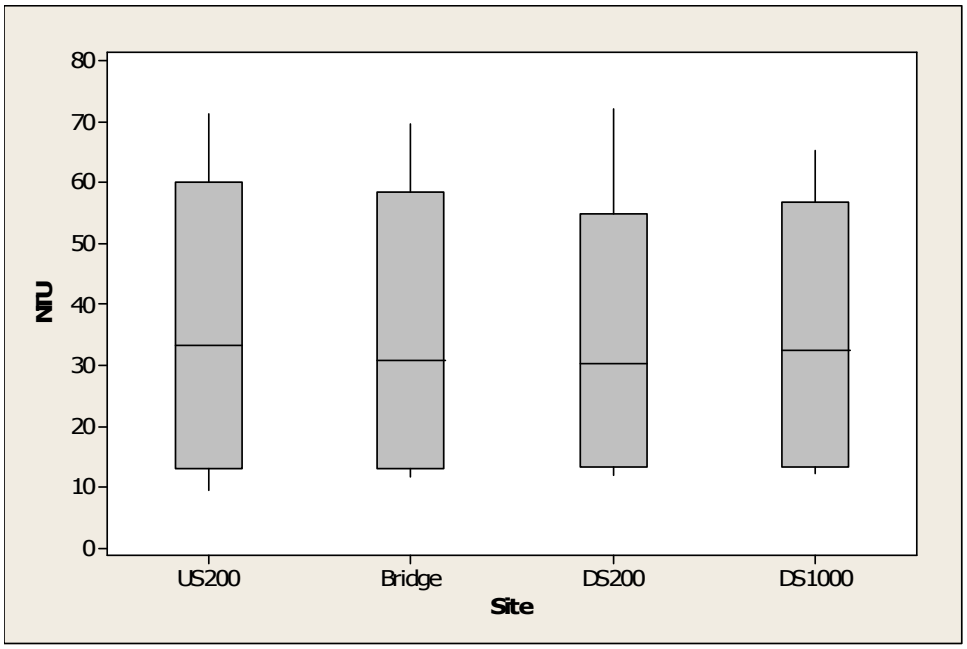

Figure 18. Range of turbidity (NTU) levels at each site during study period.

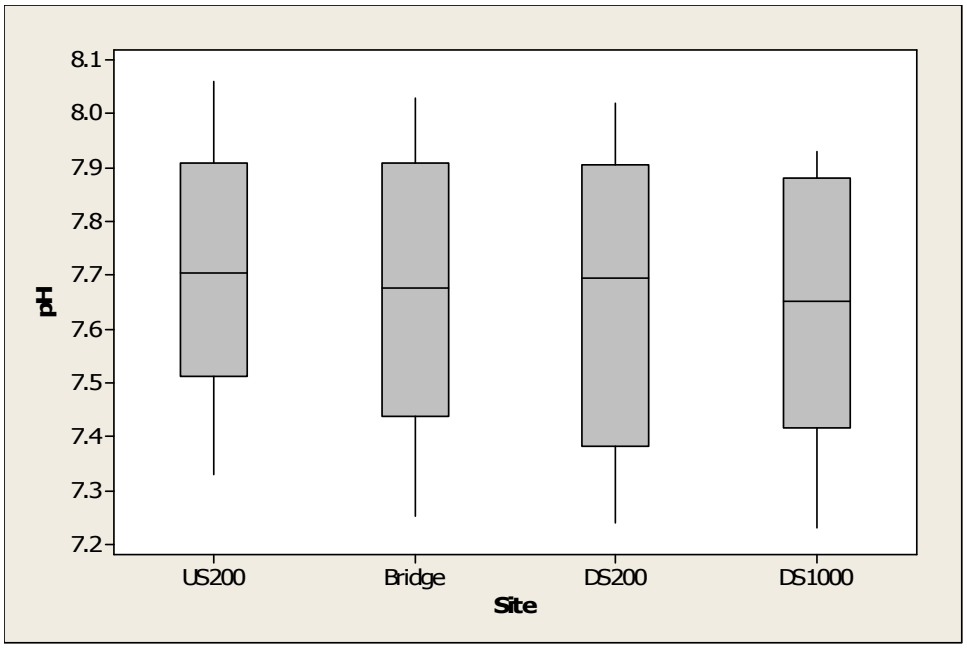

Figure 19. Range of pH levels at each site during study period.

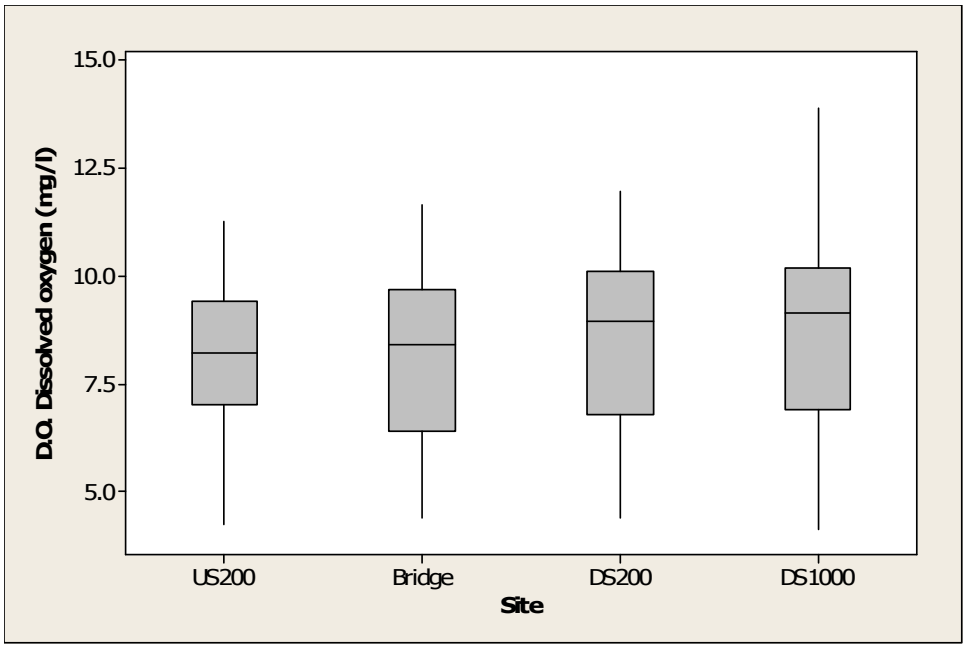

Figure 20. Range of dissolved oxygen levels at each site during study period.

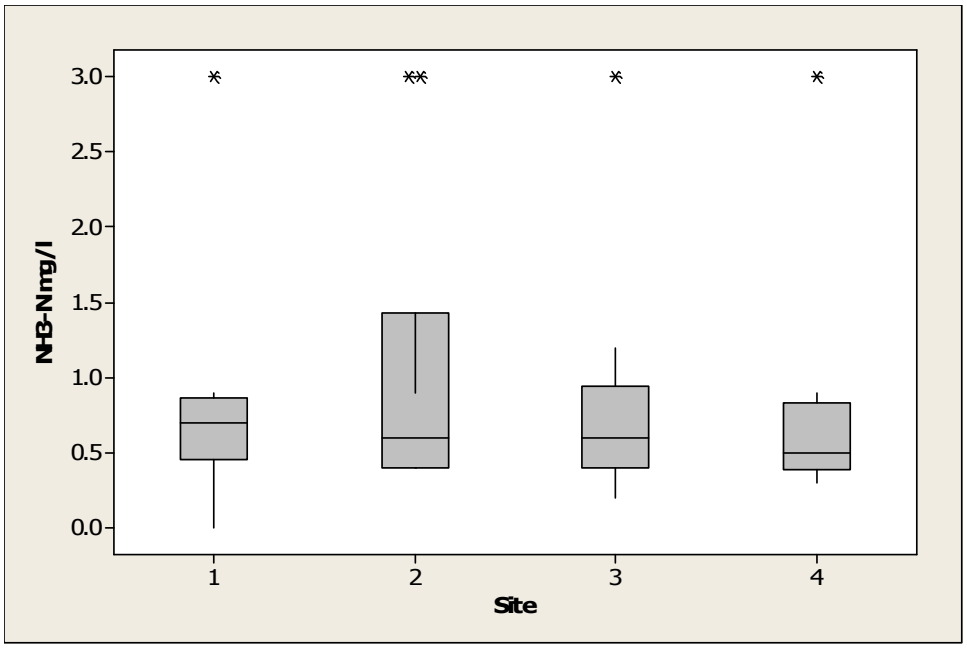

Figure 21. Range of ammonia nitrogen (N-NH3 mg/l) levels at each site during study period.

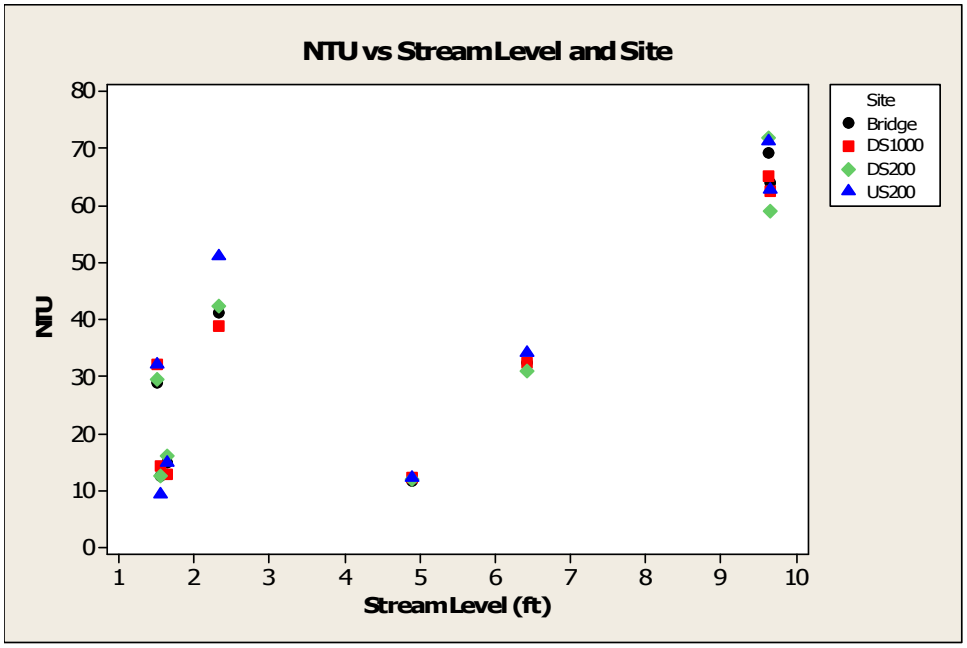

Figure 22. Relationship between measured turbidity (NTU) values versus HCOEM stream gage level.

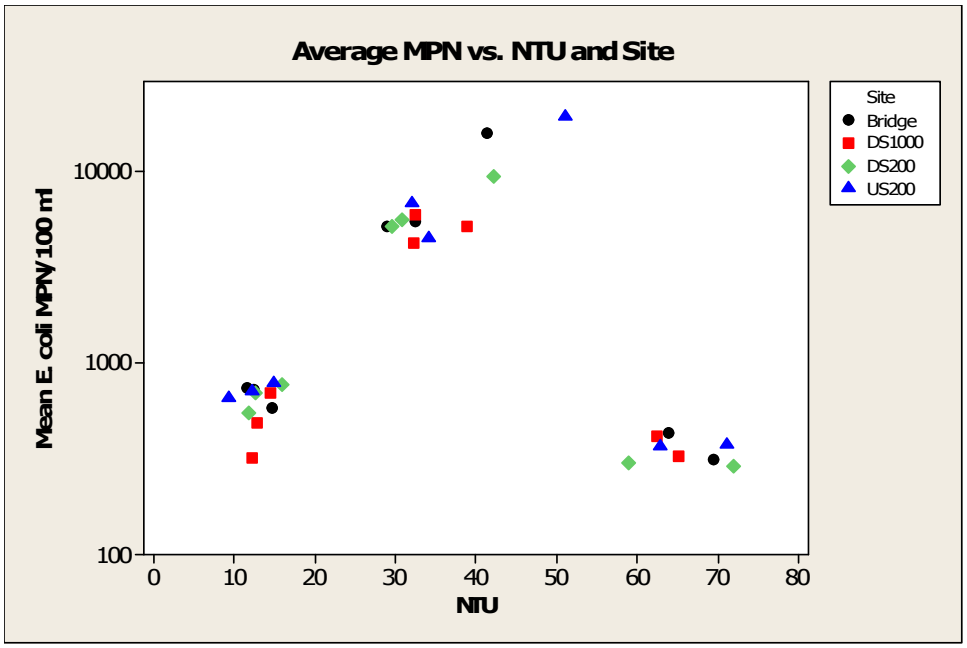

Figure 23. Relationship between measured turbidity (NTU) values versus average *E. coli* levels (MPN).

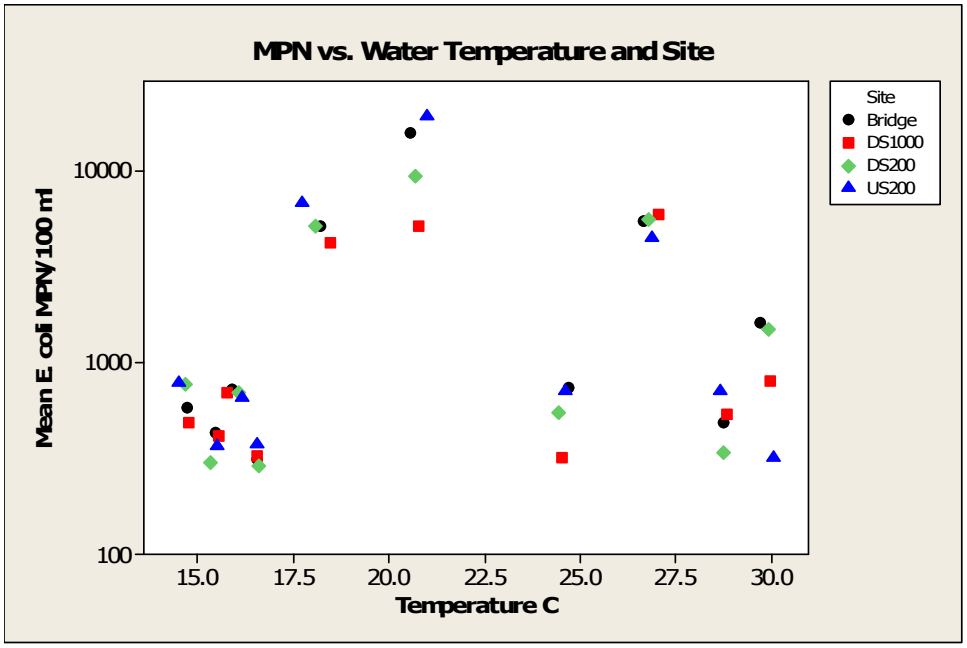

Figure 24. Relationship between measured water temperature versus average *E. coli* levels (MPN).

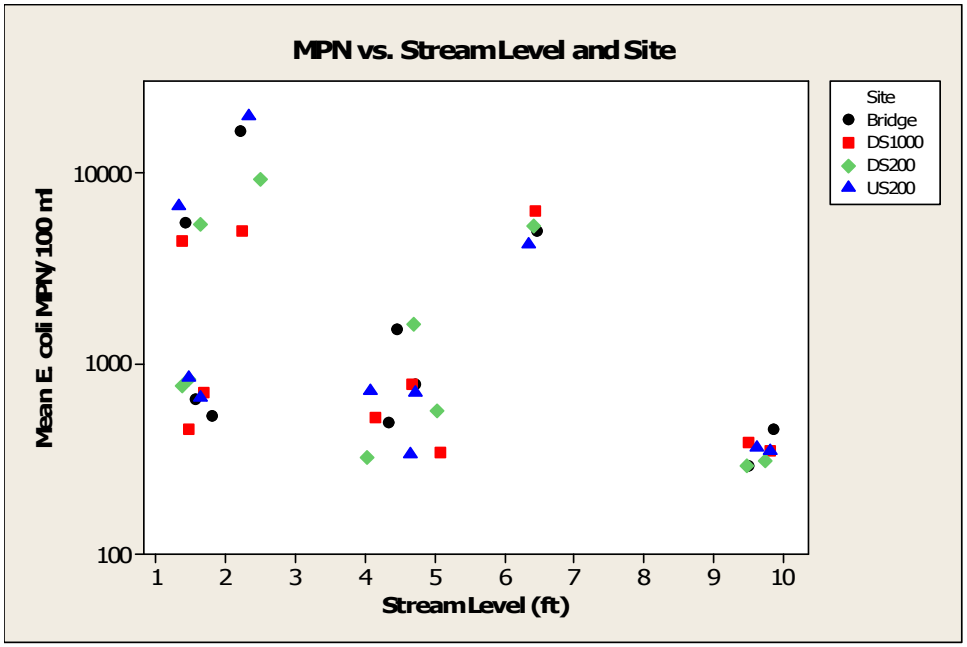

Figure 25. Relationship between measured stream level versus average *E. coli* levels (MPN).

A principal components analysis (PCA) was conducted to identify and evaluate possible linear combinations of original variables which produce a smaller number of factors that explain the majority of the variability in the data (Streiner1986). We identified two factors using PCA that explained the majority (57.8%) of the variation in the data (Table 6, Figs. 26-29). The first factor, which we describe "hydrology", primarily reflects the positive influence of water level and associated turbidity and declining levels of E.coli.(Table 6 and Fig. 26 and 29). The second factor, which we describe as "seasonality", primarily reflects the influence of water temperature and diel period and associated increasing levels of E. coli. The larger coefficients associated with the variables E. coli, water temperature and diel score suggest that this "seasonality" factor has a greater influence on bacteria levels. The site or location variable which was scaled according to relative distance in the stream (e.g. -200, 0, 200, 1000 ft) did not have a high coefficient value on either PCA axis. This suggests this variable did not contribute much to either PC equation and therefore did not explain much of the variation in the observed data. These scores and interpretation of factors agree with previous ANOVA and simple linear correlation analysis which documented a negative correlation between E. coli levels and stream flow and the higher levels observed during day and summer collections (Tables 5 and 6 and Figs. 9-10, 13-14). The location or site variable did not yield a high coefficient value, that is did not load highly on either PC 1 or PC 2, suggesting location (sites) contributed little to explaining variation in the data structure.

Table 5. Linear correlation analysis between average E. coli density, turbidity (NTU), water temperature (C), stream level (ft), cumulative 1 day rainfall (in), cumulative 3 day rainfall (in), NH3-N, dissolved oxygen (D.O.), and specific conductance.

	NTU	E. coli	Temp	Level	Day1prec	Day2prec	NH_3-N	D.O.
E. coli	0.103							
	0.575							
Temp	-0.212	0.056						
	0.243	0.730						
Level	0.765	-0.298	0.008					
	0.000	0.062	0.960					
Day1prec	0.086	0.783	-0.140	-0.449				
	0.640	0.000	0.389	0.004				
Day2prec	0.075	0.760	-0.148	-0.456	0.998			
	0.682	0.000	0.361	0.003	0.000			
NH_3-N	-0.113	0.239	0.479	0.199	-0.106	-0.113		
	0.539	0.138	0.002	0.218	0.513	0.486		
D.O.	0.002	-0.051	-0.606	-0.281	0.036	0.028	-0.544	
	0.990	0.756	0.000	0.080	0.828	0.864	0.000	
SpCond	-0.705	-0.022	0.199	-0.741	-0.058	-0.048	-0.056	0.378
	0.000	0.891	0.218	0.000	0.721	0.770	0.733	0.016

Cell Contents: Pearson correlation upper row, P-Value lower row.

Table 6. Results of principal component analysis of variables including water temperature (C) , turbidity (NTU), Average Log_e *E. coli* MPN/100 ml, stream level (ft), diel period (1 –day, 0 night), and location (upstream -200, bridge 0, downstream 200 ft (200) and downstream 1000 ft (1000).

Eigenanalysis of the Correlation Matrix

Eigenvalue	1.8450	1.6231	1.0363	0.8481	0.6142	0.0333
Proportion	0.307	0.271	0.173	0.141	0.102	0.006
Cumulative	0.307	0.578	0.751	0.892	0.994	1.000

Variable	PC1	PC2
C	-0.169	0.481
NTU	0.670	0.179
AvgLN	-0.198	0.624
level	0.683	0.021
Diel	0.128	0.565
Location	0.013	-0.162

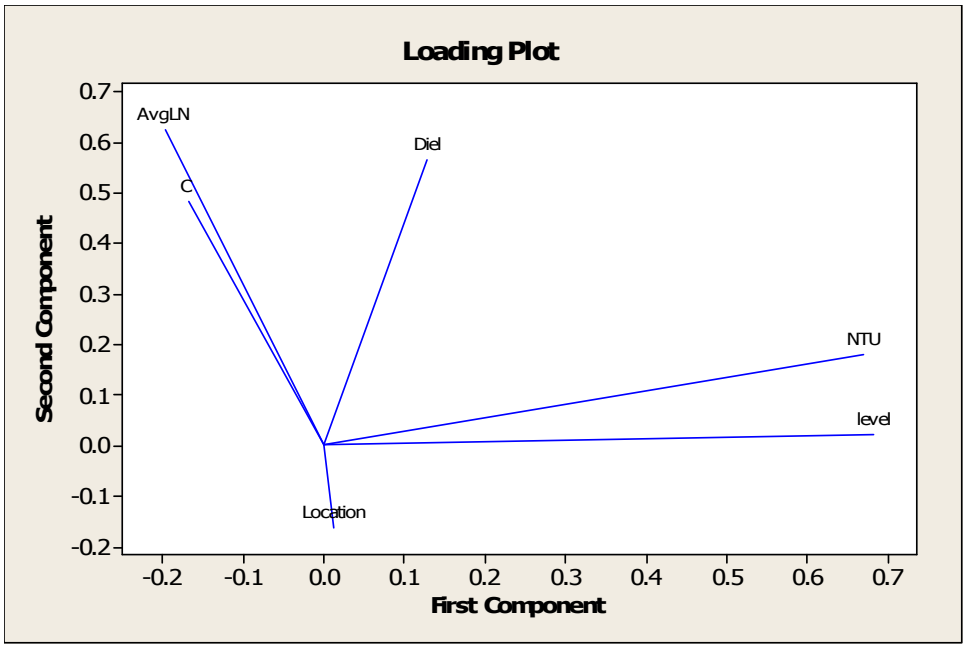

Figure 26. Principal components loading plot showing relationship of each original variable and principal components 1 and 2.

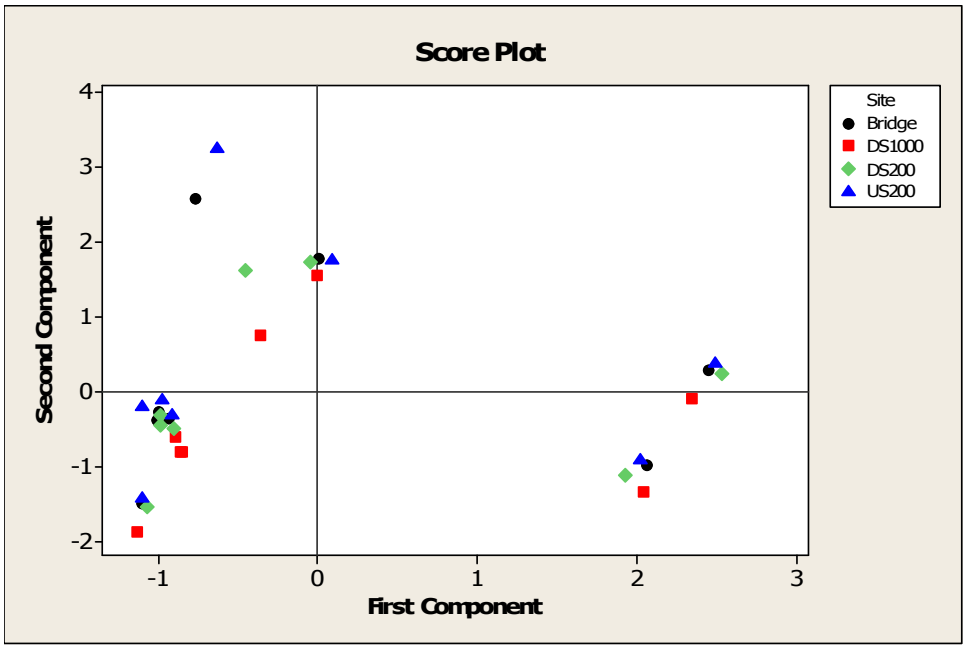

Figure 27. Principal components score plot showing relationship of collection scores and principal components 1 and 2. Legend highlights sites associated with collections.

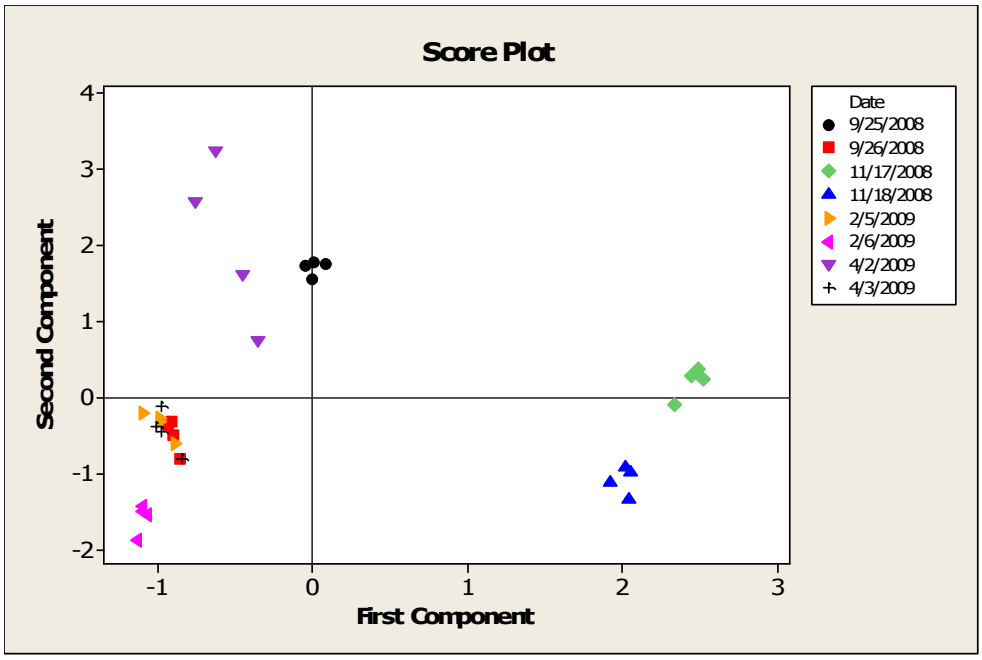

Figure 28. Principal components score plot showing relationship of collection scores and principal components 1 and 2. Legend highlights sample dates associated with collections.

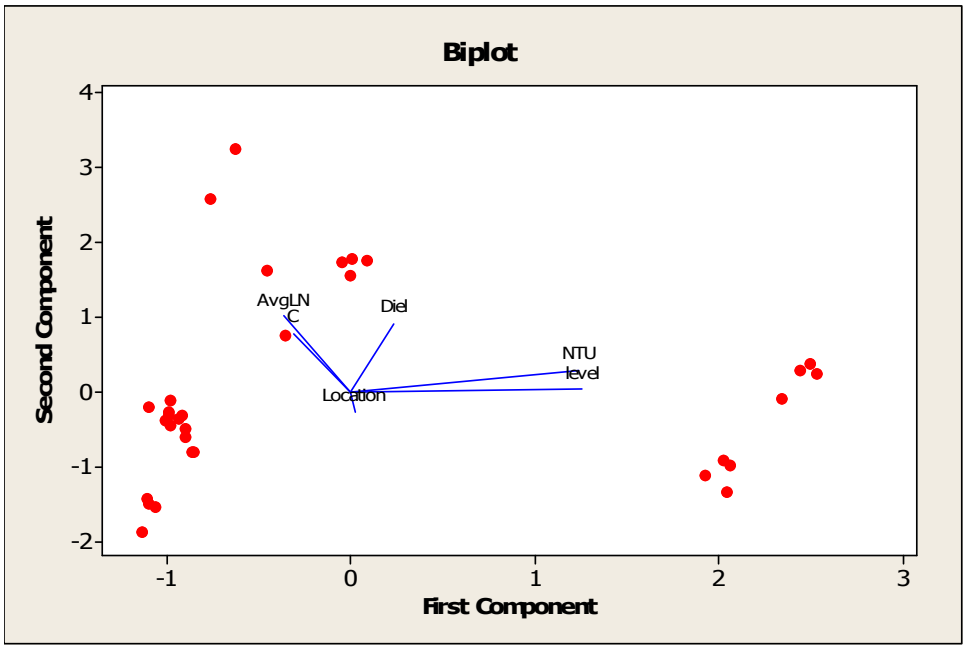

Figure 29. Principal components biplot showing relationship of collection scores, original variables and principal components 1 and 2.

PCA score plots illustrate the distribution of individual collections (date and sites) in relation to each PCA axis (Figs. 26-28). The November 2008 collections at all sites exhibited high PC1 scores in contrast to the other collections. This indicates that the November 2008 collections occurred during conditions when turbidity and water levels were high and water temperature and *E. coli* levels were low (Figure 28). Another grouping of collections included daytime September 2008 and April 2009 collections which exhibited low NTU and water levels and intermediate water temperatures and bacteria densities (Figs. 26-28). The third grouping of collections represents a mixture of collections made primarily at night and/or in February (Fig. 26-28).

E. coli Loading Estimates

Using data obtained in the laboratory from pellets collected during November 2009, and literature on fecal production rates we attempted to generate a crude estimate of the amount of fecal material produced and *E. coli* deposited by the Waugh Street bridge bat colony (Table 7)(Sgro and Wilkins 2003). Our estimates suggest that the colony produces between 524 and 628 million *E. coli* colony forming units per day. Factors such as incomplete deposition into the river, variable population size, and local rainfall which would wash stockpiled fecal pellets on the stream bank would affect these estimates. However, even after consideration of these factors the Waugh Street colony represents a significant *E. coli* source.

Table 7. Estimates of *E. coli* loading into Buffalo Bayou from Waugh Street bat colony based on *E. coli* counts taken from fecal material suspensions and literature fecal production rates (Sgro and Wilkins 2003).

ID number	mL of dilution water	Number of pellets	feces weight (g)	<=>	Extended count E. coli	1/2 extended count E. coli	<=>	E. coli per gram	Using 1/2 E. coli for < DL
1	80	2	0.0174	<	1.25	0.63	<	71.8	35.9
2	80	2	0.0132	=	3.75	3.75	=	284.1	284.1
3	80	2	0.0151	<	1.25	0.63	<	82.8	41.4
4	80	2	0.0127	<	1.25	0.63	<	98.4	49.2
5	50	10	0.0384	=	275.20	275.20	=	7,166.7	7,166.7
6	50	10	0.0475	>	4,839.20	4,839.20	>	101,877.9	101,877.9
7	50	10	0.0540	=	251.80	251.80	=	4,663.0	4,663.0
8	50	10	0.0402	<	2.00	1.00	<	49.8	24.9
9	50	18	0.0773	=	2.00	2.00	=	25.9	25.9
10	40	18	0.0863	>	6,049.00	6,049.00	>	70,092.7	70,092.7
11	40	18	0.0539	=	5.00	5.00	=	92.8	92.8
12	40	18	0.0851	>	6,049.00	6,049.00	>	71,081.1	71,081.1
13	100	Blank	0.0027	<	1.00	0.50	<	370.4	185.2
Average								21,298.9	21,286.3
Daily loading rate in grams (g) of feces based on 300,000 bats								29,500	29,500
Daily loading rate in grams (g) of feces based on 250,000 bats								24,600	24,600
Daily loading # E. coli MPN based on 300,000 bats								628,317,626	627,945,438
Daily loading # E. coli MPN based on 250,000 bats								523,953,003	523,642,636

Microbial Source Identification Results

Fluorescence in situ hybridization

We used DNA probes EUB338 specific for the domain Bacteria, EcoII specific for *E. coli*, and Enc specific for Enterococcus spp. bacteria to determine the average number of these bacteria per bat guano pellet (Table 8). The E. coli values correlate well with values independently derived by Dr. Guillen's laboratory.

Table 8. Average number and standard deviation of all bacteria, *E. coli*, and Enterococcus spp. bacteria per pellet of bat guano.

	All Bacteria	E. coli	Enterococcus spp.
Probe	EUB338	EcoII	Enc
pellet1	126.98413	49.60317	17.85714286
pellet2	18668.651	642.8571	20240.07937
pellet3	15716.27	240.0794	31.74603175
pellet4	961142.86	17.85714	7.936507937
pellet5	358382.94	71.42857	32001.98413
Average	270807.54	204.3651	10459.92063
Std. Dev.	414169	49.603	17.85

HFERP DNA Fingerprinting

E. coli isolates from bat guano and from water samples collected directly under the Waugh Avenue Bridge, 100 or 200 feet, and 1000 feet downstream were analyzed for similarity using four different cluster analyses. Examination of guano isolate HFERP fingerprints revealed a statistically relevant seasonal difference (Appendix 1: Fig. 1). Therefore, we analyzed guano and water samples separately for spring and fall groupings (August 2008 and November 2009). For spring, samples were collected in April 2009. A combination of four different cluster similarity analyses and correlation algorithms were tested yielding three approaches (Table 9; Appendix 1 Figs 2-4). The Dice Coefficient and Jaccard Index plus UPGMA similarity cluster produced identical dendrograms, however these correlation algorithms are closely related. The Dice/Neighbor Joining dendrograms are produced in a stepwise manner and therefore are not as statistically reliable as Dice or Jaccard or Pearson algorithms paired with UPGMA. Again, we used similar combinations of dendrogram construction for the "fall" samples collected on 8/14/08 (water only) and 11/17/09 (guano and water) (Table 10; Appendix 1: Figs. 5-7). Our recommendation is to use the Dice or Jaccard/UPGMA determinations of bat guano contribution to the *E. coli* counts observed downstream in Buffalo Bayou. A dendrogram with both spring and fall isolates is included (Appendix 1: Fig. 8).

Table 9. HFERP DNA fingerprint similarity coefficient and cluster analyses for 4/2/2009 for *E. coli* isolated from bat guano, and ambient water from the Waugh St. Bridge, 200 and 1000 feet downstream of the Bridge. Dendrograms for Dice Coefficient and Jaccard Index/UPGMA were identical.

Dendrogram	Sampling Location	'Guano-like' E. coli Fingerprint	Total E. coli Fingerprints	Percent 'Guano-like' Fingerprints
Dice Coefficient/ Neighbor Joining Cluster N=205	Waugh St. Bridge	42	45	93.3
	200 ft downstream	31	37	83.7
	1000 ft downstream	24	42	57.1
Dice Coefficient-Jaccard Index/ UPGMA Cluster N=205	Waugh St. Bridge	31	45	71.1
	200 ft downstream	23	37	62.2
	1000 ft downstream	5	42	11.9
Pearson Correlation Coefficient / UPGMA Cluster N=205	Waugh St. Bridge	21	45	46.6
	200 ft downstream	2	37	5.4
	1000 ft downstream	17	42	40.4

Table 10. HFERP DNA fingerprint similarity coefficient and cluster analyses for 8/14/08 and 11/17/09 for *E. coli* isolated from bat guano, and ambient water from the Waugh St. Bridge, 100 and 1000 feet downstream of the bridge. Dendrograms for Dice Coefficient and Jaccard Index/UPGMA were identical.

Dendrogram	Sampling Location	'Guano-like' E. coli Fingerprint	Total E. coli Fingerprints	Percent 'Guano-like' Fingerprints
Dice Coefficient/ Neighbor Joining Cluster N=187 G=65+9	Waugh Av. Bridge	6	20	30
	100 & 200 ft downstream	16	35	45.7
	1000 ft downstream	33	38	86.8
Dice Coefficient-Jaccard Index/ UPGMA Cluster N=187	Waugh Av. Bridge	10	20	50
	100 &200 ft downstream	14	35	40
	1000 ft downstream	7	38	8.4
Pearson Correlation Coefficient / UPGMA Cluster N=187	Waugh Av. Bridge	3	20	15
	100 & 200 ft downstream	10	35	28.5
	1000 ft downstream	20	38	52.6

Biotype Analysis of Sugar Utilization

E. coli isolates from bat guano and from samples collected directly under the Waugh Avenue Bridge, 100 and 200 feet upstream as well as downstream, and 1000 feet downstream were analyzed for similarity the Dice Coefficient/UPGMA analysis that is recommended for binary data (i.e. 1, 0) (Appendix 1:Fig. 9). Several clusters of E. coli isolated from upstream samples (100 and 200 ft) and at TCEQ water quality station 11351 (Buffalo Bayou at Shepherd Dr.) had similar sugar utilization profiles to the guano isolates that were collected downstream under the Waugh Avenue Bridge. Based upon these results, we used only the sugar utilization profiles from *E. coli* isolated from samples directly under the Waugh Avenue Bridge and at downstream stations for the percent 'guano-like' calculations in Table 11. The sugar utilization data analyses were completed prior to 11/17/09 so these November 2009 samples were not included. Between 40 and 67% *of E. coli* isolated from Buffalo Bayou water samples collected downstream of the bridge clustered with isolates from the guano.

Table 11. Sugar Utilization similarity coefficient and cluster analyses for 2008-2009 *E. coli* isolated from bat guano, the Waugh St. Bridge, 100 and 1000 feet downstream of the bridge. The Dice Coefficient/UPGMA cluster analysis was used to determine isolates similar to guano.

Dendrogram	Sampling Location	'Guano-like' *E. coli* Sugar Utilization	Total E. coli Isolates per	Percent 'Guano-like'
Dice Correlation Coefficient / UPGMA Cluster	Waugh St. Bridge	99	146	67
	100 ft downstream	22	46	47
	1000 ft downstream	22	55	40

Conclusions and Recommendations

Several lines of evidence suggest that the Waugh Street bat colony is a significant source of *E. coli* bacteria in Buffalo Bayou. Historical data obtained from the HGAC CRP and TCEQ water quality monitoring programs document long term trends in elevated *E. coli* levels downstream of the Shepard Street site. In addition, *E. coli* levels appear to increase downstream at the Sabine Street site. The Waugh Street bat colony is located in between these locations and represents the only visible large congregation of mammals and source of *E. coli* that is found between these two monitoring sites.

During our study *E. coli* levels were often similar between all sites both upstream and downstream of the Waugh Street bat colony. This would suggest that the colony is not having a strong influence on overall densities of indicator bacteria.

These data agree with past studies in Texas that have not shown any increase in indicator bacteria levels downstream of large bat colonies such as the Commerce Street Bridge in Austin (Pers comm. Herrington, Chris Herrington City of Austin chris.herrington@ci.austin.tx.us). However, it should be noted that our experimental design did not include sites as far downstream as the Sabine Street site which is located 8000 feet downstream of the bat colony in contrast to our farthest site which was only 1000 feet downstream. It is highly likely that *E. coli* deposited in fecal pellets from the bat colony might not have had sufficient time to reach increased densities historically observed at the Sabine Street site. Another explanation may be the contribution of *E. coli* from other sources including upstream bat colonies. In a related study Dr. Brinkmeyer's research team observed other congregations of bats located upstream and downstream of the Waugh Street bridge colony. Depending on their densities these colonies may represent a significant source of indicator bacteria.

A secondary source of evidence suggesting *E. coli* contributions by the bat colony is the diel and seasonal patterns in bacteria densities in ambient water observed during our study. Lowest levels in *E. coli* levels were usually seen in the early morning (night samples) while bats were out foraging. This agrees with what is known about the activity pattern of Mexican free-tailed bat (Schmidly 2004; Sgro and Wilkins. 2003). Furthermore, these diel differences were smaller or absent during the winter months when bats are in a state of torpor or have emigrated to warmer southern locations. However, this seasonal and diel pattern also manifested at the site located upstream of the bat colony suggesting another potential source. As previously mentioned other congregations of bats were located upstream of the Waugh Street bridge colony. Depending on their densities this may represent a significant source of indicator bacteria. Assuming they are also Mexican free-tailed bats, we would expect them to follow similar diel and seasonal patterns in abundance.

A third line of evidence supporting the hypothesis that the Waugh Street bat colony is a significant source of indicator bacteria is our loading projection based on a mass balance equation which utilized crude estimates of the colony population size, literature based per capita fecal production rates and estimates of *E. coli* densities per gram of feces and per pellet to generate overall estimates of fecal loading and subsequently indicator bacteria loading rates. Using these values we estimated that the colony likely produces between 524 and 628 million *E. coli* colony forming units per day. Factors such as incomplete fecal deposition into the river, inaccurate population size estimates, and local rainfall events could drastically affect these estimates. However, even after consideration of all of these factors, the Waugh Street colony represents a significant *E. coli* source.

Evidence suggesting the Waugh Street Bridge and/or Mexican free-tailed bats are a significant source of *E. coli* includes the results of our genetic analyses and related tests. The HFERP analyses determined that 15 to >50% of *E. coli* isolates at the Waugh Avenue Bridge and at downstream sampling locations had DNA

fingerprints that clustered with the those from the bat guano indicating a strong likelihood of bats as a source of *E. coli* in Buffalo Bayou. While less conclusive, the sugar utilization data produced similar trends. While working on a parallel project in 2008-2009 for the TCEQ, Dr. Brinkmeyer's research team found high numbers of bats living in bridges upstream from the Waugh Avenue Bridge bat colony that could explain the similarity of upstream sugar utilization to that of the Waugh Avenue Bridge *E. coli* isolates. It also appears that sugar utilization is a less distinctive method for determination of source for the *E. coli* isolates than the HFERP fingerprinting, however the overlap of trends observed by the two methods indicates that the bat colony guano is a source of *E. coli*, at least to 1000 ft below the Waugh Avenue Bridge. Based upon these results and our observations of the additional large bat 'nestings' in bridges upstream of Waugh Avenue (in particular South Mason Road) and downtown at the intersections of Smith and Franklin streets that create a large bridge structure and the presence of highly visible layers of guano, bats are definitely contributing to the *E. coli* loads in Buffalo Bayou. Additionally, since 2009, we have observed bats at the Waugh Avenue Bridge as well as downtown at the Smith-Franklin Street Bridge complex year round. The absolute densities and activity of these overwintering bats would determine their overall contribution to *E. coli* loads in Buffalo Bayou. These large bat populations represent a significant but poorly understood source of nutrients and *E. coli* bacteria in our urban watersheds. Additional research is critically needed to characterize the interaction of bat population dynamics, seasonal and diel movement, fecal and associated nutrient and bacteria loading, and resulting instream indicator bacteria concentrations.

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Literature Cited

Amann, R. I., W. Ludwig, K.-H. Schleifer. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. 59:143-469.

CRWQCB (State of California, California Regional Water Quality Control Board, Central Coast Region). 2004. Adoption of the San Luis Obispo Creek Total Maximum Daily Load (TMDL) for pathogens as a basin plan amendment.http://www.swrcb.ca.gov/rwqcb3/Board/Agendas/120304/Item Reports/Item28/Index.htm

Davis, R.B., C.F. Herreid and H.L. Short. 1962. Mexican Free-Tailed Bats in Texas. Ecological Monographs. 32(4): 311-346.

Gordon, D. M. and Cowling, A. 2003. The distribution and genetic structure of *Escherichia coli* in Australian vertebrates: host and geographic effects. Microbiol. 149:3575-3586.

Hassan, W. M., Ellender, R. D., and Wang, S. Y. 2006. Fidelity of bacterial source tracking: *Escherichia coli* vs. *Enterococcus* spp. and minimizing assignment of isolates from nonlibrary sources. J. Appl. Microbiol. 102:591-598.

He, Y. keen, J. E., Westerman, R. B., Littledike, E. T., and Kwang, J. 1996. Monoclonal antibodies for detection of the H7 antigen of *Escherichia coli*. Appl. Environ. Microbiol. 62:3325-3332.

HGAC (Houston Galveston Area Council) 2010. Water Resources Information Map (WRIM). http://www.h-gac.com/rds/water_quality/default.aspx

Johnson, L. K., Brown, M. B., Carruthers, E. A., Ferguson J. A., Dombek, P. E., and Sadowsky, M. 2004. Sample size, library composition, and gentypic diversity among natural populations of *Escherichia coli* from different animals influence accuracy of determining sources of fecal pollution. Appl. Environ. Microbiol. 70:4478-4485.

Keeley, B. and M. Tuttle. 1999. Bats in American Bridges. Resource Publication No. 4. Bat Conservation International, Inc.

Keeley B. and M. Tuttle. 2000. The Texas Bats and Bridges Project. Bat Conservation International and Texas Department of Transportation. http://www.batcon.org/bridge/babtop.html

Kunz, T.H. 1990. Ecological and behavioral methods for the study of bats. Smithsonian Institution Press. Washington, D.C.

Petersen, T.M., H.S. Rifai, M.P. Suarez and A.R. Stein. 2005. Bacteria loads from point and nonpoint sources in an urban watershed. Journal of Environmental Engineering 31(10):1414-1425.

Rifai, H. 2006. Total Maximum Daily Loads for fecal pathogens in Buffalo Bayou and White Oak Bayou. Final Report. Texas Commission on Environmental Quality. 100 pp.

Schmidly, D.J. 1991. The bats of Texas. Texas A&M University Press. College Station, Texas.

Schmidly, D.J. 2004. The mammals of Texas. University of Texas Press. Austin, Texas.

Sgro, M. and K. Wilkins. 2003. Roosting behavior of the Mexican free-tailed bat (Tadarida brasiliensis) in a highway overpass. Western North American Naturalist 63(3): 366-373.

Souza, V., Rocha, M., Valera, A., and Eguiarte, L. E. 1999. Genetic structure of natural populations of *Escherichia coli* in wild hosts on different continents. Appl. Environ. Microbiol. 65:3373-3385.

Stoeckel, D. M. and Harwood, V. J. 2007. Performance, Design, and Analysis in Microbial source Tracking Studies: Minireview. Appl. Environ. Microbiol. 73:2405-2415.

Streiner, N. 1986). PDQ Statistics. B.C. Decker, Inc. Philadelphia, PA.

TSL (Texas State Legislature). 2000. Texas Administrative Code. Title 30 Environmental Quality. Part 1. Texas Commission on Environmental Quality Chapter 307 Texas Surface Water Quality Standards

TCEQ (Texas Commission on Environmental Quality). 2007. DRAFT 2006 Texas 303(d) List (June 27, 2007). http://www.tceq.state.tx.us/assets/public/compliance/monops/water/06twqi/2006_303d.pdf. Austin, Texas.

TCEQ. (Texas Commission on Environmental Quality). 2008a. Eighteen Total Maximum Daily Loads for Bacteria in Buffalo and White Oak Bayous and Tributaries Segments 1013, 1013A, 1013C, 1014, 1014A, 1014B, 1014E, 1014H, 1014K, 1014L, 1014M, 1014N, 1014O, 1017, 1017A, 1017B, 1017D, and 1017E. For Public Comment: June 2008. Austin, Texas.

TCEQ. (Texas Commission on Environmental Quality). 2008b. Surface Water Quality Monitoring Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue, (RG-415)

TCEQ (Texas Commission on Environmental Quality). 2010. Surface Water Quality Monitoring Web Reporting. http://www8.tceq.state.tx.us/SwqmisWeb/public/index.faces

Tuttle, M.D. 1994. The lives of Mexican free-tailed bats. BATS 12(3):6-14.

Tuttle, M.D. 2003. Texas Bats. Bat Conservation International. Austin, Texas.

USGS. 2010. Stream gage data. Daily mean values for 08074000. http://waterdata.usgs.gov/tx/nwis/dvstat/?format=sites_selection_links&search_site_no=08074000&agency_cd=USGS&referred_module=sw

Appendix 1. Multivariate analysis of genetic data.

Figure 1. HFERP DNA Fingerprint Spring vs. Fall Bat Guano Only
Pearson Coefficient Algorithm/UPGMA Clustering

Fall


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4209 bats guano 3a
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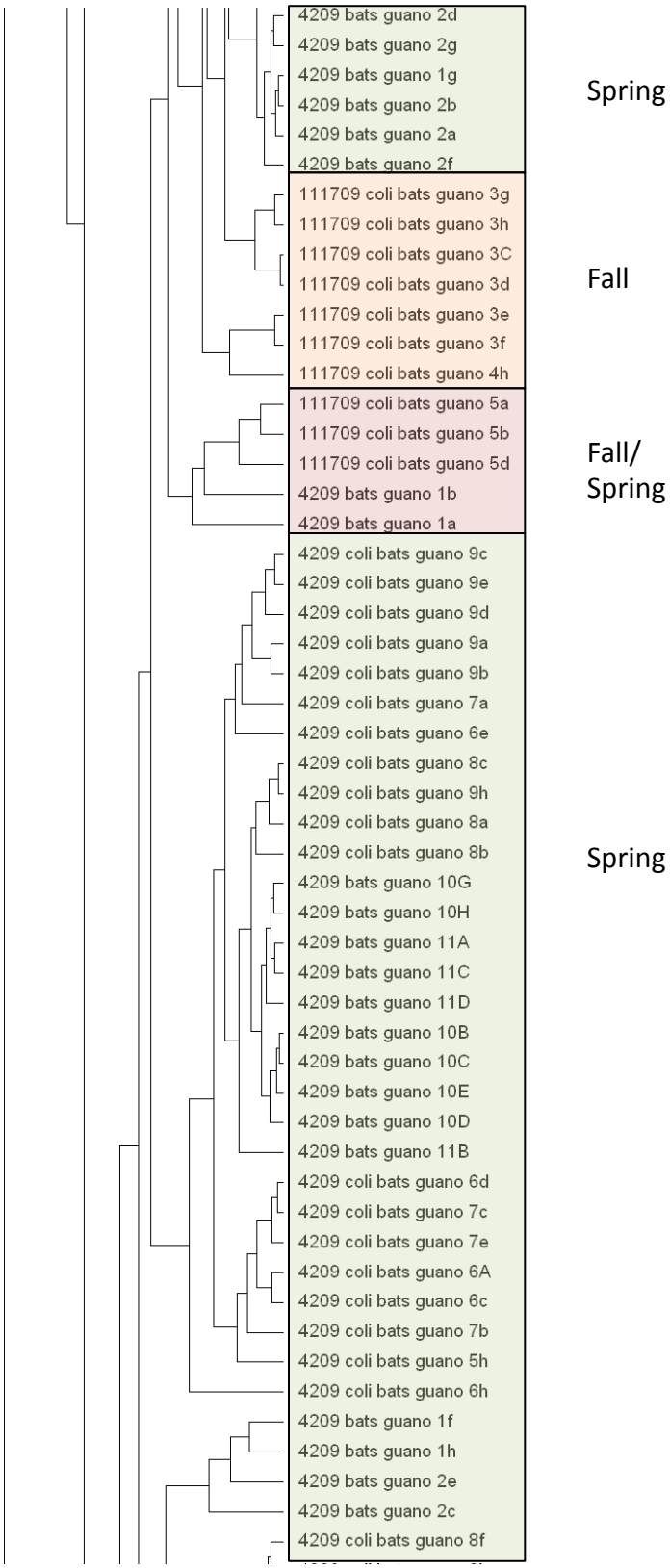
Fall
Spring
Fall
Spring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Spring
Fall
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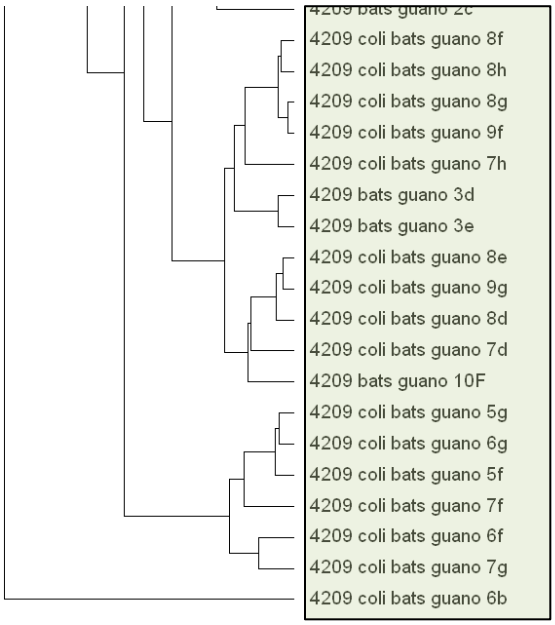
Spring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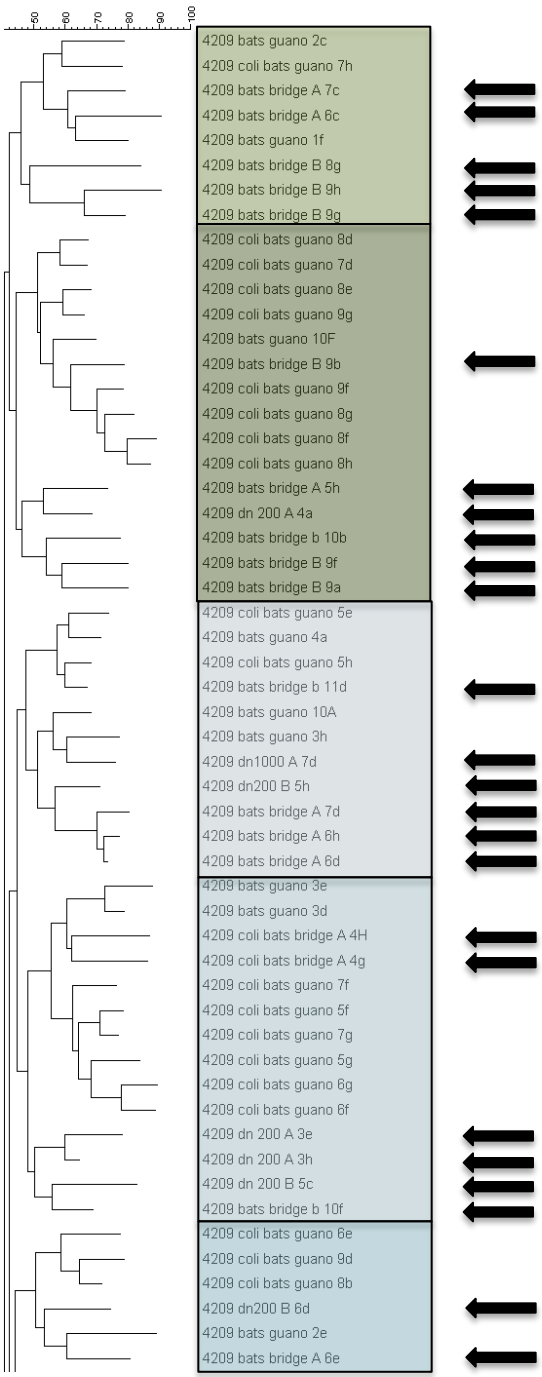
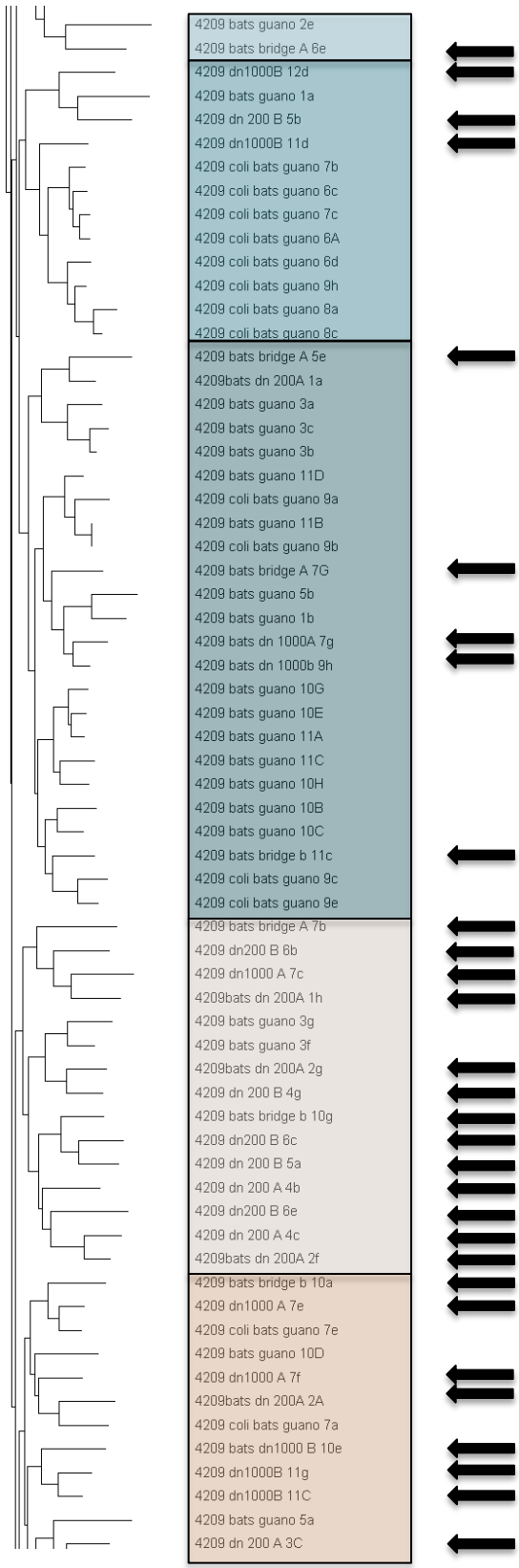
Spring

Figure 2. HFERP DNA Fingerprint April 2009
Dice Coefficient Algorithm/Neighbor Joining Clustering

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4209 coli bats guano 8c
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4209 bats guano 11B
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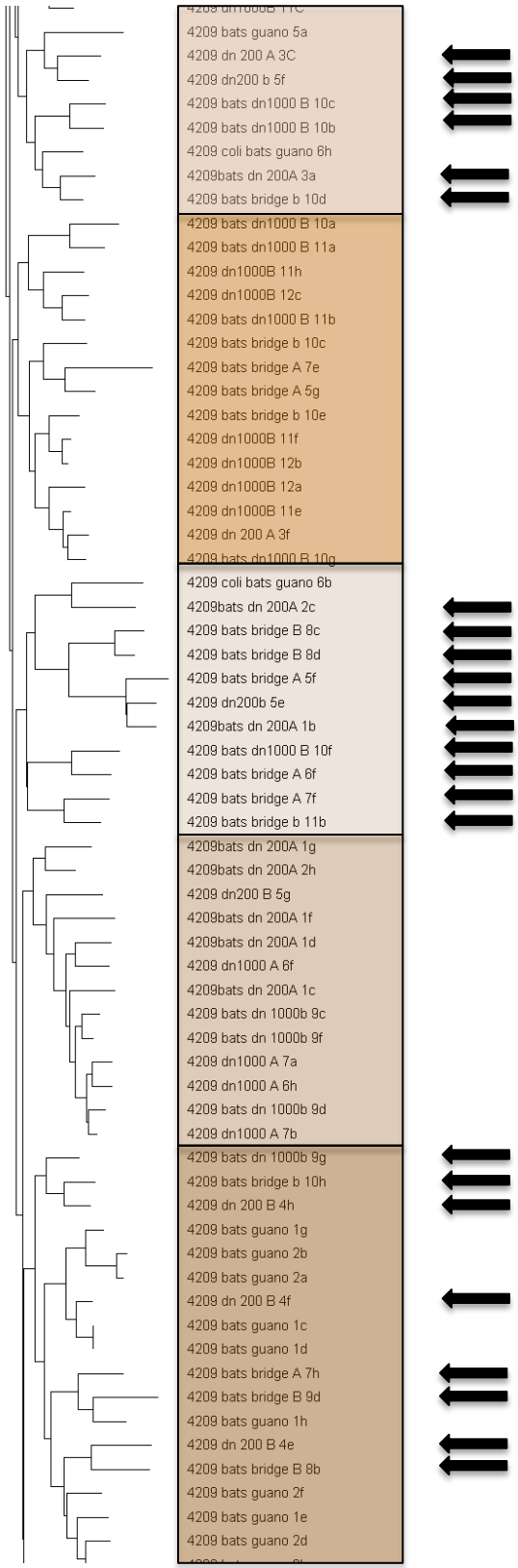
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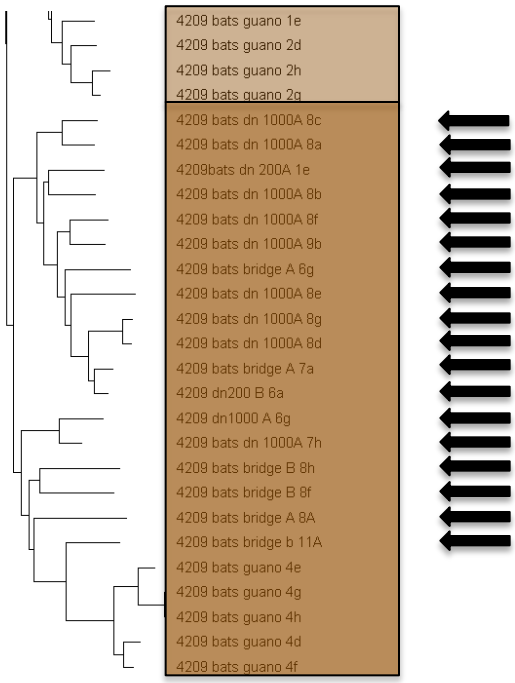
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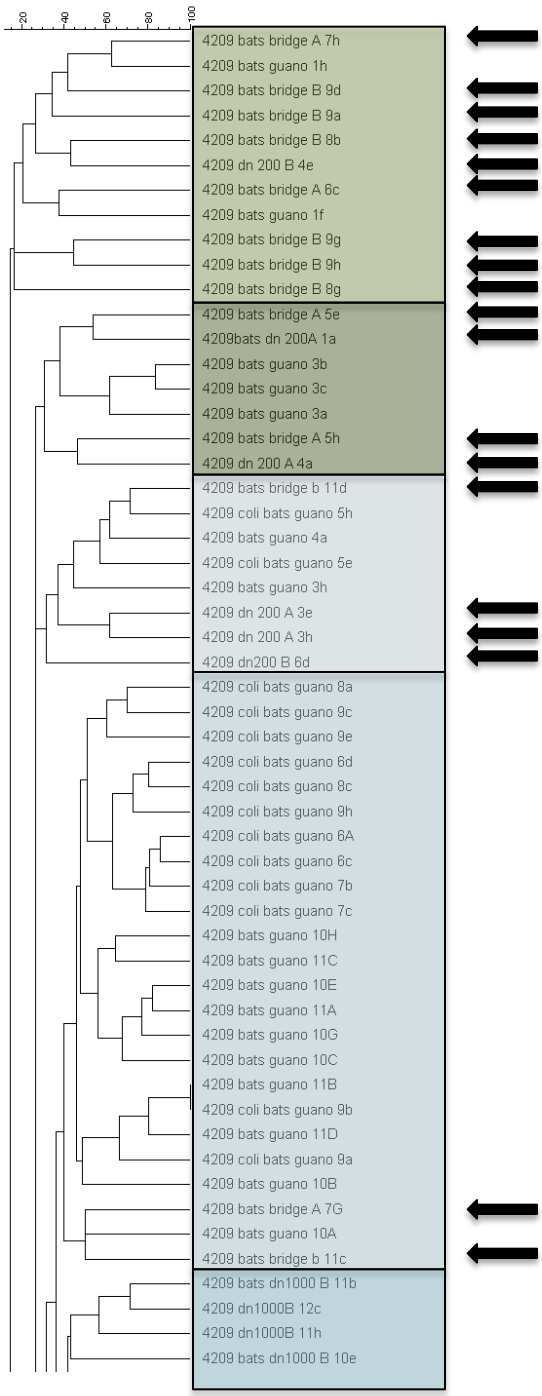
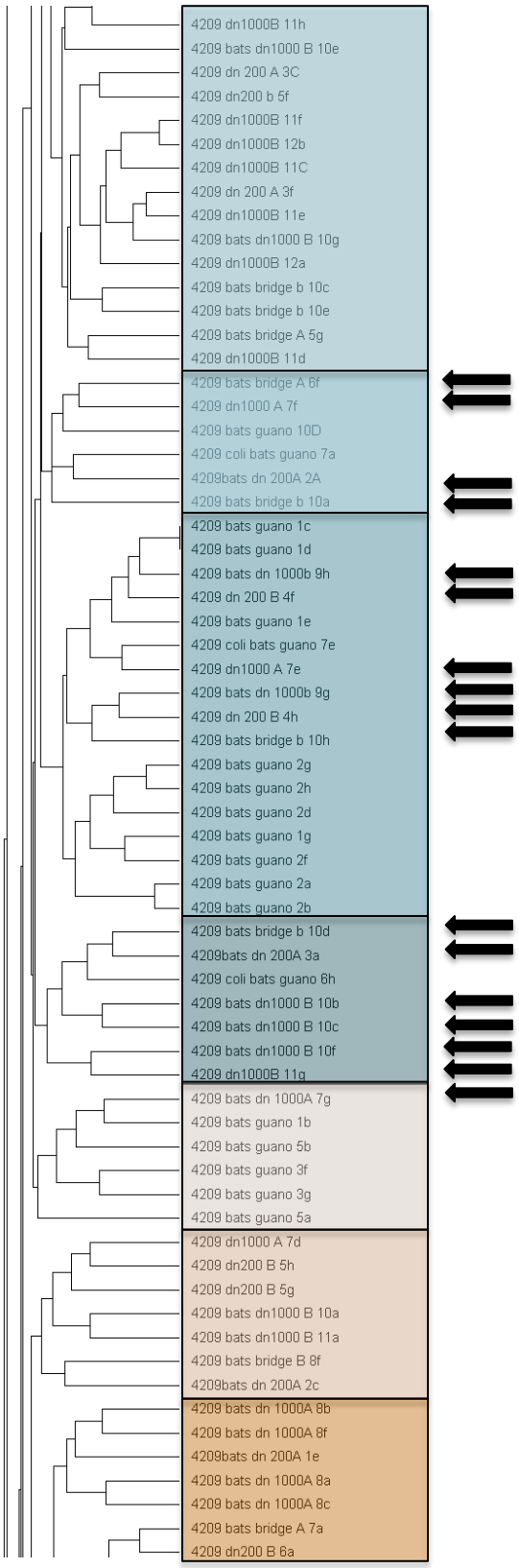
Figure 3. HFERP DNA Fingerprint April 2009
Jaccard Index Algorithm/UPGMA Clustering

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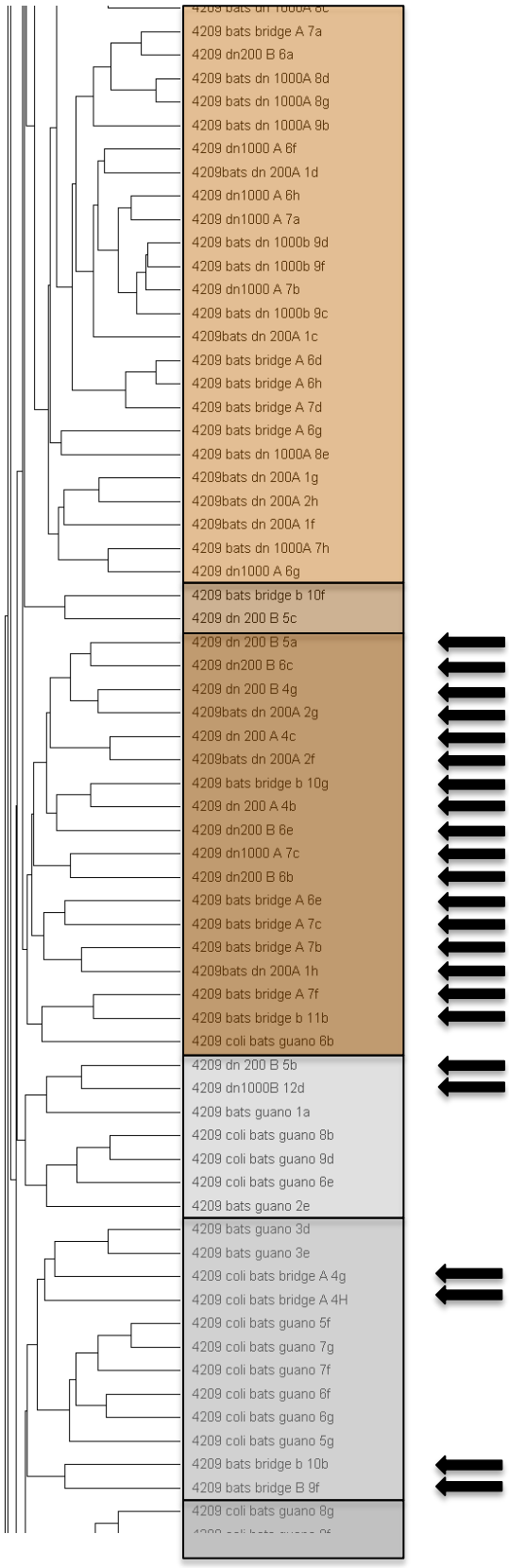
4209 dn 200 A 3f
4209 dn1000B 11e
4209 bats dn1000 B 10g
4209 dn1000B 12a
4209 bats bridge b 10c
4209 bats bridge b 10e
4209 bats bridge A 5g
4209 dn1000B 11d
4209 bats bridge A 6f
4209 dn1000 A 7f
4209 bats guano 10D
4209 coli bats guano 7a
4209bats dn 200A 2A
4209 bats bridge b 10a
4209 bats guano 1c
4209 bats guano 1d
4209 bats dn 1000b 9h
4209 dn 200 B 4f
4209 bats guano 1e
4209 coli bats guano 7e
4209 dn1000 A 7e
4209 bats dn 1000b 9g
4209 dn 200 B 4h
4209 bats bridge b 10h
4209 bats guano 2g
4209 bats guano 2h
4209 bats guano 2d
4209 bats guano 1g
4209 bats guano 2f
4209 bats guano 2a
4209 bats guano 2b
4209 bats bridge b 10d
4209bats dn 200A 3a
4209 coli bats guano 6h
4209 bats dn1000 B 10b
4209 bats dn1000 B 10c
4209 bats dn1000 B 10f
4209 dn1000B 11g
4209 bats dn 1000A 7g
4209 bats guano 1b
4209 bats guano 5b
4209 bats guano 3f
4209 bats guano 3g
4209 bats guano 5a
4209 dn1000 A 7d
4209 dn200 B 5h
4209 dn200 B 5g
4209 bats dn1000 B 10a
4209 bats dn1000 B 11a
4209 bats bridge B 8f
4209bats dn 200A 2c
4209 bats dn 1000A 8b
4209 bats dn 1000A 8f
4209bats dn 200A 1e
4209 bats dn 1000A 8a
4209 bats dn 1000A 8c
4209 bats bridge A 7a
4209 dn200 B 6a


4209 bats dn 1000A 8c
4209 bats bridge A 7a
4209 dn200 B 6a
4209 bats dn 1000A 8d
4209 bats dn 1000A 8g
4209 bats dn 1000A 9b
4209 dn1000 A 6f
4209bats dn 200A 1d
4209 dn1000 A 6h
4209 dn1000 A 7a
4209 bats dn 1000b 9d
4209 bats dn 1000b 9f
4209 dn1000 A 7b
4209 bats dn 1000b 9c
4209bats dn 200A 1c
4209 bats bridge A 6d
4209 bats bridge A 6h
4209 bats bridge A 7d
4209 bats bridge A 6g
4209 bats dn 1000A 8e
4209bats dn 200A 1g
4209bats dn 200A 2h
4209bats dn 200A 1f
4209 bats dn 1000A 7h
4209 dn1000 A 6g
4209 bats bridge b 10f
4209 dn 200 B 5c
4209 dn 200 B 5a
4209 dn200 B 6c
4209 dn 200 B 4g
4209bats dn 200A 2g
4209 dn 200 A 4c
4209bats dn 200A 2f
4209 bats bridge b 10g
4209 dn 200 A 4b
4209 dn200 B 6e
4209 dn1000 A 7c
4209 dn200 B 6b
4209 bats bridge A 6e
4209 bats bridge A 7c
4209 bats bridge A 7b
4209bats dn 200A 1h
4209 bats bridge A 7f
4209 bats bridge b 11b
4209 coli bats guano 6b
4209 dn 200 B 5b
4209 dn1000B 12d
4209 bats guano 1a
4209 coli bats guano 8b
4209 coli bats guano 9d
4209 coli bats guano 6e
4209 bats guano 2e
4209 bats guano 3d
4209 bats guano 3e
4209 coli bats bridge A 4g
4209 coli bats bridge A 4H
4209 coli bats guano 5f
4209 coli bats guano 7g
4209 coli bats guano 7f
4209 coli bats guano 6f
4209 coli bats guano 6g
4209 coli bats guano 5g
4209 bats bridge b 10b
4209 bats bridge B 9f
4209 coli bats guano 8g

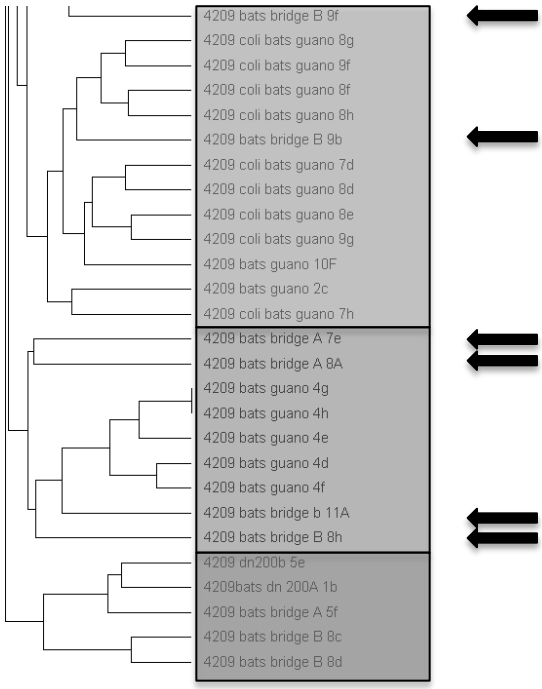
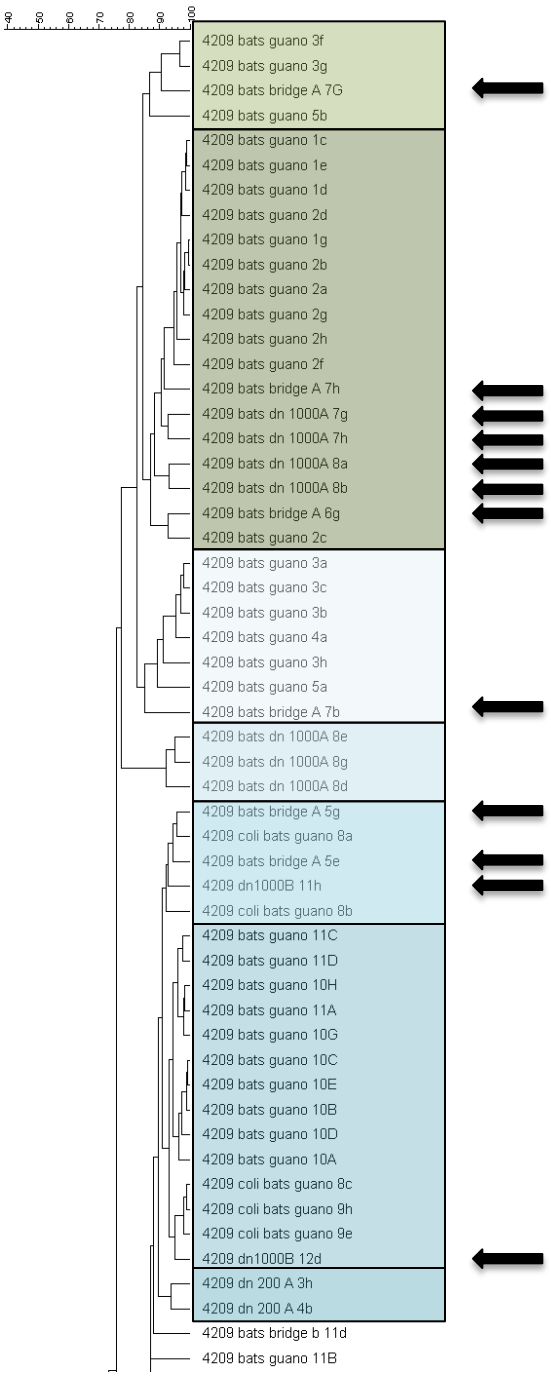
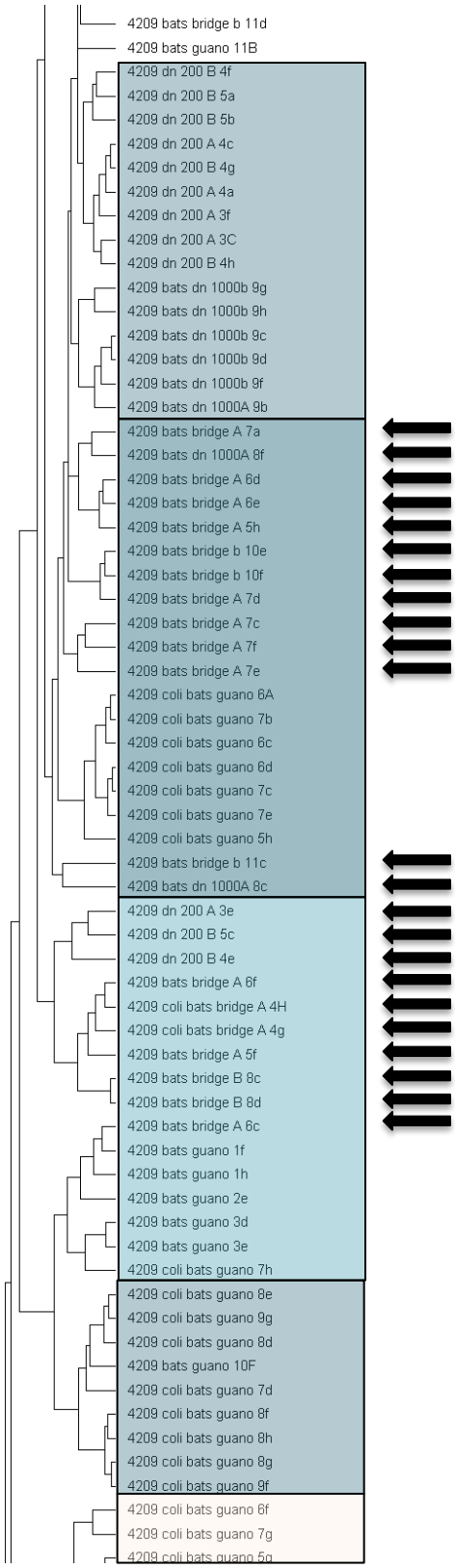
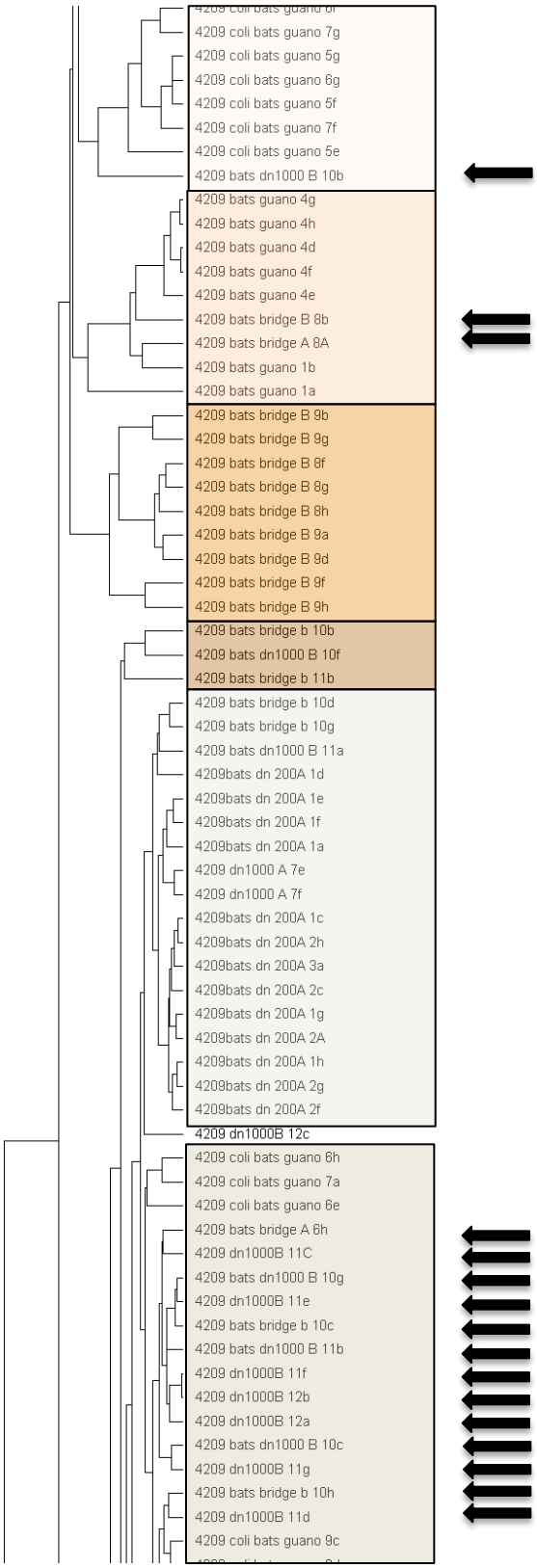

4209 bats bridge B 9f
4209 coli bats guano 8g
4209 coli bats guano 9f
4209 coli bats guano 8f
4209 coli bats guano 8h
4209 bats bridge B 9b
4209 coli bats guano 7d
4209 coli bats guano 8d
4209 coli bats guano 8e
4209 coli bats guano 9g
4209 bats guano 10F
4209 bats guano 2c
4209 coli bats guano 7h
4209 bats bridge A 7e
4209 bats bridge A 8A
4209 bats guano 4g
4209 bats guano 4h
4209 bats guano 4e
4209 bats guano 4d
4209 bats guano 4f
4209 bats bridge b 11A
4209 bats bridge B 8h
4209 dn200b 5e
4209bats dn 200A 1b
4209 bats bridge A 5f
4209 bats bridge B 8c
4209 bats bridge B 8d

Figure 4. HFERP DNA Fingerprint April 2009
Pearson Coefficient Algorithm/UPGMA Clustering




4209 bats bridge b 11d
4209 bats guano 11B
4209 dn 200 B 4f
4209 dn 200 B 5a
4209 dn 200 B 5b
4209 dn 200 A 4c
4209 dn 200 B 4g
4209 dn 200 A 4a
4209 dn 200 A 3f
4209 dn 200 A 3C
4209 dn 200 B 4h
4209 bats dn 1000b 9g
4209 bats dn 1000b 9h
4209 bats dn 1000b 9c
4209 bats dn 1000b 9d
4209 bats dn 1000b 9f
4209 bats dn 1000A 9b
4209 bats bridge A 7a
4209 bats dn 1000A 8f
4209 bats bridge A 6d
4209 bats bridge A 6e
4209 bats bridge A 5h
4209 bats bridge b 10e
4209 bats bridge b 10f
4209 bats bridge A 7d
4209 bats bridge A 7c
4209 bats bridge A 7f
4209 bats bridge A 7e
4209 coli bats guano 6A
4209 coli bats guano 7b
4209 coli bats guano 6c
4209 coli bats guano 6d
4209 coli bats guano 7c
4209 coli bats guano 7e
4209 coli bats guano 5h
4209 bats bridge b 11c
4209 bats dn 1000A 8c
4209 dn 200 A 3e
4209 dn 200 B 5c
4209 dn 200 B 4e
4209 bats bridge A 6f
4209 coli bats bridge A 4H
4209 coli bats bridge A 4g
4209 bats bridge A 5f
4209 bats bridge B 8c
4209 bats bridge B 8d
4209 bats bridge A 6c
4209 bats guano 1f
4209 bats guano 1h
4209 bats guano 2e
4209 bats guano 3d
4209 bats guano 3e
4209 coli bats guano 7h
4209 coli bats guano 8e
4209 coli bats guano 9g
4209 coli bats guano 8d
4209 bats guano 10F
4209 coli bats guano 7d
4209 coli bats guano 8f
4209 coli bats guano 8h
4209 coli bats guano 8g
4209 coli bats guano 9f
4209 coli bats guano 6f
4209 coli bats guano 7g
4209 coli bats guano 5g


4209 coli bats guano 7g
4209 coli bats guano 5g
4209 coli bats guano 6g
4209 coli bats guano 5f
4209 coli bats guano 7f
4209 coli bats guano 5e
4209 bats dn1000 B 10b
4209 bats guano 4g
4209 bats guano 4h
4209 bats guano 4d
4209 bats guano 4f
4209 bats guano 4e
4209 bats bridge B 8b
4209 bats bridge A 8A
4209 bats guano 1b
4209 bats guano 1a
4209 bats bridge B 9b
4209 bats bridge B 9g
4209 bats bridge B 8f
4209 bats bridge B 8g
4209 bats bridge B 8h
4209 bats bridge B 9a
4209 bats bridge B 9d
4209 bats bridge B 9f
4209 bats bridge B 9h
4209 bats bridge b 10b
4209 bats dn1000 B 10f
4209 bats bridge b 11b
4209 bats bridge b 10d
4209 bats bridge b 10g
4209 bats dn1000 B 11a
4209bats dn 200A 1d
4209bats dn 200A 1e
4209bats dn 200A 1f
4209bats dn 200A 1a
4209 dn1000 A 7e
4209 dn1000 A 7f
4209bats dn 200A 1c
4209bats dn 200A 2h
4209bats dn 200A 3a
4209bats dn 200A 2c
4209bats dn 200A 1g
4209bats dn 200A 2A
4209bats dn 200A 1h
4209bats dn 200A 2g
4209bats dn 200A 2f
4209 dn1000B 12c
4209 coli bats guano 6h
4209 coli bats guano 7a
4209 coli bats guano 6e
4209 bats bridge A 6h
4209 dn1000B 11C
4209 bats dn1000 B 10g
4209 dn1000B 11e
4209 bats bridge b 10c
4209 bats dn1000 B 11b
4209 dn1000B 11f
4209 dn1000B 12b
4209 dn1000B 12a
4209 bats dn1000 B 10c
4209 dn1000B 11g
4209 bats bridge b 10h
4209 dn1000B 11d
4209 coli bats guano 9c

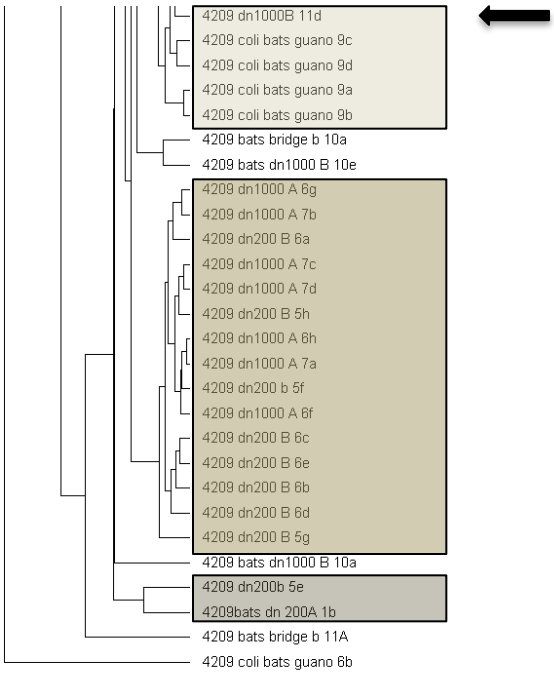
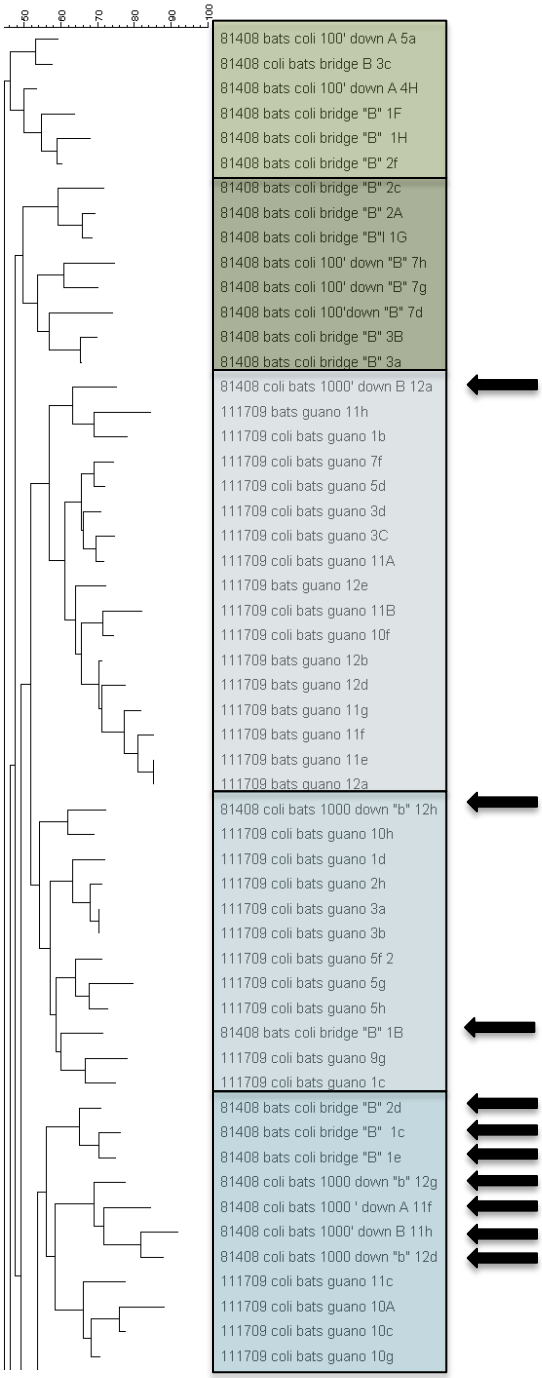
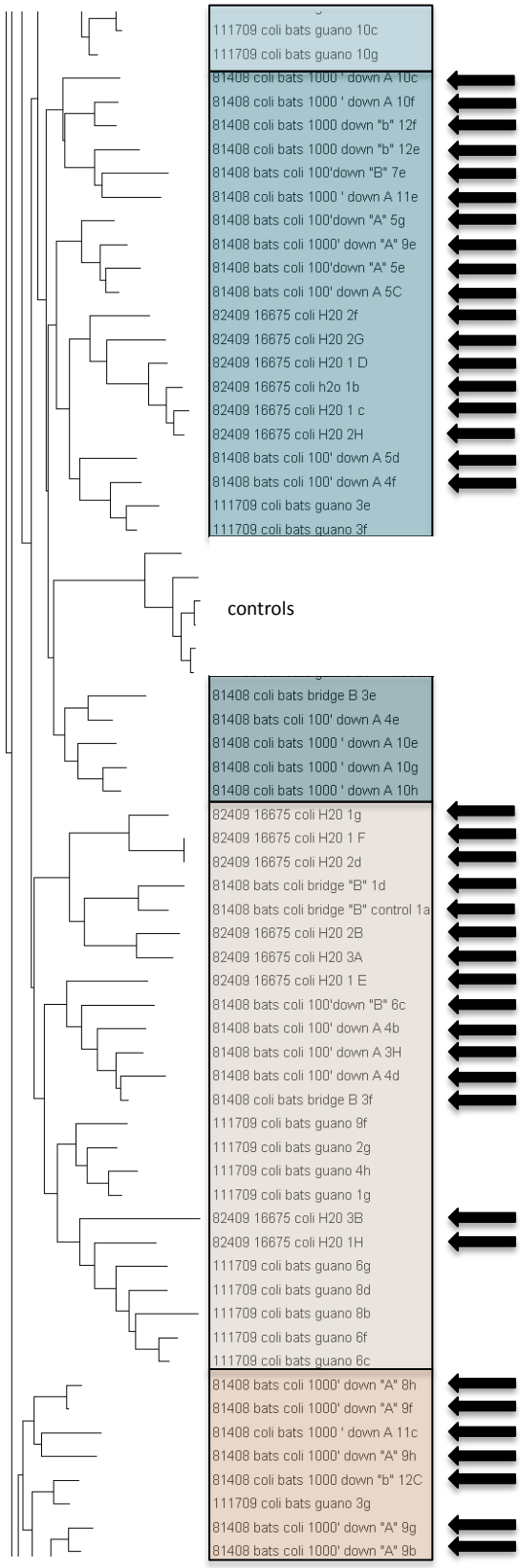
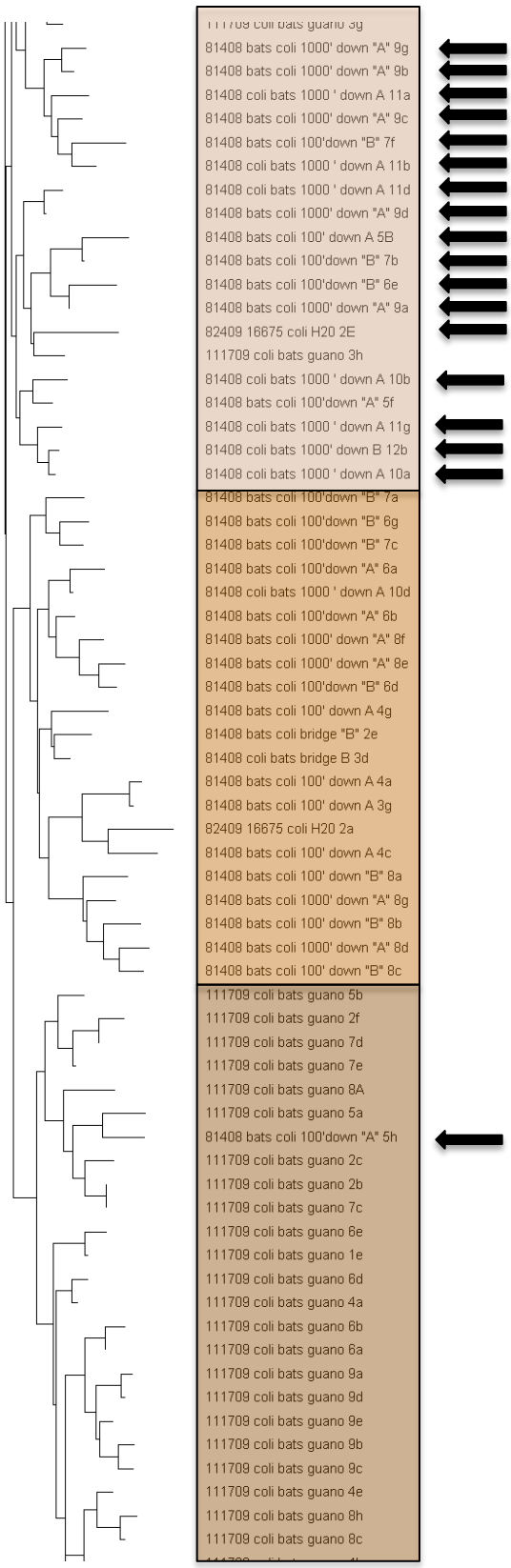

4209 dn1000B 11d
4209 coli bats guano 9c
4209 coli bats guano 9d
4209 coli bats guano 9a
4209 coli bats guano 9b
4209 bats bridge b 10a
4209 bats dn1000 B 10e
4209 dn1000 A 6g
4209 dn1000 A 7b
4209 dn200 B 6a
4209 dn1000 A 7c
4209 dn1000 A 7d
4209 dn200 B 5h
4209 dn1000 A 6h
4209 dn1000 A 7a
4209 dn200 b 5f
4209 dn1000 A 6f
4209 dn200 B 6c
4209 dn200 B 6e
4209 dn200 B 6b
4209 dn200 B 6d
4209 dn200 B 5g
4209 bats dn1000 B 10a
4209 dn200b 5e
4209bats dn 200A 1b
4209 bats bridge b 11A
4209 coli bats guano 6b

Figure 5. HFERP DNA Fingerprint August 2008-November 2009
Dice Coefficient Algorithm/Neighbor Joining Clustering




111709 coli bats guano 10c
111709 coli bats guano 10g
81408 coli bats 1000 ' down A 10c
81408 coli bats 1000 ' down A 10f
81408 coli bats 1000 down "b" 12f
81408 coli bats 1000 down "b" 12e
81408 bats coli 100'down "B" 7e
81408 coli bats 1000 ' down A 11e
81408 bats coli 100'down "A" 5g
81408 bats coli 1000' down "A" 9e
81408 bats coli 100'down "A" 5e
81408 bats coli 100' down A 5C
82409 16675 coli H20 2f
82409 16675 coli H20 2G
82409 16675 coli H20 1 D
82409 16675 coli h2o 1b
82409 16675 coli H20 1 c
82409 16675 coli H20 2H
81408 bats coli 100' down A 5d
81408 bats coli 100' down A 4f
111709 coli bats guano 3e
111709 coli bats guano 3f
controls
81408 coli bats bridge B 3e
81408 bats coli 100' down A 4e
81408 coli bats 1000 ' down A 10e
81408 coli bats 1000 ' down A 10g
81408 coli bats 1000 ' down A 10h
82409 16675 coli H20 1g
82409 16675 coli H20 1 F
82409 16675 coli H20 2d
81408 bats coli bridge "B" 1d
81408 bats coli bridge "B" control 1a
82409 16675 coli H20 2B
82409 16675 coli H20 3A
82409 16675 coli H20 1 E
81408 bats coli 100'down "B" 6c
81408 bats coli 100' down A 4b
81408 bats coli 100' down A 3H
81408 bats coli 100' down A 4d
81408 coli bats bridge B 3f
111709 coli bats guano 9f
111709 coli bats guano 2g
111709 coli bats guano 4h
111709 coli bats guano 1g
82409 16675 coli H20 3B
82409 16675 coli H20 1H
111709 coli bats guano 6g
111709 coli bats guano 8d
111709 coli bats guano 8b
111709 coli bats guano 6f
111709 coli bats guano 6c
81408 bats coli 1000' down "A" 8h
81408 bats coli 1000' down "A" 9f
81408 coli bats 1000 ' down A 11c
81408 bats coli 1000' down "A" 9h
81408 coli bats 1000 down "b" 12C
111709 coli bats guano 3g
81408 bats coli 1000' down "A" 9g
81408 bats coli 1000' down "A" 9b


111709 coli bats guano 3g
81408 bats coli 1000' down "A" 9g
81408 bats coli 1000' down "A" 9b
81408 coli bats 1000 ' down A 11a
81408 bats coli 1000' down "A" 9c
81408 bats coli 100'down "B" 7f
81408 coli bats 1000 ' down A 11b
81408 coli bats 1000 ' down A 11d
81408 bats coli 1000' down "A" 9d
81408 bats coli 100' down A 5B
81408 bats coli 100'down "B" 7b
81408 bats coli 100'down "B" 6e
81408 bats coli 1000' down "A" 9a
82409 16675 coli H20 2E
111709 coli bats guano 3h
81408 coli bats 1000 ' down A 10b
81408 bats coli 100'down "A" 5f
81408 coli bats 1000 ' down A 11g
81408 coli bats 1000' down B 12b
81408 coli bats 1000 ' down A 10a
81408 bats coli 100'down "B" 7a
81408 bats coli 100'down "B" 6g
81408 bats coli 100'down "B" 7c
81408 bats coli 100'down "A" 8a
81408 coli bats 1000 ' down A 10d
81408 bats coli 100'down "A" 6b
81408 bats coli 1000' down "A" 8f
81408 bats coli 1000' down "A" 8e
81408 bats coli 100'down "B" 6d
81408 bats coli 100' down A 4g
81408 bats coli bridge "B" 2e
81408 coli bats bridge B 3d
81408 bats coli 100' down A 4a
81408 bats coli 100' down A 3g
82409 16675 coli H20 2a
81408 bats coli 100' down A 4c
81408 bats coli 100' down "B" 8a
81408 bats coli 1000' down "A" 8g
81408 bats coli 100' down "B" 8b
81408 bats coli 1000' down "A" 8d
81408 bats coli 100' down "B" 8c
111709 coli bats guano 5b
111709 coli bats guano 2f
111709 coli bats guano 7d
111709 coli bats guano 7e
111709 coli bats guano 8A
111709 coli bats guano 5a
81408 bats coli 100'down "A" 5h
111709 coli bats guano 2c
111709 coli bats guano 2b
111709 coli bats guano 7c
111709 coli bats guano 6e
111709 coli bats guano 1e
111709 coli bats guano 6d
111709 coli bats guano 4a
111709 coli bats guano 6b
111709 coli bats guano 6a
111709 coli bats guano 9a
111709 coli bats guano 9d
111709 coli bats guano 9e
111709 coli bats guano 9b
111709 coli bats guano 9c
111709 coli bats guano 4e
111709 coli bats guano 8h
111709 coli bats guano 8c

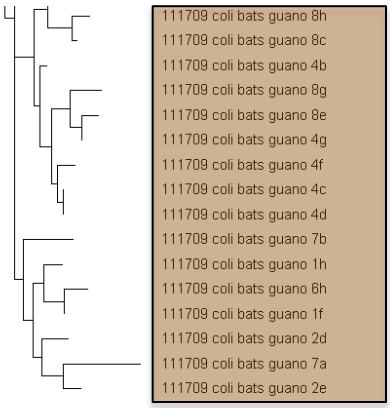
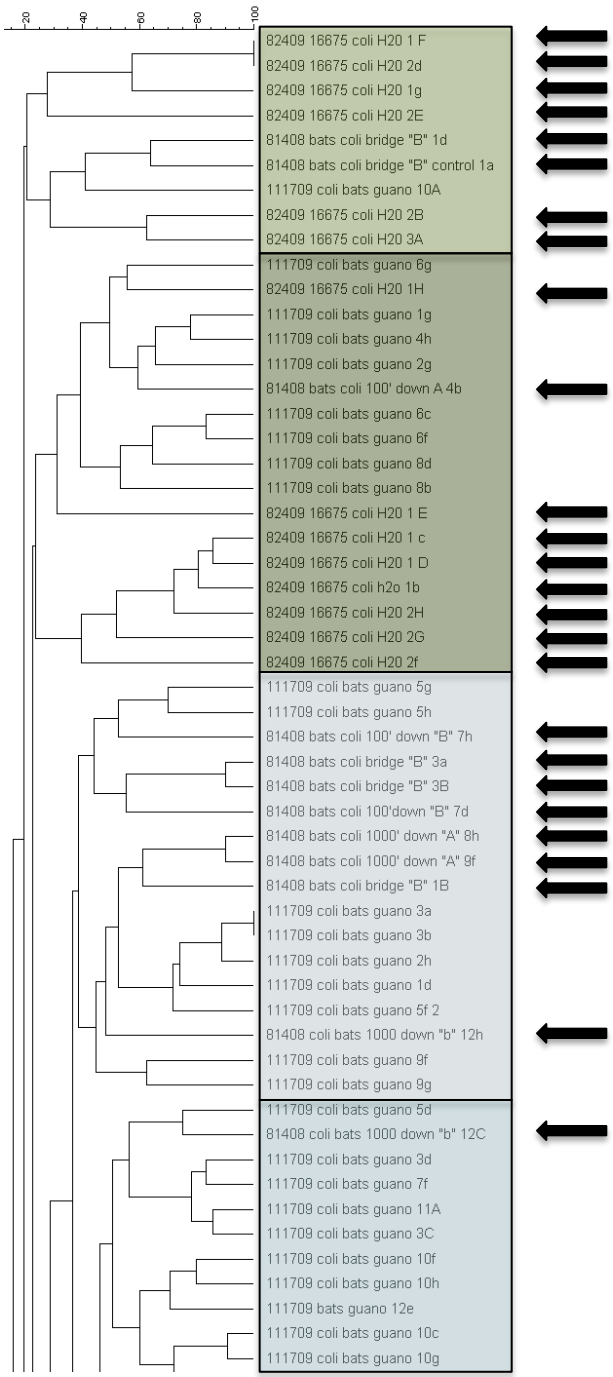
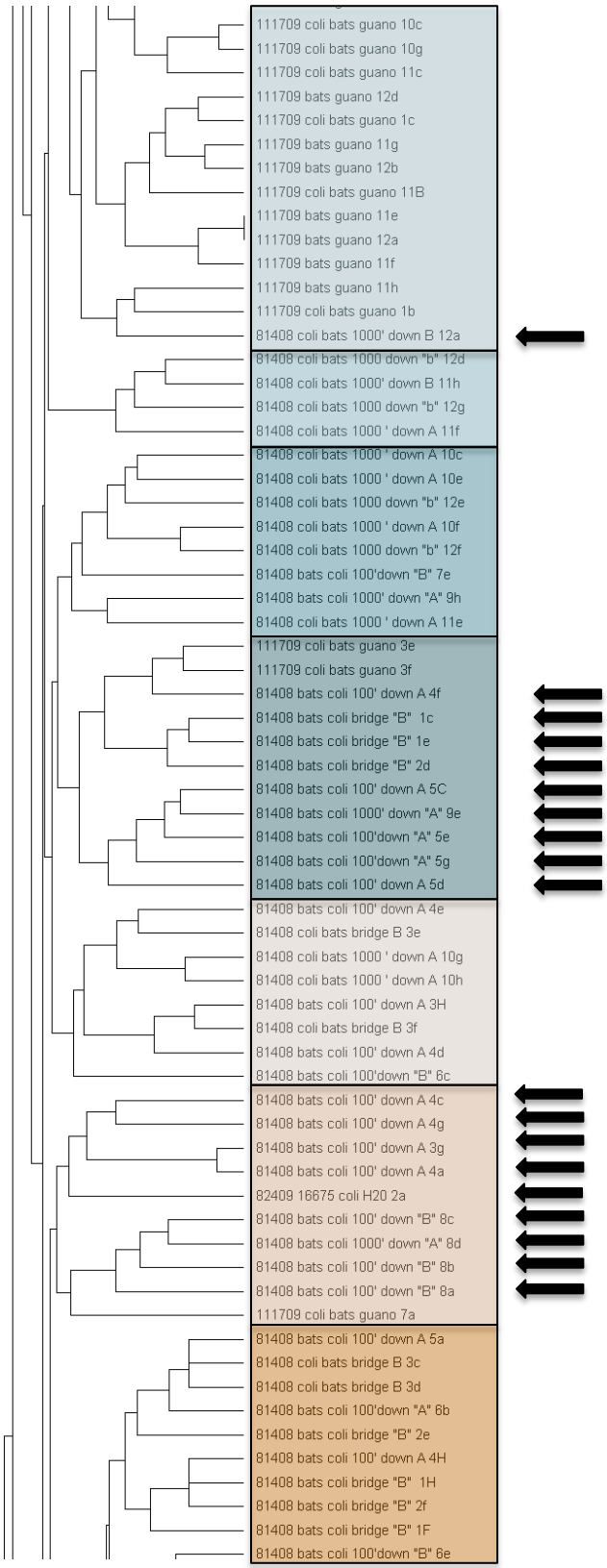
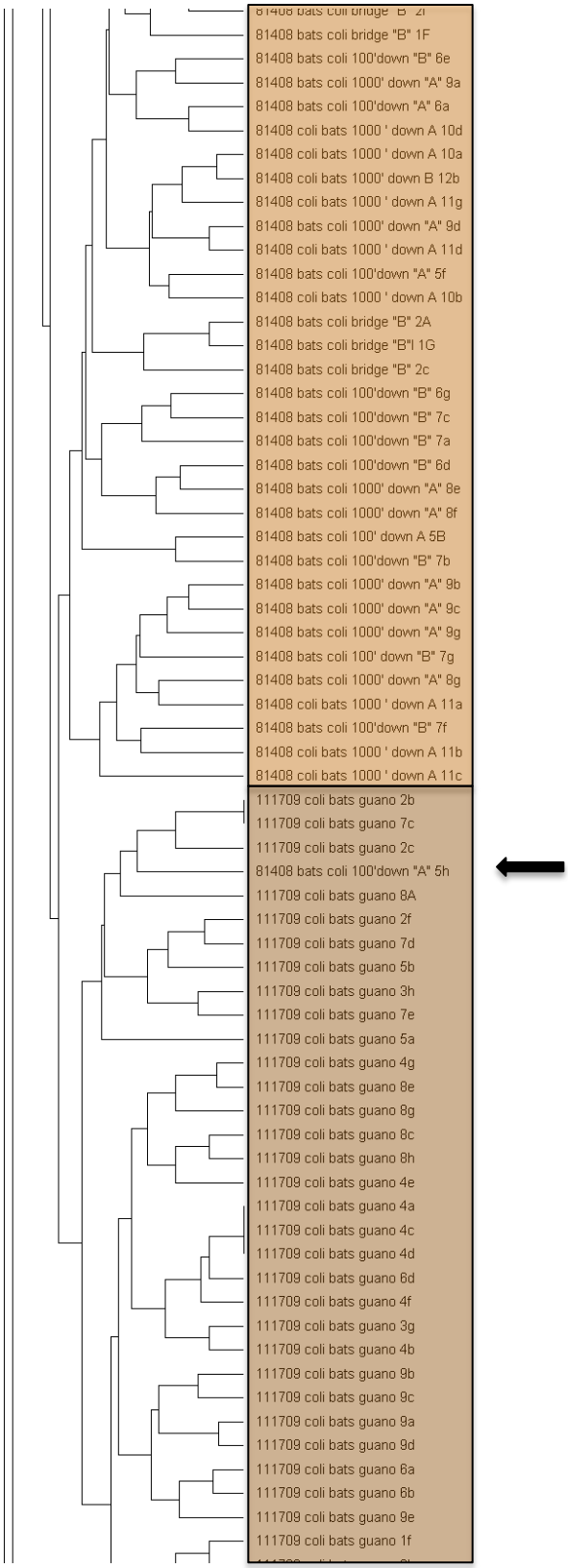

111709 coli bats guano 8h
111709 coli bats guano 8c
111709 coli bats guano 4b
111709 coli bats guano 8g
111709 coli bats guano 8e
111709 coli bats guano 4g
111709 coli bats guano 4f
111709 coli bats guano 4c
111709 coli bats guano 4d
111709 coli bats guano 7b
111709 coli bats guano 1h
111709 coli bats guano 6h
111709 coli bats guano 1f
111709 coli bats guano 2d
111709 coli bats guano 7a
111709 coli bats guano 2e

Figure 6. HFERP DNA Fingerprint August 2008-November 2009
Jaccard Index Algorithm/UPGMA Clustering




111709 coli bats guano 10c
111709 coli bats guano 10g
111709 coli bats guano 11c
111709 bats guano 12d
111709 coli bats guano 1c
111709 bats guano 11g
111709 bats guano 12b
111709 coli bats guano 11B
111709 bats guano 11e
111709 bats guano 12a
111709 bats guano 11f
111709 bats guano 11h
111709 coli bats guano 1b
81408 coli bats 1000' down B 12a
81408 coli bats 1000 down "b" 12d
81408 coli bats 1000' down B 11h
81408 coli bats 1000 down "b" 12g
81408 coli bats 1000 ' down A 11f
81408 coli bats 1000 ' down A 10c
81408 coli bats 1000 ' down A 10e
81408 coli bats 1000 down "b" 12e
81408 coli bats 1000 ' down A 10f
81408 coli bats 1000 down "b" 12f
81408 bats coli 100'down "B" 7e
81408 bats coli 1000' down "A" 9h
81408 coli bats 1000 ' down A 11e
111709 coli bats guano 3e
111709 coli bats guano 3f
81408 bats coli 100' down A 4f
81408 bats coli bridge "B" 1c
81408 bats coli bridge "B" 1e
81408 bats coli bridge "B" 2d
81408 bats coli 100' down A 5C
81408 bats coli 1000' down "A" 9e
81408 bats coli 100'down "A" 5e
81408 bats coli 100'down "A" 5g
81408 bats coli 100' down A 5d
81408 bats coli 100' down A 4e
81408 coli bats bridge B 3e
81408 coli bats 1000 ' down A 10g
81408 coli bats 1000 ' down A 10h
81408 bats coli 100' down A 3H
81408 coli bats bridge B 3f
81408 bats coli 100' down A 4d
81408 bats coli 100'down "B" 6c
81408 bats coli 100' down A 4c
81408 bats coli 100' down A 4g
81408 bats coli 100' down A 3g
81408 bats coli 100' down A 4a
82409 16675 coli H20 2a
81408 bats coli 100' down "B" 8c
81408 bats coli 1000' down "A" 8d
81408 bats coli 100' down "B" 8b
81408 bats coli 100' down "B" 8a
111709 coli bats guano 7a
81408 bats coli 100' down A 5a
81408 coli bats bridge B 3c
81408 coli bats bridge B 3d
81408 bats coli 100'down "A" 6b
81408 bats coli bridge "B" 2e
81408 bats coli 100' down A 4H
81408 bats coli bridge "B" 1H
81408 bats coli bridge "B" 2f
81408 bats coli bridge "B" 1F
81408 bats coli 100'down "B" 6e


81408 bats coli bridge "B" 2f
81408 bats coli bridge "B" 1F
81408 bats coli 100'down "B" 6e
81408 bats coli 1000' down "A" 9a
81408 bats coli 100'down "A" 6a
81408 coli bats 1000 ' down A 10d
81408 coli bats 1000 ' down A 10a
81408 coli bats 1000' down B 12b
81408 coli bats 1000 ' down A 11g
81408 bats coli 1000' down "A" 9d
81408 coli bats 1000 ' down A 11d
81408 bats coli 100'down "A" 5f
81408 coli bats 1000 ' down A 10b
81408 bats coli bridge "B" 2A
81408 bats coli bridge "B"l 1G
81408 bats coli bridge "B" 2c
81408 bats coli 100'down "B" 6g
81408 bats coli 100'down "B" 7c
81408 bats coli 100'down "B" 7a
81408 bats coli 100'down "B" 6d
81408 bats coli 1000' down "A" 8e
81408 bats coli 1000' down "A" 8f
81408 bats coli 100' down A 5B
81408 bats coli 100'down "B" 7b
81408 bats coli 1000' down "A" 9b
81408 bats coli 1000' down "A" 9c
81408 bats coli 1000' down "A" 9g
81408 bats coli 100' down "B" 7g
81408 bats coli 1000' down "A" 8g
81408 coli bats 1000 ' down A 11a
81408 bats coli 100'down "B" 7f
81408 coli bats 1000 ' down A 11b
81408 coli bats 1000 ' down A 11c
111709 coli bats guano 2b
111709 coli bats guano 7c
111709 coli bats guano 2c
81408 bats coli 100'down "A" 5h
111709 coli bats guano 8A
111709 coli bats guano 2f
111709 coli bats guano 7d
111709 coli bats guano 5b
111709 coli bats guano 3h
111709 coli bats guano 7e
111709 coli bats guano 5a
111709 coli bats guano 4g
111709 coli bats guano 8e
111709 coli bats guano 8g
111709 coli bats guano 8c
111709 coli bats guano 8h
111709 coli bats guano 4e
111709 coli bats guano 4a
111709 coli bats guano 4c
111709 coli bats guano 4d
111709 coli bats guano 6d
111709 coli bats guano 4f
111709 coli bats guano 3g
111709 coli bats guano 4b
111709 coli bats guano 9b
111709 coli bats guano 9c
111709 coli bats guano 9a
111709 coli bats guano 9d
111709 coli bats guano 6a
111709 coli bats guano 6b
111709 coli bats guano 9e
111709 coli bats guano 1f

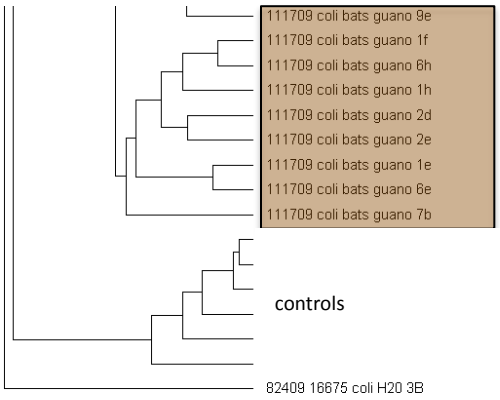
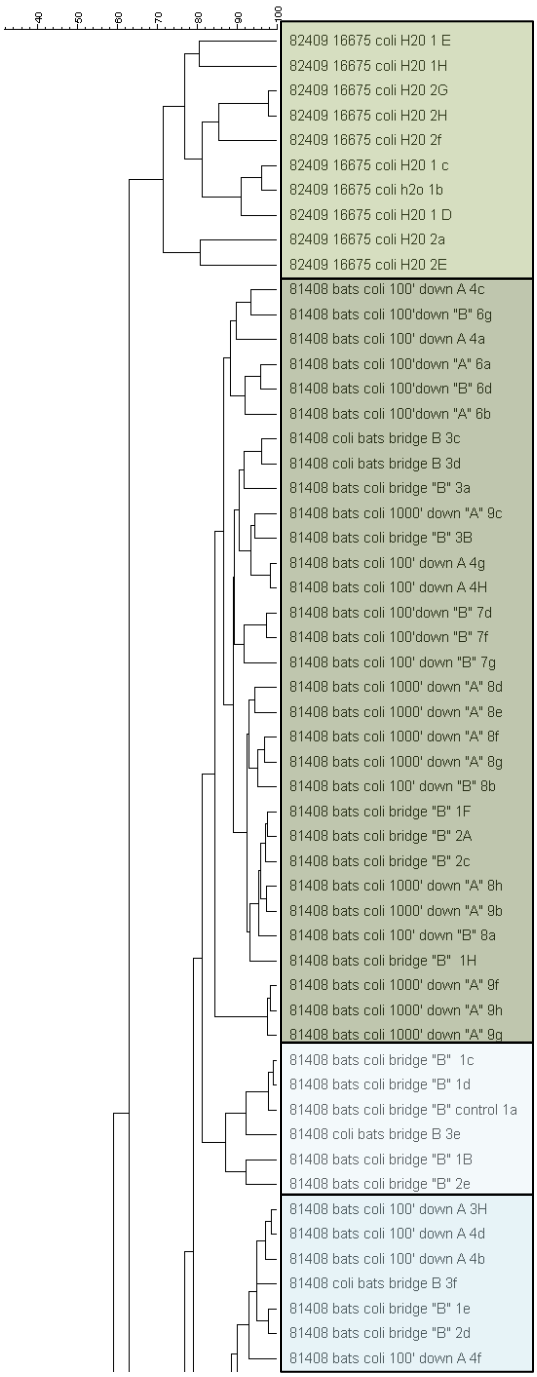
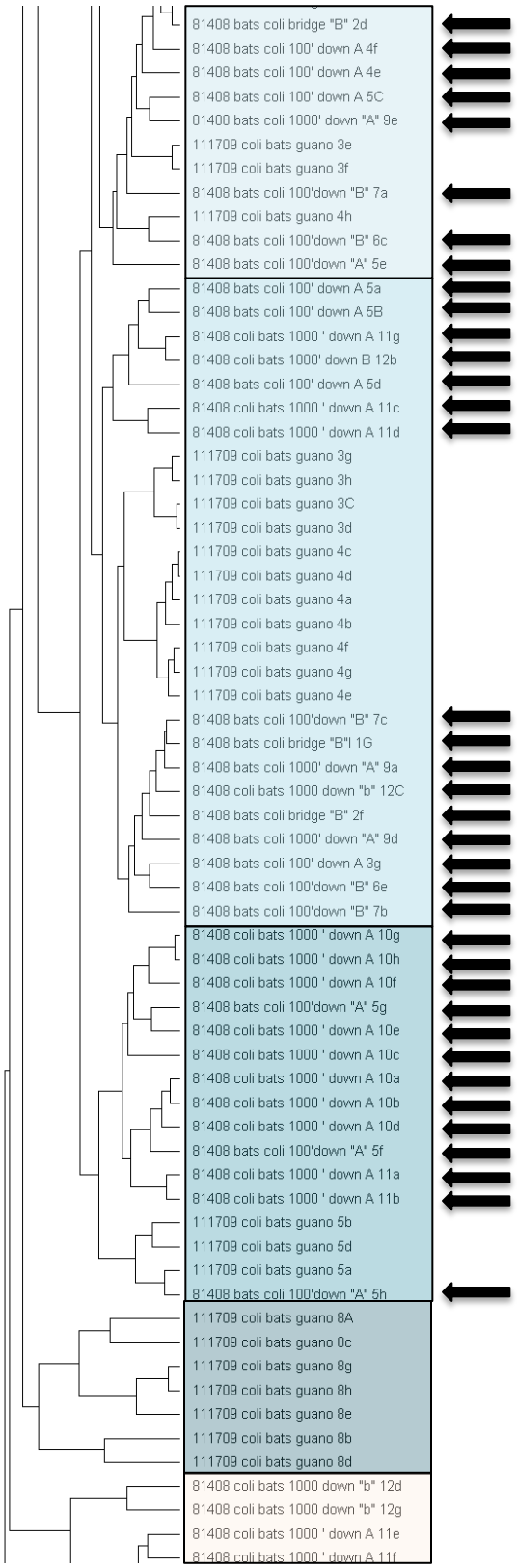
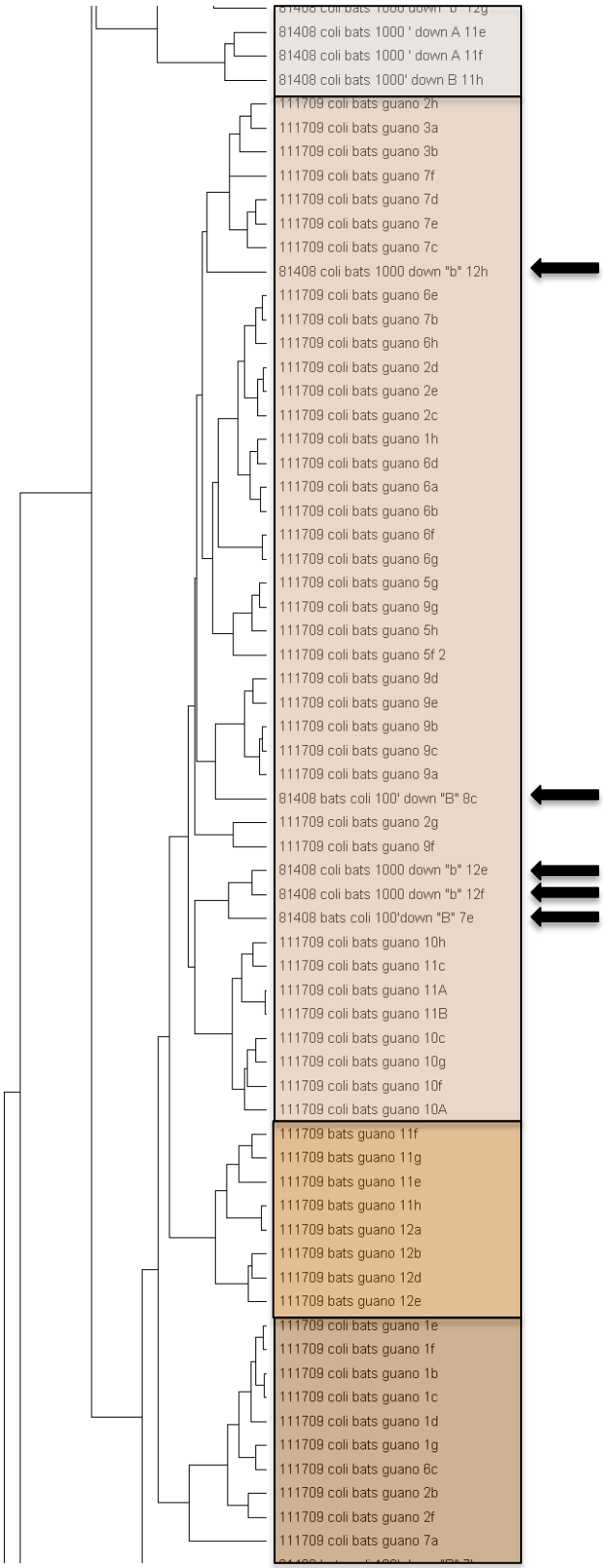

111709 coli bats guano 9e
111709 coli bats guano 1f
111709 coli bats guano 6h
111709 coli bats guano 1h
111709 coli bats guano 2d
111709 coli bats guano 2e
111709 coli bats guano 1e
111709 coli bats guano 6e
111709 coli bats guano 7b
controls
82409 16675 coli H20 3B

Figure 7. HFERP DNA Fingerprint August 2008-November 2009
Pearson Coefficient Algorithm/UPGMA Clustering




81408 bats coli bridge "B" 2d
81408 bats coli 100' down A 4f
81408 bats coli 100' down A 4e
81408 bats coli 100' down A 5C
81408 bats coli 1000' down "A" 9e
111709 coli bats guano 3e
111709 coli bats guano 3f
81408 bats coli 100'down "B" 7a
111709 coli bats guano 4h
81408 bats coli 100'down "B" 6c
81408 bats coli 100'down "A" 5e
81408 bats coli 100' down A 5a
81408 bats coli 100' down A 5B
81408 coli bats 1000 ' down A 11g
81408 coli bats 1000' down B 12b
81408 bats coli 100' down A 5d
81408 coli bats 1000 ' down A 11c
81408 coli bats 1000 ' down A 11d
111709 coli bats guano 3g
111709 coli bats guano 3h
111709 coli bats guano 3C
111709 coli bats guano 3d
111709 coli bats guano 4c
111709 coli bats guano 4d
111709 coli bats guano 4a
111709 coli bats guano 4b
111709 coli bats guano 4f
111709 coli bats guano 4g
111709 coli bats guano 4e
81408 bats coli 100'down "B" 7c
81408 bats coli bridge "B"I 1G
81408 bats coli 1000' down "A" 9a
81408 coli bats 1000 down "b" 12C
81408 bats coli bridge "B" 2f
81408 bats coli 1000' down "A" 9d
81408 bats coli 100' down A 3g
81408 bats coli 100'down "B" 6e
81408 bats coli 100'down "B" 7b
81408 coli bats 1000 ' down A 10g
81408 coli bats 1000 ' down A 10h
81408 coli bats 1000 ' down A 10f
81408 bats coli 100'down "A" 5g
81408 coli bats 1000 ' down A 10e
81408 coli bats 1000 ' down A 10c
81408 coli bats 1000 ' down A 10a
81408 coli bats 1000 ' down A 10b
81408 coli bats 1000 ' down A 10d
81408 bats coli 100'down "A" 5f
81408 coli bats 1000 ' down A 11a
81408 coli bats 1000 ' down A 11b
111709 coli bats guano 5b
111709 coli bats guano 5d
111709 coli bats guano 5a
81408 bats coli 100'down "A" 5h
111709 coli bats guano 8A
111709 coli bats guano 8c
111709 coli bats guano 8g
111709 coli bats guano 8h
111709 coli bats guano 8e
111709 coli bats guano 8b
111709 coli bats guano 8d
81408 coli bats 1000 down "b" 12d
81408 coli bats 1000 down "b" 12g
81408 coli bats 1000 ' down A 11e
81408 coli bats 1000 ' down A 11f


81408 coli bats 1000 ' down A 11e
81408 coli bats 1000 ' down A 11f
81408 coli bats 1000' down B 11h
111709 coli bats guano 2h
111709 coli bats guano 3a
111709 coli bats guano 3b
111709 coli bats guano 7f
111709 coli bats guano 7d
111709 coli bats guano 7e
111709 coli bats guano 7c
81408 coli bats 1000 down "b" 12h
111709 coli bats guano 6e
111709 coli bats guano 7b
111709 coli bats guano 8h
111709 coli bats guano 2d
111709 coli bats guano 2e
111709 coli bats guano 2c
111709 coli bats guano 1h
111709 coli bats guano 6d
111709 coli bats guano 8a
111709 coli bats guano 6b
111709 coli bats guano 6f
111709 coli bats guano 6g
111709 coli bats guano 5g
111709 coli bats guano 9g
111709 coli bats guano 5h
111709 coli bats guano 5f 2
111709 coli bats guano 9d
111709 coli bats guano 9e
111709 coli bats guano 9b
111709 coli bats guano 9c
111709 coli bats guano 9a
81408 bats coli 100' down "B" 8c
111709 coli bats guano 2g
111709 coli bats guano 9f
81408 coli bats 1000 down "b" 12e
81408 coli bats 1000 down "b" 12f
81408 bats coli 100'down "B" 7e
111709 coli bats guano 10h
111709 coli bats guano 11c
111709 coli bats guano 11A
111709 coli bats guano 11B
111709 coli bats guano 10c
111709 coli bats guano 10g
111709 coli bats guano 10f
111709 coli bats guano 10A
111709 bats guano 11f
111709 bats guano 11g
111709 bats guano 11e
111709 bats guano 11h
111709 bats guano 12a
111709 bats guano 12b
111709 bats guano 12d
111709 bats guano 12e
111709 coli bats guano 1e
111709 coli bats guano 1f
111709 coli bats guano 1b
111709 coli bats guano 1c
111709 coli bats guano 1d
111709 coli bats guano 1g
111709 coli bats guano 6c
111709 coli bats guano 2b
111709 coli bats guano 2f
111709 coli bats guano 7a

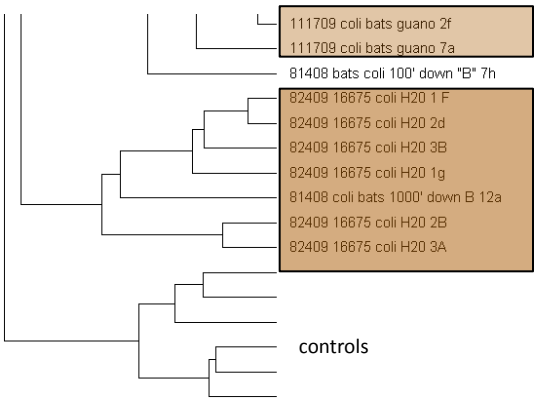
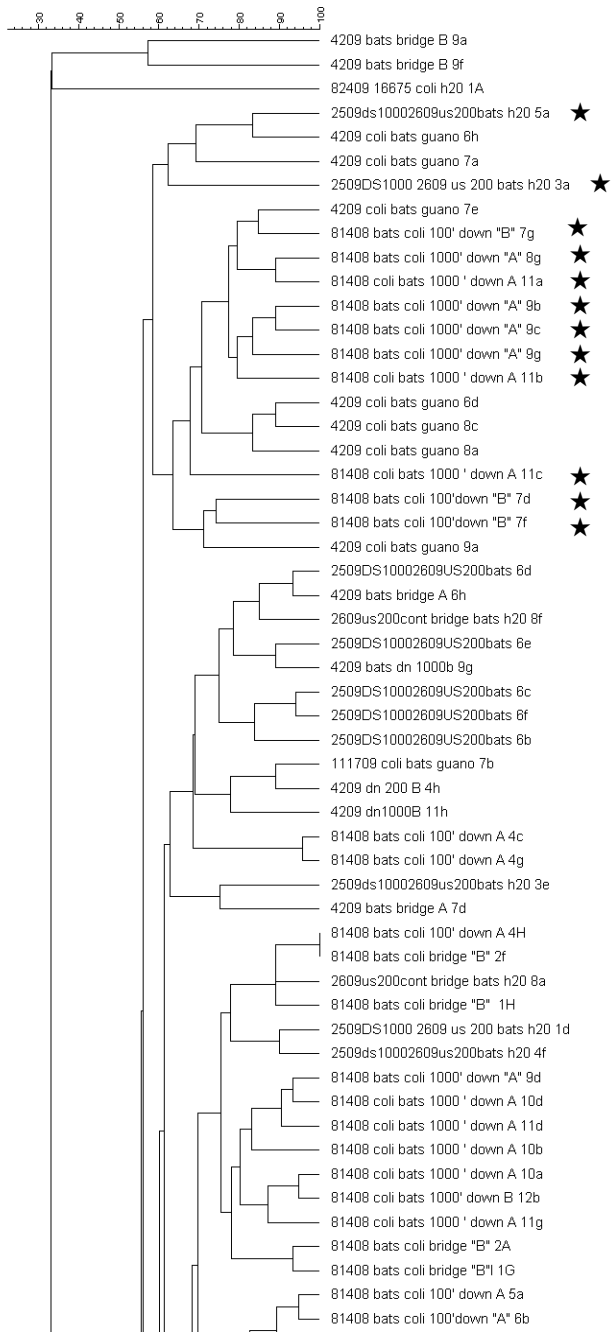
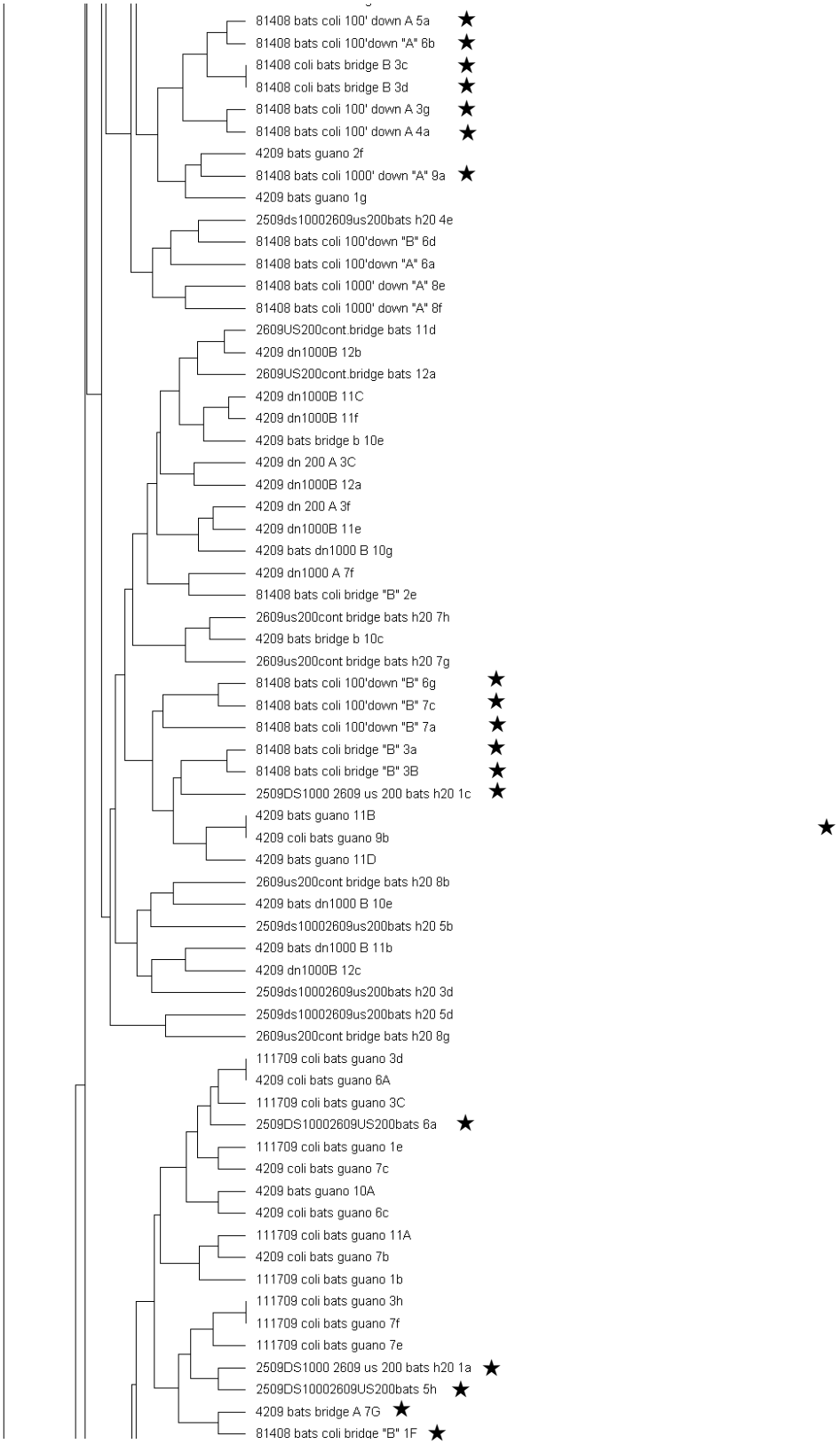
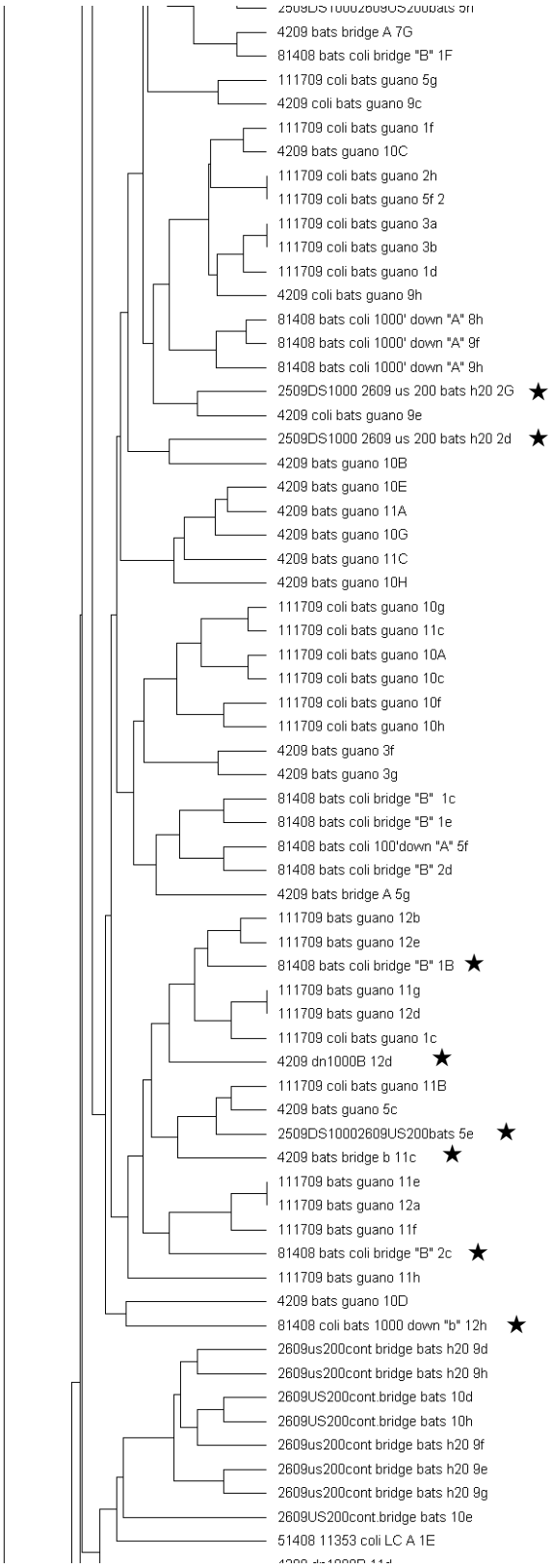
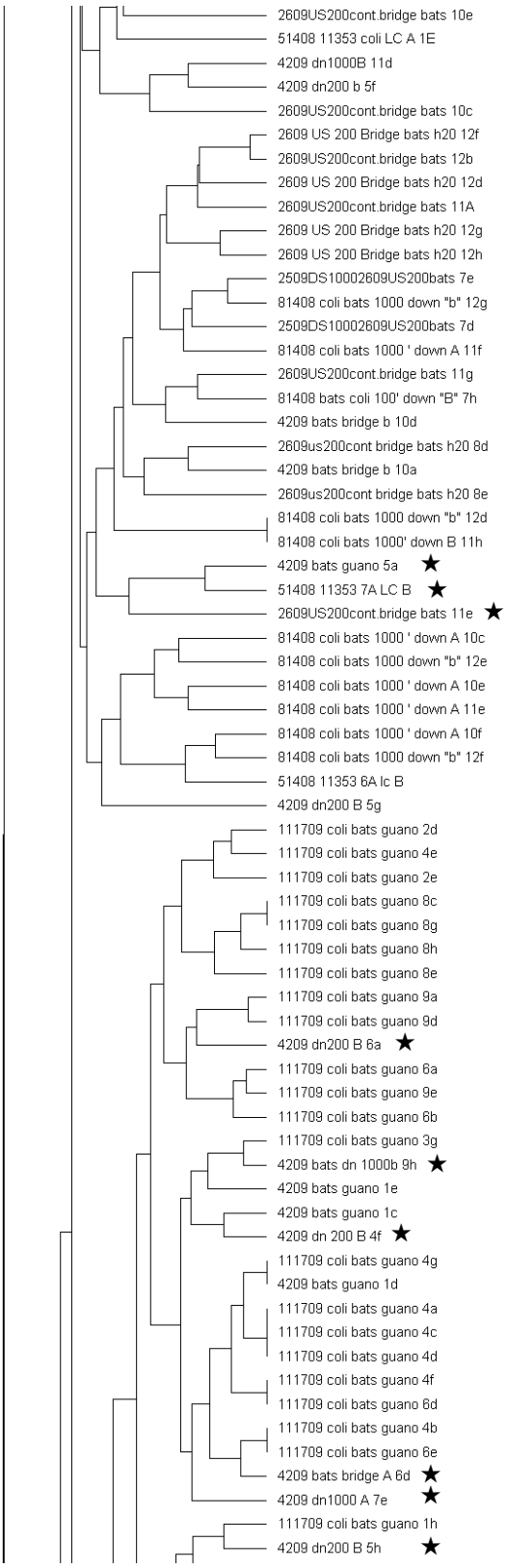

111709 coli bats guano 2f
111709 coli bats guano 7a
81408 bats coli 100' down "B" 7h
82409 16675 coli H20 1 F
82409 16675 coli H20 2d
82409 16675 coli H20 3B
82409 16675 coli H20 1g
81408 coli bats 1000' down B 12a
82409 16675 coli H20 2B
82409 16675 coli H20 3A
controls

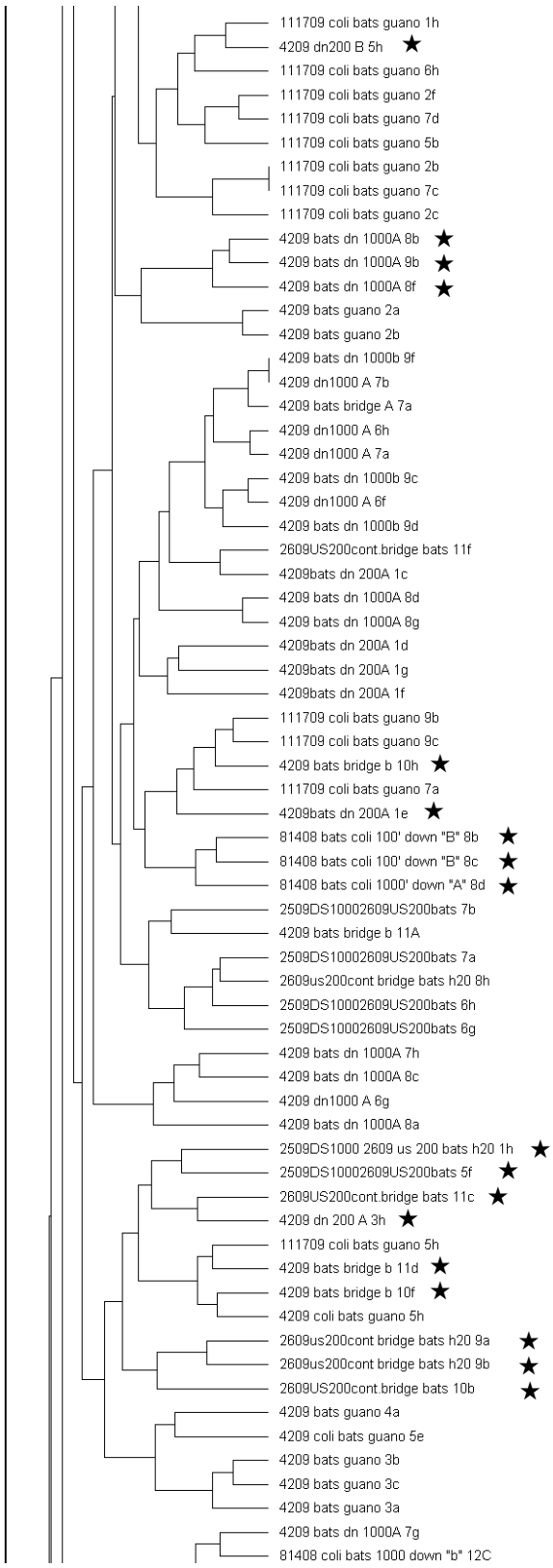
Figure 8. HFERP DNA Fingerprint for Spring (Apr 2009) and Fall (Aug 2008, Nov 2009)
Dice Coefficient Algorithm-UPGMA Cluster Analysis
Stars denote isolates from water samples with high similarity to bat guano isolates

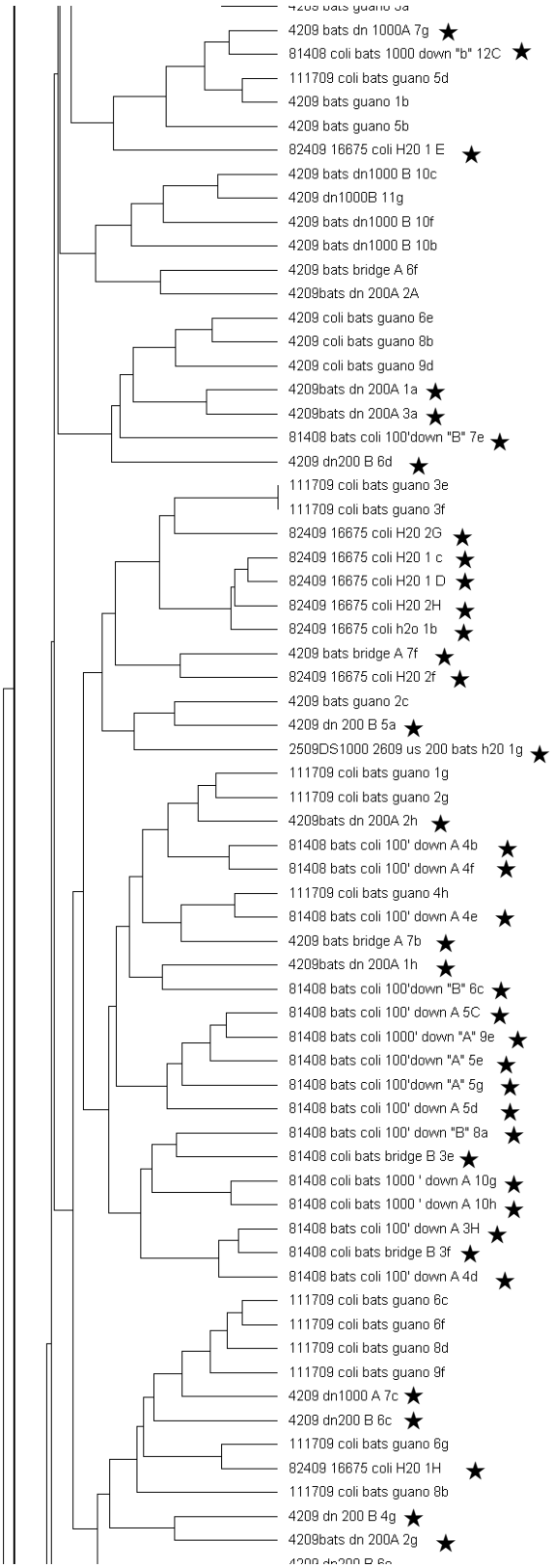


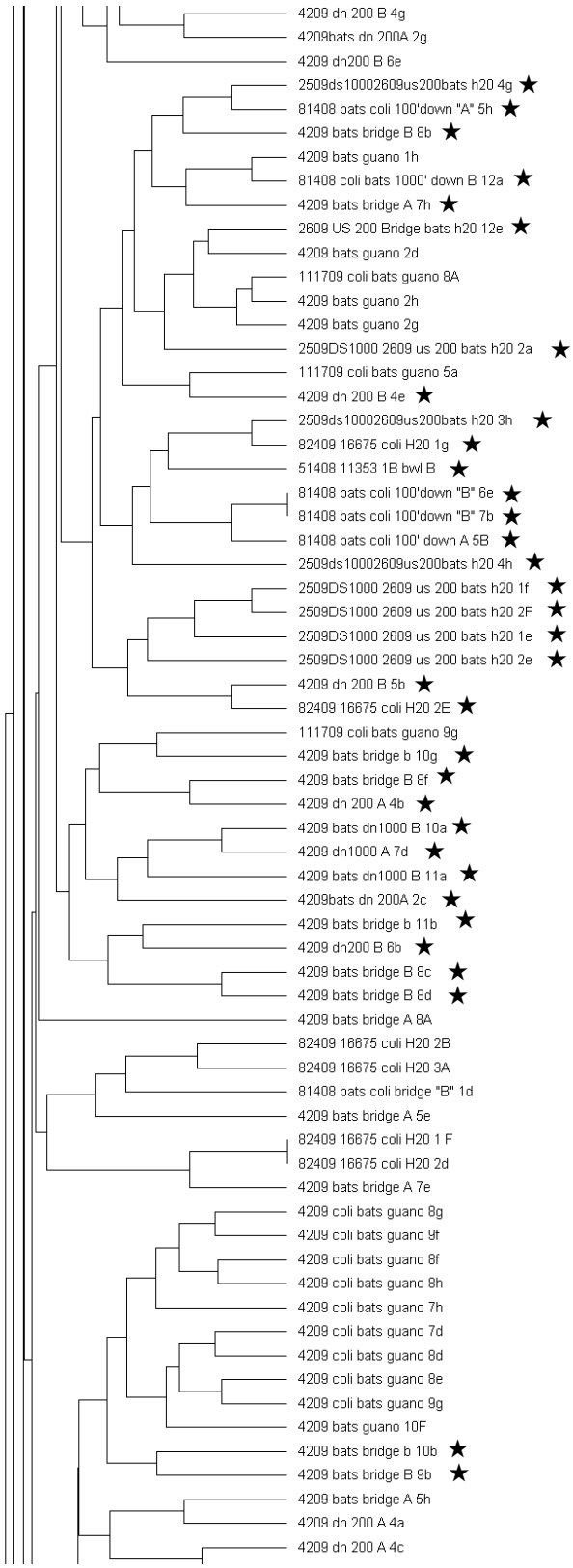

81408 bats coli 100' down A 5a
81408 bats coli 100'down "A" 6b
81408 coli bats bridge B 3c
81408 coli bats bridge B 3d
81408 bats coli 100' down A 3g
81408 bats coli 100' down A 4a
4209 bats guano 2f
81408 bats coli 1000' down "A" 9a
4209 bats guano 1g
2509ds10002609us200bats h20 4e
81408 bats coli 100'down "B" 6d
81408 bats coli 100'down "A" 8a
81408 bats coli 1000' down "A" 8e
81408 bats coli 1000' down "A" 8f
2609US200cont.bridge bats 11d
4209 dn1000B 12b
2609US200cont.bridge bats 12a
4209 dn1000B 11C
4209 dn1000B 11f
4209 bats bridge b 10e
4209 dn 200 A 3C
4209 dn1000B 12a
4209 dn 200 A 3f
4209 dn1000B 11e
4209 bats dn1000 B 10g
4209 dn1000 A 7f
81408 bats coli bridge "B" 2e
2609us200cont bridge bats h20 7h
4209 bats bridge b 10c
2609us200cont bridge bats h20 7g
81408 bats coli 100'down "B" 6g
81408 bats coli 100'down "B" 7c
81408 bats coli 100'down "B" 7a
81408 bats coli bridge "B" 3a
81408 bats coli bridge "B" 3B
2509DS1000 2609 us 200 bats h20 1c
4209 bats guano 11B
4209 coli bats guano 9b
4209 bats guano 11D
2609us200cont bridge bats h20 8b
4209 bats dn1000 B 10e
2509ds10002609us200bats h20 5b
4209 bats dn1000 B 11b
4209 dn1000B 12c
2509ds10002609us200bats h20 3d
2509ds10002609us200bats h20 5d
2609us200cont bridge bats h20 8g
111709 coli bats guano 3d
4209 coli bats guano 6A
111709 coli bats guano 3C
2509DS10002609US200bats 6a
111709 coli bats guano 1e
4209 coli bats guano 7c
4209 bats guano 10A
4209 coli bats guano 6c
111709 coli bats guano 11A
4209 coli bats guano 7b
111709 coli bats guano 1b
111709 coli bats guano 3h
111709 coli bats guano 7f
111709 coli bats guano 7e
2509DS1000 2609 us 200 bats h20 1a
2509DS10002609US200bats 5h
4209 bats bridge A 7G
81408 bats coli bridge "B" 1F

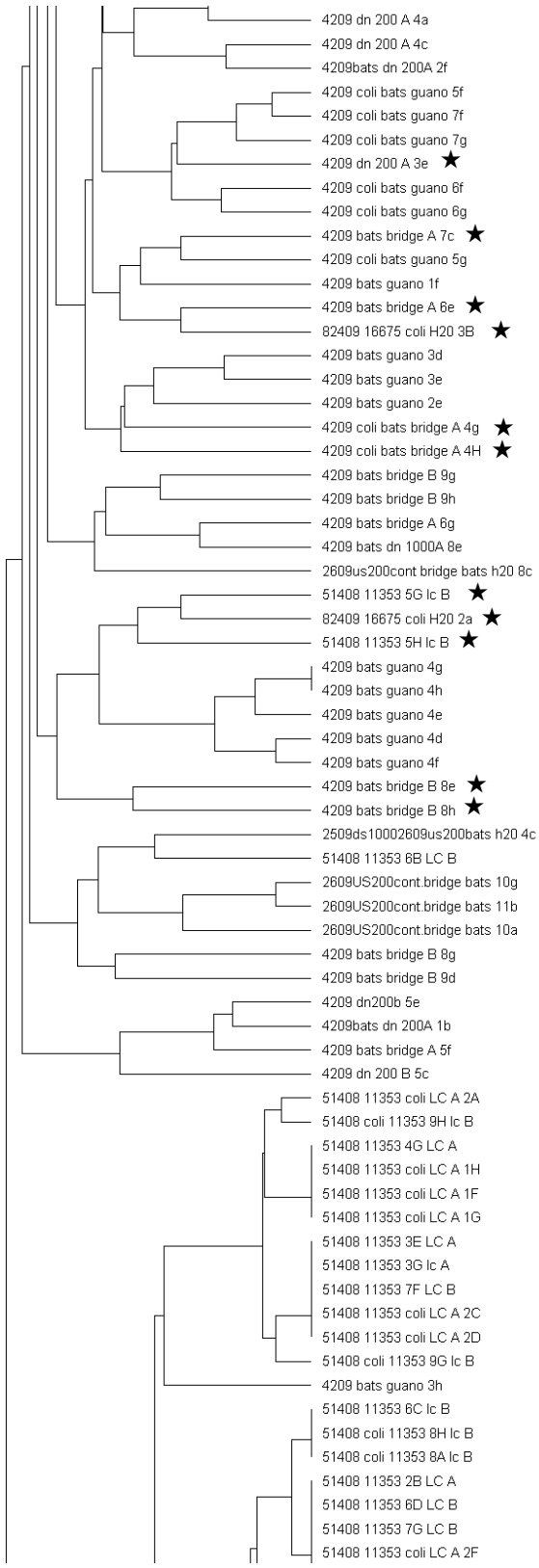

2509DS10002609US200bats 5h
4209 bats bridge A 7G
81408 bats coli bridge "B" 1F
111709 coli bats guano 5g
4209 coli bats guano 9c
111709 coli bats guano 1f
4209 bats guano 10C
111709 coli bats guano 2h
111709 coli bats guano 5f 2
111709 coli bats guano 3a
111709 coli bats guano 3b
111709 coli bats guano 1d
4209 coli bats guano 9h
81408 bats coli 1000' down "A" 8h
81408 bats coli 1000' down "A" 9f
81408 bats coli 1000' down "A" 9h
2509DS1000 2609 us 200 bats h20 2G ★
4209 coli bats guano 9e
2509DS1000 2609 us 200 bats h20 2d ★
4209 bats guano 10B
4209 bats guano 10E
4209 bats guano 11A
4209 bats guano 10G
4209 bats guano 11C
4209 bats guano 10H
111709 coli bats guano 10g
111709 coli bats guano 11c
111709 coli bats guano 10A
111709 coli bats guano 10c
111709 coli bats guano 10f
111709 coli bats guano 10h
4209 bats guano 3f
4209 bats guano 3g
81408 bats coli bridge "B" 1c
81408 bats coli bridge "B" 1e
81408 bats coli 100'down "A" 5f
81408 bats coli bridge "B" 2d
4209 bats bridge A 5g
111709 bats guano 12b
111709 bats guano 12e
81408 bats coli bridge "B" 1B ★
111709 bats guano 11g
111709 bats guano 12d
111709 coli bats guano 1c
4209 dn1000B 12d ★
111709 coli bats guano 11B
4209 bats guano 5c
2509DS10002609US200bats 5e ★
4209 bats bridge b 11c ★
111709 bats guano 11e
111709 bats guano 12a
111709 bats guano 11f
81408 bats coli bridge "B" 2c ★
111709 bats guano 11h
4209 bats guano 10D
81408 coli bats 1000 down "b" 12h ★
2609us200cont bridge bats h20 9d
2609us200cont bridge bats h20 9h
2609US200cont.bridge bats 10d
2609US200cont.bridge bats 10h
2609us200cont bridge bats h20 9f
2609us200cont bridge bats h20 9e
2609us200cont bridge bats h20 9g
2609US200cont.bridge bats 10e
51408 11353 coli LC A 1E


2609US200cont.bridge bats 10e
51408 11353 coli LC A 1E
4209 dn1000B 11d
4209 dn200 b 5f
2609US200cont.bridge bats 10c
2609 US 200 Bridge bats h20 12f
2609US200cont.bridge bats 12b
2609 US 200 Bridge bats h20 12d
2609US200cont.bridge bats 11A
2609 US 200 Bridge bats h20 12g
2609 US 200 Bridge bats h20 12h
2509DS10002609US200bats 7e
81408 coli bats 1000 down "b" 12g
2509DS10002609US200bats 7d
81408 coli bats 1000 ' down A 11f
2609US200cont.bridge bats 11g
81408 bats coli 100' down "B" 7h
4209 bats bridge b 10d
2609us200cont bridge bats h20 8d
4209 bats bridge b 10a
2609us200cont bridge bats h20 8e
81408 coli bats 1000 down "b" 12d
81408 coli bats 1000' down B 11h
4209 bats guano 5a
51408 11353 7A LC B
2609US200cont.bridge bats 11e
81408 coli bats 1000 ' down A 10c
81408 coli bats 1000 down "b" 12e
81408 coli bats 1000 ' down A 10e
81408 coli bats 1000 ' down A 11e
81408 coli bats 1000 ' down A 10f
81408 coli bats 1000 down "b" 12f
51408 11353 6A lc B
4209 dn200 B 5g
111709 coli bats guano 2d
111709 coli bats guano 4e
111709 coli bats guano 2e
111709 coli bats guano 8c
111709 coli bats guano 8g
111709 coli bats guano 8h
111709 coli bats guano 8e
111709 coli bats guano 9a
111709 coli bats guano 9d
4209 dn200 B 6a
111709 coli bats guano 6a
111709 coli bats guano 9e
111709 coli bats guano 6b
111709 coli bats guano 3g
4209 bats dn 1000b 9h
4209 bats guano 1e
4209 bats guano 1c
4209 dn 200 B 4f
111709 coli bats guano 4g
4209 bats guano 1d
111709 coli bats guano 4a
111709 coli bats guano 4c
111709 coli bats guano 4d
111709 coli bats guano 4f
111709 coli bats guano 6d
111709 coli bats guano 4b
111709 coli bats guano 6e
4209 bats bridge A 6d
4209 dn1000 A 7e
111709 coli bats guano 1h
4209 dn200 B 5h


111709 coli bats guano 1h
4209 dn200 B 5h
111709 coli bats guano 6h
111709 coli bats guano 2f
111709 coli bats guano 7d
111709 coli bats guano 5b
111709 coli bats guano 2b
111709 coli bats guano 7c
111709 coli bats guano 2c
4209 bats dn 1000A 8b
4209 bats dn 1000A 9b
4209 bats dn 1000A 8f
4209 bats guano 2a
4209 bats guano 2b
4209 bats dn 1000b 9f
4209 dn1000 A 7b
4209 bats bridge A 7a
4209 dn1000 A 6h
4209 dn1000 A 7a
4209 bats dn 1000b 9c
4209 dn1000 A 6f
4209 bats dn 1000b 9d
2609US200cont.bridge bats 11f
4209bats dn 200A 1c
4209 bats dn 1000A 8d
4209 bats dn 1000A 8g
4209bats dn 200A 1d
4209bats dn 200A 1g
4209bats dn 200A 1f
111709 coli bats guano 9b
111709 coli bats guano 9c
4209 bats bridge b 10h
111709 coli bats guano 7a
4209bats dn 200A 1e
81408 bats coli 100' down "B" 8b
81408 bats coli 100' down "B" 8c
81408 bats coli 1000' down "A" 8d
2509DS10002609US200bats 7b
4209 bats bridge b 11A
2509DS10002609US200bats 7a
2609us200cont bridge bats h20 8h
2509DS10002609US200bats 6h
2509DS10002609US200bats 6g
4209 bats dn 1000A 7h
4209 bats dn 1000A 8c
4209 dn1000 A 6g
4209 bats dn 1000A 8a
2509DS1000 2609 us 200 bats h20 1h
2509DS10002609US200bats 5f
2609US200cont.bridge bats 11c
4209 dn 200 A 3h
111709 coli bats guano 5h
4209 bats bridge b 11d
4209 bats bridge b 10f
4209 coli bats guano 5h
2609us200cont bridge bats h20 9a
2609us200cont bridge bats h20 9b
2609US200cont.bridge bats 10b
4209 bats guano 4a
4209 coli bats guano 5e
4209 bats guano 3b
4209 bats guano 3c
4209 bats guano 3a
4209 bats dn 1000A 7g
81408 coli bats 1000 down "b" 12C


4209 bats dn 1000A 7g
81408 coli bats 1000 down "b" 12C
111709 coli bats guano 5d
4209 bats guano 1b
4209 bats guano 5b
82409 16675 coli H20 1 E
4209 bats dn1000 B 10c
4209 dn1000B 11g
4209 bats dn1000 B 10f
4209 bats dn1000 B 10b
4209 bats bridge A 6f
4209bats dn 200A 2A
4209 coli bats guano 6e
4209 coli bats guano 8b
4209 coli bats guano 9d
4209bats dn 200A 1a
4209bats dn 200A 3a
81408 bats coli 100'down "B" 7e
4209 dn200 B 6d
111709 coli bats guano 3e
111709 coli bats guano 3f
82409 16675 coli H20 2G
82409 16675 coli H20 1 c
82409 16675 coli H20 1 D
82409 16675 coli H20 2H
82409 16675 coli h2o 1b
4209 bats bridge A 7f
82409 16675 coli H20 2f
4209 bats guano 2c
4209 dn 200 B 5a
2509DS1000 2609 us 200 bats h20 1g
111709 coli bats guano 1g
111709 coli bats guano 2g
4209bats dn 200A 2h
81408 bats coli 100' down A 4b
81408 bats coli 100' down A 4f
111709 coli bats guano 4h
81408 bats coli 100' down A 4e
4209 bats bridge A 7b
4209bats dn 200A 1h
81408 bats coli 100'down "B" 6c
81408 bats coli 100' down A 5C
81408 bats coli 1000' down "A" 9e
81408 bats coli 100'down "A" 5e
81408 bats coli 100'down "A" 5g
81408 bats coli 100' down A 5d
81408 bats coli 100' down "B" 8a
81408 coli bats bridge B 3e
81408 coli bats 1000 ' down A 10g
81408 coli bats 1000 ' down A 10h
81408 bats coli 100' down A 3H
81408 coli bats bridge B 3f
81408 bats coli 100' down A 4d
111709 coli bats guano 6c
111709 coli bats guano 6f
111709 coli bats guano 8d
111709 coli bats guano 9f
4209 dn1000 A 7c
4209 dn200 B 6c
111709 coli bats guano 6g
82409 16675 coli H20 1H
111709 coli bats guano 8b
4209 dn 200 B 4g
4209bats dn 200A 2g


4209 dn 200 B 4g
4209bats dn 200A 2g
4209 dn200 B 8e
2509ds10002609us200bats h20 4g
81408 bats coli 100'down "A" 5h
4209 bats bridge B 8b
4209 bats guano 1h
81408 coli bats 1000' down B 12a
4209 bats bridge A 7h
2609 US 200 Bridge bats h20 12e
4209 bats guano 2d
111709 coli bats guano 8A
4209 bats guano 2h
4209 bats guano 2g
2509DS1000 2609 us 200 bats h20 2a
111709 coli bats guano 5a
4209 dn 200 B 4e
2509ds10002609us200bats h20 3h
82409 16675 coli H20 1g
51408 11353 1B bwl B
81408 bats coli 100'down "B" 6e
81408 bats coli 100'down "B" 7b
81408 bats coli 100' down A 5B
2509ds10002609us200bats h20 4h
2509DS1000 2609 us 200 bats h20 1f
2509DS1000 2609 us 200 bats h20 2F
2509DS1000 2609 us 200 bats h20 1e
2509DS1000 2609 us 200 bats h20 2e
4209 dn 200 B 5b
82409 16675 coli H20 2E
111709 coli bats guano 9g
4209 bats bridge b 10g
4209 bats bridge B 8f
4209 dn 200 A 4b
4209 bats dn1000 B 10a
4209 dn1000 A 7d
4209 bats dn1000 B 11a
4209bats dn 200A 2c
4209 bats bridge b 11b
4209 dn200 B 6b
4209 bats bridge B 8c
4209 bats bridge B 8d
4209 bats bridge A 8A
82409 16675 coli H20 2B
82409 16675 coli H20 3A
81408 bats coli bridge "B" 1d
4209 bats bridge A 5e
82409 16675 coli H20 1 F
82409 16675 coli H20 2d
4209 bats bridge A 7e
4209 coli bats guano 8g
4209 coli bats guano 9f
4209 coli bats guano 8f
4209 coli bats guano 8h
4209 coli bats guano 7h
4209 coli bats guano 7d
4209 coli bats guano 8d
4209 coli bats guano 8e
4209 coli bats guano 9g
4209 bats guano 10F
4209 bats bridge b 10b
4209 bats bridge B 9b
4209 bats bridge A 5h
4209 dn 200 A 4a
4209 dn 200 A 4c


4209 dn 200 A 4a
4209 dn 200 A 4c
4209bats dn 200A 2f
4209 coli bats guano 5f
4209 coli bats guano 7f
4209 coli bats guano 7g
4209 dn 200 A 3e
4209 coli bats guano 6f
4209 coli bats guano 6g
4209 bats bridge A 7c
4209 coli bats guano 5g
4209 bats guano 1f
4209 bats bridge A 6e
82409 16675 coli H20 3B
4209 bats guano 3d
4209 bats guano 3e
4209 bats guano 2e
4209 coli bats bridge A 4g
4209 coli bats bridge A 4H
4209 bats bridge B 9g
4209 bats bridge B 9h
4209 bats bridge A 6g
4209 bats dn 1000A 8e
2609us200cont bridge bats h20 8c
51408 11353 5G lc B
82409 16675 coli H20 2a
51408 11353 5H lc B
4209 bats guano 4g
4209 bats guano 4h
4209 bats guano 4e
4209 bats guano 4d
4209 bats guano 4f
4209 bats bridge B 8e
4209 bats bridge B 8h
2509ds10002609us200bats h20 4c
51408 11353 6B LC B
2609US200cont.bridge bats 10g
2609US200cont.bridge bats 11b
2609US200cont.bridge bats 10a
4209 bats bridge B 8g
4209 bats bridge B 9d
4209 dn200b 5e
4209bats dn 200A 1b
4209 bats bridge A 5f
4209 dn 200 B 5c
51408 11353 coli LC A 2A
51408 coli 11353 9H lc B
51408 11353 4G LC A
51408 11353 coli LC A 1H
51408 11353 coli LC A 1F
51408 11353 coli LC A 1G
51408 11353 3E LC A
51408 11353 3G lc A
51408 11353 7F LC B
51408 11353 coli LC A 2C
51408 11353 coli LC A 2D
51408 coli 11353 9G lc B
4209 bats guano 3h
51408 11353 6C lc B
51408 coli 11353 8H lc B
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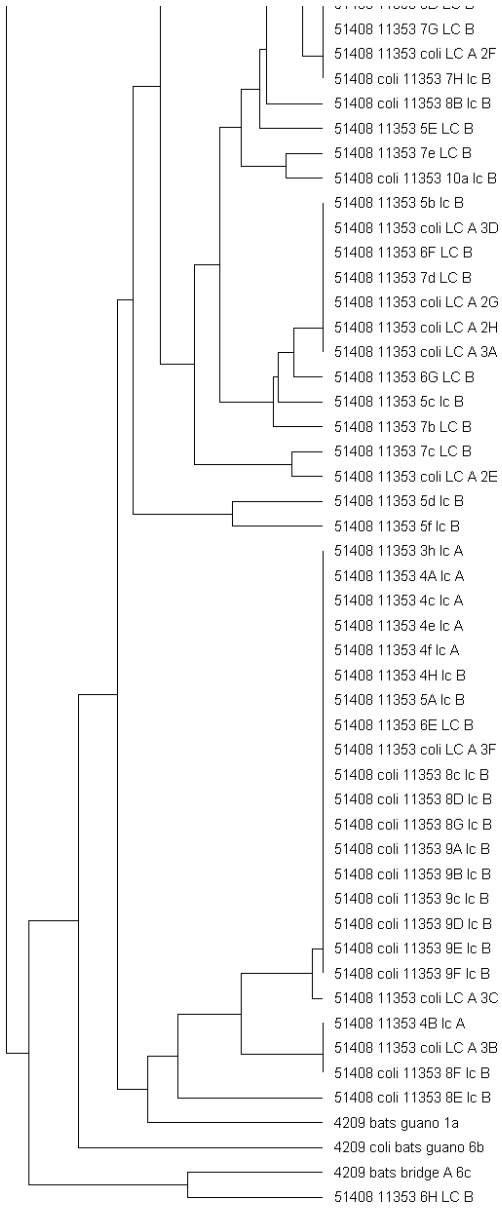
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51408 coli 11353 10a lc B
51408 11353 5b lc B
51408 11353 coli LC A 3D
51408 11353 6F LC B
51408 11353 7d LC B
51408 11353 coli LC A 2G
51408 11353 coli LC A 2H
51408 11353 coli LC A 3A
51408 11353 6G LC B
51408 11353 5c lc B
51408 11353 7b LC B
51408 11353 7c LC B
51408 11353 coli LC A 2E
51408 11353 5d lc B
51408 11353 5f lc B
51408 11353 3h lc A
51408 11353 4A lc A
51408 11353 4c lc A
51408 11353 4e lc A
51408 11353 4f lc A
51408 11353 4H lc B
51408 11353 5A lc B
51408 11353 6E LC B
51408 11353 coli LC A 3F
51408 coli 11353 8c lc B
51408 coli 11353 8D lc B
51408 coli 11353 8G lc B
51408 coli 11353 9A lc B
51408 coli 11353 9B lc B
51408 coli 11353 9c lc B
51408 coli 11353 9D lc B
51408 coli 11353 9E lc B
51408 coli 11353 9F lc B
51408 11353 coli LC A 3C
51408 11353 4B lc A
51408 11353 coli LC A 3B
51408 coli 11353 8F lc B
51408 coli 11353 8E lc B
4209 bats guano 1a
4209 coli bats guano 6b
4209 bats bridge A 6c
51408 11353 6H LC B

Figure 9. Sugar Utilization Testing for all Isolates
Dice Coefficient Algorithm-UPGMA of Binary Data

1000' Down 1F-AUG 14 08
1000' Down 1G-AUG 14 08
1000' Down 2A-FEB 05 09
1000' Down 2B-FEB 05 09
1000' Down 2C-AUG 14 08
1000' Down 2C-FEB 05 09
1000' Down 2F-FEB 05 09
1000' Down 2H-FEB 05 09
1000' Down 3B-FEB 05 09
1000' Down 3C-FEB 05 09
1000' Down 3D-FEB 05 09
1000' Down 3E-FEB 05 09
1000' Down 3F-FEB 05 09
1000' Down 3G-FEB 05 09
1000' Down 3H-FEB 05 09
1000' Down 4A-FEB 05 09
1000' Down 4B-FEB 05 09
1000' Down 4C- FEB 06 09
1000' Down 4D- FEB 06 09
1000' Down 4D-FEB 05 09
1000' Down 4E- FEB 06 09
1000' Down 4E-FEB 05 09
1000' Down 4F- FEB 06 09
1000' Down 4F-FEB 05 09
1000' Down 4G- FEB 06 09
1000' Down 4G-FEB 05 09
1000' Down 4H- FEB 06 09
1000' Down 4H-FEB 05 09
1000' Down 5A- FEB 06 09
1000' Down 5B- FEB 06 09
1000' Down 5C- FEB 06 09
1000' Down 5D- FEB 06 09
1000' Down 5D-FEB 05 09
1000' Down 5E- FEB 06 09
1000' Down 5E-FEB 05 09
1000' Down 5F- FEB 06 09
1000' Down 5F-FEB 05 09
1000' Down 5G- FEB 06 09
1000' Down 5H- FEB 06 09
1000' Down 6A- FEB 06 09
1000' Down 6A-FEB 05 09
1000' Down 6B- FEB 06 09
1000' Down 6B-FEB 05 09
1000' Down 6C- FEB 06 09
1000' Down 6C-FEB 05 09
1000' Down 6D- FEB 06 09
1000' Down 6D-FEB 05 09
1000' Down 6E- FEB 06 09
1000' Down 6E-FEB 05 09
1000' Down 6F- FEB 06 09
1000' Down 6F-FEB 05 09
1000' Down 6G- FEB 06 09
1000' Down 6G-APR 02 09
1000' Down 6H- FEB 06 09
1000' Down 6H-APR 02 09
1000' Down 7A- FEB 06 09
1000' Down 7A-FEB 05 09
1000' Down 7B- FEB 06 09
1000' Down 7B-APR 02 09
1000' Down 7B-AUG 14 08
1000' Down 7B-FEB 05 09
1000' Down 7C- FEB 06 09
1000' Down 7C-APR 02 09
1000' Down 7C-AUG 14 08

1000' Down 7C- FEB 06 09
1000' Down 7C-APR 02 09
1000' Down 7C-AUG 14 08
1000' Down 7C-FEB 05 09
1000' Down 7D-APR 02 09
1000' Down 7D-AUG 14 08
1000' Down 7E-APR 02 09
1000' Down 7F-APR 02 09
1000' Down 7F-AUG 14 08
1000' Down 7G-APR 02 09
1000' Down 7G-AUG 14 08
1000' Down 7H-APR 02 09
1000' Down 8A-APR 02 09
1000' Down 8B- FEB 06 09
1000' Down 8B-APR 02 09
1000' Down 8B-AUG 14 08
1000' Down 8C- FEB 06 09
1000' Down 8C-APR 02 09
1000' Down 8D- FEB 06 09
1000' Down 8E- FEB 06 09
1000' Down 8E-APR 02 09
1000' Down 8F- FEB 06 09
1000' Down 8F-APR 02 09
1000' Down 8G- FEB 06 09
1000' Down 8G-APR 02 09
1000' Down 8H- FEB 06 09
1000' Down 8H-APR 02 09
1000' Down 9A- FEB 06 09
1000' Down 9A-APR 02 09
1000' Down 9B- FEB 06 09
1000' Down 9B-APR 02 09
1000' Down 9C- FEB 06 09
1000' Down 9D- FEB 06 09
1000' Down 9E- FEB 06 09
1000' Down 9E-APR 02 09
1000' Down 9F- FEB 06 09
1000' Down 9G- FEB 06 09
1000' Down 9G-APR 02 09
1000' Down 9G-AUG 14 08
1000' Down 9H- FEB 06 09
1000' Down 9H-APR 02 09
11353 H2O 10A0May 14 08
11353 H2O 10B0May 14 08
11353 H2O 1B0Aug 06 08
11353 H2O 1B0May 14 08
11353 H2O 1C0Aug 06 08
11353 H2O 1D0Aug 06 08
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11353 H2O 1E0May 14 08
11353 H2O 1F0May 14 08
11353 H2O 1G0Aug 06 08
11353 H2O 1G0May 14 08
11353 H2O 2A0May 14 08
11353 H2O 2B0Aug 06 08
11353 H2O 2B0May 14 08
11353 H2O 2C0Aug 06 08
11353 H2O 2D0Aug 06 08
11353 H2O 2D0May 14 08
11353 H2O 2E0Aug 06 08
11353 H2O 2E0May 14 08
11353 H2O 2F0Aug 06 08
11353 H2O 2F0May 14 08

11353 H2O 2F0Aug 06 08
11353 H2O 2F0May 14 08
11353 H2O 2G0Aug 06 08
11353 H2O 2G0May 14 08
11353 H2O 2H0May 14 08
11353 H2O 3A0Aug 06 08
11353 H2O 3A0May 14 08
11353 H2O 3B0May 14 08
11353 H2O 3C0May 14 08
11353 H2O 3D0Aug 06 08
11353 H2O 3D0May 14 08
11353 H2O 3E0Aug 06 08
11353 H2O 3E0May 14 08
11353 H2O 3F0Aug 06 08
11353 H2O 3F0May 14 08
11353 H2O 3G0Aug 06 08
11353 H2O 3G0May 14 08
11353 H2O 3H0Aug 06 08
11353 H2O 3H0May 14 08
11353 H2O 4A0Aug 06 08
11353 H2O 4A0May 14 08
11353 H2O 4B0May 14 08
11353 H2O 4C0May 14 08
11353 H2O 4D0May 14 08
11353 H2O 4E0Aug 06 08
11353 H2O 4E0May 14 08
11353 H2O 4F0Aug 06 08
11353 H2O 4F0May 14 08
11353 H2O 4G0Aug 06 08
11353 H2O 4G0May 14 08
11353 H2O 4H0Aug 06 08
11353 H2O 4H0May 14 08
11353 H2O 5A0Aug 06 08
11353 H2O 5B0Aug 06 08
11353 H2O 5B0May 14 08
11353 H2O 5C0May 14 08
11353 H2O 5D0Aug 06 08
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11353 H2O 5E0May 14 08
11353 H2O 5F0Aug 06 08
11353 H2O 5F0May 14 08
11353 H2O 5G0Aug 06 08
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11353 H2O 7C0May 14 08
11353 H2O 7D0May 14 08
11353 H2O 7E0May 14 08
11353 H2O 7F0May 14 08
11353 H2O 7G0May 14 08
11353 H2O 7H0May 14 08
11353 H2O 8A0May 14 08
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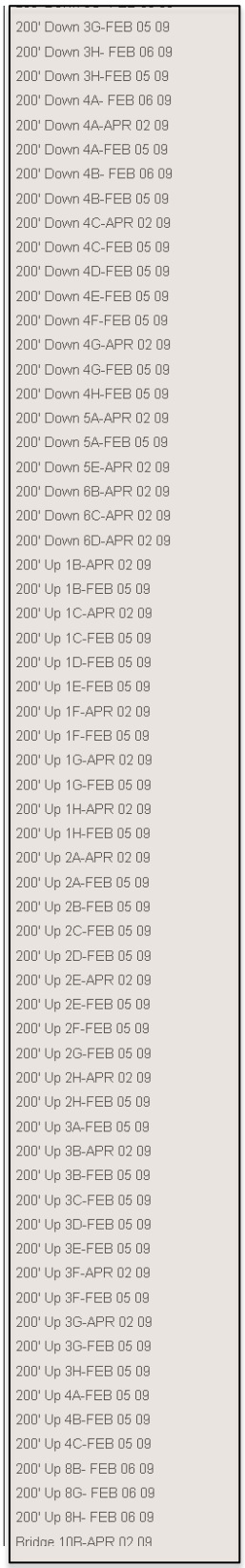
11353 H2O 8B0May 14 08
11353 H2O 8C0May 14 08
11353 H2O 8D0May 14 08
11353 H2O 8E0May 14 08
11353 H2O 8F0May 14 08
11353 H2O 8G0May 14 08
11353 H2O 8H0May 14 08
11353 H2O 9A0May 14 08
11353 H2O 9B0May 14 08
11353 H2O 9C0May 14 08
11353 H2O 9D0May 14 08
11353 H2O 9E0May 14 08
11353 H2O 9F0May 14 08
11353 H2O 9G0May 14 08
11353 H2O 9H0May 14 08
200' Down 1B- FEB 06 09
200' Down 1B-APR 02 09
200' Down 1B-FEB 05 09
200' Down 1C- FEB 06 09
200' Down 1C-FEB 05 09
200' Down 1D- FEB 06 09
200' Down 1D-FEB 05 09
200' Down 1E- FEB 06 09
200' Down 1E-FEB 05 09
200' Down 1F- FEB 06 09
200' Down 1F-FEB 05 09
200' Down 1G- FEB 06 09
200' Down 1G-FEB 05 09
200' Down 1H- FEB 06 09
200' Down 1H-FEB 05 09
200' Down 2A- FEB 06 09
200' Down 2A-APR 02 09
200' Down 2A-FEB 05 09
200' Down 2B- FEB 06 09
200' Down 2B-FEB 05 09
200' Down 2C- FEB 06 09
200' Down 2C-FEB 05 09
200' Down 2D- FEB 06 09
200' Down 2E- FEB 06 09
200' Down 2E-APR 02 09
200' Down 2E-FEB 05 09
200' Down 2F- FEB 06 09
200' Down 2F-APR 02 09
200' Down 2F-FEB 05 09
200' Down 2G- FEB 06 09
200' Down 2G-FEB 05 09
200' Down 2H- FEB 06 09
200' Down 2H-FEB 05 09
200' Down 3A- FEB 06 09
200' Down 3A-APR 02 09
200' Down 3A-FEB 05 09
200' Down 3B- FEB 06 09
200' Down 3B-FEB 05 09
200' Down 3C- FEB 06 09
200' Down 3C-FEB 05 09
200' Down 3D- FEB 06 09
200' Down 3E- FEB 06 09
200' Down 3E-APR 02 09
200' Down 3F- FEB 06 09
200' Down 3F-FEB 05 09
200' Down 3G- FEB 06 09
200' Down 3G-FEB 05 09
200' Down 3H- FEB 06 09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200' Down 3H- FEB 06 09
200' Down 3H-FEB 05 09
200' Down 4A- FEB 06 09
200' Down 4A-APR 02 09
200' Down 4A-FEB 05 09
200' Down 4B- FEB 06 09
200' Down 4B-FEB 05 09
200' Down 4C-APR 02 09
200' Down 4C-FEB 05 09
200' Down 4D-FEB 05 09
200' Down 4E-FEB 05 09
200' Down 4F-FEB 05 09
200' Down 4G-APR 02 09
200' Down 4G-FEB 05 09
200' Down 4H-FEB 05 09
200' Down 5A-APR 02 09
200' Down 5A-FEB 05 09
200' Down 5E-APR 02 09
200' Down 6B-APR 02 09
200' Down 6C-APR 02 09
200' Down 6D-APR 02 09
200' Up 1B-APR 02 09
200' Up 1B-FEB 05 09
200' Up 1C-APR 02 09
200' Up 1C-FEB 05 09
200' Up 1D-FEB 05 09
200' Up 1E-FEB 05 09
200' Up 1F-APR 02 09
200' Up 1F-FEB 05 09
200' Up 1G-APR 02 09
200' Up 1G-FEB 05 09
200' Up 1H-APR 02 09
200' Up 1H-FEB 05 09
200' Up 2A-APR 02 09
200' Up 2A-FEB 05 09
200' Up 2B-FEB 05 09
200' Up 2C-FEB 05 09
200' Up 2D-FEB 05 09
200' Up 2E-APR 02 09
200' Up 2E-FEB 05 09
200' Up 2F-FEB 05 09
200' Up 2G-FEB 05 09
200' Up 2H-APR 02 09
200' Up 2H-FEB 05 09
200' Up 3A-FEB 05 09
200' Up 3B-APR 02 09
200' Up 3B-FEB 05 09
200' Up 3C-FEB 05 09
200' Up 3D-FEB 05 09
200' Up 3E-FEB 05 09
200' Up 3F-APR 02 09
200' Up 3F-FEB 05 09
200' Up 3G-APR 02 09
200' Up 3G-FEB 05 09
200' Up 3H-FEB 05 09
200' Up 4A-FEB 05 09
200' Up 4B-FEB 05 09
200' Up 4C-FEB 05 09
200' Up 8B- FEB 06 09
200' Up 8G- FEB 06 09
200' Up 8H- FEB 06 09
Bridge 10R-APR 02 09

200' Up 8G- FEB 06 09
200' Up 8H- FEB 06 09
Bridge 10B-APR 02 09
Bridge 10C- FEB 06 09
Bridge 10C-APR 02 09
Bridge 10D- FEB 06 09
Bridge 10F- FEB 06 09
Bridge 10F-APR 02 09
Bridge 10G- FEB 06 09
Bridge 10H- FEB 06 09
Bridge 11B- FEB 06 09
Bridge 11C- FEB 06 09
Bridge 11C-APR 02 09
Bridge 11D- FEB 06 09
Bridge 11D-APR 02 09
Bridge 11F- FEB 06 09
Bridge 11G- FEB 06 09
Bridge 11H- FEB 06 09
Bridge 12A- FEB 06 09
Bridge 12B- FEB 06 09
Bridge 12C- FEB 06 09
Bridge 12D- FEB 06 09
Bridge 12F- FEB 06 09
Bridge 12G- FEB 06 09
Bridge 12H- FEB 06 09
Bridge 3A-AUG 14 08
Bridge 3C-AUG 14 08
Bridge 4D-FEB 05 09
Bridge 4E-FEB 05 09
Bridge 4F-FEB 05 09
Bridge 4G-APR 02 09
Bridge 4G-FEB 05 09
Bridge 4H-FEB 05 09
Bridge 5A-FEB 05 09
Bridge 5B-APR 02 09
Bridge 5B-FEB 05 09
Bridge 5C-APR 02 09
Bridge 5C-FEB 05 09
Bridge 5D-APR 02 09
Bridge 5D-FEB 05 09
Bridge 5E-FEB 05 09
Bridge 5F-APR 02 09
Bridge 5F-FEB 05 09
Bridge 5G-FEB 05 09
Bridge 5H-APR 02 09
Bridge 5H-FEB 05 09
Bridge 6A-APR 02 09
Bridge 6A-FEB 05 09
Bridge 6B-APR 02 09
Bridge 6B-FEB 05 09
Bridge 6C-APR 02 09
Bridge 6C-FEB 05 09
Bridge 6D-APR 02 09
Bridge 6D-FEB 05 09
Bridge 6E-APR 02 09
Bridge 6E-FEB 05 09
Bridge 6F-APR 02 09
Bridge 6F-FEB 05 09
Bridge 6G-APR 02 09
Bridge 6G-FEB 05 09
Bridge 6H-APR 02 09
Bridge 6H-FEB 05 09
Bridge 7A-FEB 05 09
Bridge 7B-APR 02 09

Bridge 8H-FEB 05 09
Bridge 7A-FEB 05 09
Bridge 7B-APR 02 09
Bridge 7C-APR 02 09
Bridge 7D- FEB 06 09
Bridge 7D-APR 02 09
Bridge 7E- FEB 06 09
Bridge 7E-APR 02 09
Bridge 7F-APR 02 09
Bridge 7G-APR 02 09
Bridge 7H-APR 02 09
Bridge 8A-APR 02 09
Bridge 8B-APR 02 09
Bridge 8D-APR 02 09
Bridge 8F-APR 02 09
Bridge 9A- FEB 06 09
Bridge 9A-APR 02 09
Bridge 9B- FEB 06 09
Bridge 9B-APR 02 09
Bridge 9D-APR 02 09
Bridge 9G- FEB 06 09
Bridge 9H- FEB 06 09
Guano 10A-APR 02 09
Guano 10A-FEB 06 09
Guano 10B-APR 02 09
Guano 10B-FEB 06 09
Guano 10C-APR 02 09
Guano 10C-FEB 06 09
Guano 10D-APR 02 09
Guano 10D-FEB 06 09
Guano 10E-APR 02 09
Guano 10E-FEB 06 09
Guano 10F-APR 02 09
Guano 10F-FEB 06 09
Guano 10G-APR 02 09
Guano 10H-APR 02 09
Guano 11A-APR 02 09
Guano 11B-APR 02 09
Guano 11C-APR 02 09
Guano 11D-APR 02 09
Guano 11E-APR 02 09
Guano 11F-APR 02 09
Guano 11G-APR 02 09
Guano 11H-APR 02 09
Guano 12A-APR 02 09
Guano 12B-APR 02 09
Guano 12C-APR 02 09
Guano 12D-APR 02 09
Guano 12E-APR 02 09
Guano 12F-APR 02 09
Guano 12G-APR 02 09
Guano 12H-APR 02 09
Guano 1F-APR 02 09
Guano 1H-APR 02 09
Guano 2B-APR 02 09
Guano 2E-APR 02 09
Guano 2G-APR 02 09
Guano 3A-APR 02 09
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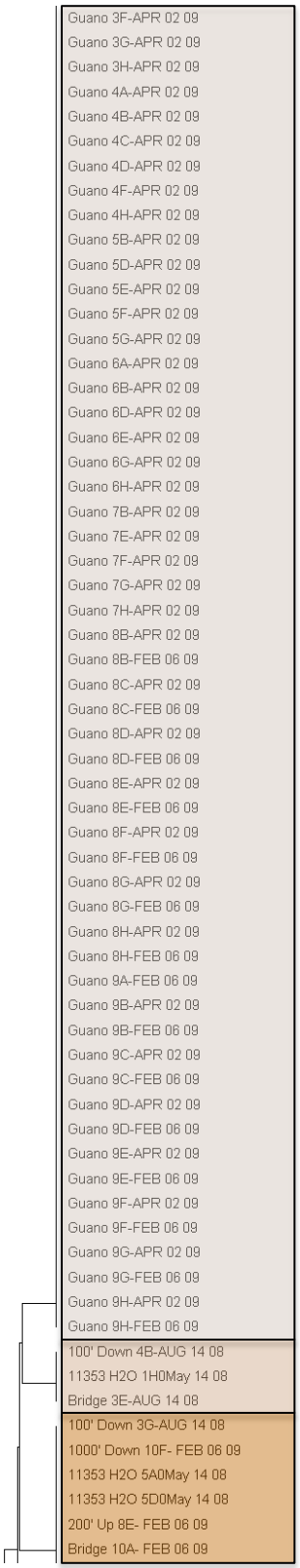
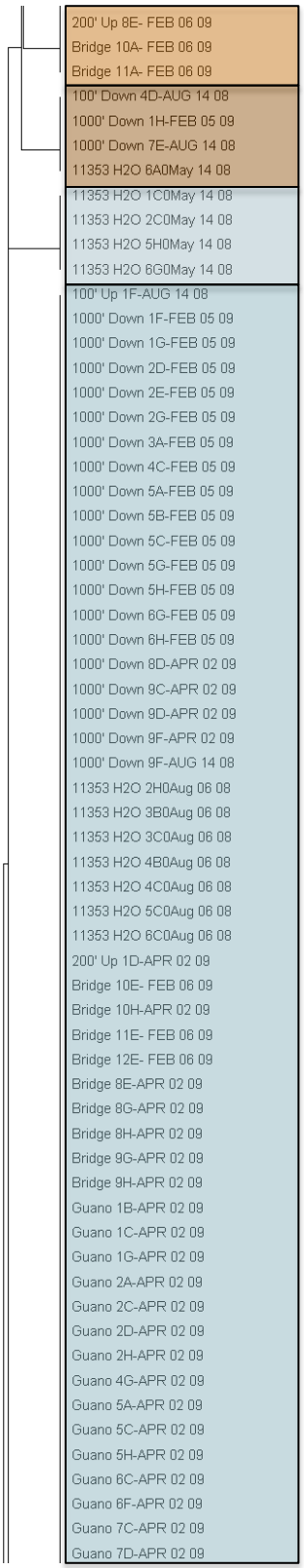
Guano 3C-APR 02 09
Guano 3E-APR 02 09
Guano 3F-APR 02 09
Guano 3G-APR 02 09


Guano 3F-APR 02 09
Guano 3G-APR 02 09
Guano 3H-APR 02 09
Guano 4A-APR 02 09
Guano 4B-APR 02 09
Guano 4C-APR 02 09
Guano 4D-APR 02 09
Guano 4F-APR 02 09
Guano 4H-APR 02 09
Guano 5B-APR 02 09
Guano 5D-APR 02 09
Guano 5E-APR 02 09
Guano 5F-APR 02 09
Guano 5G-APR 02 09
Guano 6A-APR 02 09
Guano 6B-APR 02 09
Guano 6D-APR 02 09
Guano 6E-APR 02 09
Guano 6G-APR 02 09
Guano 6H-APR 02 09
Guano 7B-APR 02 09
Guano 7E-APR 02 09
Guano 7F-APR 02 09
Guano 7G-APR 02 09
Guano 7H-APR 02 09
Guano 8B-APR 02 09
Guano 8B-FEB 06 09
Guano 8C-APR 02 09
Guano 8C-FEB 06 09
Guano 8D-APR 02 09
Guano 8D-FEB 06 09
Guano 8E-APR 02 09
Guano 8E-FEB 06 09
Guano 8F-APR 02 09
Guano 8F-FEB 06 09
Guano 8G-APR 02 09
Guano 8G-FEB 06 09
Guano 8H-APR 02 09
Guano 8H-FEB 06 09
Guano 9A-FEB 06 09
Guano 9B-APR 02 09
Guano 9B-FEB 06 09
Guano 9C-APR 02 09
Guano 9C-FEB 06 09
Guano 9D-APR 02 09
Guano 9D-FEB 06 09
Guano 9E-APR 02 09
Guano 9E-FEB 06 09
Guano 9F-APR 02 09
Guano 9F-FEB 06 09
Guano 9G-APR 02 09
Guano 9G-FEB 06 09
Guano 9H-APR 02 09
Guano 9H-FEB 06 09
100' Down 4B-AUG 14 08
11353 H2O 1H0May 14 08
Bridge 3E-AUG 14 08
100' Down 3G-AUG 14 08
1000' Down 10F- FEB 06 09
11353 H2O 5A0May 14 08
11353 H2O 5D0May 14 08
200' Up 8E- FEB 06 09
Bridge 10A- FEB 06 09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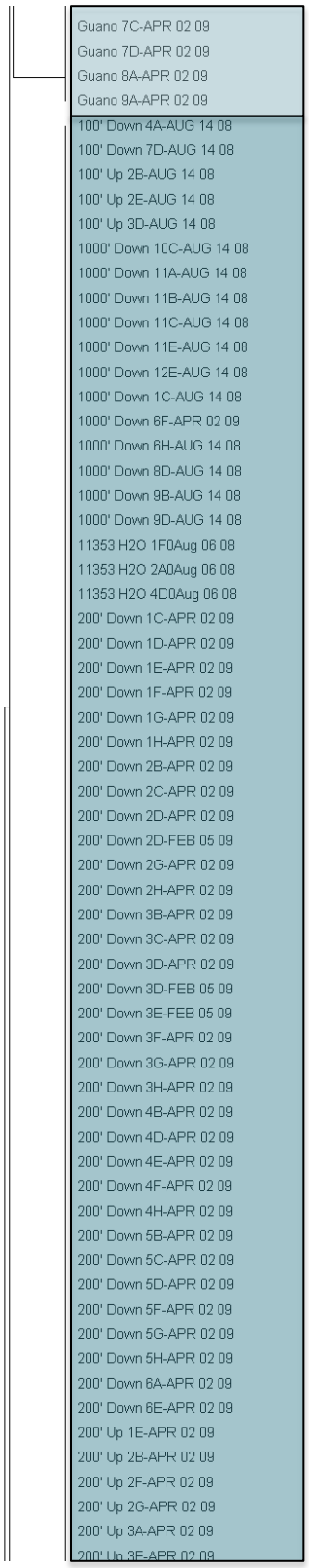
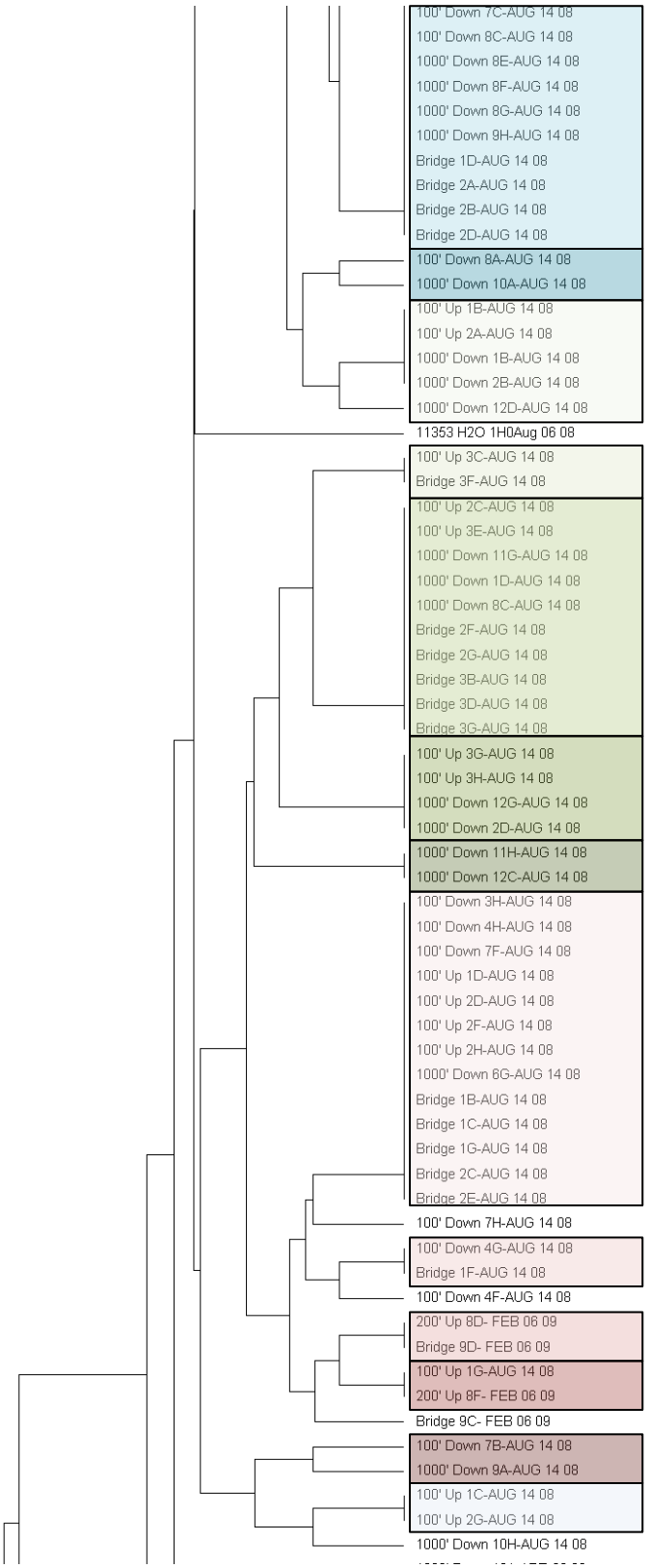
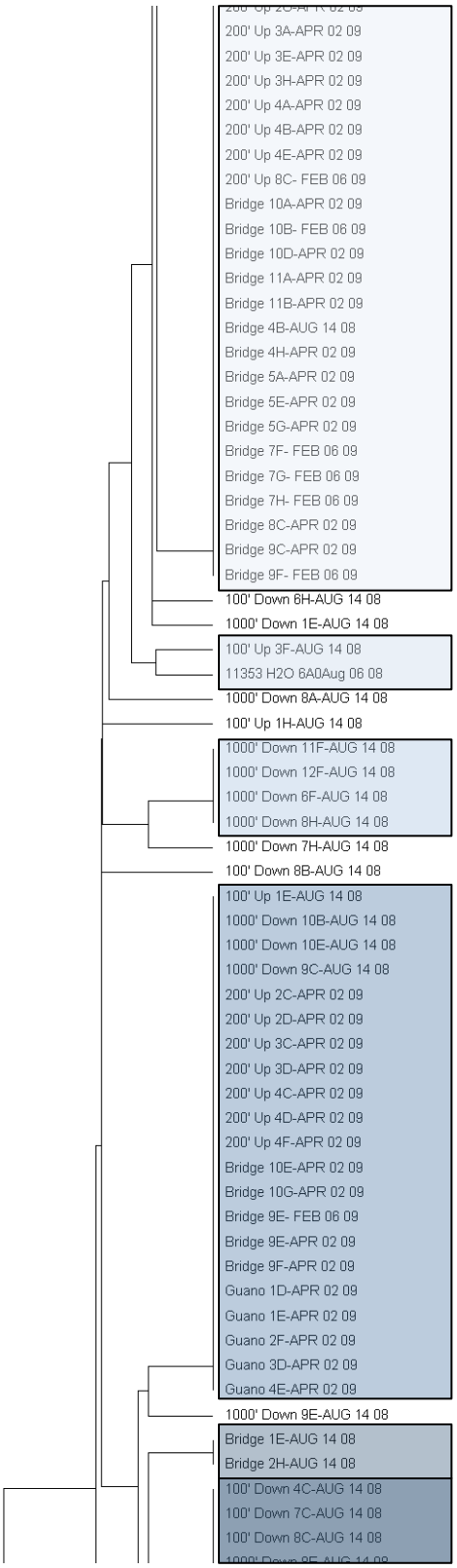
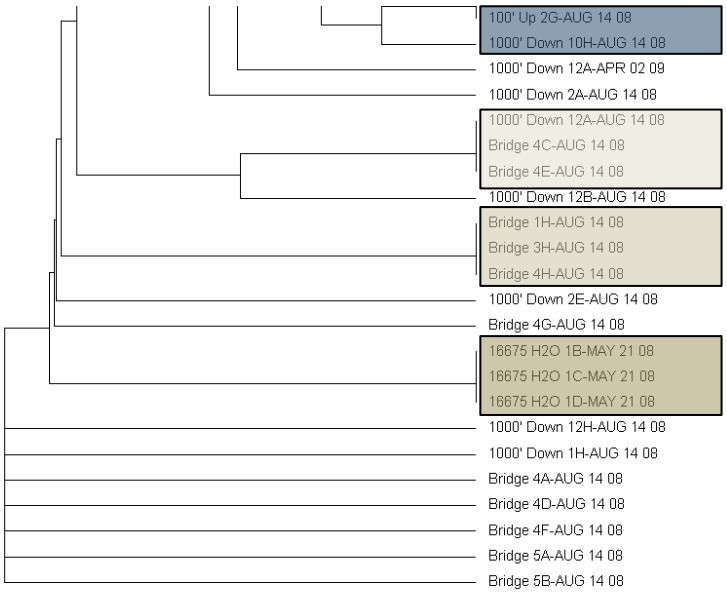
Bridge 10A- FEB 06 09
Bridge 11A- FEB 06 09
100' Down 4D-AUG 14 08
1000' Down 1H-FEB 05 09
1000' Down 7E-AUG 14 08
11353 H2O 6A0May 14 08
11353 H2O 1C0May 14 08
11353 H2O 2C0May 14 08
11353 H2O 5H0May 14 08
11353 H2O 6G0May 14 08
100' Up 1F-AUG 14 08
1000' Down 1F-FEB 05 09
1000' Down 1G-FEB 05 09
1000' Down 2D-FEB 05 09
1000' Down 2E-FEB 05 09
1000' Down 2G-FEB 05 09
1000' Down 3A-FEB 05 09
1000' Down 4C-FEB 05 09
1000' Down 5A-FEB 05 09
1000' Down 5B-FEB 05 09
1000' Down 5C-FEB 05 09
1000' Down 5G-FEB 05 09
1000' Down 5H-FEB 05 09
1000' Down 6G-FEB 05 09
1000' Down 6H-FEB 05 09
1000' Down 8D-APR 02 09
1000' Down 9C-APR 02 09
1000' Down 9D-APR 02 09
1000' Down 9F-APR 02 09
1000' Down 9F-AUG 14 08
11353 H2O 2H0Aug 06 08
11353 H2O 3B0Aug 06 08
11353 H2O 3C0Aug 06 08
11353 H2O 4B0Aug 06 08
11353 H2O 4C0Aug 06 08
11353 H2O 5C0Aug 06 08
11353 H2O 6C0Aug 06 08
200' Up 1D-APR 02 09
Bridge 10E- FEB 06 09
Bridge 10H-APR 02 09
Bridge 11E- FEB 06 09
Bridge 12E- FEB 06 09
Bridge 8E-APR 02 09
Bridge 8G-APR 02 09
Bridge 8H-APR 02 09
Bridge 9G-APR 02 09
Bridge 9H-APR 02 09
Guano 1B-APR 02 09
Guano 1C-APR 02 09
Guano 1G-APR 02 09
Guano 2A-APR 02 09
Guano 2C-APR 02 09
Guano 2D-APR 02 09
Guano 2H-APR 02 09
Guano 4G-APR 02 09
Guano 5A-APR 02 09
Guano 5C-APR 02 09
Guano 5H-APR 02 09
Guano 6C-APR 02 09
Guano 6F-APR 02 09
Guano 7C-APR 02 09
Guano 7D-APR 02 09


Guano 7C-APR 02 09
Guano 7D-APR 02 09
Guano 8A-APR 02 09
Guano 9A-APR 02 09
100' Down 4A-AUG 14 08
100' Down 7D-AUG 14 08
100' Up 2B-AUG 14 08
100' Up 2E-AUG 14 08
100' Up 3D-AUG 14 08
1000' Down 10C-AUG 14 08
1000' Down 11A-AUG 14 08
1000' Down 11B-AUG 14 08
1000' Down 11C-AUG 14 08
1000' Down 11E-AUG 14 08
1000' Down 12E-AUG 14 08
1000' Down 1C-AUG 14 08
1000' Down 6F-APR 02 09
1000' Down 6H-AUG 14 08
1000' Down 8D-AUG 14 08
1000' Down 9B-AUG 14 08
1000' Down 9D-AUG 14 08
11353 H2O 1F0Aug 06 08
11353 H2O 2A0Aug 06 08
11353 H2O 4D0Aug 06 08
200' Down 1C-APR 02 09
200' Down 1D-APR 02 09
200' Down 1E-APR 02 09
200' Down 1F-APR 02 09
200' Down 1G-APR 02 09
200' Down 1H-APR 02 09
200' Down 2B-APR 02 09
200' Down 2C-APR 02 09
200' Down 2D-APR 02 09
200' Down 2D-FEB 05 09
200' Down 2G-APR 02 09
200' Down 2H-APR 02 09
200' Down 3B-APR 02 09
200' Down 3C-APR 02 09
200' Down 3D-APR 02 09
200' Down 3D-FEB 05 09
200' Down 3E-FEB 05 09
200' Down 3F-APR 02 09
200' Down 3G-APR 02 09
200' Down 3H-APR 02 09
200' Down 4B-APR 02 09
200' Down 4D-APR 02 09
200' Down 4E-APR 02 09
200' Down 4F-APR 02 09
200' Down 4H-APR 02 09
200' Down 5B-APR 02 09
200' Down 5C-APR 02 09
200' Down 5D-APR 02 09
200' Down 5F-APR 02 09
200' Down 5G-APR 02 09
200' Down 5H-APR 02 09
200' Down 6A-APR 02 09
200' Down 6E-APR 02 09
200' Up 1E-APR 02 09
200' Up 2B-APR 02 09
200' Up 2F-APR 02 09
200' Up 2G-APR 02 09
200' Up 3A-APR 02 09
200' Up 3E-APR 02 09


100' Down 7C-AUG 14 08
100' Down 8C-AUG 14 08
1000' Down 8E-AUG 14 08
1000' Down 8F-AUG 14 08
1000' Down 8G-AUG 14 08
1000' Down 9H-AUG 14 08
Bridge 1D-AUG 14 08
Bridge 2A-AUG 14 08
Bridge 2B-AUG 14 08
Bridge 2D-AUG 14 08
100' Down 8A-AUG 14 08
1000' Down 10A-AUG 14 08
100' Up 1B-AUG 14 08
100' Up 2A-AUG 14 08
1000' Down 1B-AUG 14 08
1000' Down 2B-AUG 14 08
1000' Down 12D-AUG 14 08
11353 H2O 1H0Aug 06 08
100' Up 3C-AUG 14 08
Bridge 3F-AUG 14 08
100' Up 2C-AUG 14 08
100' Up 3E-AUG 14 08
1000' Down 11G-AUG 14 08
1000' Down 1D-AUG 14 08
1000' Down 8C-AUG 14 08
Bridge 2F-AUG 14 08
Bridge 2G-AUG 14 08
Bridge 3B-AUG 14 08
Bridge 3D-AUG 14 08
Bridge 3G-AUG 14 08
100' Up 3G-AUG 14 08
100' Up 3H-AUG 14 08
1000' Down 12G-AUG 14 08
1000' Down 2D-AUG 14 08
1000' Down 11H-AUG 14 08
1000' Down 12C-AUG 14 08
100' Down 3H-AUG 14 08
100' Down 4H-AUG 14 08
100' Down 7F-AUG 14 08
100' Up 1D-AUG 14 08
100' Up 2D-AUG 14 08
100' Up 2F-AUG 14 08
100' Up 2H-AUG 14 08
1000' Down 6G-AUG 14 08
Bridge 1B-AUG 14 08
Bridge 1C-AUG 14 08
Bridge 1G-AUG 14 08
Bridge 2C-AUG 14 08
Bridge 2E-AUG 14 08
100' Down 7H-AUG 14 08
100' Down 4G-AUG 14 08
Bridge 1F-AUG 14 08
100' Down 4F-AUG 14 08
200' Up 8D- FEB 06 09
Bridge 9D- FEB 06 09
100' Up 1G-AUG 14 08
200' Up 8F- FEB 06 09
Bridge 9C- FEB 06 09
100' Down 7B-AUG 14 08
1000' Down 9A-AUG 14 08
100' Up 1C-AUG 14 08
100' Up 2G-AUG 14 08
1000' Down 10H-AUG 14 08


200' Up 3A-APR 02 09
200' Up 3E-APR 02 09
200' Up 3H-APR 02 09
200' Up 4A-APR 02 09
200' Up 4B-APR 02 09
200' Up 4E-APR 02 09
200' Up 8C- FEB 06 09
Bridge 10A-APR 02 09
Bridge 10B- FEB 06 09
Bridge 10D-APR 02 09
Bridge 11A-APR 02 09
Bridge 11B-APR 02 09
Bridge 4B-AUG 14 08
Bridge 4H-APR 02 09
Bridge 5A-APR 02 09
Bridge 5E-APR 02 09
Bridge 5G-APR 02 09
Bridge 7F- FEB 06 09
Bridge 7G- FEB 06 09
Bridge 7H- FEB 06 09
Bridge 8C-APR 02 09
Bridge 9C-APR 02 09
Bridge 9F- FEB 06 09
100' Down 6H-AUG 14 08
1000' Down 1E-AUG 14 08
100' Up 3F-AUG 14 08
11353 H2O 6A0Aug 06 08
1000' Down 8A-AUG 14 08
100' Up 1H-AUG 14 08
1000' Down 11F-AUG 14 08
1000' Down 12F-AUG 14 08
1000' Down 6F-AUG 14 08
1000' Down 8H-AUG 14 08
1000' Down 7H-AUG 14 08
100' Down 8B-AUG 14 08
100' Up 1E-AUG 14 08
1000' Down 10B-AUG 14 08
1000' Down 10E-AUG 14 08
1000' Down 9C-AUG 14 08
200' Up 2C-APR 02 09
200' Up 2D-APR 02 09
200' Up 3C-APR 02 09
200' Up 3D-APR 02 09
200' Up 4C-APR 02 09
200' Up 4D-APR 02 09
200' Up 4F-APR 02 09
Bridge 10E-APR 02 09
Bridge 10G-APR 02 09
Bridge 9E- FEB 06 09
Bridge 9E-APR 02 09
Bridge 9F-APR 02 09
Guano 1D-APR 02 09
Guano 1E-APR 02 09
Guano 2F-APR 02 09
Guano 3D-APR 02 09
Guano 4E-APR 02 09
1000' Down 9E-AUG 14 08
Bridge 1E-AUG 14 08
Bridge 2H-AUG 14 08
100' Down 4C-AUG 14 08
100' Down 7C-AUG 14 08
100' Down 8C-AUG 14 08


100' Up 2G-AUG 14 08
1000' Down 10H-AUG 14 08
1000' Down 12A-APR 02 09
1000' Down 2A-AUG 14 08
1000' Down 12A-AUG 14 08
Bridge 4C-AUG 14 08
Bridge 4E-AUG 14 08
1000' Down 12B-AUG 14 08
Bridge 1H-AUG 14 08
Bridge 3H-AUG 14 08
Bridge 4H-AUG 14 08
1000' Down 2E-AUG 14 08
Bridge 4G-AUG 14 08
16675 H2O 1B-MAY 21 08
16675 H2O 1C-MAY 21 08
16675 H2O 1D-MAY 21 08
1000' Down 12H-AUG 14 08
1000' Down 1H-AUG 14 08
Bridge 4A-AUG 14 08
Bridge 4D-AUG 14 08
Bridge 4F-AUG 14 08
Bridge 5A-AUG 14 08
Bridge 5B-AUG 14 08