

**Upper Fish River Bacterial Source  
Tracking Project  
October 2008 - March 2011**

**Addendum for the Mobile Bay National  
Estuary Program**

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## Addendum

### Results of Additional Polymerase Chain Reaction-based Source Identification

Two additional sampling events for polymerase chain reaction (PCR) source identification analyses were undertaken on February 16, 2012 and March 19, 2012. Sampling locations were at US Highway 90, County Road 54 and Woodhaven Dairy Road on Fish River (Figure, page 4). *Bacteroidetes* identification was selected as the indicator analysis because Source Molecular Corporation (SMC) offers this analysis for human, bovine and equine host markers. SMC only offers human and bovine markers for *Enterococcus*. On February 16, 2012, a high water event following at least three days of dry weather was tested. A total of 1.1 in rain was recorded at the USGS gage on Fish River and AL Highway 104 ([http://waterdata.usgs.gov/al/nwis/uv?site\\_no=02378500](http://waterdata.usgs.gov/al/nwis/uv?site_no=02378500)). Baseflow conditions were sampled on March 19, 2012. No human or bovine markers were detected in February 2012 high water samples, yet the equine marker was detected at all three locations. A strong positive result was detected at Fish River at US90 with trace positives detected at both CR54 and Woodhaven Dairy Rd (Table, page 5). The 2012 result are similar to results from high water sampling event in 2010 and 2011. Human *Bacteroidetes* markers were detected at each sample location in one of two high water events in 2010-11. Bovine *Bacteroidetes* markers were not detected in any sampling event, high water or baseflow, at any sampling location throughout the source tracking study. A single human *Bacteroidetes* marker was detected in Fish River at Woodhaven Dairy Rd under baseflow conditions.

### Conclusions

When comparing the results from multiple antibiotic resistance source detection results and the PCR-based *Bacteroidetes* tests, the strongest similarities occur for equine sources. Equine sources were detected at high water flows under analysis by both source identification methods. As demonstrated by the enumeration techniques used in this study, higher levels of *E. coli*, a fecal bacterium, are present in Fish River during high water caused by rain events. Few *E. coli* are present as baseflow levels. Many factors influence the survival of *Bacteroidetes* in the freshwater rivers (Kreader C.A., 1998 and Fiksdal, L. et.al, 1985). Also, fecal bacteria like *E. coli* may persist longer in the environment than do *Bacteroidetes* (Bell, A. et.al., 2009 and Ballesté, E. and A. R. Blanch. 2010). Equine *Bacteroidetes* could persist longer in the water than those from human or bovine sources. This could account for the difference in detection rates of the three markers. The frequency of detection of the human and bovine DNA markers could be influenced by collecting samples earlier in runoff flush. Based upon PCR-based testing and the antibiotic resistance work included in the study, equine sources of pathogens are present in the upper Fish River watershed and should be accounted for in any management effort.

## Literature

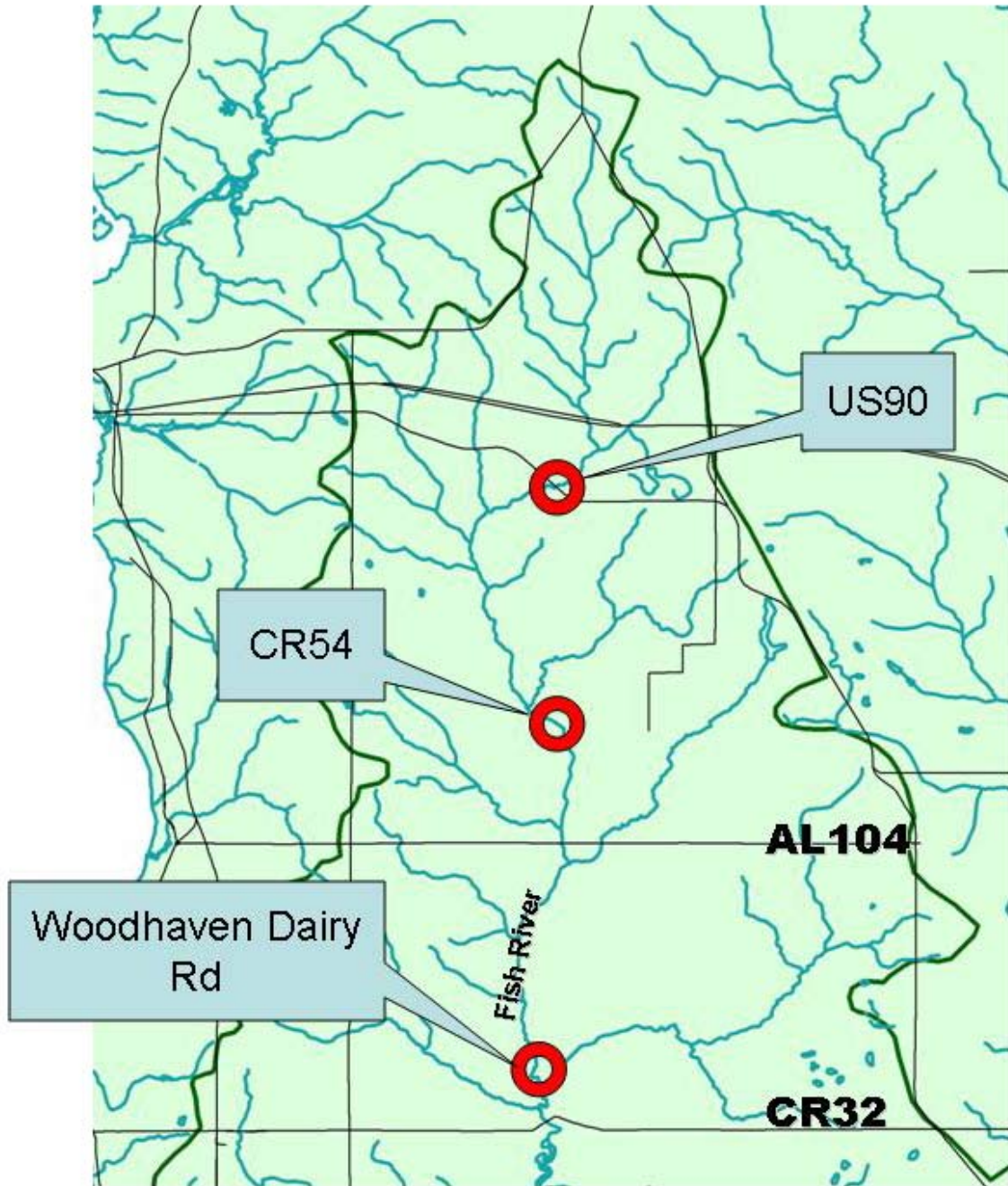
**Ballesté, E. and A. R. Blanch.** 2010. Persistence of *Bacteroides* Species Populations in a River as Measured by Molecular and Culture Techniques, *Appl. Environ. Microbiol.*, vol. 76 no. 22 7608-7616

**Bell, A., A. C. Layton, L. McKay, D. Williams, R. Gentry, and G. S. Sayler.** 2009. Factors influencing the persistence of fecal *Bacteroides* in stream water. *J. Environ. Qual.* 38:1224-1232.

**Fiksdal, L., J. S. Maki, S. J. LaCroix, and J. T. Staley.** 1985. Survival and detection of *Bacteroides* spp., prospective indicator bacteria. *Appl. Environ. Microbiol.* 49:148-150.

**Kreader, C. A.** 1998. Persistence of PCR-detectable *Bacteroides distasonis* from human feces in river water. *Appl. Environ. Microbiol.* 64:4103-4105.

**PCR-based source identification sampling locations on Fish River.**



**Results of Bacteroidetes PCR-based source identification analyses for three locations on Fish River**

		Sampling Locations		
		US 90	CR 54	Woodhaven Dairy Rd
<b>Human Bacteroidetes</b>				
Date Sampled	Condition	Detected (☞) or Not Detected (X)		
4/7/2010	Baseflow	X	X	X
5/17/2010	Rain	X	X	X
1/1/2011	Rain	☞	☞	☞
2/16/2012	Rain	X	X	X
3/19/2012	Baseflow	X	X	☞
<b>Bovine Bacteroidetes</b>				
Date Sampled	Condition	Detected (☞) or Not Detected (X)		
4/7/2010	Baseflow	X	X	X
5/17/2010	Rain	X	X	X
1/1/2011	Rain	X	X	X
2/16/2012	Rain	X	X	X
3/19/2012	Baseflow	X	X	X
<b>Equine Bacteroidetes</b>				
Date Sampled	Condition	Detected (☞) or Not Detected (X)		
2/5/2011	Rain	☞	☞	☞
3/30/2011	Rain	X	☞	X
2/16/2012	Rain	☞	☞*	☞*
3/19/2012	Baseflow	X	X	X

\* Result Qualified: Equine bacteroidetes markers were detected at a level less than the method ascribes for a full positive result