Investigations Into Coliform Bacteria

Coliform bacteria, as typified by *Escherichia coli* (*E. coli*) and fecal streptococci (enterococci), have for decades been used as indicator organisms. An indicator organism is a microorganism whose presence is evidence that water has been polluted with the feces of humans or other warm-blooded animals. The coliform group of bacteria, commonly used as an indicator, is defined as aerobic and facultative anaerobic, nonspore forming, Gram-stain negative rods that ferment lactose with gas production within 48 hours of incubation at 35°C. Coliforms reside in the intestinal tract, and are excreted in large numbers in feces, averaging about 50 million coliforms per gram. Pathogenic bacteria and viruses causing enteric diseases in humans originate from fecal discharges of diseased persons. Pathogenic bacteria, however, are normally present at very low levels, and are expensive and difficult to isolate and identify. Isolation of disease causing organisms is further complicated by their low survival rate in the ambient environment. Coliform bacteria, on the other hand, have a relatively high survival rate in the ambient environment and are easily and inexpensively identified with a minimum of laboratory equipment. Consequently, water contaminated by fecal pollution is identified as being potentially dangerous by the presence of coliform bacteria.

Elevated coliform counts frequently close the Bay to shellfish harvesting. Levels increase in the Bay during local rainfall and when the Apalachicola River rises. It is assumed that rainfall transports bacteria from the land into the river, and that the river transports bacteria to the Bay. The high coliform counts observed in the base flows of the selected sites led NWFWMD to investigate the distribution and sources of coliform bacteria entering the bay. Long-term fecal coliform data collected from specific sites within the Apalachicola Bay shellfish harvesting area were obtained from the FDEP Division of Marine Resources. In addition to coliform data, the files also contained corresponding data on local rainfall and river stages. These data were plotted onto a map of the Bay utilizing the District's GIS system. Figure 35 depicts average coliform count isoconcentration lines within the Apalachicola Bay, developed using the same techniques

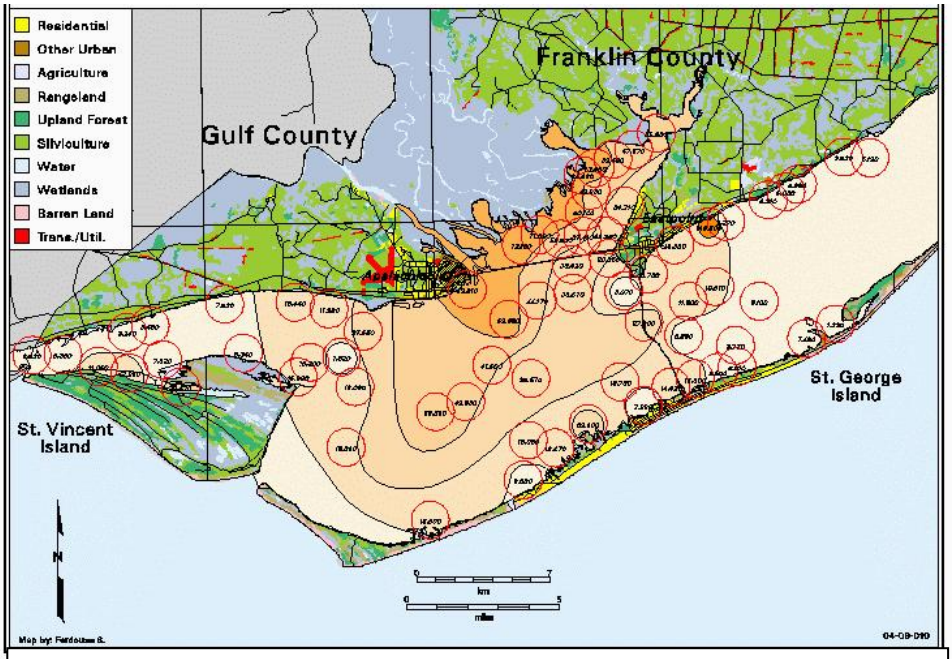

Figure 35: Distribution of Coliform Concentrations

used to depict contamination plumes in groundwater contamination analysis. Higher average concentrations are indicated in the figure by darker colors; conversely, lower average concentrations are indicated by lighter colors. The figure suggests that the source of coliform contamination is closely associated to the Apalachicola River inflow. Local nonpoint discharges adjacent to the bay at Apalachicola, Eastpoint and St. George Island also appear to contribute to the coliform contamination within the bay. This observation seems consistent with the land-based sampling efforts previously described. A statistical summary of the long-term fecal coliform data from the Apalachicola Bay shellfish harvesting areas collected from January 1979 through December 1995 is located as a table in Appendix C. The number of samples taken was highly variable, ranging from single samples taken in some areas to several hundred in others. The sampling results were highly variable as well, with means ranging from less than one to thousands of colonies measured in samples from a single site.

In late fall of 1996, FDEP initiated a water quality study to identify sources of fecal coliform bacteria. Fecal coliform levels were monitored by FDEP at selected locations in the Apalachicola River and its tributaries from the Chipola River cutoff south, beginning in November 1996 and ending in March 1998. River and tributary samples were collected on dates selected to coincide as closely as possible with sampling done by FDEP's Shellfish Environmental Assessment Section in Apalachicola Bay. A total of 11 coordinated river and bay sampling excursions were conducted during the study period. Elevated levels of fecal coliform were observed throughout the study area. Observed levels varied widely by location and time of year, with no single location or suite of locations having elevated levels during each sampling event. The most frequent high coliform levels were found in the upper reaches of tributaries in the immediate vicinity of the City of Apalachicola, including Breakaway Canal, Poorhouse Creek, and Scipio Creek. Brothers River and Jackson River above and below Huckleberry Creek also demonstrated frequently elevated coliform levels.

All tributaries demonstrated high coliform levels periodically during the study. In the Chipola, Brothers, and Jackson Rivers, levels at or above 200 MPN/100 ml at tributary mouths were observed in February 1997 and 1998, and in May 1997 in the Chipola River. At the mouths of smaller tributaries in the upper portion of the study area, such as Kennedy, Brushy, Scott, Owl and Smith Creeks, levels at or above 200 MPN/100 ml also occurred in February 1997 and 1998, and in May 1997 at Kennedy Creek. A similar pattern was observed at smaller tributary mouths from Jackson River south, including Grassy, Poorhouse, and Scipio Creeks and Breakaway Canal, with very high February coliforms in both 1997 and 1998. However, high coliform levels in May 1997, as noted for both the Chipola River and Kennedy Creek in the upper portion of the study area, were not evident at any tributaries in the lower portion of the study area.

Frequent high coliform levels, with less clear seasonal patterns, were observed at sites in the upper reaches of the tributaries, including Brothers River in January 1997, February 1998, and March 1998; at Jackson River and Huckleberry Creek in February, May, and August 1997 and January and February 1998; at Breakaway Canal in November 1996, February and August 1997, and February 1998; at Scipio Creek in November 1996, February, May, and August 1997, and February 1998.

Fecal coliform levels in the mainstem of the river varied considerably by time of year, and generally showed a pattern of either being low to moderate and stable throughout the study area, declining from upriver to downriver, or increasing from upriver to downriver. Consistently low river coliform levels were found throughout the study area in November 1996 and August 1997, accompanied by a very low river flow. Moderate coliform levels were found in the river in December 1997 and January 1998, accompanied by a moderate to high river stage, which increased by about four feet between December 16, 1997 and January 20, 1998.

Results of the FDEP study indicate that sources of fecal coliform are widespread throughout the lower portion of the Apalachicola River drainage basin. It seems clear that a comprehensive approach to source identification and reduction is needed. This approach will require that the cumulative impacts of multiple sources be understood and dealt with effectively. Elevated levels of fecal coliforms were observed in several tributaries that drain watersheds with very little human development, suggesting non-human sources. The study suggested that the most prudent approach would be to identify human sources of coliform contamination, and implement strategies to address these sources. (Marx, 1998)

One of the main characteristics of an indicator organism, such as coliform bacteria, is that it must be present at a higher concentration than the pathogens it infers. For this reason, methods that can discriminate the source of coliforms may have greater predictive and useful value, compared to developing multiple tests that must target specific pathogens. It would be useful to identify the source of fecal pollution during regular water analysis, so that potential remediation efforts can be more focused and effective. Several attempts have been made to develop methods that differentiate sources of fecal pollution, including the use of fecal streptococci. Initially, the ratio of fecal coliform to fecal streptococci was used as an indicator of fecal source. A ratio of four or greater was considered to indicate a human source, while a ratio of 0.7 or less indicated an animal source. This ratio has since proven unreliable, and the method has been abandoned. Other methods under consideration or under development include DNA fingerprinting, *Cryptosporidium* oocyst viability assays in cell cultures, and microbial source tracking.

It has been reported (Tamplin, 1997) that discriminate analysis of multiple antibiotic resistance (MAR) and ribotype profiles of *E. coli* could differentiate human and nonhuman sources of fecal pollution, and permit an estimation of the proportion from each source. These applications were initially limited to human versus nonhuman, and not a specific nonhuman species, although work is proceeding on differentiation of non-human species. MAR differentiates *E. coli* from different sources using antibiotics commonly associated with human and animal therapy, as well as animal feed. Human origin isolates are typically more resistant to antibiotics than nonhuman origin isolates. Examples of single antibiotics which differentiate human and nonhuman *E. coli* at a P value less than 0.05 (two-sided binomial test) are ampicillin, chlortetracycline, kanamycin, nalidixic acid, neomycin, oxytetracycline, streptomycin, sulfathiazole and tetracycline. The results of the research indicate that the MAR profile of *E. coli* is associated with source. MAR profiles of *E. coli* isolated directly from human and animal feces showed high similarity with MAR profiles of human and nonhuman sources. Importantly, discriminate analysis of MAR profiles showed that 82% of human isolates were correctly classified.

Samples were collected by standard methods, labeled and placed on ice inside coolers, and transported to the laboratory by overnight courier. Because the number of bacteria per sample is less critical than the actual types of bacteria isolated, the traditional six-hour holding time associated with coliform sampling may be expanded. Sample preparation and bacteriological tests for isolation of *E. coli* were performed using established procedures. A predetermined water volume, based on an initial measurement of the *E. coli* Most Probable Number (MPN), was filtered through a 0.2 *u*m pore sized filter. Filters were placed on MacConkey agar, incubated at 35^{o}C for 18 hours, and all lactose-fermenting *E. coli* were screened for presumptive identification. Presumptive *E. coli* isolates were confirmed by standard biochemical tests (Indole, Methyl red, Voges-Proskaur and Citrate).

MAR were performed by established procedures using selected antibiotics typically associated with animal feed and/or clinical treatments. Concentrations of antibiotics used include: 10 *u*g/ml ampicillin, 25 *u*g/ml chlortetracycline, 75 U/ml penicillin G, and 500 *u*g/ml sulfathiazole. Aliquots of stock solutions were added to tempered Mueller-Hinton agar, mixed, poured into petri dishes and stored at 5^{0}C for no longer than two weeks. *E. coli* isolates were grown in 96 well plates containing Tryptic Soy Broth at 35^{o}C for four to six hours, replica-plated onto antibiotic containing agar and control plates without antibiotic, and incubated at 35^{o}C for 18 hours. *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 were used as positive (resistant to all antibiotics except for sulfathiazole; *E. coli* ATCC 25922 or *Klebsiella Pneumoniae* ATCC 13883 was used as positive control for sulfathiazole) and negative (sensitive to all antibiotics tested) controls, respectively. Isolates were recorded as resistant to an antibiotic if growth, measured with a metric ruler, was indistinguishable from that on the control plate without antibiotic; more than 10 to 15% reduced growth was recorded as a sensitive reaction to the antibiotic, although growth was normally reduced greater than 90%.

The District contracted with Dr. Tamplin to perform MAR analysis on samples collected from the river and bay. Funding permitted only a limited number of samples to be analyzed, and it was decided to sample in and near likely coliform sources in the bay, during both a low and a high flow period. Additional sampling was performed on the Apalachicola River, beginning at the base of the Jim Woodruff Dam and proceeding south, sampling above and below major tributaries and communities.

The sampling sites were chosen with the intent to gather a "snapshot" of the distribution of coliforms and an estimation of their origins. Site descriptions are presented in Table 4. Sites C1, C2, and C3 were chosen to evaluate potential runoff from Eastpoint vicinity, as the results of sampling presented earlier in this report indicated elevated total and fecal coliform counts. C4 was chosen due to its proximity with the oyster beds, while C5 was chosen for its proximity to a developed portion of St. George Island. C6 was located to sample the background runoff entering East Bay from an undeveloped area, and C7 is proximate to both the Eastpoint sewage treatment plant outfall and the Sportsman Lodge Motel and Marina. C8 was chosen to represent an undeveloped portion of the Apalachicola River discharge, and C9 again represented the oyster beds. C10, C11, and C12 sampled developed portions of St. George Island, where four "package" sewage treatment plants are located, and C13, C14, and C15 represent "clear" portions of the Bay. St. Vincent's Island is uninhabited, so C16 was expected to display non-human origin bacteria. Sites C17 through C22 sampled runoff and discharge from the City of

Apalachicola, proximate to a number of marinas, landfills, stormwater outfalls, and sewage disposal sites. Site C23 sampled Huckleberry Creek at its confluence with Jackson River, to evaluate the effects of the City of Apalachicola sewage treatment plant discharge into it. Finally, Site C24 sampled the Apalachicola River upstream of the confluence of Jackson River.

Table 4: Coliform Sampling Sites in Apalachicola Bay

Station Number	Description
C1	St. George Sound at Highway 65
C2	Off patrol station at Porters Bar
C3	Mouth of jetties and channel marker
C4	Over oyster beds, by 5 pole wooden structure
C5	East Hole off Church Street
C6	East Bay near the data log station
C7	East Point Causeway anchor
C8	East Bay River
C9	East of the causeway
C10	St. George Island, third channel west
C11	Plantation East
C12	Nick's Hole
C13	Turn buoy
C14	Little St. George, Marshall House between docks
C15	Dry Bar near data log station
C16	Big Bayou
C17	Mouth of 2 Mile Channel
C18	2 Mile Channel, Mile Marker 12
C19	Between TM marker and west bank
C20	by Number 4 channel mark out from marina
C21	Scipio boat basin
C22	Scipio Creek north of boat basin
C23	mouth of Huckleberry Creek
C24	Apalachicola River, mile marker 6.6

On April 27 and 28, 1999 the District conducted its first "snapshot" sample of the bay, at the sites previously described. The river flow during this period was uncharacteristically low, and was dropping due to an ongoing drought situation, contrary to typical historical seasonal flow. River flow, as measured at the Chattahoochee gage, was 7030 cubic feet per second (cfs) on April 27, and 6950 cfs on April 28. April is typically one of the rivers high flow months. Samples were collected by standard methods over the two-day period, and shipped on ice to the University of Florida Food Safety Laboratory each evening via overnight mail. For each sample where *E. coli* was isolated, ten strains were identified to allow a ratio of human source to nonhuman source to be calculated. The results of the sampling and analyses are presented numerically in Table 5, and graphically in Figure 36. Figure 36 also provides an indication of the distribution of the identification of source among the ten strains isolated, by utilizing pie charts

where coliforms were isolated. It should be noted that the nature of the tests allows for instances where even below the detection limit of less than two Most Probable Number per 100 milliliter of sample (<2 MPN/100 mL), it is still possible to isolate bacterial colonies. This allows isolation of strains to differentiate even when the MPN is reported to be below the detection limit.

Table 5: Discriminate Analysis of MAR Profiles
(Apalachicola Bay Samples Taken April 27 and 28, 1999)

Sample Site	MPN/100 mL	Source of Pollution	Probability of Correct Identification	Number of Strains Isolated
C1	<2	NA	NA	NA
C2	<2	NA	NA	NA
C3	<2	NA	NA	NA
C4	<2	NA	NA	NA
C5	<2	NA	NA	NA
C6	<2	NH	0.98	H(0), NH(10), ND(0)
C7	<2	NA	NA	NA
C8	23	NH	0.98	H(0), NH(10), ND(0)
C9	<2	NA	NA	NA
C10	<2	NA	NA	NA
C11	<2	NA	NA	NA
C12	2	ND	<0.95	H(0), NH(0), ND(10)
C13	<2	NA	NA	NA
C14	<2	NA	NA	NA
C15	<2	NA	NA	NA
C16	<2	NH	0.98	H(0), NH(10), ND(0)
C17	13	NH	0.95	H(0), NH(8), ND(2)
C18	10	NH/H	0.98/0.99	H(3), NH(2), ND(5)
C19	31	NH/H	0.98/0.99	H(1), NH(9), ND(0)
C20	13	NH	0.98	H(0), NH(9), ND(1)
C21	130	NH	0.98	H(0), NH(8), ND(2)
C22	170	NH	0.98	H(0), NH(10), ND(0)
C23	8	NH	0.98	H(0), NH(10), ND(0)
C24	5	NH	0.98	H(0), NH(10), ND(0)

H = Human Source Pollution
NH = Non-human Source Pollution
NA = Not Available
ND = Not Determined

Due perhaps in part to the low river flow, sufficient coliforms were not isolated from half the sites sampled (C1 through C5, C7, C9 through C11, and C13 through C15) to allow MAR testing (MPN less than two). These sites were, for the most part, either within the main body of the Bay or along St. George Island. With only a few exceptions, the remaining sites, taken from the

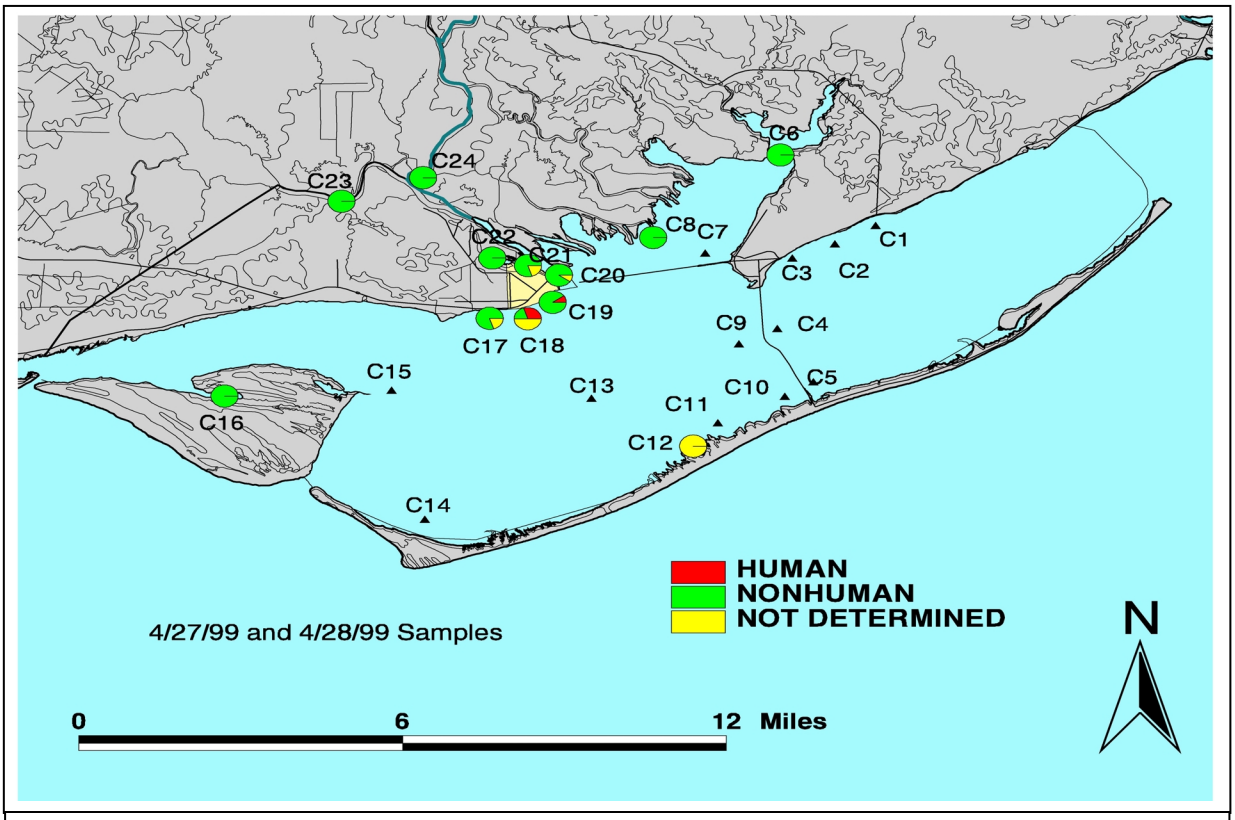

Figure 36: Results of April MAR Coliform Sampling

Apalachicola River, East Bay and St. Vincent Island, returned low coliform counts, of nonhuman and indeterminate sources. Two of the notable exceptions, Site C18 (2-Mile Channel at Mile Marker 12), and Site C19 (between TM marker and west bank), both indicated mixed human/nonhuman sources. Three human origin strains were isolated from Site C18, accompanied by two strains of nonhuman origin, while one human origin strain was isolated from Site C19, accompanied by nine nonhuman origin strains. Of those sites where coliforms could be isolated, all, with the exception of C8 (East Bay River) and C24 (Apalachicola River, mile marker 6.6) were associated with fishing or marina activities or with the sewage treatment plant discharging into Huckleberry Creek. It is of interest to note that in this sampling run, approximately 17% of the strains of *E. coli* sampled could not be differentiated.

On June 29 and 30, 1999 the District conducted its second "snapshot" sample of the bay. The river stage during the period sampled was higher than the previous sampling event in April, and was increasing, although typically and historically June is not a high river flow month. Again, the current drought situation is likely a cause. River flow, again measured at the Chattahoochee gage, was 12,700 cfs on June 29 and 14,100 cfs on June 30. Samples were again collected by standard methods over the two-day period, and shipped on ice to the University of Florida Food Safety Laboratory each evening via overnight mail. For each sample where *E. coli* was isolated, ten strains were identified to allow a ratio of human source to nonhuman source to be calculated. The results of the sampling and analyses are presented numerically in Table 6, and graphically in

Figure 37. Figure 37 also provides an indication of the distribution of the identification of source among the ten strains isolated, by utilizing pie charts where coliforms were isolated.

Table 6: Discriminate Analysis of MAR Profiles
(Apalachicola Bay Samples Taken June 29 and 30, 1999)

Sample Site	MPN/100 mL	Source of Pollution	Probability of Correct Identification	Number of Strains Isolated
C1	<2	NA	NA	NA
C2	<2	H	0.99	H(9), NH(0), ND(1)
C3	2	ND	<0.95	H(0), NH(0), ND(10)
C4	<2	NA	NA	NA
C5	<2	NA	NA	NA
C6	5	H	0.99	H(4), NH(0), ND(6)
C7	4	H	0.99	H(4), NH(0), ND(6)
C8	7	H	0.99	H(1), NH(0), ND(9)
C9	2	ND	<0.95	H(0), NH(0), ND(10)
C10	8	NH/H	0.98/0.99	H(5), NH(1), ND(4)
C11	2	NH	0.98	H(0), NH(10), ND(0)
C12	<2	ND	<0.95	H(0), NH(0), ND(10)
C13	<2	NA	NA	NA
C14	<2	NA	NA	NA
C15	<2	NA	NA	NA
C16	5	H	0.99	H(9), NH(0), ND(1)
C17	2	ND	<0.95	H(0), NH(0), ND(10)
C18	5	NH	0.98	H(0), NH(2), ND(8)
C19	33	NH/H	0.98/0.99	H(2), NH(1), ND(7)
C20	13	H	0.99	H(1), NH(0), ND(10)
C21	130	H	0.99	H(1), NH(0), ND(9)
C22	79	NH/H	0.98/0.99	H(2), NH(1), ND(7)
C23	8	NH/H	0.98/0.99	H(1), NH(1), ND(8)
C24	8	NH/H	0.98/0.99	H(1), NH(1), ND(8)

H = Human Source Pollution
N = Non-human Source Pollution
NA = Not Available
ND = Not Determined

This sampling event, taken during a higher river flow than the previous one, presents a different picture of the river and bay. Only six sites, again within the body of the bay, failed to produce sufficient coliforms for MAR analysis. The river sites all returned strains from both human and nonhuman sources, as did two of the Eastpoint sites and one of the St. George Island sites. Surprisingly, both the East Bay and the St. Vincent Island sites returned human origin strains, the St. Vincent Island site strongly so with nine out of ten strains isolated being of human origin. Oddly, this site gave the strongest reading of any site for human origins. Only one site sampled,

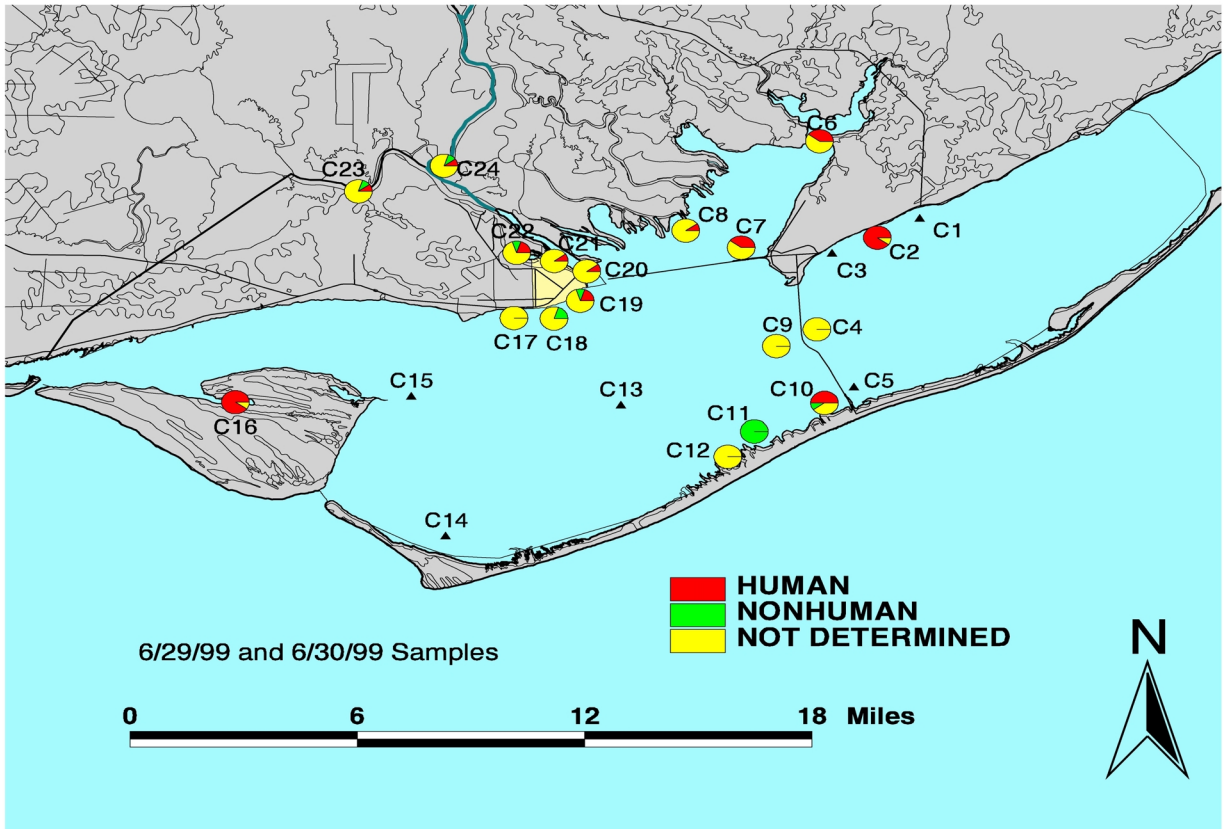

Figure 37: Results of June MAR Coliform Sampling

Site C11 (Plantation East on St. George Island), returned strains exclusively nonhuman in origin, although the low number of strains isolated suggests limited human involvement. It should be noted, however, that the indeterminate strains isolated throughout the sampling area might well be of either human or nonhuman origin. The greater number of sites where coliforms were isolated (although the MPN's were lower) may lend credence to the theory that the river is a source of coliform bacteria to the Bay. The results also suggest that stormwater runoff and/or sewer or septic tank overflows during wet periods may be a significant source of human origin coliforms. From this sampling run, approximately 69% of the strains isolated could not be differentiated.

To complete the limited discriminate coliform sampling events scheduled by the District, a screen of the Apalachicola River was needed. Accordingly, on September 28 and 29, 1999 the District sampled the length of the river. River flow at the time of this sampling was again low and dropping, measured at the Chattahoochee gage as 6090 cfs on September 28 and 6000 cfs on September 29 (provisional data at the time of this writing). Table 7 presents descriptions of the sample sites. The goal in choosing the sites was to gather information above and below major tributary inflows and settlements, where coliform bacteria might be introduced into the mainstem of the river. Sampling began at the base of the Jim Woodruff Dam and proceeded south to the bay. In addition to the river samples, the river/bay interface sampling sites and those adjoining

the City of Apalachicola previously described were included, as were two sites bracketing Eastpoint. Results of the sampling event are presented numerically in Table 8, and graphically in Figure 38.

Table 7: Coliform Sampling Sites on the Apalachicola River

Station Number	Description
R1	Near Jim Woodruff Dam Outfall, upstream of US 90
R2	Above Flat Creek, above I-10
R3	Below Flat Creek, above I-10
R4	Above Graves Creek (Thomas Mill and Wilson Mill Tributaries)
R5	Below Graves Creek (Thomas Mill and Wilson Mill Tributaries)
R6	Below Stafford Creek
R7	Above Sutton Creek
R8	Below Sutton Creek
R9	Above Iamonia Lake
R10	Below Florida River (above cutoff)
R11	Dead Lake at County Road 22 Bridge
R12	Below Chipola River inflow
R13	Above Brothers River
R14	Below Brothers River
R15	Apalachicola River, mile marker 6.6 (C24 above)
R16	Mouth of Huckleberry Creek (C23 above)
R17	Scipio Creek, North of boat basin (C22 above)
R18	Scipio Creek boat basin (C21 above)
R19	Apalachicola River, by No. 4 channel marker (C20 above)
R20	Apalachicola Bay, between TM marker and west bank (C19 above)
R21	2 Mile Channel mile marker 12 (C18 above)
R22	Mouth of 2 Mile Channel (C17 above)
R23	Bay, East Point Causeway anchor (C7 above)
R24	Bay near Eastpoint, mouth of jetties and channel marker (C3 above)

Table 8: Discriminate Analysis of MAR Profiles
(Apalachicola River and Bay Samples Taken September 28 and 29, 1999)

Sample Site	MPN/100 mL	Source of Pollution	Probability of Correct Identification	Number of Strains Isolated
R1	2	H/NH	0.99/0.98	H(3), NH(7), ND(0)
R2	2	NH	0.98	H(0), NH(1), ND(9)
R3	2	NH	0.98	H(0), NH(10), ND(0)
R4	17	NH	0.98	H(0), NH(4), ND(6)
R5	22	NH	0.98	H(0), NH(2), ND(8)
R6	33	H/NH	0.99/0.98	H(1), NH(5), ND(4)
R7	79	NH	0.98	H(0), NH(6), ND(4)
R8	33	NH	0.98	H(0), NH(6), ND(4)
R9	170	ND	<0.95	H(0), NH(0), ND(10)
R10	17	ND	<0.95	H(0), NH(0), ND(10)
R11	23	ND	<0.95	H(0), NH(0), ND(10)
R12	23	ND	<0.95	H(0), NH(0), ND(10)
R13	23	ND	<0.95	H(0), NH(0), ND(10)
R14	49	ND	<0.95	H(0), NH(0), ND(10)
R15	23	ND	<0.95	H(0), NH(0), ND(10)
R16	49	ND	<0.95	H(0), NH(0), ND(10)
R17	49	ND	<0.95	H(0), NH(0), ND(10)
R18	350	H	0.99	H(2), NH(0), ND(8)
R19	23	ND	<0.95	H(0), NH(0), ND(10)
R20	33	ND	<0.95	H(0), NH(0), ND(10)
R21	13	ND	<0.95	H(0), NH(0), ND(10)
R22	31	ND	<0.95	H(0), NH(0), ND(10)
R23	NA	NA	NA	NA
R24	350	H	0.99	H(1), NH(0), ND(9)

H = Human Source Pollution
N = Non-human Source Pollution
NA = Not Available
ND = Not Determined

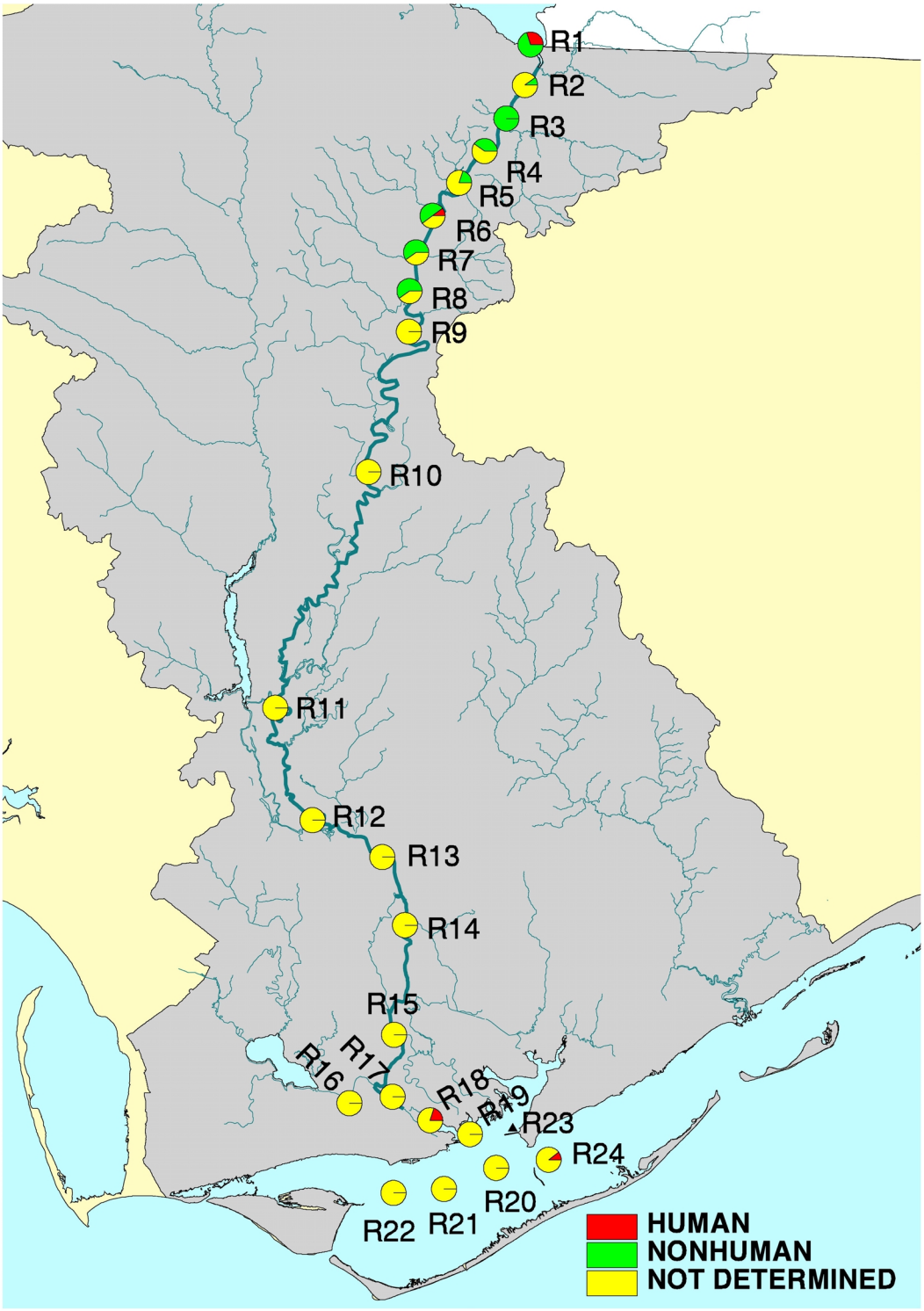

Figure 38: Results of September MAR Coliform Sampling

Site R1 at the base of the Jim Woodruff dam outfall returned a low count (MPN of two) of coliform strains of mixed human (three strains) and nonhuman (seven strains) origin. This suggests that Lake Seminole may be a limited source of human contamination, possibly through boaters, marinas, or septic tanks. Discharges from the town of Chattahoochee may also contribute. The MPN does not increase downriver, indicating that during this time the Flat Creek inflow had little effect. The MAR analysis, however, did not return strains from human sources. Above Flat Creek, only one "certain" (with a probability of 98%) nonhuman source strain was isolated. Below Flat Creek, however, every strain isolates was "certainly" nonhuman in origin. The indeterminate strains may be of either human or nonhuman origin, indicating a very limited human involvement. Therefore, little significance should be placed on this observation.

Samples taken above and below the entry of Graves Creek, which drains both Thomas Mill and Wilson Mill tributaries, also returned strains identified as having nonhuman origins. It is noteworthy, however, that the coliform MPN increased from Flat Creek to Graves Creek, and increased again below the confluence, suggesting the creek may be a source of coliforms. Stafford Creek is possibly a source of human origin coliforms, as one strain was isolated below its confluence. Coliforms are introduced into the mainstem of the river between Stafford Creek and Sutton Creek, as the MPN increased significantly, all apparently of nonhuman origin. The MPN dropped below Sutton Creek, while still returning nonhuman origin strains.

Sampling down the remainder of the river to the bay did not isolate strains that could be differentiated as originating from human or nonhuman sources. Therefore, no clear conclusions could be drawn concerning sources in this region. In fact, over 79% of the strains of *E. coli* isolated were indeterminate. With the exception of the sample taken above Iamonia Lake, the MPN's were all relatively low and consistent, with the exception of a few elevated (relatively so) values. The Scipio Creek boat basin, for example, returned a coliform MPN of 350, of human origin. The June 29 and 30 sampling event also returned strains of human origin. It would appear (based, of course, on only two sampling events) that there are significant sources of human waste contamination within the boat basin. One other source of human contamination was isolated off East Point, at the mouth of the jetties and channel marker. Again, the results of this sampling event point to boating activities and sewage treatment plants.

While the results of these sampling events and the discrimination of sources are interesting, they are obviously far too limited to draw concrete conclusions. It is clear, however, that human fecal contamination is present, both in the river and in the bay, which comes as no surprise. The study presented here suggests that likely sources to the Bay include stormwater runoff from both Apalachicola and Eastpoint, the City of Apalachicola sewage treatment plant discharge to Huckleberry Creek and treatment plants on St. George Island, and from the lower section of the Apalachicola River. Human source coliforms were also isolated from East Bay and St. Vincent Island, which warrants further investigation. The St. Vincent Island findings also indicates the need for further testing of the MAR procedure, as the island is uninhabited and therefore is not expected to be a source of human origin coliforms. Possible river sources suggested by this study include water released from Lake Seminole and Stafford Creek. The Scipio Creek boat basin also appears to be a hot spot. These results agree with suspected or observed sources of contamination. It should be noted that these river sources were "identified" with a single screen of the river, which unfortunately resulted in a significant number of strains that could not be

differentiated. There may be other sources of human contamination that were not identified by this limited screen. Despite the shortcomings inherent to such a limited sampling base, it would appear that there may be some merit to the method. However, with over half the strains of *E. coli* isolated (61.4%) indeterminate as to source, further testing will be needed to insure the test is conveying expected results.