LOWER RINCON CREEK WATERSHED STUDY

A Field Investigation into the Source of Fecal Contamination
in the
Lower Rincon Creek Watershed and Ocean Interface (Surfzone)

County of Santa Barbara
In Cooperation with
Heal the Ocean

October 1999

Prepared for:

Santa Barbara County
Public Health Department

Santa Barbara County
Water Agency (Project Clean Water)

Heal the Ocean

Prepared by:
Santa Barbara County
Environmental Health Services Division

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1.0 INTRODUCTION

1.1 BACKGROUND INFORMATION

Long-term surfzone monitoring, conducted by the Santa Barbara County Public Health Department Ocean Water Monitoring Program and elsewhere throughout the State, have indicated that levels of total coliform, fecal coliform and enterococcus are elevated in runoff from urban areas. Elevated coliform bacteria levels pose a public health risk to beachgoers (surfers, swimmers, and waders) that have contact with ocean or creek water. In Santa Barbara County, bacteria levels exceeding recreational standards have forced the closure of many public beach areas, including the popular Rincon Beach. Since coliform bacteria are an indicator of possible human health risk from water contact, it is commonly used as the primary factor in determining whether beaches should be closed. In addition, studies conducted to date have focused primarily on the concentrations of coliform bacteria, rather than identifying their host organism sources. Identification of potential sources of these bacteria is the next logical step in focusing on future remediation efforts. DNA analysis has been used in a number of recent studies along the West Coast:

- Coronado Island, San Diego
- Little Soos Creek, Washington State
- a proposed wet weather study for the Aqua Hedionda watershed in San Diego County

Heal the Ocean (a nonprofit organization dedicated to improving ocean water quality), the Santa Barbara County Public Health Department, "Clean Up Rincon Effluents" (CURE- a nonprofit group dedicated to improving ocean water quality in the Rincon Creek area), as well as a number of other public and private organizations were interested in determining the source or sources of bacterial contamination in the lower Rincon Creek Watershed and adjacent ocean surfzone. Although there is speculation that septic systems of the residential community at Rincon Point are contributing to high bacterial levels in the lower sections of the watershed and the ocean, previous indicator testing of coliform bacteria has failed to produce empirical evidence of this relationship.

Project Clean Water is a community coordinated effort which has been identifying potential sources of contamination through focused: (a) physical examinations of the watershed, (b) water testing at specific locations within the watershed, and (c) constituent testing of ocean water. In order to better understand the sources of water contamination at Rincon Creek, Heal the Ocean and Santa Barbara County agencies combined efforts to fund a detailed study that focused on identifying the sources of bacteriological contamination in the lower Rincon Creek Watershed. Although the selected watershed for the study makes up the boundary between Santa Barbara County and Ventura County, the overall study is believed to be relevant and applicable to other similar rural watersheds.

Dr. Mansour Samadpour, a professor with the University of Washington Department of Environmental Health, has developed a technique for identification of fecal sources using *Escherichia coli* (*E. coli*) bacteria. *E.coli* is a coliform bacterium that makes up approximately 80% of the coliform group in normal intestinal flora. *E.coli* is easily modified and adaptive to various host environments. It is this adaptive ability that is thought to lead to changes to the genetic material that is species specific. Comparison of *E.coli* genetic material extracted from creek water to a database of known fecal source samples allows for the identification of the source of the *E.coli* and hence the bacterial contamination.

1.2 STUDY OBJECTIVES AND SCOPE

The Rincon Creek watershed begins in the Los Padres National Forest located in both Santa Barbara and Ventura Counties. For several miles, Rincon Creek forms the border between the two counties. The watershed encompasses about 14 miles from tributary sources in the local mountains, to the ocean just southeast of the City of Carpinteria. The creek has several major tributaries including Casitas Creek. For the most part, the major land uses are agricultural with a few isolated residential areas. The beach area around Rincon Creek has shown elevated bacterial levels on a consistent basis even during periods of low flow.

The Rincon Watershed was selected for the following reasons:

- The watershed has limited land uses (residential, agricultural)
- Interest from both the public and governmental entities
- Extensive recreational use (surfing, fishing, and swimming) in ocean water near the creek mouth
- A focused section of the watershed could act as a "pilot" study for other watersheds
- Fewer potential sources of human bacterial contamination
- A typical lagoon impoundment that occurs in several other Santa Barbara County watersheds
- Sufficient access to creek areas for conducting the monitoring

The Lower Rincon Creek Watershed Study was designed to identify the sources of coliform bacteria in discharges from dry weather flows. A map depicting the stream configuration is shown in Figure 1 and Figure 1A. The lower portion of Rincon Creek Watershed forms a lagoon that is under tidal influences which creates an interface between freshwater, brackish water and ocean water. The land uses of the upper sections of the watershed are predominantly natural chaparral with the middle reaches being mainly agricultural (avocado and lemon orchards), with small sections of residential. The lowest portion of the watershed surrounding the lagoon area is predominantly residential. Three sample locations were chosen in an attempt to isolate the upper and middle sections of the watershed from the lower residential section:

- Sample site RC-007, which is just upstream of the lagoon and residential areas, represents everything flowing down the watershed up until that point.
- Sample location RC-002 is in the lower end of the lagoon and represents the mixing of salt and freshwater as well as any contributions from the lower residential area to the watershed.
- Sample RC-OC is located in the surfzone, at the mouth of the creek, and represents the watershed's contribution to the oceans.

The bacteriological study was also designed to characterize the sources of *Escherichia coli* (*E. coli*) and the presence of human pathogens in runoff from the Rincon watershed. In order to determine coliform sources, we retained the services of Dr. Mansour Samadpour of the University of Washington. Dr. Samadpour conducted DNA testing on fecal coliforms in water samples obtained from the watershed to determine their host organisms and therefore, sources, of the bacteria. Specifically, ribosomal RNA typing using two restriction enzymes produced genetic fingerprinting of the cultures. These fingerprints were then compared to known sources within Dr. Samadpour's *E. coli* DNA library, which is composed of over 24,000 previously identified DNA fingerprints representing thousands of source species. Dr. Samadpour's library was supplemented with fecal samples collected from local species.

2.0 STUDY METHODS

The following investigations were conducted for this study:

1. Major mammal and avian species were identified which may be contributing to the fecal coliform loading in the watershed.
2. Water samples were collected from three discrete locations in the watershed: in the surfzone at the mouth of the Rincon Creek Watershed (labeled as sampling point RC-OC on Figure 1); in the lagoon (labeled as sampling point RC-002); and just upstream of the lagoon (labeled as sampling point RC – 007).
3. Sources of fecal coliform present in the water samples were determined by comparing E.coli genetic material extracted from the water samples to previously established genetic ribotyping of *E.coli* bacteria. Two databases were used for this comparison:
   - fecal samples collected from species in the watershed and/or
   - an extensive library of previously ribotyped *E.coli* that exists at the University of Washington.

2.1 WATER AND FECAL SOURCE SAMPLE COLLECTION

Under baseflow (non-storm) conditions, water sample collection was performed over 10 sampling events at each of the 3 sample locations, with 5 water samples collected per sampling event at each location. Fifty (50) discrete water samples were collected from each location for a total of 150 water samples collected in order to perform the DNA analyses. The samples were collected from the flowing water stream, lagoon, and ocean surfzone at 10-minute intervals, and the timing of sample collection was varied in order to capture both high and low tide events.

Fecal "source" samples were also collected from both mammal and bird species that were determined to be the most likely contributors. A total of 208 fecal samples were collected over the course of this study, of which 80 were bird species and 128 were mammal species. A maximum of 20 fecal samples per species was collected. Source samples were collected from the Rincon Watershed, other field locations within the Santa Barbara County, the Santa Barbara animal shelter and from pumper truck tanks of local septic system maintenance companies. Water samples were collected as grab samples using sterile containers. Quality Assurance and Quality Control (QA/QC) measures were followed and all samples were immediately placed in a cooler with ice and promptly transported to the Santa Barbara County Public Health Laboratory for analysis. All fecal samples were collected using sterile instruments and containers, placed immediately in Cary Blair transport media, placed in a cooler with ice and promptly transported to the Santa Barbara County Public Health Laboratory. From the laboratory, source samples were boxed and shipped to Dr. Samadpour's laboratory at the University of Washington for further analysis.

2.2 PUBLIC HEALTH LABORATORY PROCEDURES

Water samples were analyzed at the Santa Barbara County Public Health Laboratory for fecal coliform enumeration using membrane filtration and direct plate count. Water samples were serially diluted to 10^{-1}, 10^{-2}, and 10^{-3}. Membrane filters from these water samples were then plated on MacConkey media and incubated overnight. Water samples were then shipped the day after sample submission for overnight delivery to Dr. Samadpour's laboratory at the University of Washington.

Concurrent with sample processing for analysis of the *E.coli* genome, culturing of *E.coli* was performed at the Santa Barbara County Public Health Department Laboratory to determine concentration of fecal coliform and *E.coli*. Two testing methods were used: membrane filtration and direct plate counts of *E.coli* colony forming units (cfu's); and Colilert (Idexx) which analyzes for *E.coli* using statistical analysis which is reported as most probable number (mpn) of *E.coli*.

2.3 UNIVERSITY OF WASHINGTON LABORATORY PROCEDURES

Once shipments were received at Dr. Samadpour's laboratory, water and source samples underwent testing to isolate *E.coli* bacteria. Morphologically appropriate colonies (round, blue and flat) were chosen from these plates and streaked for isolation onto MacConkey media and incubated at 37°C for 24 hours.

Isolated colonies that fermented lactose on MacConkey media were then restreaked onto Tripticase Soy Agar (TSA). An average of sixteen isolates were obtained from each water and fecal sample. Biochemical analysis was done to positively identify *E.coli*. This was accomplished by inoculating each isolate into a tryptophane broth and onto a sodium citrate slant and incubating at 37°C for 24 hours. Isolates that were able to produce indole from tryptophane and not able to utilize sodium citrate as a sole source of carbon were positively identified as *E. coli*. These isolates were then assigned an isolate number and cultured again on TSA to obtain enough cells for storage in LB-15% glycerol freezing media at -70°C and for genomic (chromosomal) DNA isolation.

3.0 RESULTS

3.1 SAMPLES PROCESSED

A total of 208 source samples (fecal samples) from over 20 species of birds and mammals were collected by Santa Barbara County staff and sent to Dr. Samdadpour's laboratory. For a listing of samples per species see Table 1. Attempts were made to extract *E.coli* from these fecal source samples. A total of 150 fecal samples produced isolates that were processed to characterize the genetic material. These results now comprise the database for the local animal population.

A total of 150 water samples were collected in the field. After membrane filtration and culturing, 138 water samples of the total 150 water samples produced a minimum of 2-19 bacterial colonies. These were then packaged and shipped to Dr. Samadpour's laboratory. Unfortunately, due to communication errors between the laboratories, thirty filter plates containing the bacterial isolates were not processed, leaving isolates from only 108 of a possible 150 water samples. From each of these filter plates, a minimum of 2 colony isolates were collected and processed to ensure *E.coli* identification. Those that were positive for *E.coli* were processed for ribosomal RNA matches. One hundred eighty four *E.coli* isolates were processed and produced a total of 124 matches. These isolates were found to match either Dr. Samadpour's laboratory (91 total matches to library) or the library created from source samples collected by Environmental Health Services (50 matches) and a slight percentage of the matches were to both databases.

Of the 124 total matches, 4 matches to Dr. Samadpour's laboratory appear to be "transitory" *E.coli*. This is terminology used to differentiate *E.coli* ribotypes that are common to a group of species, or that match source samples from more than one species. Therefore the 4 matches that were identified as beaver and river otter, which are not known to inhabit the watershed were deemed to be transitory. All other species identified are known to inhabit the watershed.

The 124 matches were generated from 82 of the 108 water samples and were fairly evenly divided between the three sample locations:

- Site 1 (ocean surfzone) 23 water samples produced 31 matches
- Site 2 (lagoon) 31 water samples produced 48 matches
- Site 3 (culvert) 28 water samples produced 43 matches
- Site 4 (Long Creek Tributary) one water sample produced 2 matches

As previously indicated, communication problems led to an overall reduction in the number of water samples processed at the University of Washington Laboratory. Three of the ten sampling dates had no water samples processed. As a result, the overall data set was reduced, but the distribution of water samples collected at each of the three main sample locations was unchanged from the original study design.

3.2 DISTRIBUTION OF MATCHES

Figure 2 depicts the number of matches per species at each of the three sampling locations- excluding species that constitute less than 4% of the total matches. Table 2 delineates the percentage of matches attributed to each species. Twenty different species matches were identified from analysis of the water samples. As noted above, 4 of these matches (otter and beaver) appear to be transitory, but as they represent a small change to the overall distribution, no efforts were made to remove these matches from the data sets.

Matches to human species showed the highest percentage (20%; 25/124 matches) but were noted in only the lagoon (14 matches) and the surfzone (11 matches) sample locations (Figure 3). No human matches were noted in the area of the culvert. Matches to duck species showed the widest distribution among sampling locations. Duck species were the second most prevalent match, (accounting for 14% of the total matches -11/124 matches) and matches were distributed over all sampling locations (Figure 4).
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Human matches were found for each sampling event (Figure 3). However, 40% (10) of the human matches occurred as a result of one sampling event (6/4/99). Opossum species were identified in 6 out of 7 sampling events (Figure 5) but accounted for only 6% (8) of the total matches (Table 2). Domestic species accounted for 46% (57) of the total matches (Table 2a).

3.3 FECAL COLIFORM (*E.COLI*) CONCENTRATIONS

Table 3 displays a comparison of results of these two testing methodologies. Table 4 depicts median and average values for *E.coli* for each of the sample dates derived from Colilert test results (reported in mpn). These values from each method for each particular water sample are not outside of the 95% confidence limits and are therefore interchangeable for concentration levels for all water samples for fecal coliform. “Recreation 1” bathing standards found in the Central Coast Regional Water Quality Control Board Basin Plan indicate that waters that are used for recreational purposes where full body contact (swimming, surfing, and wading) is likely, should have levels of fecal coliform below 400 mpn (or cfu’s). Only one water sample of the 150 collected tested at a level above 400 cfu’s (Sample identification number RC-1-K5 collected on 6/4/99- see Table 3).

Comparison of *E.coli* averages and median values with the relative numbers of matches of species did not show any consistent correlation (i.e. the number of matches did not increase or decrease as concentrations of coliform bacteria increased or decreased). This may be due to the relatively low coliform levels and relatively low species matches. In addition, Dr. Samadpour’s technique produces a qualitative analysis of species contributing to coliform levels whereby correlations to quantity (concentration of bacteria) are unlikely.

3.4 TIDAL INFLUENCES

Tidal cycles occurring during sampling periods were recorded. Table 5 displays field measurements of temperature, pH and the tidal changes occurring over the sampling period. For tide changes, a positive number indicates the relative increase in feet of the tide. A negative number indicates the relative decrease in feet of the tide when the tide was going out and water levels were decreasing. Attempts were made to compare number of matches to incoming and outgoing tides; no correlation could be ascertained. In addition, tidal cycles were compared to coliform levels. A slight correlation was noted for two sampling dates in relation to negative tides. On May 25 and May 26, negative tides (-0.7 and –1.1) were recorded; on these dates coliform levels increased from the culvert sample location to the lagoon and increased further at the surfzone sample location. This may be a result of the receding tide drawing water from the culvert into the lagoon, and eventually reaching the ocean and thereby increasing the bacterial load in the surfzone. However, this same phenomenon was not noted for May 24, 1999 when there was a –0.8 tidal fluctuation during the sampling period.

Temperature and pH variations were not considered significant between sampling locations on a particular date. Changes in pH over various sampling dates are indicative of a natural environment and the interaction of a number of factors such as organic loading, flow regimes, and sunlight.

Five water samples were collected at the same sampling location at ten-minute intervals for each sampling event. When a species match was made, these water samples were analyzed to determine a trend over these time intervals, such as an increase or decrease in the matches compared to the time of collection; no obvious trends were noted. In addition, water samples were collected at different times of day for different sampling events. Once again, as the number of matches for each species were relatively low, no correlation could be ascertained which indicated that the time of sample collection had a bearing on the number of matches obtained.

4.0 DISCUSSIONS AND CONCLUSIONS

The findings that human species matches were the most prevalent identified source among those samples tested and were also present in water samples from each sampling date indicate an additional source for a potential public health risk associated with recreational water contact. Although zoonotic illnesses and their sources (human illness attributed to animal sources) are not uncommon under natural conditions, there is also a potential for transmission of human illness when human waste products (e.g. sewage) are present. Unfortunately, the limitations inherent with this particular scientific method and application in this setting does little to identify pathogens, or measure any significant infectious levels (doses) within the watershed. *E.coli* is a coliform bacterium that has many subspecies; the majority are hosts of normal intestinal flora. Only a few, such as *E. coli* 0157:H7 have been found to be pathogenic. However, while not specifically a component of the workplan, Dr. Samadpour did examine each of the species matches to determine if the *E.coli* isolates were pathogenic *E.coli* 0157:H7. Dr. Samadpour reported that no *E.coli* 0157:H7 was present in the isolates tested from the Lower Rincon Creek Watershed.

The assessment of the risk of human illness is a very complex issue and involves a multitude of factors. Certainly, the infectious nature, quantity (infectious dose levels) and pathogenicity of the organism are all critical considerations. Although this DNA methodology provides a better landscape of potential sources of *E.coli* organisms than traditional methodologies, it has limitations with providing other significant risk assessment information. The routine standard fecal coliform, *E.coli* and enteroccocci measurements currently provide the best standardized measurement of some of these other key parameters (e.g. quantity levels of organisms). As indicated above, the presence of *E.coli* alone does not address pathogenicity per se. Additional compounding factors such as exposure timeframe, path of infection (e.g. ingestion.), concentration at time of exposure (dosage), viability of pathogen, and many others are also incorporated in determing an individuals health risk. Future studies are needed to address how molecular DNA methodologies can be applied to answer some of the above concerns.

Matches to human species were only noted in the lagoon and ocean sample locations. No human species matches were associated with water samples collected at the culvert sample location. Previous Project Clean Water creek surveys performed and this pilot study did not identify a current problem with human encampments in the Rincon Watershed. As there are only isolated residential areas further back into the watershed, it appears that a link between septic systems in the Rincon Point residential area and the adjacent lagoon and beach areas is likely to be the cause of contamination. It is not possible from this study to ascertain how that link may be occurring or the significance, since this technique cannot quantify the extent or amount of this contribution. However, since there is no evidence of septic system effluent surfacing and entering the creek, it may be occurring through a subsurface connection to the creek and/or ocean water.

The presence of human waste and other domesticated animal sources in the watershed and at the surfzone may pose a potential health risk to bathers in this immediate area. Although coliform concentrations do not exceed current ocean water testing standards, it is recommended that the lagoon and creek mouth areas (where body contact with these recreational waters is likely) be posted with signs that warn the general public of this increased health risk.

Water sampling occurred during periods of low creek water flows. Under these conditions, it is likely that fecal coliform sources from the watershed upstream of the culvert sample location were reduced. If a link exists from the community septic systems to the watershed during these low flow conditions, the relative number of human matches (due to a constant supply from ongoing septic system usage) compared to non-human matches could be increased. And, the relative percentage of fecal coliform concentrations attributed to human sources may be increased.

Previous indicator organism (coliform bacteria) testing in the ocean surfzone at the mouth of Rincon Creek has indicated that bacterial levels increase with creek flows. Water and source sample collections occurred during the late spring and early summer months (May and June, 1999). Creek flow at this time was relatively low. As a result, low coliform concentrations obtained from water samples were not unexpected. Previous wet weather studies, such as the South Coast Watershed Characterization Study, have shown large increases in coliform concentrations as a result of storm water runoff. Storm water runoff transports coliform bacteria from large areas of the watershed into the creek and eventually to the ocean.

Recent changes to the California Health and Safety Code have established more restrictive bacteriological standards for recreational waters. This provides additional public health risk protection as the standards now monitor for three types of bacterial contamination: total coliform, fecal coliform, and enterococcus. The new regulations mandate ocean water testing at this beach location with a minimum frequency of weekly from April to October. Rather than trying to supplant this minimum program, it is further recommended to increase the amount of monitoring in this area to prevent potential risks to the beachgoing population.

This may take the form of monitoring in the lagoon, sampling further east and west of the creek mouth and/or monitoring on more than a weekly frequency. Previous sampling at this and other beach areas where creeks empty to the ocean have shown elevated concentrations of bacteria adjacent to the creek mouth/surfzone interface. Warning signs permanently posted in this area would alert beachgoers about the health risks associated with contact with ocean and creek waters in this area. Increased frequency or extent of monitoring provides a better assurance of actual conditions and helps to inform beachgoers of changing bacterial loading in the creek area.

Sign posting, locations of signs and ongoing monitoring will be re-evaluated after the community's connection to a sanitary sewer system.

In general, it appears that the use of the Lower Rincon Creek Watershed in a pilot study is appropriate; based on the limited number of land uses and ability to focus the study on several narrowly defined goals. Previous knowledge of potential sources in the watershed, from Project Clean Water surveys performed in the fall of 1998, provided insight into understanding potential sources of *E.coli*. Although almost all source species collected had matches from the water samples, the identification of where these matches occurred in the watershed provides insight into source reduction strategies.

Although the majority of species matches were due to wild animal population, 46% of the species matches were attributed to domestic animal sources (which includes human sources). The observance of domestic animal waste in the watershed, especially in the lagoon and surfzone area, suggests that source reduction strategies should initially be targeted in these areas.

Matches of dogs and cats suggest simple reduction strategies such as the need for pet owners to be cognizant of the proper disposal methods of pet waste material. Project Clean Water, a community effort to improve water quality in our creeks and oceans, has developed several educational brochures to assist owners of domestic pets and horses in the proper disposal of animal waste. Appendix A contains excerpts from these brochures.

What is also clear is that there is a wide variety of native species contributing to the fecal coliform levels in the watershed. As indicated above, there are health risks associated with animal species. Outbreaks attributed to *Giardia* and *E.coli* 0157H7, in other areas, have been attributed to native species such as ducks, bears, etc. Source reduction strategies for these animals are much more difficult to implement than for domestic sources. However, discouraging the attraction of certain non-native species (such as migratory bird populations) by providing unnatural food sources should be discouraged. Maintenance of the natural contours and flora is important in the ability of the watershed to act as a filtering mechanism to trap and in some cases remove fecal coliform bacteria. Channelization activities in creek settings may be effective to control the movement of water, but are often destructive to the watershed's ability to effectively remove contaminants. Sterilization of the creek, via removal of all fecal coliform bacteria would devastate the ecosystem of the creek. Some level of nonpathogenic coliform is essential to preserving creek biota. The challenge is to understand the loading capacity of the watershed and to reduce or eliminate all non-natural pollutant contributions.

Dr. Samadpour's laboratory at the University of Washington has been using ribosomal typing of *E.coli* to identify sources of coliform concentrations for a number of years. The establishment of a library of over 24,000 *E.coli* RNA ribotypes provides a valuable resource and increases the potential for matches of unknown water sample ribotypes to previously identified source samples. This study increased the database for Dr. Samadpour's ongoing work. Unfortunately, due to sample processing difficulties occurring at both laboratories, thirty water samples were not processed. It is difficult to speculate how these unprocessed samples would have impacted the overall distribution of the species matches. However, as there was a wide diversity of species matches that were noted from the remainder of the data, it is unlikely that the overall species diversity would change, but rather a potential for minor changes among the percentage matches for each species is possible.

Recently, the Rincon Point homeowners approved a plan for annexation of their homes to public sewer lines of the Carpinteria Sanitary District. The plan, currently in the environmental planning stage, would bring sanitary sewer infrastructure to the community and allow for the conversion of all 74 residences from septic to sewer. Projected completion of connection to the Sanitary District wastewater treatment plant is scheduled for approximately the Fall of 2001. In an area that is known to have septic system problems, the most prudent source reduction strategy is conversion to sanitary sewer whenever possible. This study confirms the need for the Rincon Point community to establish sanitary sewer disposal for domestic wastewater. This process will also include the proper destruction of existing septic systems during the conversion process. Careful monitoring of water quality conditions should be carried out after the conversion process is completed to ascertain any improvements in overall water quality.

5.0 Summary

Although all study goals were achieved, there still remain many obstacles to reliance on this technique for widespread usage. Some of the problems that will need to be overcome include:

- Potential interference from many sources in the watershed. It is critical that the scope of the application in a natural environment be as focused as possible.
- Relatively high expense for ongoing monitoring or extensive studies.
- Protracted timeframe between sample submission and test results.
- Inability to address potential public health risk based on findings.

The study was effective in:

- ✓ Identifying sources of fecal coliform pollution.
- ✓ Providing a rough assessment of location within the watershed of these source contributions within the scope of this study workplan.
- ✓ Providing focus and guidance to potential source reduction strategies.
- ✓ Increasing experience of local staff with source and water collection techniques.
- ✓ Development of the first component of a local database for *E.coli* ribotypes if future studies and technique application are contemplated.
- ✓ Providing reassurance to the local community that conversion of septic systems, in this particular watershed, to a sanitary sewer collection system will likely reduce the amount of human waste in the watershed.

Table 1

Rincon Creek Watershed Fecal Sample Sources

Genera	Common Name	Number of Fecal Samples Collected (208 Total)
	Birds	
RALLIDAE	Coots	4
COLUMBIDAE	Dove- Pigeons	
	Rock (Pigeons)	10
	Mourning	10
ANATIDAE	Ducks- Mallard	11
ARDEIDAE	Egret-	5
	Great	
	Snowy	
ARDEIDAE	Heron	2
	Great-Blue	8
	Green-Backed	
	Night	
PELECANIDAE	Pelicans	10
LARIDAE	Sea Gulls-	7
	Western	3
	California	
	Ringbilled	
HIRUNDINIDAE	Swallows	10
	Mammals	
URSIDAE	Bear	1
FELIDAE	Cats	
	Bobcat	3
	Domestic	20
BOVIDAE	Cattle	20
CANIDAE	Coyote	5
CANIDAE	Dogs	20
EQUIDAE	Horses	20
HOMO SAPIENS	Humans	20
DIDELPHICAE	Opossums	5
PROCYANIDAE	Raccoon	4
HETEROMYIDAE	Rats	6
MUSTELIDAE	Skunks	6

Table 2

Matches of water samples to species at each sample location

Species	# of Matches	% of Total Matches	Ocean	Lagoon	Culvert
Human	25	20%	11	14	0
Duck	14[1]	11%	3	6	3
Dog	12	10%	3	7	2
Seagull	11	9%	2	5	4
Raccoon	8	6%	1	4	3
Opossum	8	6%	3	2	3
Horse	7	6%	2	0	5
Cat	7	6%	1	3	3
Coyote	6	5%	1	0	5
Cow	5	4%	1	0	4
Bobcat	3	2%	0	2	1
Raven	3	2%	0	0	3
Rodent	3	2%	1	0	2
Pelican/Seagull	3	2%	1	2	0
Otter	3	2%	0	0	3
Skunk	2	2%	0	2	0
Sheep	1	1%	0	1	0
Fox	1	1%	0	0	1
Beaver	1	1%	0	0	1
Swallow	1	1%	1	0	0
TOTAL	**124**	**100%**	**31**	**48**	**43**

Table 2A

Matches to Domestic Sources

Species	# of Matches	% of Total Matches
Human	25	20%
Dog	12	10%
Horse	7	6%
Cat	7	6%
Cow	5	4%
Sheep	1	1%
TOTAL	**57**	**46%**

[1] Site 4 (located in Long Creek- a tributary to Rincon Creek) accounted for 2 matches. Both were for the duck species. These matches were accounted for in total matches but the site location is not shown on this chart.

Table 3
Fecal Coliform Concentrations

Date	**Sample ID**	Fecal Coliform		**Date**	**Sample ID**	Fecal Coliform		**Date**	**Sample ID**	Fecal Coliform	
		Colilert (mpn)	CFU's/ 100ml			Colilert (mpn)	CFU's/ 100ml			Colilert (mpn)	CFU's/ 100ml
5/19/99	RC-1-A1	74	40	5/24/99	RC-1-C1	10	40	5/26/99	RC-1-E1	359	300
5/19/99	RC-1-A2	187	160	5/24/99	RC-1-C2	<10	10	5/26/99	RC-1-E2	131	110
5/19/99	RC-1-A3	142	80	5/24/99	RC-1-C3	41	50	5/26/99	RC-1-E3	135	170
5/19/99	RC-1-A4	131	50	5/24/99	RC-1-C4	20	70	5/26/99	RC-1-E4	201	80
5/19/99	RC-1-A5	31	60	5/24/99	RC-1-C5	10	90	5/26/99	RC-1-E5	211	230
5/19/99	RC-2-A1	158	190	5/24/99	RC-2-C1	63	50	5/26/99	RC-2-E1	97	80
5/19/99	RC-2-A2	98	70	5/24/99	RC-2-C2	41	90	5/26/99	RC-2-E2	85	120
5/19/99	RC-2-A3	98	80	5/24/99	RC-2-C3	74	80	5/26/99	RC-2-E3	197	40
5/19/99	RC-2-A4	109	50	5/24/99	RC-2-C4	73	70	5/26/99	RC-2-E4	74	50
5/19/99	RC-2-A5	246	140	5/24/99	RC-2-C5	52	60	5/26/99	RC-2-E5	97	100
5/19/99	RC-3-A1	10	20	5/24/99	RC-3-C1	<10	20	5/26/99	RC-3-E1	52	60
5/19/99	RC-3-A2	52	<10	5/24/99	RC-3-C2	<10	10	5/26/99	RC-3-E2	31	20
5/19/99	RC-3-A3	<10	20	5/24/99	RC-3-C3	20	60	5/26/99	RC-3-E3	30	10
5/19/99	RC-3-A4	<10	10	5/24/99	RC-3-C4	20	80	5/26/99	RC-3-E4	<10	50
5/19/99	RC-3-A5	10	40	5/24/99	RC-3-C5	<10	80	5/26/99	RC-3-E5	<10	40
5/20/99	RC-1-B1	<10	20	5/24/99	RC-1-C5DUP	31	10	5/26/99	RC-4-E1	132	70
5/20/99	RC-1-B2	10	20	5/25/99	RC-1-D1	98	90	5/27/99	RC-1-F1	<10	30
5/20/99	RC-1-B3	63	70	5/25/99	RC-1-D2	109	100	5/27/99	RC-1-F2	52	30
5/20/99	RC-1-B4	10	110	5/25/99	RC-1-D3	84	70	5/27/99	RC-1-F3	31	20
5/20/99	RC-1-B5	10	30	5/25/99	RC-1-D4	201	180	5/27/99	RC-1-F4	20	140
5/20/99	RC-2-B1	<10	80	5/25/99	RC-1-D5	52	30	5/27/99	RC-1-F5	73	80
5/20/99	RC-2-B2	<10	90	5/25/99	RC-2-D1	52	30	5/27/99	RC-2-F1	52	80
5/20/99	RC-2-B3	41	80	5/25/99	RC-2-D2	10	30	5/27/99	RC-2-F2	74	50
5/20/99	RC-2-B4	31	50	5/25/99	RC-2-D3	52	40	5/27/99	RC-2-F3	52	80
5/20/99	RC-2-B5	20	120	5/25/99	RC-2-D4	20	40	5/27/99	RC-2-F4	108	140
5/20/99	RC-3-B1	20	70	5/25/99	RC-2-D5	41	40	5/27/99	RC-2-F5	226	100
5/20/99	RC-3-B2	20	70	5/25/99	RC-3-D1	31	10	5/27/99	RC-3-F1	122	120
5/20/99	RC-3-B3	<10	160	5/25/99	RC-3-D2	10	20	5/27/99	RC-3-F2	73	20
5/20/99	RC-3-B4	<10	80	5/25/99	RC-3-D3	<10	90	5/27/99	RC-3-F3	132	190
5/20/99	RC-3-B5	<10	30	5/25/99	RC-3-D4	<10	30	5/27/99	RC-3-F4	85	170
				5/25/99	RC-3-D5	<10	20	5/27/99	RC-3-F5	31	130

Table 3 continued

Date	Sample ID	Fecal Coliform		Date	Sample ID	Fecal Coliform		Date	Sample ID	Fecal Coliform	
		Colilert (mpn)	CFU's/ 100ml			Colilert (mpn)	CFU's/ 100ml			Colilert (mpn)	CFU's/ 100ml
6/01/99	RC-1-G1	<10	<10	6/03/99	RC-1-J1	30	<10	6/04/99	RC-1-K1	10	30
6/01/99	RC-1-G2	10	40	6/03/99	RC-1-J2	10	10	6/04/99	RC-1-K2	74	60
6/01/99	RC-1-G3	10	<10	6/03/99	RC-1-J3	<10	<10	6/04/99	RC-1-K3	145	180
6/01/99	RC-1-G4	10	<10	6/03/99	RC-1-J4	10	10	6/04/99	RC-1-K4	118	230
6/01/99	RC-1-G5	20	<10	6/03/99	RC-1-J5	<10	<10	6/04/99	RC-1-K5	285	430
6/01/99	RC-2-G1	85	90	6/03/99	RC-2-J1	63	130	6/04/99	RC-2-K1	31	140
6/01/99	RC-2-G2	41	90	6/03/99	RC-2-J2	20	90	6/04/99	RC-2-K2	63	140
6/01/99	RC-2-G3	309	230	6/03/99	RC-2-J3	10	140	6/04/99	RC-2-K3	108	130
6/01/99	RC-2-G4	233	80	6/03/99	RC-2-J4	62	130	6/04/99	RC-2-K4	73	300
6/01/99	RC-2-G5	52	80	6/03/99	RC-2-J5	84	100	6/04/99	RC-2-K5	85	330
6/01/99	RC-3-G1	10	30	6/03/99	RC-3-J1	10	40	6/04/99	RC-3-K1	<10	50
6/01/99	RC-3-G2	<10	30	6/03/99	RC-3-J2	20	40	6/04/99	RC-3-K2	<10	70
6/01/99	RC-3-G3	20	40	6/03/99	RC-3-J3	20	20	6/04/99	RC-3-K3	<10	20
6/01/99	RC-3-G4	<10	40	6/03/99	RC-3-J4	20	20	6/04/99	RC-3-K4	<10	30
6/01/99	RC-3-G5	10	30	6/03/99	RC-3-J5	10	30	6/04/99	RC-3-K5	<10	60
6/02/99	RC-1-H1	<10	<10								
6/02/99	RC-1-H2	10	10								
6/02/99	RC-1-H3	<10	<10								
6/02/99	RC-1-H4	10	<10								
6/02/99	RC-1-H5	<10	<10								
6/02/99	RC-2-H1	97	40								
6/02/99	RC-2-H2	160	130								
6/02/99	RC-2-H3	74	220								
6/02/99	RC-2-H4	52	130								
6/02/99	RC-2-H5	120	210								
6/02/99	RC-3-H1	41	60								
6/02/99	RC-3-H2	10	60								
6/02/99	RC-3-H3	31	50								
6/02/99	RC-3-H4	52	50								
6/02/99	RC-3-H5	20	30								

Table 4

Fecal Coliform Concentrations per Sample Site

Date	Fecal Coliform (mpn)/100ml					
	Surfzone		Lagoon		Culvert	
	Median	Average	Median	Average	Median	Average
05/20/1999	10	23.3	31	30.7	20	20
05/24/1999	15	20.3	63	60.6	20	20
05/25/1999	98	108.8	41	35	20.5	20.5
05/26/1999	201	207.4	97	110	31	37.7
05/27/1999	41.5	44	74	102.4	85	88.6
06/01/1999	10	12.5	85	144	10	13.3
06/04/1999	118	126.4	73	72	<10	<10

Table 5

Field Measurements (pH, Temperature and Tidal Cycles) at various sampling locations and sampling dates

pH			Temperature °C			Tide (ft)
Ocean	Lagoon	Culvert	Ocean	Lagoon	Culvert	Changes
8.23	8.02	8.14	17.5	17.5	16.4	+0.5
8.24	8.26	8.2	18.6	17	17.3	+0.5
7.85	7.83	8.15	15.6	14.7	14	-0.8
7.7	8.06	7.77	15.8	15.6	15.6	-0.7
8.03	8.04	8.16	16.7	16.9	15.9	-1.1
8.17	8.18	7.9	16.5	16.8	15.6	-0.5
8.45	8.22	8.14	16.8	15.6	14.7	+0.5
8.35	8.33	8.06	15.7	15.5	15.2	+0.5
8.16	8.22	8.43	19.6	22	16.3	+0.5
8.37	8.41	8.22	14.9	15.6	13.8	+0.5

Figure 1

Aerial Photo of the Rincon Creek Watershed

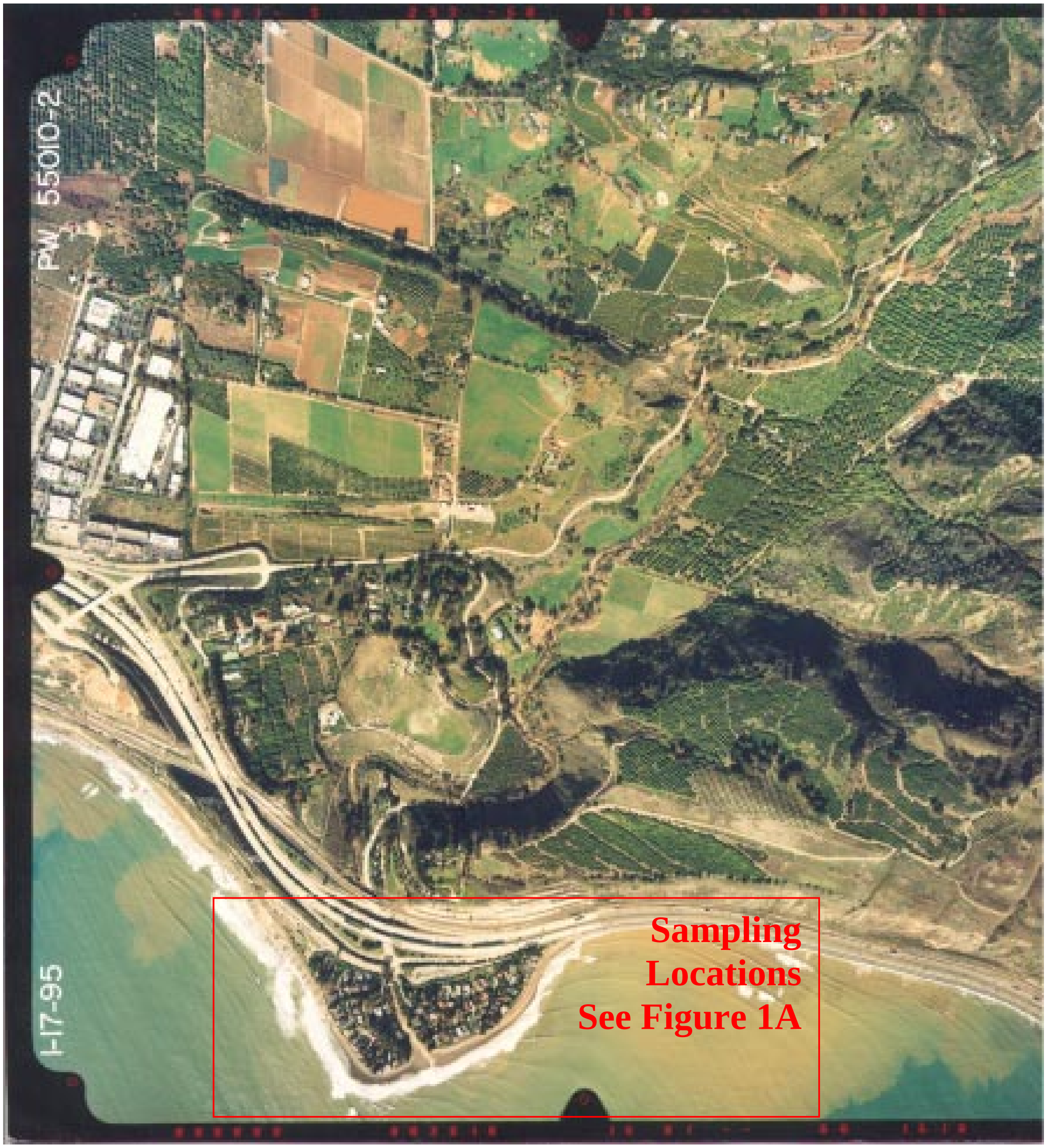
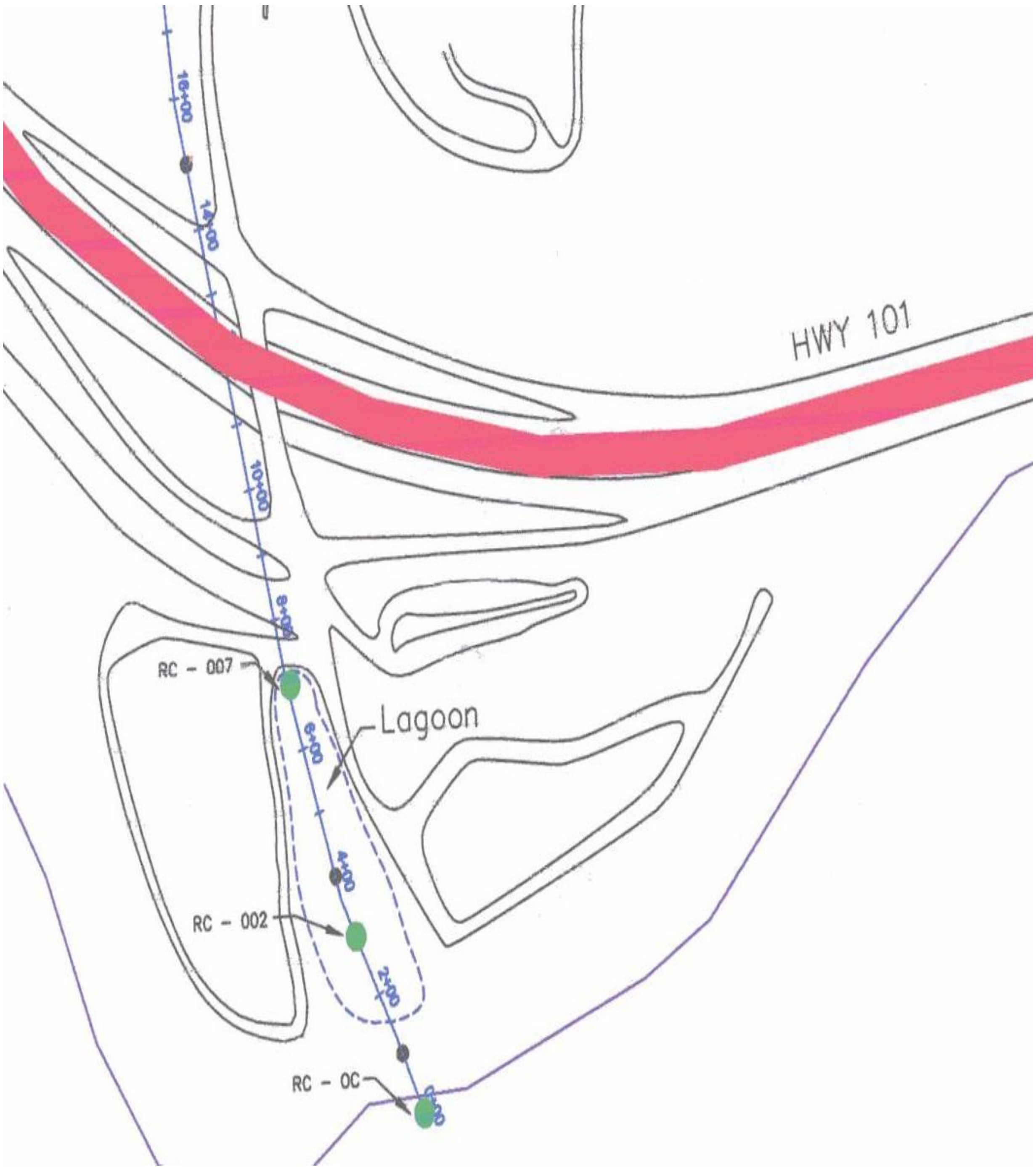


Figure 1A

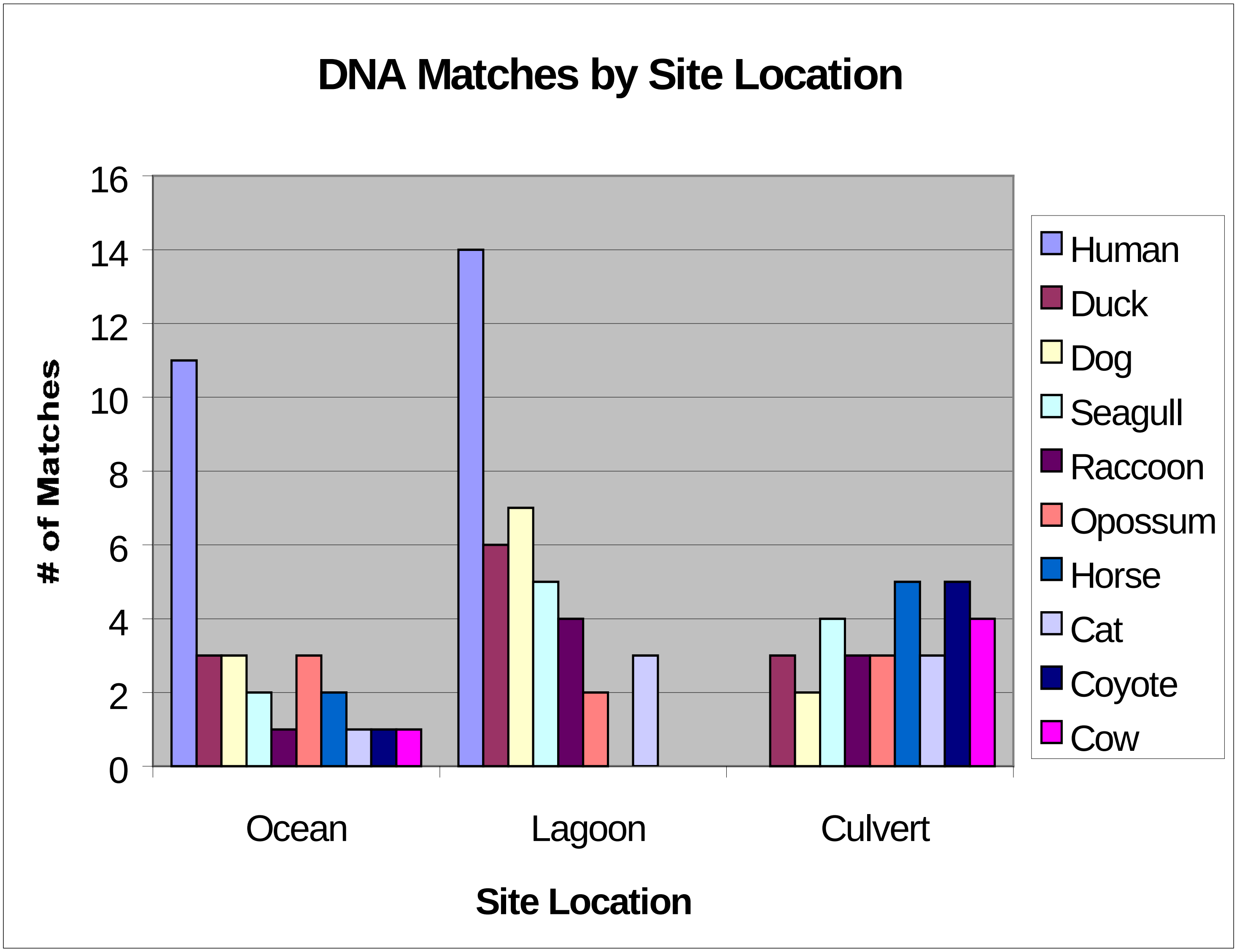
Sampling Locations in Lower Rincon Creek Watershed



OCEAN

Figure 2

Summary of species matches per sample locations
(Note, only species with over 4% of matches are presented in figure)



Note: Sample Site #4 not depicted on this figure

Figure 3

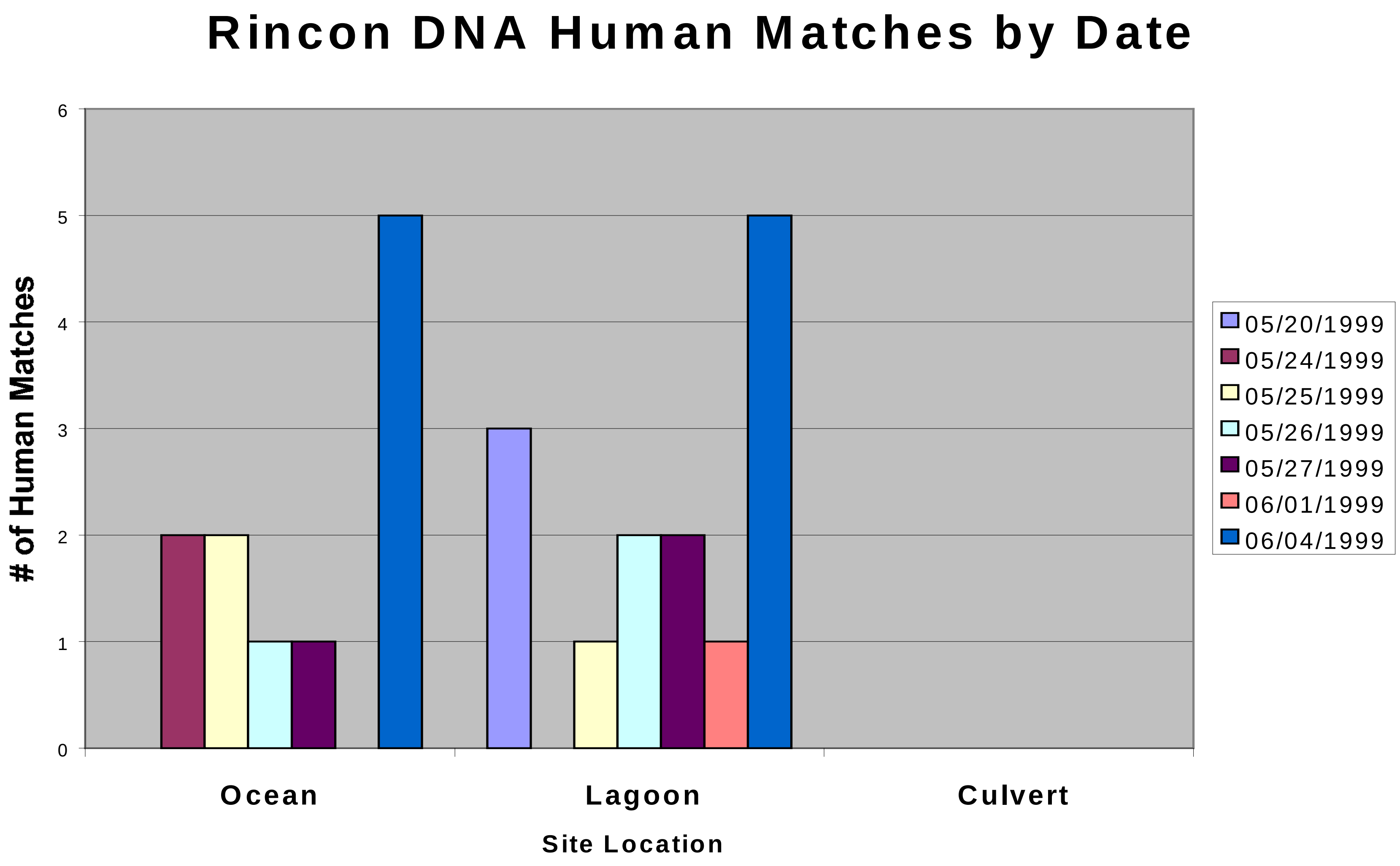
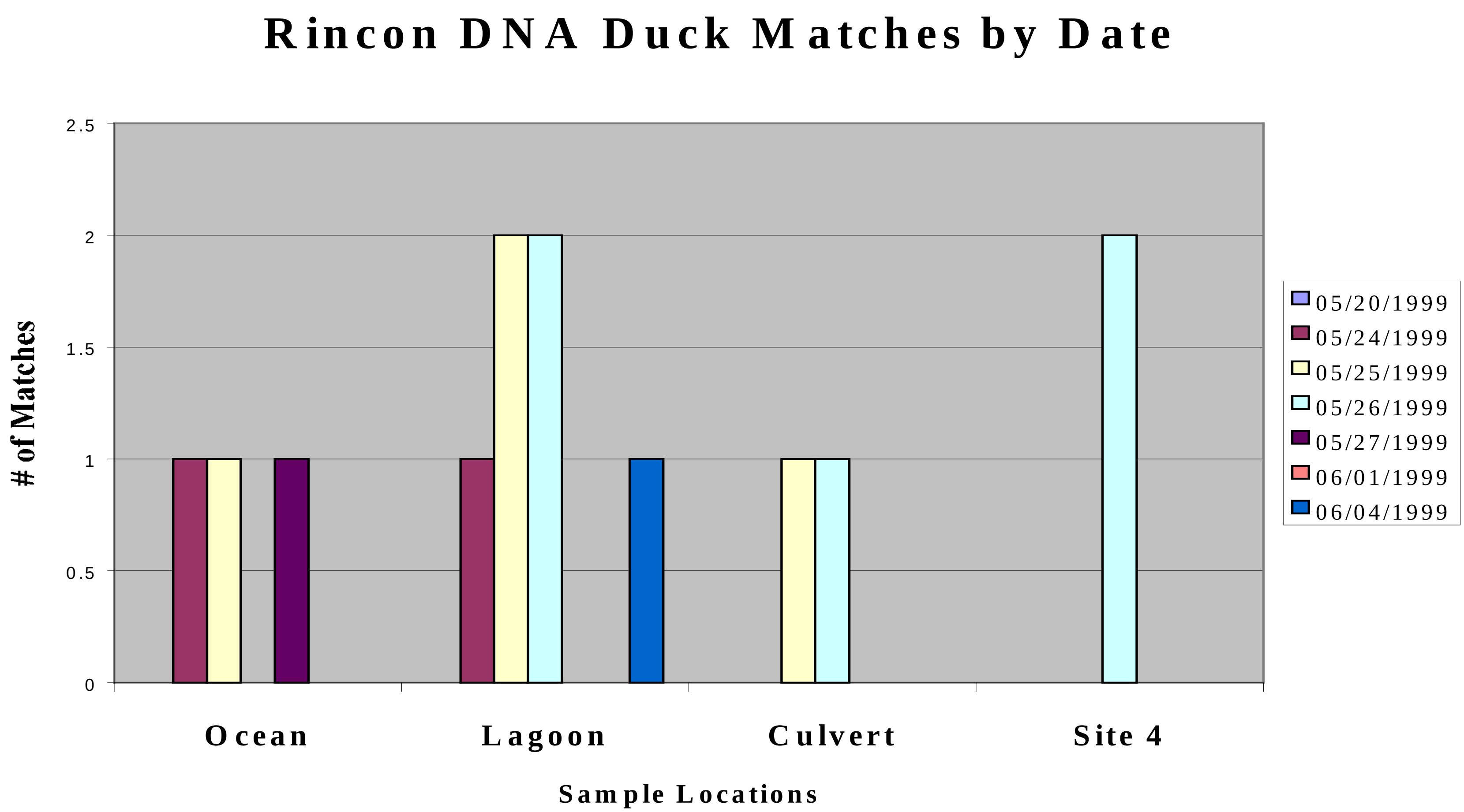
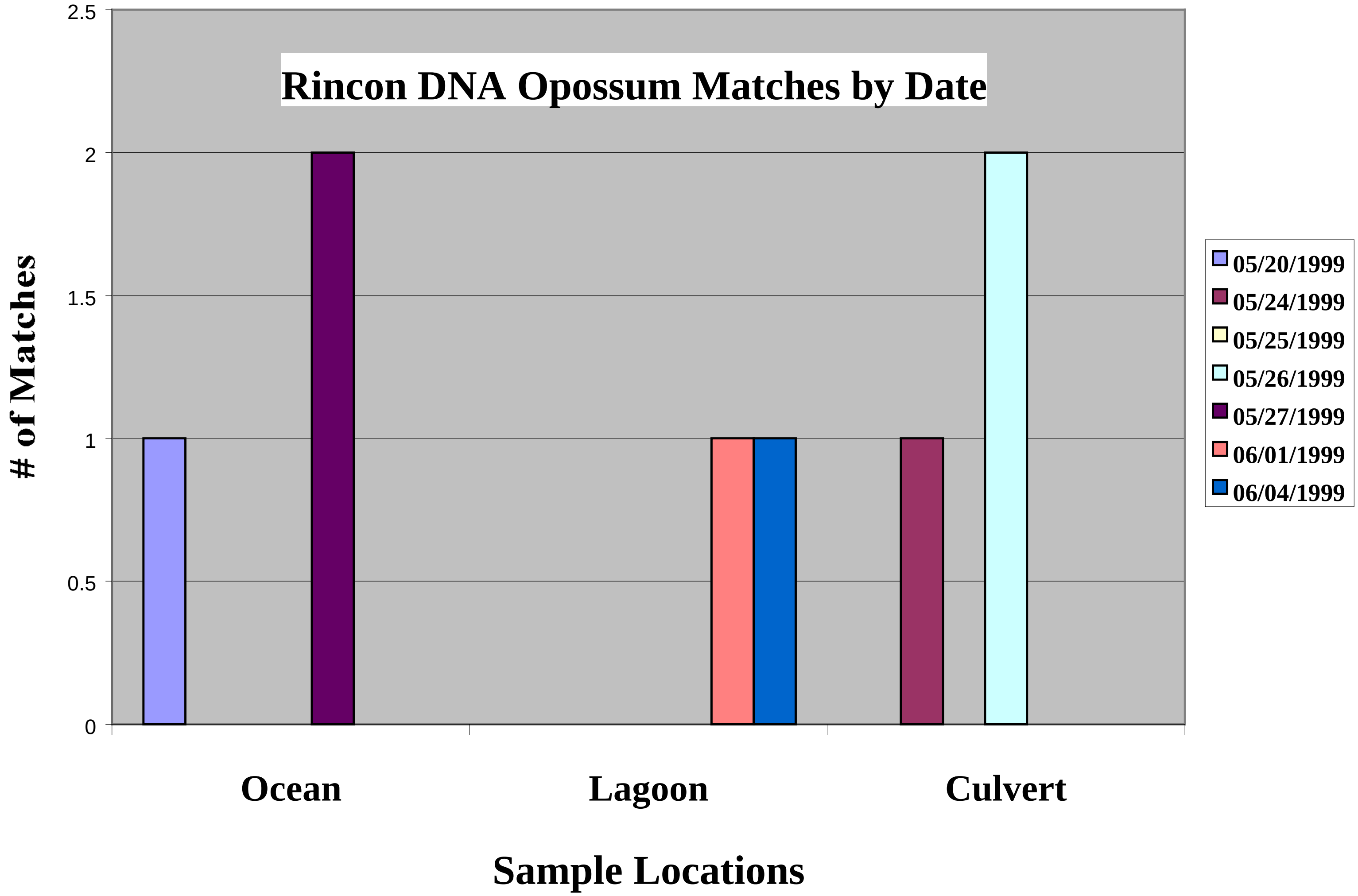

Rincon DNA Human Matches by Date
# of Human Matches
0
1
2
3
4
5
6
Ocean
Lagoon
Culvert
Site Location
05/20/1999
05/24/1999
05/25/1999
05/26/1999
05/27/1999
06/01/1999
06/04/1999

Figure 4



Site 4 = Long Creek Tributary

FIGURE 5


Rincon DNA Opossum Matches by Date
# of Matches
2.5
2
1.5
1
0.5
0
Ocean
Lagoon
Culvert
Sample Locations
05/20/1999
05/24/1999
05/25/1999
05/26/1999
05/27/1999
06/01/1999
06/04/1999

APPENDIX A

Project Clean Water
Source Control Recommendations
For Pet and Domestic Animal Waste Management

PETS

On a walk
Picking up after your pet while out on a walk insures that waste will not wash into catch basins that drain to creeks and eventually to the ocean. Picking up after your pet on the beach stops the direct flow of waste to the ocean.
Remember, it is illegal not to pick up your dog's waste.

- Always carry a plastic bag with you when you take your dog for a walk. Plastic grocery and vegetable bags work well. Place your hand in the bag, pick up the waste, then turn the bag inside out.
- Commercial "scoopers" can also be purchased to make proper disposal of pet waste even easier. Check pet stores and catalogs.
- Dogs can be trained to "take care of business" at the beginning of a walk so you can dispose of the waste right away.

Clean up at home
Pet owners have many different ways of cleaning up after their pets at home. Here are a few that are creek and ocean friendly:

- Smaller quantities of pet waste can be left to decompose slowly on permeable surfaces.
- Larger amounts should be scooped and placed in the trash. One method suggested for this is to place a plastic bag in a 5-gallon trash can with a lid. When you pick up dog droppings in your yard, put them in that trash can, then cover them with a sprinkling of powdered lime (available at building supply stores) and close the lid. Each time you clean up the yard and add droppings to the container, add more lime. When the container is full, tie the bag closed and put it in your regular trash container.
- A more innovative method, which not only disposes of pet waste but also creates fertilizer, is to install a disposal system, commercially available through pet owners' and gardening catalogs. These systems use bacterial and enzymatic cultures which reduce the waste to a liquid, which is then absorbed into the soil.

Domestic Animal Waste

Animal waste contains nutrients that make great fertilizer, but cause problems for aquatic life in creeks, wetlands and the ocean. It also contains bacteria, which can cause gastro-intestinal disorders and other medical problems for swimmers and anyone who may come in contact with the water. When it rains, or sometimes during irrigation, animal waste left uncovered or stored improperly near creeks and storm drains can flow, untreated, directly to the ocean. As a result:

- Nutrients in animal waste fertilize aquatic plants; they grow and take oxygen away from other aquatic life, which then causes the aquatic life to die.
- Sediment in runoff from livestock facilities harms aquatic life by clogging the gills of fish, blocking sunlight, and raising water temperatures.
- When the bacterial level in ocean water gets too high, beaches close to protect the public.
- Caring for domestic animals sometimes requires the use of pesticides. These chemicals may also wash into creeks and flow to the ocean, harming aquatic life and people.

Planning Ahead

- Place barns, corrals and other high-use areas so that rain or irrigation will carry runoff away from the nearest creek. Surround the area with pasture, if possible.
- Divert runoff from your property so that it doesn't cross livestock areas.
- If possible, design diversion terraces which allow runoff to be filtered through vegetation.

Grazing

- Divide grazing areas into 3 or more units of equal size and rotate animals.
- When grass is grazed down to 3 or 4 inches move them to another section, allowing the grass to grow to 8 to 10 inches before allowing regrazing.
- Keep animals away from wet fields, and indoors if possible, during rainfall.

Clean-up and Storage

- Gather up soiled bedding and manure on a daily basis from stalls and paddocks.
- Place it in sturdy, insect resistant, leak-proof containers:
    - Plastic garbage cans with lids
    - Fly-tight wooden or concrete storage sheds
    - Composters
    - Pits or trenches lined with an impermeable layer

Then What?

- Compost the material for your own use or donate it to local nurseries or botanic gardens.
- Use it to fertilize pastures, but **not just before or during a rainstorm.**
- Transport the material to topsoil companies or composting centers.