Draft Report of the University of California, Irvine
Water Quality Survey 1996

The Spatial Distribution of Waterborne Microbial Contaminants in Channels Feeding Talbert Marsh in Huntington Beach, California

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Forward

The University of California, Irvine (UCI) Water Quality Survey is a group of undergraduate students majoring in Environmental Engineering at UCI. As part of their design experience, these students designed and implemented a field project aimed at assessing the source and fate of microbial pollutants in a local watershed. The entire project was subjected to two external reviews -- one midquarter review to discuss the field plans, and a final review to report the results obtained. The external reviewers were made up of individuals from local government agencies, environmental groups, and universities. This report describes the Survey's 1996 study of the microbial water quality in Talbert Marsh, located in Huntington Beach, California.

This project would not have been possible without the enthusiastic participation of the reviewers, technical support of the County Sanitation Districts of Orange County and the Orange County Water Districts, and the financial support of the National Water Research Institute and the National Science Foundation. We thank all the following people for serving on the reviewing committee and/or for their invaluable input during the course of this project.

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This report is a compilation of the group and individual reports submitted by the participating students at the end of the school quarter. Each student played an important role in executing this project and completing this report.

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Abstract

This study investigated the sources and sinks of bacterial contamination in two channels feeding Talbert Marsh, a coastal wetland located in Huntington Beach, California. Bacterial contaminants were present in urban runoff feeding the channels and were subsequently diluted by ocean water as they traveled downstream. For the time frame encompassed by this study (March 1, 1996), dilution appeared to be the single most important mechanism for the "purification" of channel water entering the Talbert Marsh ecosystem.

Introduction

Coastal wetland systems represent an important environmental resource because of their importance as a habitat for rare and endangered species, their ability to buffer shorelines against erosion, the many recreational opportunities they afford, and their natural aesthetic beauty. There is additional evidence that well-managed wetlands can also act as "natural" treatment systems for the removal of pollutants in runoff and treated sewage effluent (Raisin and Mitchell, 1995; Patruno and Russell, 1994; Soukup et al., 1994).

The mechanisms of bacterial contaminant removal by wetlands are not fully understood; however, a combination of physical, chemical and biological processes appears to be involved (Vincent, 1994). Possible physical processes include filtration by the bottom sediments and attached biofilms, and coagulation and sedimentation in the water column (O'Melia, 1995; Rivera et al., 1995). Chemical processes include oxidation, exposure to biocides excreted by some aquatic plants and microbial populations, and adsorption to organic matter (Batchelor et al., 1990). Biological removal mechanisms include predation by higher trophic levels, attack by lytic bacteria and viruses, and natural die-off (Gersberg et al., 1989).

While a number of studies have examined how wetland systems influence the water quality of surface runoff, no studies have been carried out, to our knowledge, which examine the distribution of bacterial contamination in channel systems that feed marsh ecosystems. In the channels we would not expect plants to play a major role in bacterial removal since vegetative growth is minimal. In rivers, one of the primary

mechanisms for particle -- and by extension bacteria -- removal is coagulation combined with sedimentation, and this mechanism is strongly dependent on water salinity (O'Melia, 1995). We reasoned that coagulation and sedimentation might also be responsible for removing biological contaminants in channels carrying urban runoff to marsh ecosystems. To test this hypothesis, we collected samples from channels feeding into Talbert Marsh in Huntington Beach, California and analyzed them for total coliform (TC), fecal coliform (FC), fecal streptococcus (FS), total heterotrophic bacteria (H), acute cytotoxicity, and a set of physical parameters including pH, salinity, turbidity, and temperature. We found a strong inverse correlation between the concentration of biological indicators and the salinity of the water, indicating that the channel water was "purified" as it approached the marsh. The purpose of this report is to: i) document the data collected for the Talbert Marsh watershed, ii) provide preliminary interpretation of these data, and iii) serve as a starting point for further investigations of the Talbert Marsh ecosystem.

Materials and Methods

Location. Talbert Marsh is located in Huntington Beach, California, adjacent to the intersection of Pacific Coast Highway and the Santa Ana River Channel outlet (see Figure 1). It is a restored marine estuary subject to diurnal tidal flushings. The marsh is fed by fresh water from the Talbert and Huntington Beach flood control channels that, in turn, receive urban runoff from the surrounding cities. The areal extent of the Talbert Marsh watershed investigated in this report is approximately 25 acres. Land use in the watershed is primarily medium to high density residential with

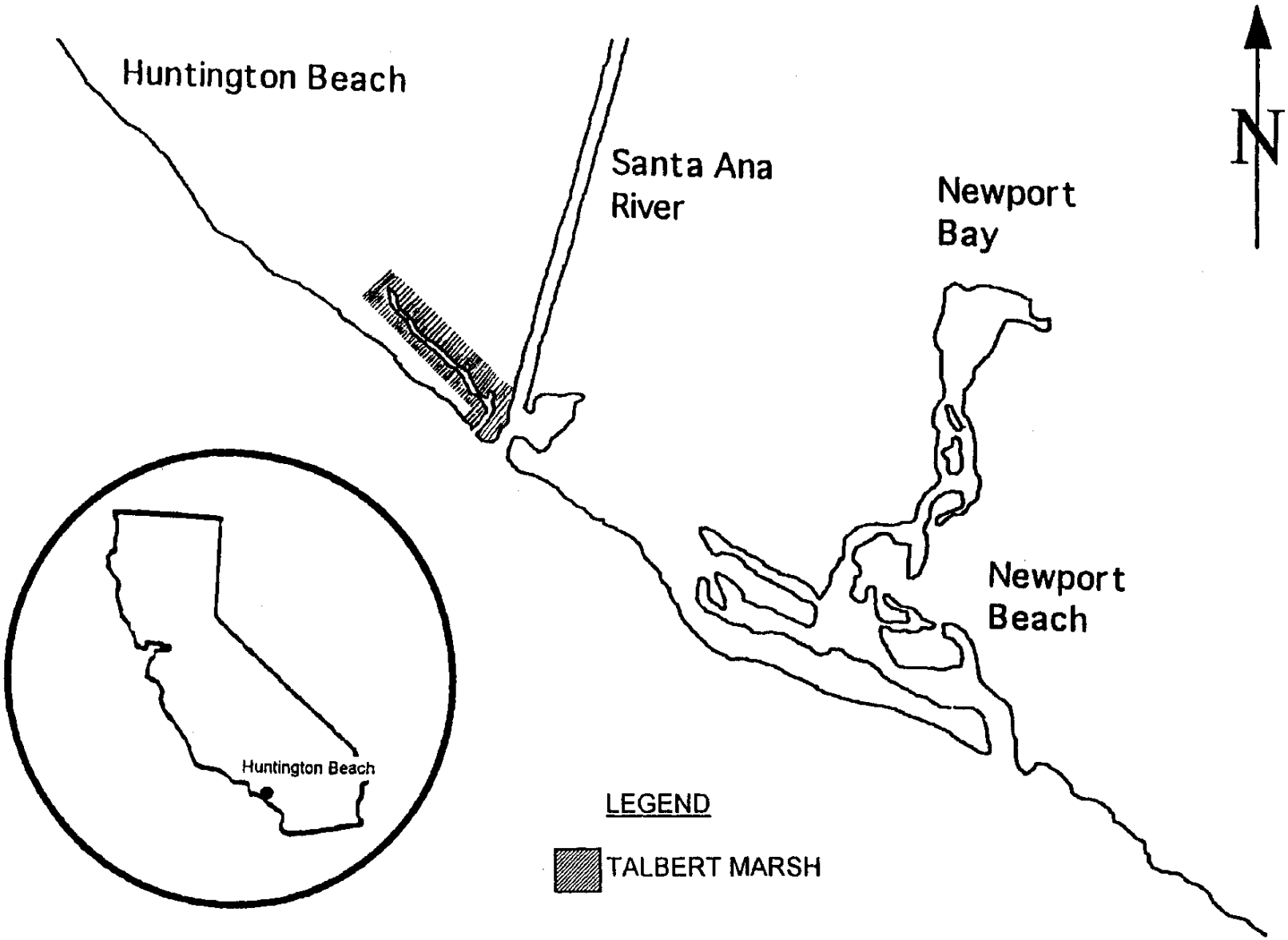

Figure 1. Location of Talbert Marsh.

some light commercial use. As a result of the very low elevation of the watershed, the lower reaches of the channel, which are earthen bottom, receive runoff from storm drain lift stations located along the channel. The upper reaches, which are characterized by concrete sides and bottom, are fed primarily by gravity flow.

Sampling Sites and Time. All five sampling sites chosen for this study are shown in Figure 2. Site 1 is located in Talbert Marsh on the downstream side of the Brookhurst street overpass. Sites 2,3 and 4 are located in the Talbert Channel on the downstream side of the Banning street overpass, Hamilton street overpass, and Adams street overpass, respectively. Sites 3 and 4 are located adjacent to storm drain lift stations. Site 5 is located in the Fountain Valley Channel which drains directly into the Talbert Channel. Talbert Channel is earthen bottom between sites 1 and 4, and completely concrete lined upstream of site 4. Sampling occurred between the hours of 7:00 a.m. and 9:00 a.m. on March 1, 1996, and between 6:00 a.m. and 8:00 a.m. on March 4, 1996. No rainfall occurred within 48 hours prior to each sampling time. However, a total of 2.95 inches of rainfall precipitated in Huntington Beach between February 19 and February 28 (Orange County Register). The flushing cycle of the channel was estimated using Xtide Tide and Current Prediction Program Version 1.4 (available on the internet at http://universe.digex.net/~dave/xtide/xtide.html). Actual tidal data for the two sampling dates were obtained from a real-time tide gauge located in Talbert Channel at the downstream side of the Brookhurst street overpass and operated by the Orange County Environmental Management Agency. Sampling on

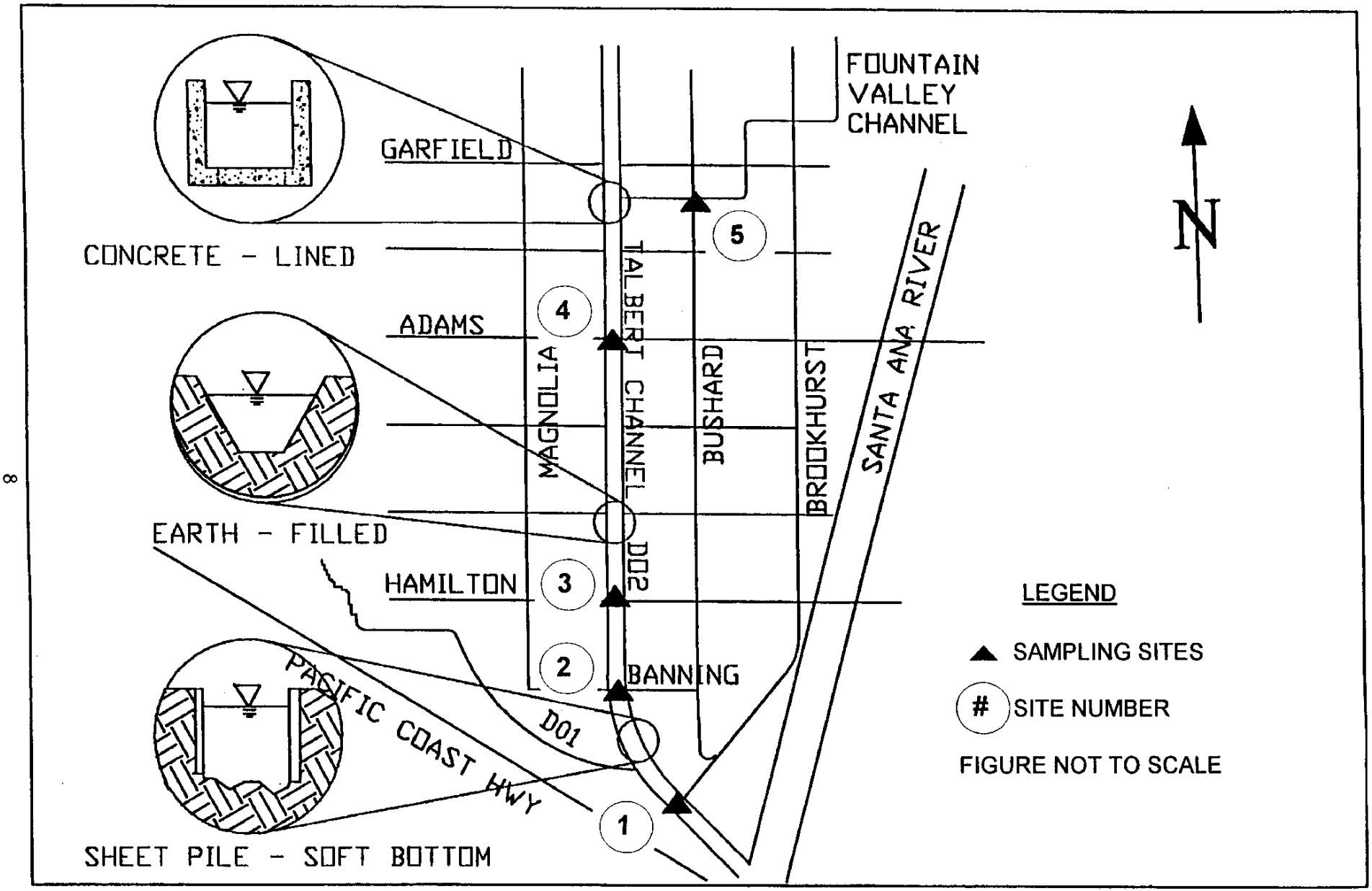

Figure 2. Location of sampling sites and characteristic cross-sections along Talbert Channel.

March 1 and March 4 occurred during receding and incoming tides, respectively (see Figure 3).

Sampling Procedure. Water samples were collected using Corning (Pittsburgh, Pennsylvania) 250 mL, non-toxic, sterile plastic bottles. Samples used for acute cytotoxicity test were collected in 25 mL borosilicate glass bottles. Each bottle was attached to a long pole and was lowered from the street overpass into the channel water. Only near surface waters were collected to prevent the disturbance of the bottom sediments. All water samples were placed immediately on ice, and transported to either UCI or the Orange County Water District for analysis. The extraction, storage, and transport of samples conformed with recommendations set forth in the Standard Methods for the Examination of Water and Wastewater, 18th edition (American Public Health Association 1992).

Physical Parameters. Temperature and pH measurements were taken on-site. The temperature was measured using a mercury-filled Celsius thermometer. The pH measurements were taken using a calibrated Orion (Boston, Massachusetts), Model 290A portable pH meter. The salinity measurements were taken in the laboratory immediately upon arrival using an Orion, Model 160 conductivity meter. The turbidity measurements were taken approximately one hour after arrival at UCI using a Hach (Ames, Iowa), Model 2100A Turbidimeter. The nephelometer was turned on twelve hours in advance of sample analysis, and calibrated using 0.5, 5, and 40 nephelometric turbidity unit (NTU) primary standards obtained from AMCO (Pittsburgh, Pennsylvania).

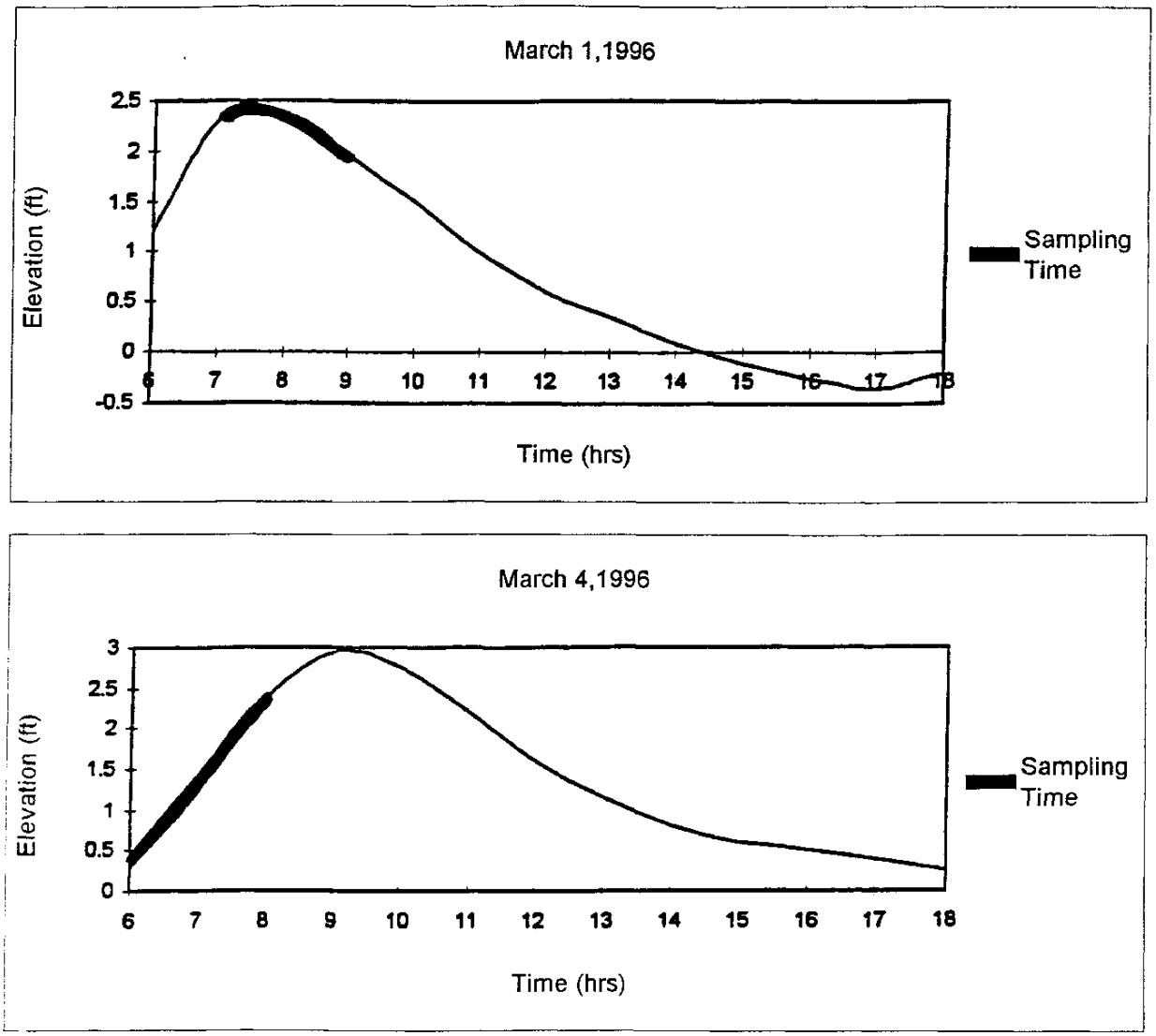

Figure 3. Elevation of water in Talbert Channel at the downstream side of Brookhurst street overpass (data obtained from O.C. Environmental Management Agency).

Total Coliform (TC), Fecal Coliform (FC), and Fecal Streptococcus (FS). The membrane filtration technique performed in the laboratory was taken from Standard Methods (American Public Health Association 1992). For membrane filter analysis, bacteria were isolated by passing the water sample through a membrane filter and then culturing the retained bacteria on a selective medium. All glassware and filtration devices used were washed and then sterilized by autoclaving. The Millipore filter apparatus (Bedford, Massachusetts) was used with 0.45 micrometer pore, 47 mm diameter, gridded membranes that were aseptically transferred with flamed sterile forceps. The filters were then moistened with 20 mL of sterile distilled water. The sample volumes chosen for filtering were 0.1, 1.0, and 5.0 mL for TC analysis. For FS and FC analysis, the sample volumes were 1.0, 10.0, and 40.0 mL. All samples less than 10.0 mL in volume were diluted to 10.0 mL with sterile distilled water prior to filtration. The water sample was shaken vigorously before the sample was pipeted into the filter apparatus. The filter was then rinsed three times with 20-30 mL of sterile distilled water. After filtering and rinsing were completed, the membranes were aseptically transferred to 60 x 15 mm sterilized disposable plastic petri dishes with selective medium. MF endo broth (Difco, Detroit, Michigan) on absorbent pads was used to isolate and tabulate TC, M-FC broth (Difco, Detroit, Michigan) on absorbent pads was used for the analysis of FC, and KF streptococcus agar (Difco, Detroit, Michigan) was used for FS. The plates were incubated for 24 hours at 35°C for TC and 44.5°C for FC. The FS plates were incubated for 48 hours at 35°C. To enable statistical analysis, each sample was filtered in triplicate. Acceptable colony counts for

TC were 20-80 red colonies with metallic sheen per plate; for FC 20-60 blue colonies per plate; and for FS 20-200 colonies demonstrating a light pink to deep red color per plate.

Verification Tests. Verification tests for TC, FC, and FS were carried out in accordance with Standard Methods (American Public Health Association 1992). The cytochrome oxidase (CO) and O-Nitrophenyl Beta-galactopyranoside (ONPG) tests were used to verify TC counts. A negative result from the CO test and a positive result from the ONPG test constituted a positive test for TC. The FC verification consists of i) gas production in lauryl tryptose broth (Difco, Detroit, Michigan) after 48 hours at 35°C and ii) gas production in EC (*E. coli*) broth (Difco, Detroit, Michigan) after 24 hours at 44.5°C. The FS verification test included i) growth on BHI agar (Difco, Detroit, Michigan) for 24 hours at 35°C, ii) 3% hydrogen peroxide test, and iii) Gram staining. No bubble formation after the addition of hydrogen peroxide and a Gram-positive stain was indicative of FS.

Total Heterotrophic Bacteria (H). The spread plate method used to determine H concentration in the samples was taken from Standard Methods (American Public Health Association 1992). Samples were plated in triplicate on R2A agar (Difco, Detroit, Michigan). Sample volumes of 0.1, 0.01, and 0.001 mL were plated, and all samples were thoroughly mixed before filtration to ensure an even distribution of cells. Plates were incubated at 28°C for 48 hours. Only the plates containing fewer than 300 colonies were enumerated and used to determine bacterial densities.

Acute Toxicity Assay. Each of the samples obtained on March 4, 1996, was analyzed for acute cell toxicity using the Microbics (Carlsbad, California) Microtox Toxicity Test System located at Orange County Water District in Fountain Valley, California. The freeze-dried Microtox Reagent was rehydrated by mixing a vile of reagent with 1000 μL of Microtox Reconstitution Solution. The rehydrated reagent was then used to create a reference scale against which the test sample readings were compared. 2500 μL of the original sample was pipeted into a well inside of the test system and four 1:2 serial dilutions were pipeted into adjacent wells. Each sample diluent was then mixed with 10 μL of the test reagent. The Microtox Data Collection and Reduction System and corresponding software were used to measure the reduction in light of each sample, as compared to the standard, after 5 min. and 15 min.

Results

Physical characteristics of the water sampled from Talbert and Fountain Valley channels are summarized in Figure 4. Raw data for all four physical parameters are tabulated in Appendix A. The temperatures recorded on 3/1/96 ranged from 12.5°C at site 1 to 16.0°C at site 5. On 3/4/96 the temperature increased less rapidly with upstream distance from the marsh, from 13.5°C at site 1 to 15.0°C at site 5. The pH of all the water samples was relatively constant, and averaged 7.79 ± 0.13. The salinity and turbidity decreased and increased, respectively, with increasing upstream distance from the marsh; at a particular site, these parameters were relatively constant on the two sampling days. On both days, the salinity increased rapidly from approximately 1 part per thousand (ppt) at the furthermost upstream station (site 5) to 32 ppt at site 3;

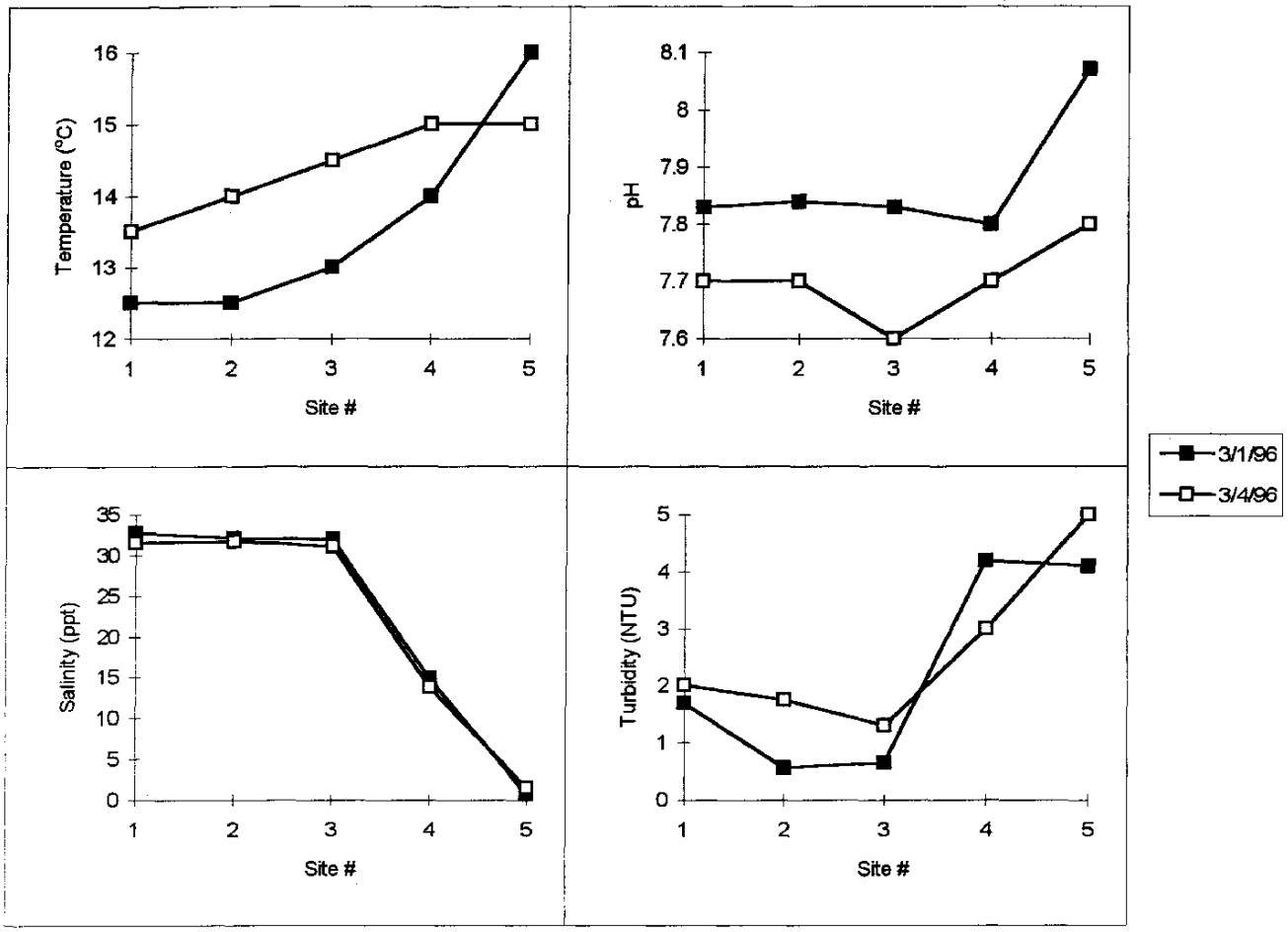

Figure 4. Physical properties of channel water at each sampling site.

downstream of site 3 the salinity remained relatively constant. The salinity of ocean water is typically in the range of 33-35 ppt (Fischer et al., 1979), and therefore the water downstream of site 3 is probably derived primarily from the intrusion of ocean water through Talbert Marsh. On both sampling days, the highest turbidity levels (3 to 5 NTU) were recorded at sites 4 and 5. We noticed that water at these sites was visibly green; hence, algal growth in the relatively low saline waters at sites 4 and 5 may partially account for their higher turbidity.

Figure 5 shows the distribution of total coliform (TC), fecal coliform (FC), fecal streptococcus (FS), and total heterotrophic bacteria (H) in Talbert and Fountain Valley channels on 3/1/96 (bars in the figure). For comparison, the salinity of the water at each site is also shown in this figure (squares). Upstream of site 3, there is a marked increase in the concentration of all bacterial indicators with increasing distance from the marsh. This increase in bacterial concentration is correlated, at least qualitatively, with a decrease in water salinity. Downstream of site 3, the concentration of bacteria decreases with proximity to the marsh, although the magnitude of the decrease is different for the different classes of bacteria. FC exhibited the largest percentage decrease, dropping from 111 CFU/100 mL at site 3 to 8 CFU/100 mL at site 1. The smallest percentage decrease was exhibited by FS which decreased from 31 CFU/100 mL at site 3 to 17 CFU/100 mL at site 1.

A fraction of the bacteria isolated from each of the samples were also subjected to verification tests, the results of which are tabulated in Appendix B. These verification tests can be summarized as follows: 16 out of 18 (or 89%) presumptive

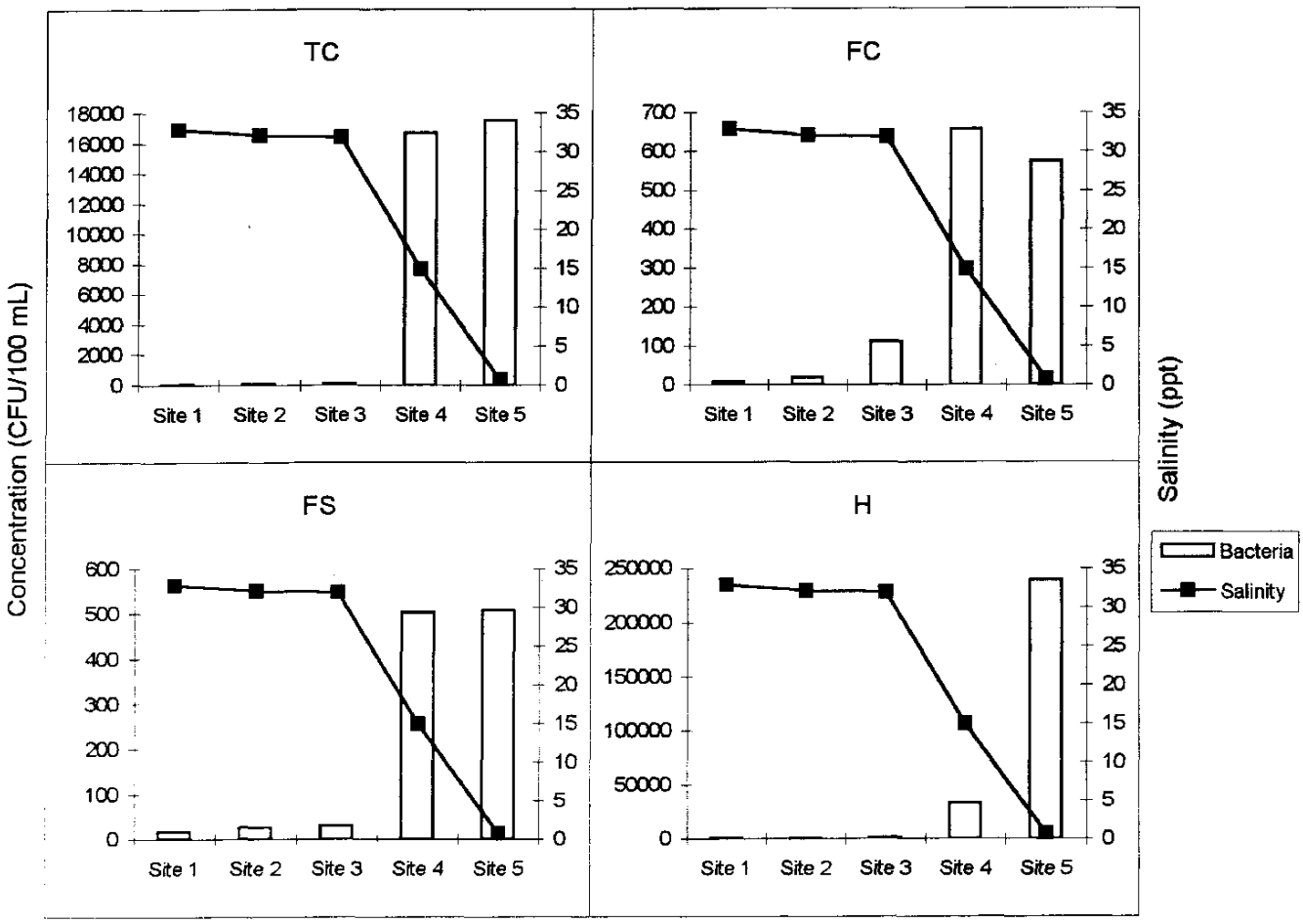

Figure 5. Bacterial concentrations and salinity at each site on March 1, 1996.

TC were verified by negative CO and positive ONPG tests; 28 out of 30 (or 93%) presumptive FC were verified by positive growth in lauryl tryptose and EC broths; and 10 out of 10 (or 100%) of presumptive FS were verified by negative hydrogen peroxide tests and positive Gram stains.

The acute cytotoxicity analysis revealed slight levels of toxicity at sites 1 through 3 and no toxicity at sites 4 and 5.

Discussion

Figure 5 indicates that the concentration of all four bacterial indicators tested for in this study (FC, TC, FS, H) decreased with increasing proximity to the marsh. This trend suggests an upstream source for the bacteria. As the bacteria are transported downstream, they are eliminated by some combination of physical, chemical and/or biological processes. In the following discussion, we consider possible sources and sinks for bacteria in Talbert and Fountain Valley channels.

Potential Sources of Bacteria

Possible sources of bacterial contamination in Talbert Channel include urban runoff, underground contamination, and upstream point sources. Figure 6 shows the cumulative rainfall in Huntington Beach by month starting in January of 1992 and ending in November of 1994 (Orange County Register). Also plotted in this figure are monthly average TC concentrations measured over the same period of time in Talbert Channel at the Banning bridge overpass by the County Sanitation Districts of Orange County (CSDOC). Based on Figure 6, there appears to be a general correlation between periods of high rainfall and elevated TC concentrations in the channel. There

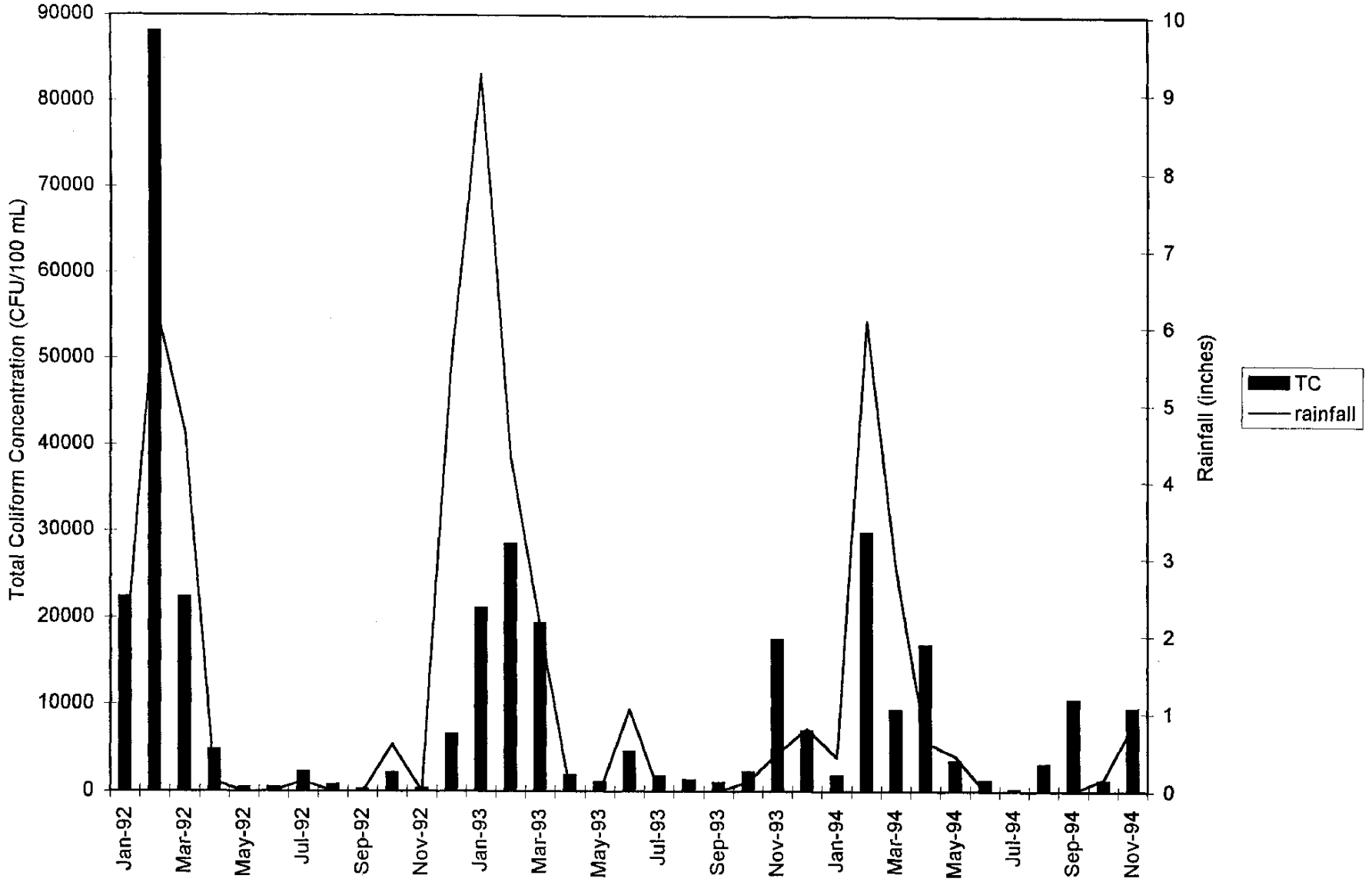

Figure 6. Comparison between rainfall and total coliform concentrations in Talbert Channel at Banning Street from January 1992 to November 1994 (data obtained from CSDOC).

are at least three possible mechanisms that might give rise to this historical correlation between rainfall and TC, including (i) rainfall increases the volume of urban runoff entering the channels, and this runoff may be contaminated with high concentrations of TC and other bacterial contaminants (Gannon and Busse, 1988; Geldreich et al., 1968), (ii) the increased volume of fresh water may enhance the survival of fecally derived bacteria (Ketchum et al., 1949), and/or (iii) the decrease in salinity of channel waters following a rainfall event may stabilize bacteria with respect to coagulation and sedimentation (O'Melia, 1995) resulting in higher concentrations of bacteria in the water column and more rapid transport downstream. It is also interesting to note that there are periods of high TC that do not correlate with high rainfall in Figure 6 (e.g., September 1994). Because water is pumped from the catch basins into the channel on an intermittent basis, contaminated water in the catch basins may be responsible for the occasional spikes in TC observed during non-rainfall periods.

Another possible source of bacteria examined in this study is underground contamination. Bacteria released from a leaking sewer line could, in theory, contaminate the channel water through the earthen bottom portion of the channels. Indeed, there are several sewer lines that run under and alongside the channels and marsh, as illustrated in Figure 7 (heavy dark lines). This scenario, however, is inconsistent with the high levels of bacteria found in the upper reaches of the channel which are completely concrete lined.

Point sources of bacterial contamination -- including illegal dumping of sewage or other biological wastes -- are likely to be intermittent in space and time. The clear

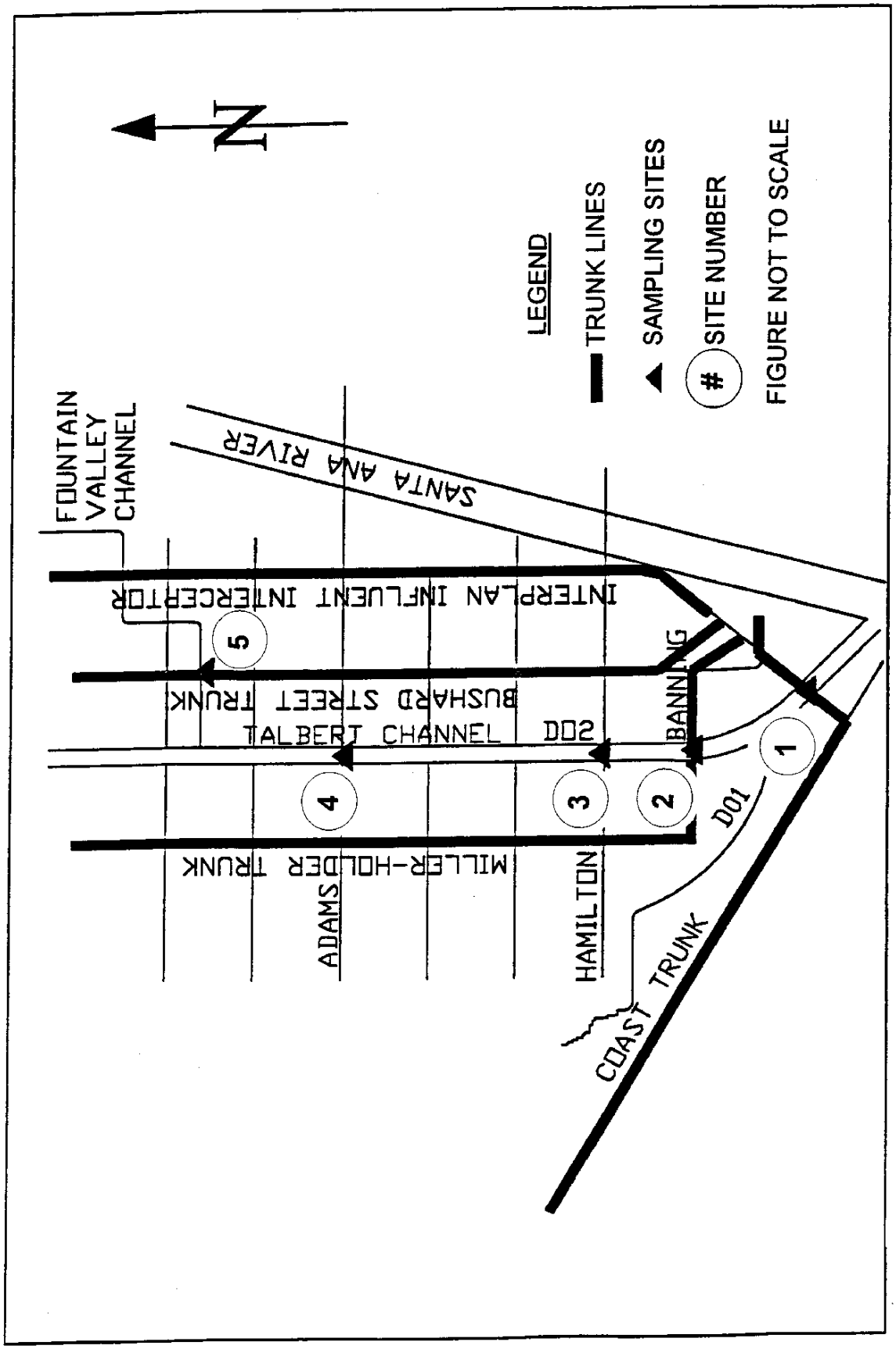

Figure 7. Sewage trunk lines located near Talbert Channel.

trend in the spatial distribution of bacteria observed in Figure 5, together with the strong correlation between rainfall and TC concentration illustrated in Figure 6, are inconsistent with point sources of bacterial contamination.

Potential Sinks of Bacteria

There are several lines of evidence to suggest that the decline in bacterial concentration with proximity to the marsh is due primarily to dilution of bacterially contaminated fresh water (most likely derived from surface runoff, see last section) with relatively pristine ocean water. Evidence to support this hypothesis includes: i) the water salinity data described earlier in this report suggest that the channel water below site 3 consists primarily of ocean water, ii) on both sampling dates we noticed that sites closer to the ocean harbored a larger overall volume of water, although this trend was not quantified, and iii) there appears to be an approximately inverse correlation between the concentration of bacteria at a particular site and water salinity (see Figure 5). If dilution is the only factor influencing the concentration of bacteria in the channel, a plot of the bacterial concentration against water salinity should yield a straight line relationship. This relationship can be expressed mathematically as follows,

$$c = -\left[\frac{c_f - c_s}{s_s - s_f}\right]s + \left[\frac{c_f s_s - c_s s_f}{s_s - s_f}\right] \qquad (1)$$

where c is the concentration of bacteria (either TC, FC, FS, or H) and s is the salinity of the water. This model assumes that relatively fresh water of salinity s_f and bacterial concentration c_f mixes with saline water of salinity s_s and bacterial concentration c_s; the model only applies if the salinity s is in the range, $s_f \le s \le s_s$.

Figure 8 shows the observed relationship between bacterial concentration and salinity (squares) measured at sites 1 through 4 along Talbert Channel; also shown in this figure are theoretical predictions based on equation 1 (dashed lines). Site 5 was excluded from this analysis because it is located in the Fountain Valley Channel, and water in this channel mixes with both ocean water (during high tide) and water originating upstream of the junction between Talbert and Fountain Valley Channels (see Figure 2). Based on Figure 8, the concentrations of TC, FS, and H agree relatively well with the straight line relationship predicted by equation 1, suggesting that dilution is the single most important factor affecting the distribution of these particular bacterial contaminants in the Talbert Channel. In contrast, the concentration of FC at sites 2 and 3 is significantly higher than the model prediction, perhaps due to i) an additional source of FC at these locations (e.g., from lift station water) or ii) bacterial growth in the water column. It is interesting to note that Hanes et al. (1964) and Kenner (1978) found that FC is more likely to multiply in channel waters than FS, consistent with hypothesis (ii).

We also investigated whether water turbidity followed the dilution relationship predicted by equation 1, as shown in Figure 9. In contrast to the FC data, water turbidity plotted below the dilution line at sites 2 and 3, suggesting that turbidity is being removed from the water column faster than can be accounted for by dilution alone. Earlier we noted that the water in the upper reaches of the channel appeared to have significant algal growth. Hence, the drop in turbidity noted at sites 2 and 3 may be due to salt-induced algal mortality.

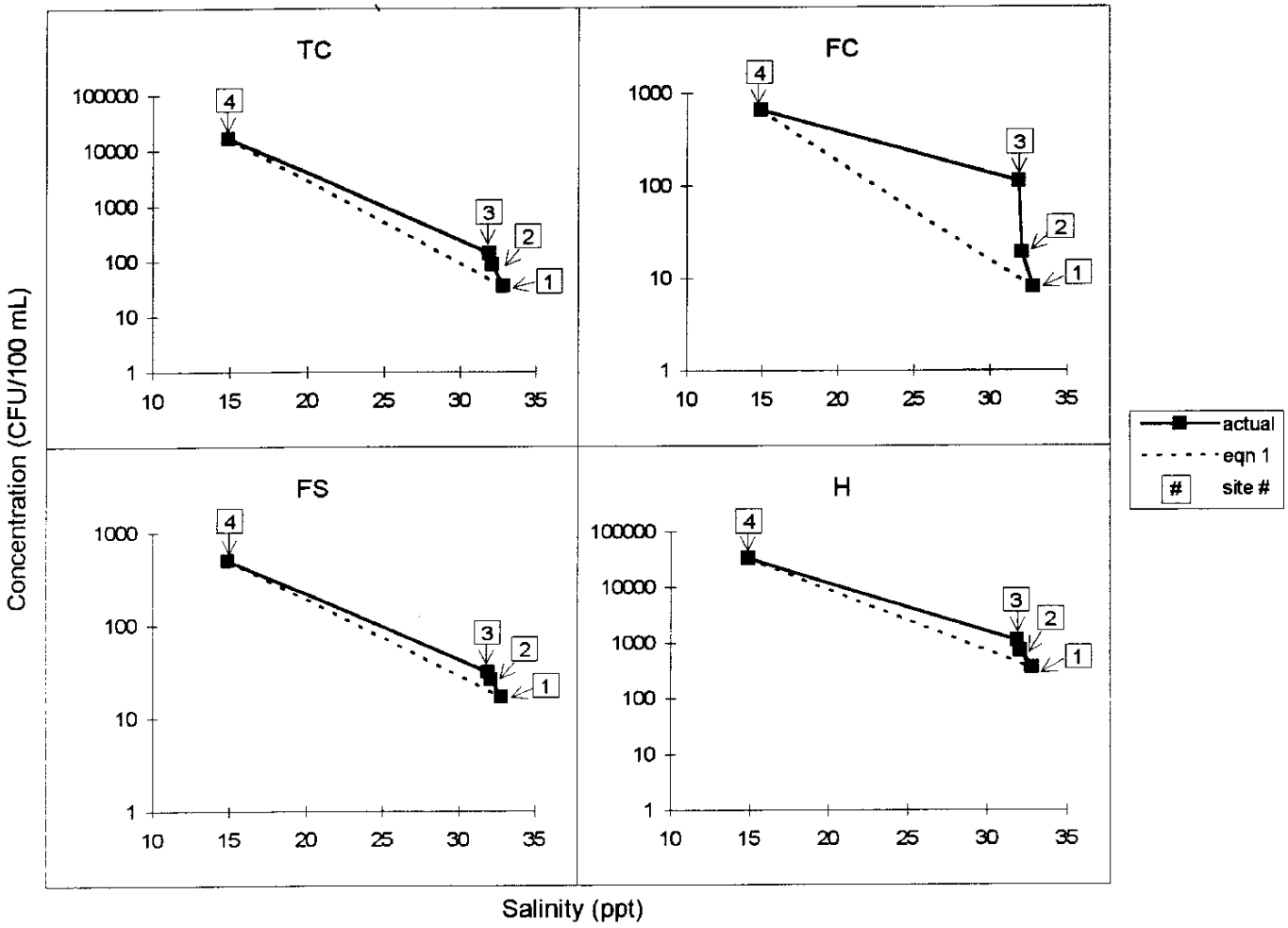

Figure 8. Relationship between bacterial concentrations and salinity.

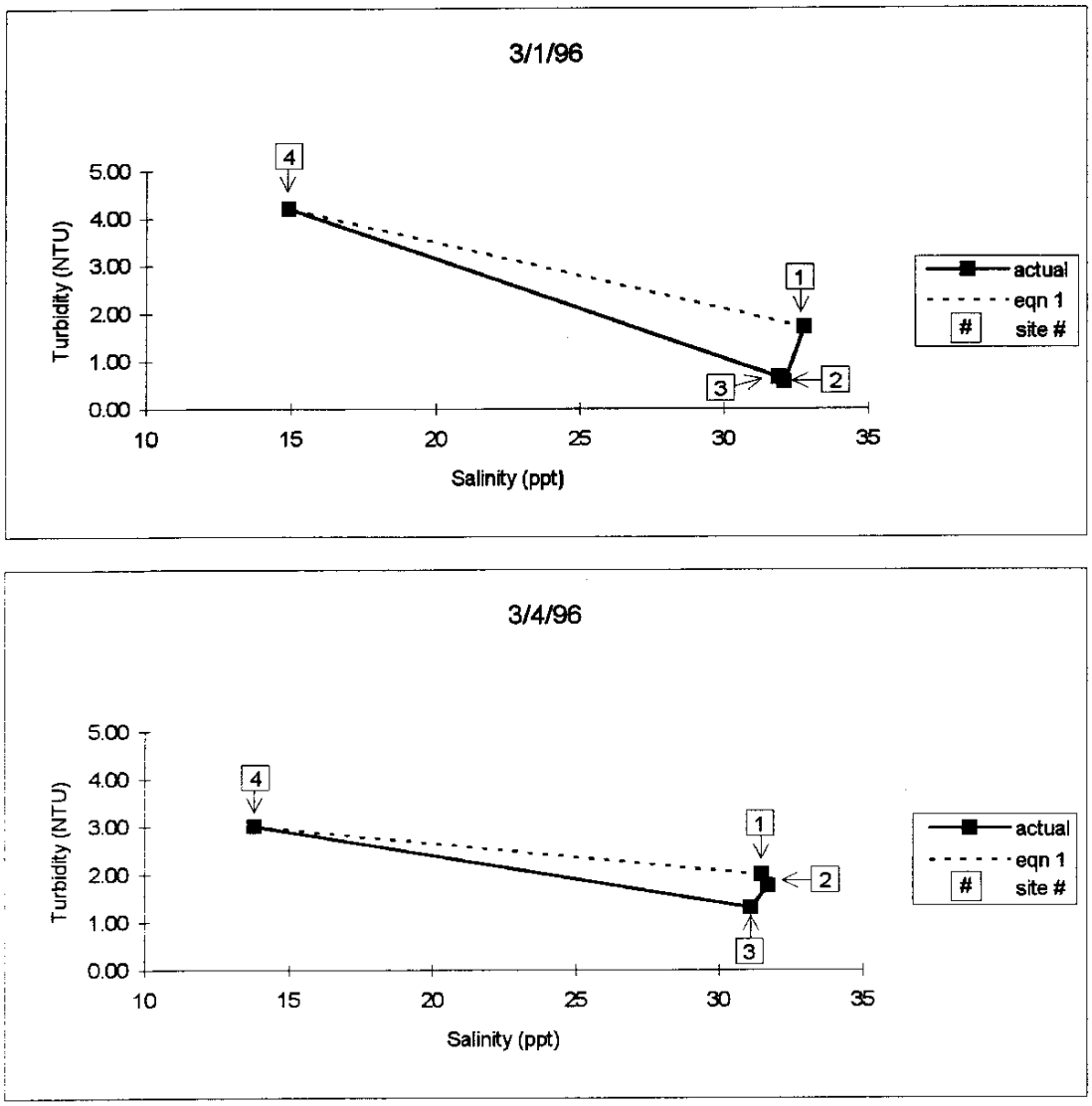

Figure 9. Relationship between turbidity and salinity.

One of the objectives of this study was to determine if coagulation and sedimentation influence the distribution of bacteria in Talbert Channel. The fact that TC, FS, and H all followed the dilution line predicted by equation 1 (see Figure 8) is strong evidence that dilution, and not coagulation plus sedimentation, is the dominant process influencing bacterial concentrations in the channel. FC plotted above the dilution line at sites 2 and 3, but this result is also inconsistent with the existence of a removal mechanism (like coagulation and sedimentation) which would tend to reduce the water phase concentration of bacteria *below* that predicted by dilution.

Biological factors that might affect bacteria in the channel include natural die-off, predation by higher trophic organisms, attack by lytic viruses, and toxin-induced death. None of the above factors, except for toxin-induced death, were examined for in this study, although their potential influence can be estimated based on previous investigations. The typical period for 90% die-off of *E. coli* in sea water is on the order of 3 to 5 days (Mitchell, 1968); in fresh water it has been shown to be strongly dependent on temperature ranging from approximately 2 days at 20°C to 10 days at 10°C (Geldreich et al., 1968). The residence time for water in Talbert Channel is on the order of hours, and thus inactivation due to either natural die-off, predation, and/or lytic attack is probably not significant. Toxins produced by biological organisms (e.g., algae, plants, and bacteria) are probably a minor factor in the bacterial removal process because the water at sites 1,2, and 3 were judged to be "slightly toxic" based on the Microtox acute toxicity assays. In fact, the slight toxicity at these sites may not be due to biological toxins, but rather the high salt concentration that exists at these sites.

Conclusion

This study provides new insights into the mechanisms responsible for the purification of urban runoff in marsh ecosystems such as Talbert Marsh. In particular, we found that bacterial contaminants enter the channels feeding Talbert Marsh primarily by urban runoff, and these bacteria are diluted by ocean water as they travel downstream. Dilution was found to be an effective way of reducing bacterial concentrations below levels required by water quality regulations for coastal recreational water

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Appendices

Appendix A

Physical Parameters

Table A-1. Results of physical parameter tests for both sampling dates.

Sampling Site	March 1,1996				March 4,1996			
	T(°C)	pH	Salinity (ppt)	Turbidity (NTU)	T(°C)	pH	Salinity (ppt)	Turbidity (NTU)
1	12.5	7.83	32.8	1.70	13.5	7.70	31.5	2.00
2	12.5	7.84	32.1	0.56	14.0	7.70	31.7	1.75
3	13.0	7.83	31.9	0.65	14.5	7.60	31.1	1.30
4	14.0	7.80	14.9	4.20	15.0	7.70	13.8	3.00
5	16.0	8.07	0.7	4.10	15.0	7.80	1.5	5.00

ppt = parts per thousand
NTU = nephelometric turbidity unit

Appendix B

Microbiological Data

Table B-1. Results of total coliform analysis.

		Volume (mL)	# of colonies	Conc. (CFU/100ml)	Avg Conc. (CFU/100 mL)	Std. Dev.
March 1,1996	site 1	10	*	*	35	n/a
		10	5	50		
		10	2	20		
	site 2	10	8	80	87	21
		10	11	110		
		10	7	70		
	site 3	10	19	190	140	n/a
		10	9	90		
		10	*	*		
	site 4	0.1	13	13000	16667	3215
		0.1	18	18000		
		0.1	19	19000		
	site 5	0.1	2*	*	17500	n/a
		0.1	17	17000		
		0.1	18	18000		
March 4,1996	site 1	20	8	40	45	18
		20	6	30		
		20	13	65		
	site 4	0.05	16	32000	21333	10066
		0.05	10	20000		
		0.05	6	12000		

24 +/- 2 hrs incub. w/ mEndo broth
ideal count = 20-80 CFU
all blanks = 0 CFU/100ml
n/a = not available
(*) = not counted

Table B-2. Results of total coliform verification.

3/1/96	CO test	ONPG test
Site 1	3(-)/3	3(+)/3
Site 2	3(-)/3	3(+)/3
Site 3	3(-)/4	4(+)/4
Site 4	3(-)/3	3(+)/3
Site 5	4(-)/5	5(+)/5

Note: positive TC verification consists of (-) CO test and (+) ONPG test

Table B-3. Results of fecal coliform analysis.

		Volume (mL)	# of colonies	Conc. (CFU/100ml)	Avg Conc. (CFU/100 mL)	Std. Dev.
March 1,1996	site 1	40	3	8	8	0
		40	3	8		
		40	3	8		
	site 2	40	8	20	19	n/a
		40	4*	*		
		40	7	18		
	site 3	40	44	110	111	n/a
		40	45	113		
		40	70*	*		
	site 4	10	76	760	657	105
		10	55	550		
		10	66	660		
	site 5	10	57	570	575	n/a
		10	58	580		
		10	101*	*		
March 4,1996	site 1	200	36	18	23	7
		200	38	19		
		200	61	31		
	site 2	200	19*	*	27	n/a
		200	49	25		
		200	55	28		

24 +/- 2 hrs incub. w/ mFC broth
ideal count = 20-60 CFU
all blanks = 0 CFU/100ml
n/a = not available
(*) = not counted

Table B-4. Results of fecal coliform verification.

3/1/96	LT Broth	EC Broth
Site 1	10(+)/10	8(+)/10
Site 4	10(+)/10	10(+)/10
Site 5	10(+)/10	10(+)/10

Note: positive FC verification consists of (+) lauryl tryptose broth and (+) EC broth

Table B-5. Results of fecal streptococcus analysis.

		Volume (mL)	# of colonies	Conc. (CFU/100ml)	Avg Conc. (CFU/100 mL)	Std. Dev.
March 1,1996	site 1	40	12	30	17	12
		40	3	8		
		40	5	13		
	site 2	40	9	23	26	3
		40	11	28		
		40	11	28		
	site 3	40	13	33	31	1
		40	12	30		
		40	12	30		
	site 4	10	54	540	503	47
		10	52	520		
		10	45	450		
	site 5	10	56	560	507	55
		10	51	510		
		10	45	450		
March 4,1996	site 1	150	30	20	19	5
		150	34	23		
		150	20	13		
	site 2	100	22	22	30	7
		100	36	36		
		100	33	33		

48 +/- 2 hrs incub. on KF agar
ideal count = 20-60 CFU
all blanks = 0 CFU/100ml

Table B-6. Results of fecal streptococci verification.

3/1/96	3% Hydrogen Peroxide	Gram Stain
Site 4	5(-)/5	5(+)/5
Site 5	5(-)/5	5(+)/5

Note: positive FS verification consists of (-) hydrogen peroxide test and (+) Gram stain

Table B-7. Results of heterotrophic bacteria analysis.

		Volume (mL)	# of colonies	Conc. (CFU/100ml)	Avg Conc. (CFU/100 mL)	Std. Dev.
March 1,1996	site 1	0.1	32	320	355	n/a
		0.1	39	390		
		0.1	*	*		
	site 2	0.1	60	600	717	104
		0.1	75	750		
		0.1	80	800		
	site 3	0.1	131	1310	1110	195
		0.1	110	1100		
		0.1	92	920		
	site 4	0.01	332	33200	33200	n/a
		0.01	*	*		
		0.01	*	*		
	site 5	0.001	251	251000	239000	n/a
		0.001	227	227000		
		0.001	*			

48 +/- 2 hrs incub. on R2A agar
ideal count = 30-300 CFU
all blanks = 0 CFU/100ml
n/a = not available
(*) = not counted

Appendix C

Encroachment Permit

COUNTY PROPERTY ENCROACHMENT PERMIT

Gene Holum 567-6243

[illegible] PRIOR TO commencing permitted use. FAILURE TO OBTAIN INSPECTION SHALL VOID THIS PERMIT.

COUNTY OF ORANGE
ENVIRONMENTAL MANAGEMENT AGENCY
BUILDING & DEVELOPMENT SERVICES
COUNTY PROPERTY PERMITS
Main Office—300 North Flower Street, Room 122
SANTA ANA, CALIFORNIA 92703-5001
OR—P.O. BOX 4048, SANTA ANA, CA 92702-4048
PHONE: 834-3432 or 834-5238
FAX: (714) 835-7425

PERMIT NO. 96-00536 FT
EFFECTIVE DATE 03/11/96
EXPIRATION DATE 03/18/96

PERMITTEE

UCI
P.O.Box 6050
Irvine, CA 92717-2175

Contact person Daniel Telephone No. (714)854-3410

TYPE	FACILITY NAME	NUMBER
	Talbert Channel	D02
	Fountain Valley Channel	D05

PERMITTED USE: Use of County property is hereby authorized as follows, subject to provisions on reverse hereof and attached hereto:

Temporary access to Orange County Flood Control District's Talbert Channel (D02) and Founatin Valley Channel (D05) right-of-ways for marine biological investigation to collect water samples, per plans and provisions attached and to the satisfaction of EMA inspection personnel.

PERMIT NOT EFFECTIVE UNTIL PERMITTED USE APPROVED BY ASSIGNED INSPECTOR.

PWO# EF68010 CEQA: CLASS 1 CAT.EXEMPT /

LOCATION OF WORK: Talbert Channel (D02) & Fountain Valley Channel (D05)

Dimension/Type. Thos. Bros. 858;C-6 Huntington Beach AREA

CONSIDERATION:

Permit Fees 0.00 (2074) Surety None TOTAL $ 0.00

Check No ____ Cash ____ Receipt ____ Date ____ Trust Fund ____ Invoice ____

Check No ____ Cash ____ Receipt ____ Date ____ Trust Fund ____ Invoice ____

Fees paid by ____

Contractor ____

Engineer ____

INSPECTION PW/Operations CC. PW/Construction

PERMITTEE'S ACCEPTANCE:

SIGNATURE ON FILE

Permittee SIGN / PRINT

COUNTY APPROVAL

BEN

2/23/96 Date

PERMIT AND APPROVED PLANS SHALL BE MAINTAINED ON JOB SITE.

PERMITTEE SHALL COMPLY WITH REGULATIONS PRINTED ON REVERSE SIDE OF PERMIT AND ATTACHMENTS.

ALL UNDERGROUND WORK REQUIRES PRIOR 'UNDERGROUND SERVICE ALERT' COMPLIANCE.

THIS PERMIT SHALL BE NON-TRANSFERABLE.

2/23/96

CASHIER DATE REGISTER NO. FEE + CODE TOTAL

COUNTY OF ORANGE
ENVIRONMENTAL MANAGEMENT AGENCY
BUILDING & DEVELOPMENT SERVICES / COUNTY PROPERTY PERMITS

SPECIAL PROVISIONS

96-00536-FT
Permit No.

A. Concurrently with the filing of this application, permittee shall pay a non-refundable fee in the amount of Seventy-Seven Dollars ($77) to the County for the granting of this permit and use of flood control district right of way.

Annual renewal of this permit may be granted by the Director of the Environmental Management Agency, upon receipt of a written application and the established fee.

B. The granting of this permit implies no warranty as to whether or not any form of sea slugs may be safe for human consumption.

C. Ingress and egress shall be at designated locations and the permitted use shall be subject to the control and satisfaction of the County's Public Facilities Maintenance Superintendent, or his authorized representative, who can be contacted at (714)567-6243.

D. Permittee acknowledges that this permit is granted only for temporary use of County properties outlined on attached sketches.

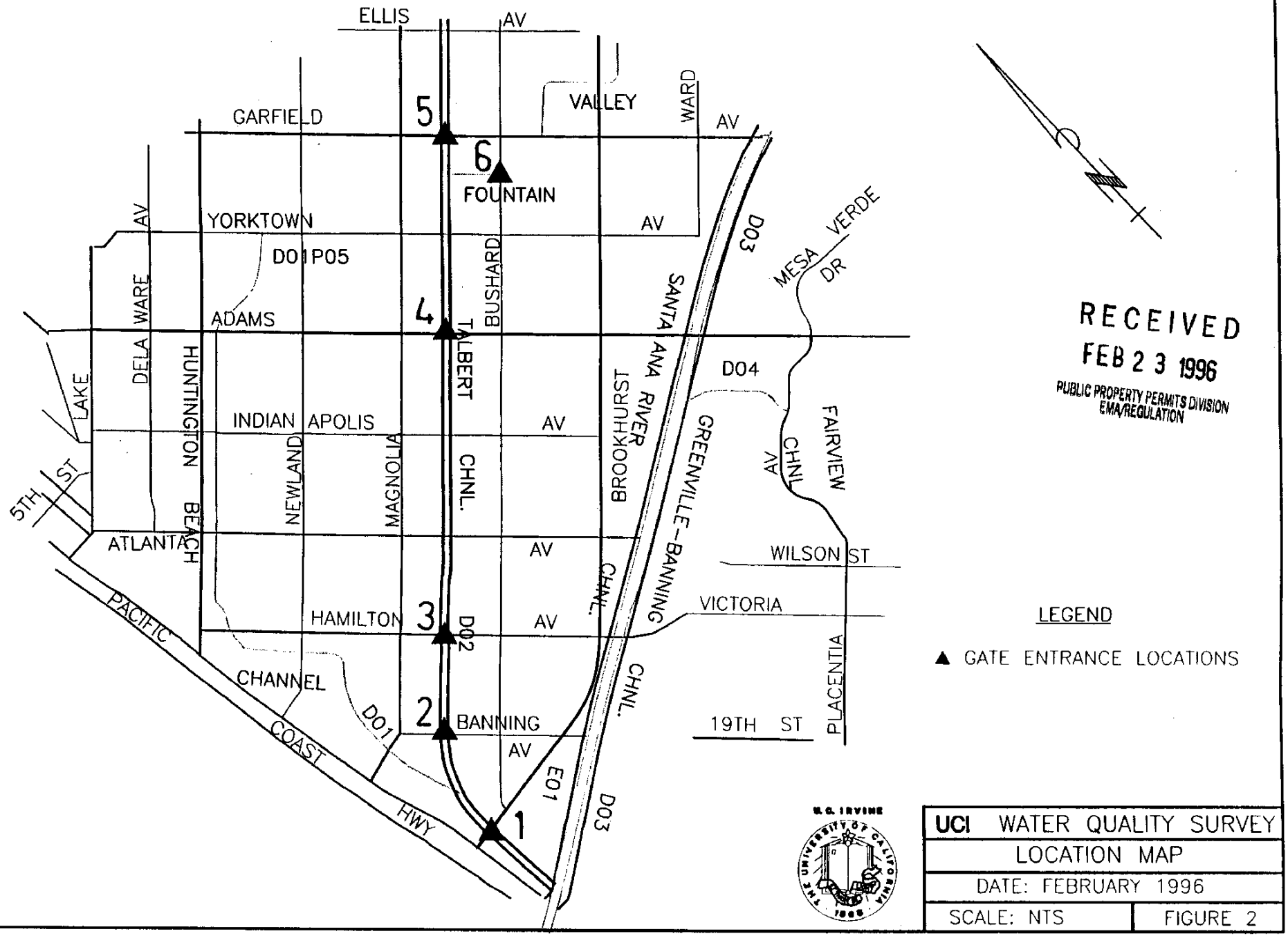
E. The County possess limited rights in the area of this permit which may not be sufficient for permittee's purposes. Permittee acknowledges the limitations of County's rights and agrees to secure permission from any other owners required for exercise for rights permittee needs.

F. This permit is non-exclusive and the County reserves the right to issue permits to others for similar or different purposes upon or including the area of this permit and when two or more such permits have been issued, the permittees shall coordinate their activities as directed by County's representative/inspector.

G. This permit may be revoked at any time for abuse of privileges, for violation of the permit provisions or reasons in the best interest of the County. In the event of such revocation, all of permittee's rights shall immediately ceased.

End

96 005 FT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DELA WARE AV
HUNTINGTON BEACH
LAKE
5TH ST
INDIAN APOLIS AV
NEWLAND
MAGNOLIA
CHNL.
BROOKHURST
SANTA ANA RIVER
D03
MESA VERDE DR
D04
FAIRVIEW
CHNL AV
GREENVILLE-BANNING
ATLANTA AV
WILSON ST
HAMILTON
3
D02
AV
VICTORIA
PACIFIC COAST HWY
CHANNEL
D01
2
BANNING
AV
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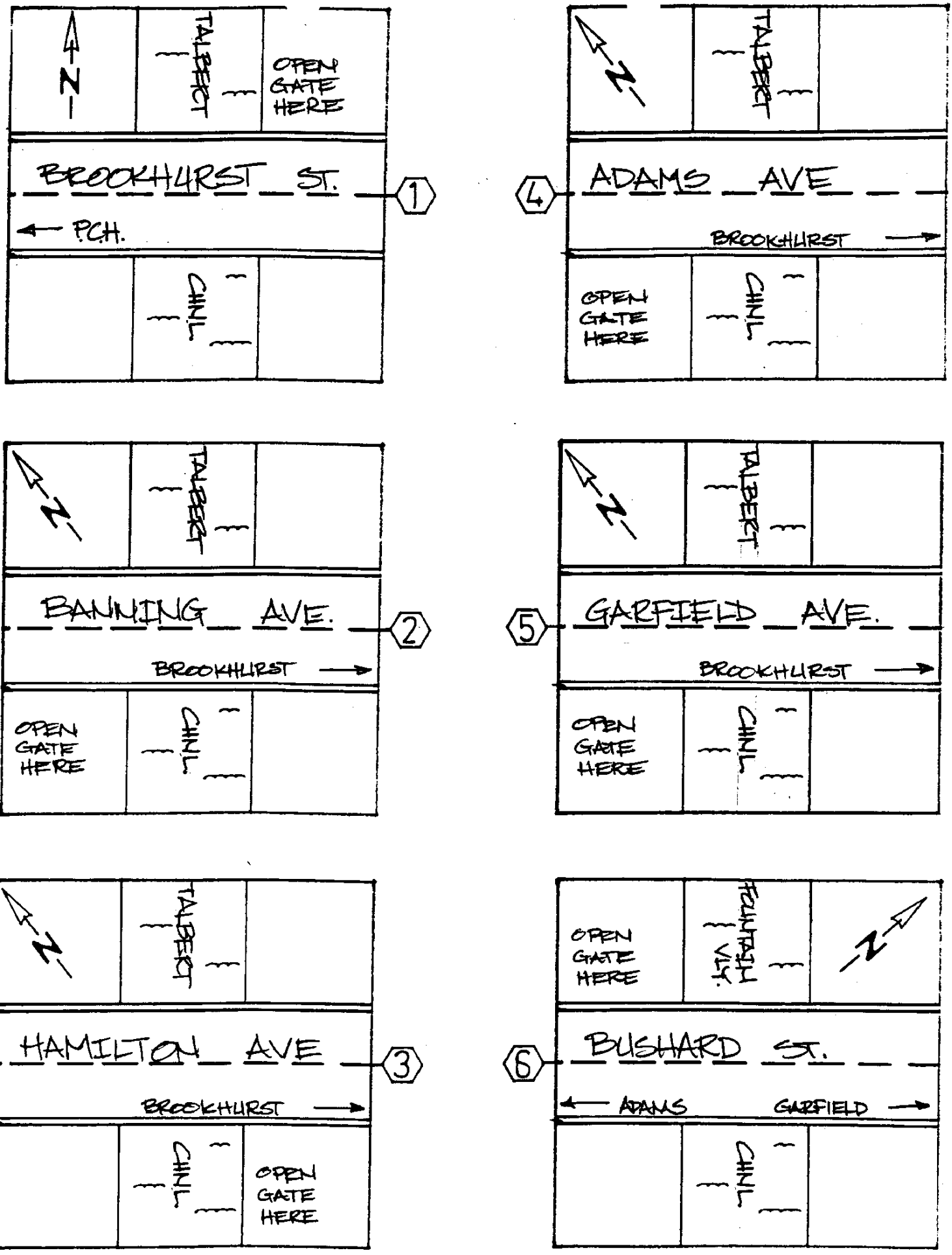
RECEIVED
FEB 23 1996
PUBLIC PROPERTY PERMITS DIVISION
EMA/REGULATION
LEGEND
▲ GATE ENTRANCE LOCATIONS
U.C. IRVINE
THE UNIVERSITY OF CALIFORNIA
1965
UCI WATER QUALITY SURVEY
LOCATION MAP
DATE: FEBRUARY 1996
SCALE: NTS
FIGURE 2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ADAMS
GARFIELD
CHNL.