

Fecal Pollution in Guam's Coastal Waters and Sediments

ERNEST A. MATSON

*Division of Natural Sciences and
Marine Laboratory
The University of Guam
Mangilao, GU 96923*

Abstract—Waters near three of Guam's wastewater treatment plants (STPs) were sampled monthly during 1989 for fecal coliform bacteria (FCs) at shoreline stations. Also, both shoreline and offshore sites were sampled quarterly for FCs and several other water quality parameters (pH, salinity, temperature, oxygen, turbidity, nitrates, and phosphates). In 1990-1991, densities of fecal coliforms and fecal streptococci were measured in sediments of coastal recreational waters, as well as in urban street water runoff.

Of all water quality parameters measured, only fecal coliform bacteria, reactive phosphate, and ammonium were detectable in the receiving waters once the effluents had been sufficiently mixed with seawater so as to be undetectable with salinity measurements. Fecal bacteria were detected further from effluents and for longer periods than phosphate or ammonium. Non-point sources of fecal contamination (e.g. street runoff) are by far the worse source of fecal contamination of Guam's coastal waters.

High numbers of indicator bacteria are commonly encountered (46% frequency) in sediments that underlie otherwise indicator-free recreational waters and therefore pose an unacknowledged and unmonitored potential health risk to recreational users. *In vitro* laboratory experiments showed *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella* spp., and *Vibrio* spp. could survive for up to three weeks in autoclaved coastal sediments that normally underlie indicator-free waters. Simple weekly monitoring of surface waters does not supply an adequate warning of the possibility of exposure to potential hazards, often misses contamination events due to street runoff, and may mislead the public into believing that health hazards occur only in surface waters and not in the underlying sediments.

Introduction

The presence of indicator microorganisms in recreational and drinking waters is used by public health officials to evaluate potential health risks. A useful and accurate indicator of the presence of recent human fecal pollution should be

easily and accurately enumerated, unequivocally identified, always present in the feces of humans or other warm-blooded animals (regardless of whether pathogens are also present), and not otherwise present in the environment. In addition, an indicator should not occur or survive for extended periods outside its primary reservoir.

Recently, however, both accepted indicators and pathogens have been isolated from otherwise "pristine" tropical mountain streams and coastal waters (Bermudez & Hazen 1988, Rivera et al. 1988). Cruz-Cruz et al. (1988) and Yoshpe-Purer & Golderman (1987) concluded that the potential pathogen *Pseudomonas aeruginosa* was endemic to tropical waters. Hazen's group (Perez-Rosas & Hazen 1988) also compared the survival of both *Escherichia coli* and *Vibrio cholera* in waters and sediments of a Puerto Rican coral reef and found that *E. coli* survived for at least 4.5 days in high numbers in water, and that *V. cholera* survived even longer and at higher densities. While the sediment studies lasted only two days, densities of both organisms did not change in that period. They concluded that, due to the extended survival of these two pathogens, the widely accepted indicators (i.e., fecal coliforms) were generally poor predictors of their occurrence. *Candida albicans* has also been shown to survive for extended periods in both temperate (Buck 1978) and tropical waters (Valdes-Collazo et al. 1987).

This study was initiated because, other than the work of Tsuda & Grosenbaugh (1977), no studies have been performed on either the distribution or occurrence of sewage in Guam's coastal waters. The scope of initial studies was expanded when it became obvious that regulated wastewater effluents were not the only source of sewage contamination.

Materials and Methods

PRELIMINARY RECONNAISSANCE

Several initial studies were performed in November and December 1988 and January 1989 to determine the distribution, residence time, and dilution rate of the effluents within the coastal waters and their chemical and bacterial content. Fluorescein was injected into the effluents and its dispersion was recorded on videotape using SCUBA and from low-flying aircraft. Because of its relatively low chemical reactivity, ammonium content was also measured. These preliminary results were used to determine sample volumes necessary and the precision required for subsequent chemical and bacteriological monitoring.

THE STUDY SITES

Guam's three largest sewage treatment plants (STPs) are situated on the island's west coast (Fig. 1) and discharge in the leeward coastal zone either within or on the fringing to barrier reefs. The Agat STP in southwestern Guam discharges primarily-treated sewage immediately offshore (ca. 2 m) in less than 2 m of water at Gaan Point. The effluent of the aerobically treated primary sewage at Agana is pumped offshore to a depth of about 26 m below MLW. Anaerobically, primarily-treated effluent of the Northern District (ND) STP in Harmon is piped

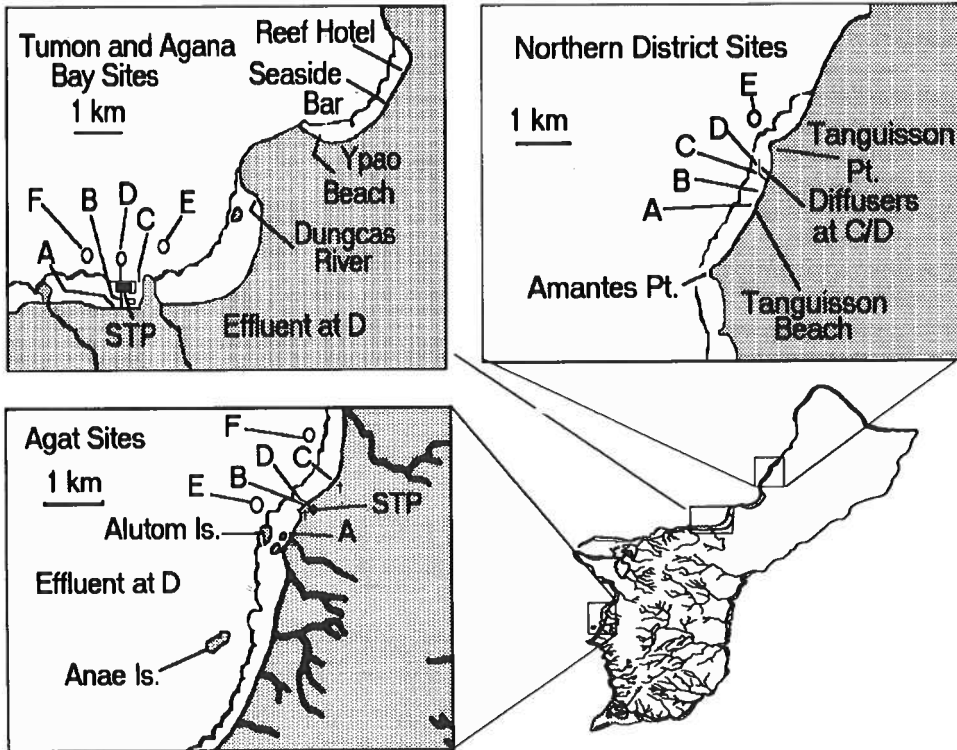


Figure 1. The study areas.

offshore north of Tanguisson Beach and discharged through at least 15 diffusers at ca. 18 m below MLW. Within about 10 minutes, effluent plumes from both the Agana and ND STPs rise intact to the surface and diffuse horizontally in the top ca. 0.5 m of surface water. The exact location of the surface plume at the Northern District is undetectable from a boat and only becomes obvious when a diver enters the water and observes the degassing, turbid waters. Prevailing winds (from ENE) and currents at both these sites usually direct the plume offshore and to the WSW.

ROUTINE SAMPLING IN 1989

The stations, water quality indices, and choice of bacteriological parameters were determined by U.S. Environmental Protection Agency (EPA) and the Guam EPA (GEPA). All samples were taken between 11:00 and 13:30 local time, in the same station sequence, regardless of tide, sea state or cloud cover. Periods of heavy surf often precluded boat trips (especially for about 7 weeks in the Fall of 1989). To avoid sampling waters that had been contaminated with storm runoff, trips were postponed if heavy rain had occurred within 3 days. Sampling commenced in February 1989 and continued for one year.

During this period, quarterly water quality monitoring was performed at all sites, including the top ca. 20 cm of surface waters (s), 5m (mid, m), and 10 m (bottom, b). Offshore station F at both Agat and Agana and station E at Northern District were in water greater than 60 m deep, so at these sites the 10 m samples are referred to as bottom water. Because the effluent is discharged at a depth of 26 m at Agana, mid and bottom water sample depths (5 and 10 m) were chosen at the suggestion of the Guam EPA in order to make comparisons with data taken during other routine coastal water quality monitoring. Monthly shoreline samples were taken for fecal coliform analysis in ca. 0.2–0.5 m of water.

All water samples were taken in initially acid-cleaned 500 ml polypropylene (PPE) bottles either with SCUBA or with a Van Dorn sampler, proceeding from the cleanest to the most impacted station, and were immediately stored in the shade at ambient temperature.

Water Quality Monitoring

Salinity (‰), O₂ (mg L⁻¹), and temperature (°C) were measured directly in the field with Yellow Springs Instrument Co. Meters. Field salinity measurements were corroborated in the lab using Cl⁻ analysis, which was performed on a Haake-Buchler Chloridometer ($\pm 0.9\%$ relative precision). All salinity data presented were calculated from measured Cl⁻ concentrations using the relationship

$$\text{Salinity (‰)} = (\text{Cl}^-/550) \times 35$$

where 550 mM is the Cl⁻ content of full-strength seawater (Stumm & Morgan 1981). Oxygen meters were pre-calibrated in the lab with O₂-saturated freshwater and meter-compensated in the field with a seawater-wetted probe. pH meters were calibrated each time with standard buffers (pH 6.9 and 9.08). Nitrate plus nitrite (i.e. NO_x) were analyzed by an improved cadmium reduction technique (Jones 1984), ammonium with an Orion[®] specific ion probe, and reactive phosphate (RP) by molybdate (Parsons et al. 1984). Except for samples of visible effluent, no samples were filtered for nutrient analysis because of the general lack of measurable weight of particles in 100 mL seawater. Subsamples for chemical analyses were refrigerated and performed within 48 hours. Secchi depth was recorded vertically and horizontally underwater with a 0.5 m diameter disc. Later, in the fourth quarter, nephelometric turbidity units (NTU) were measured with as Hach Turbidimeter.

Bacteriology

Fecal coliform bacteria were quantified in duplicate on Millipore[®] type HAWG 047SO 0.45 μm gridded membrane filters placed on pads saturated with Difco Bacto mFC broth using accepted procedures (A.P.H.A. et al. 1985). Fecal coliforms and pH were always measured immediately upon return to the laboratory and analyses were completed that day.

Between August 1990 and May 1991, surface samples (ca. top 2–3 cm) of subtidal coastal sediment were collected from 10 sites in Tumon Bay, 9 sites in Agana Bay, and, infrequently at 11 sites around the southern perimeter of Guam. Both fecal coliforms (as above) and fecal streptococci (Difco Bacto mFS agar)

were enumerated in sediments collected in sterile Whirl-Pak® bags. Densities are expressed per gram of whole wet sediment and as densities that would occur if the sediment were to be resuspended into and thoroughly dispersed in the overlying waters at the water depths that occurred at the time samples were taken.

Randomly chosen blue colonies on mFC and yellow colonies from TCBS (Tryptone-Citrate-Bile Salts, Difco, for *Vibrio* spp.) media were identified by restreaking on selective and differential media. This provided a collection of several wild-type isolates of *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella* spp., and *Vibrio* spp. for later use in tests of survival in artificial microcosms. In these tests, log phase pure cultures were added to PVC "flow-through" chambers in which the cultures were physically separated from ambient seawaters by 0.22 μm pore size Nuclepore® filters. Subsamples were taken daily and enumerated on appropriate media. In another version, log phase pure cultures were added to sterile 500 mL Erlenmeyer flasks that had been filled with 300 mL of seawater and ca. 100 mL of Tumon Bay sediment. Daily enumerations were made for up to 21 days.

Organic Nitrogen in Algae and Feces

At Agat, samples of the common red alga, *Galaxaura marginata*, were collected along two transects parallel to shore ca. 75 and 100 m offshore in ca. 3 and 6 m of water, respectively. At the Northern District, samples of the calcareous green alga, *Halimeda opuntia*, were collected along transects parallel to the diffusers at depths of ca. 14 m, 10 m, and 6 m that were 5, 20, and 50 m, respectively, from the diffusers. All algal samples were collected from clumps attached to the substratum and placed in Whirl-Pak® bags.

Additionally, at the Northern District STP outfall, feces of the sea cucumber *Holothuria atra* were collected along a transect perpendicular to the diffuser pipe at decreasing depths (toward shore). Samples of freshly deposited fecal pellets were gathered from directly behind the animals and placed in Whirl-Pak® bags. The samples were dried to constant weight, homogenized in a pestle, and stored for subsequent N and C analysis. Water content of algal samples was determined by weighing subsamples before and after drying at 50 °C. Total organic nitrogen (TON) and carbon (TOC) were determined with a Carlo Erba Model 1500 NCS elemental analyzer in duplicate against *p*-amino benzoic acid standards.

Results and Discussion

CALCULATED IMPACT ZONES OF WASTEWATER EFFLUENTS

Impact and dilution zones, as well as the diameter of a circle required to dilute the effluent to levels undetectable with salinity measurements were calculated from discharge data (Table 1). This is done for each of the three effluents based on their maximum allowable daily flows, not their actual flows. The immediate impact area (ha) is the area of water occupied by a layer of effluent that is 1 m deep after 1 day of effluent discharge. This depth is approximately that observed in the field when the subsurface effluent boils to the surface, dissipates,

Table 1. Maximum effluent discharge, calculated impact zones, dissipation areas (dilution zone), and distances (diameter) for complete dilution of maximum allowable daily flow.

Site	Discharge m ³ d ⁻¹	Impact ha d ⁻¹	Dilution m d ⁻¹	Diameter m
Agat	5.7 × 10 ³	0.57	63	900
Agana	45 × 10 ³	4.5	495	2510
N. Dist.	23 × 10 ³	2.3	253	1790

and degasses. The effluent water is both less dense and apparently contains gases that are supersaturated (at pH ca. 7.4) in the seawater once mixing occurs. Effluent waters "float" until thorough mixing occurs and the effluent is no longer detectable ($\pm 0.9\%$ at 35‰ salinity) using Cl⁻ content. This is the second statistic in the Table, and is defined at that area (ha) of water 1 m deep that is required to dilute the effluent to full strength seawater. The calculation is based on an average effluent salinity of 0.32‰ (5 mM Cl⁻). The diameter of this dilution zone is given as though the zone were a perfect circle, although in reality the effluent dissipates downwind in the surface current. For the three effluents, the distances (diameters) to complete mixing with seawater are on the order of a few thousand meters. For the Agana and Northern District effluents, this is usually in an obliquely offshore direction. At Agat, however, the effluent is discharged in shallow nearshore waters and it therefore may be transported either offshore or along shore depending upon tidal stage and wind. It should be noted that both the Agat and Agana STPs often meet or exceed their design capacity limits (1.5 and 12 MGD, respectively) during heavy rain, while the Northern District STP (at the time this report was written) discharges about a third of its 6 MGD capacity.

WATER QUALITY MONITORING

Results of preliminary reconnaissance of FC density and salinity in the impact zones are in Table 2. FCs ranged from undetectable (<1 in 100 mL) to >10⁷ 100 mL⁻¹. All subsequent monitoring data are listed in Table 3, and summaries and relationships between water quality parameters and the occurrence of fecal coliforms are given in Figure 2. Also, a summary of linear regression coefficients of variation (R²) is given in Table 4. With respect to salinity, essentially all of the data are well clustered around 35‰, with few exceptions. Low salinities occurred only directly in the effluent boils and near aquifer or surface water discharge sites. Oxygen content was never observed to be below saturation at any sites and were frequently well above saturation, especially at shoreline stations where active benthic photosynthesis occurs. High oxygen values (>10 mg L⁻¹) always occurred at shoreline stations and lower ones were always above saturation in the effluent boils and receiving waters. pH rapidly increased to ambient levels upon mixing of the effluent with seawater.

Table 2. Preliminary reconnaissance (n = 1) of fecal coliforms and salinity within the predicted impact zones.

	Log ₁₀ FC 100 mL ⁻¹	Salinity (‰)
9 February 1989:		
Northern District		
Diffuser #1	7.4	1.7
Diffuser #2	7.0	1.1
Plume-1	<5	35.1
Plume-2	4.0	36.1
Surface Boil	3.0	35.6
Upwind water	<3	35.3
Bottom water	2.6	35.9
Agana		
Effluent	6.8	3.5
Plume-1	4.6	33.2
Upwind water	4.1	35.5
Downwind water	3.4	35.5
Bottom water	1.0	35.7
23 March 1989:		
Agana		
Effluent	6.9	0.5
AGA	0.0	34.0
AGB	0.0	34.0
AGC	0.84	34.0
AGD	3.6	30.0
AGE	0.0	33.9
AGF	0.84	33.5
Northern District		
Effluent	5.8	<1
NDA	1.0	33.8
NDB	0.0	34.0
NDC	3.8	33.6
NDD	0.0	33.9
NDE	0.0	33.8

Table 3. Water quality data.

Station/ Depth ^a	Temp °C	Salinity ‰	pH	O ₂ mg L ⁻¹	Turbidity m	NOx mg L ⁻¹	RP mg L ⁻¹
25 April 1989							
NDA	27.2	32.9	8.42		>10	0.032	0.0042
NDB	27.0	32.8	8.41		>10	0.032	0.0090
NDCs	27.5	32.8	8.40		3	0.032	0.013
NDCm							
NDCb							
NDDs	26.8	32.9	8.40		>10	0.038	0.0080

Table 3. Continued.

Station/ Depth*	Temp °C	Salinity ‰	pH	O ₂ mg L ⁻¹	Turbidity m	NOx mg L ⁻¹	RP mg L ⁻¹
NDDm							
NDDb	26.8	32.9	8.39		>10	0.030	0.0076
NDEs	26.8	34.0	8.38		>10	0.0015	0.0066
NDEm	27.0	34.0	8.39		>10	0.0011	0.0097
NDEb	27.0	34.0	8.38		>10	0.0023	0.0035
AGA	31.0	32.9	8.42		>10	0.071	0.0042
AGB	27.8	33.7	8.40		9	0.016	0.0055
AGCs	27.4	33.2	8.40		3	0.026	0.0080
AGCm							
AGCb							
AGDs	27.0	32.0	8.31		2	0.013	0.11
AGDm							
AGDb							
AGEs	27.2	33.5	8.38		>10	0.013	0.0049
AGEm	27.1	33.4	8.37		>10	0.012	0.0084
AGEb	27.1	33.2	8.38		>10	0.010	0.0070
AGFs	27.0	33.6	8.38		>10	0.0064	0.0061
AGFm	27.0	33.5	8.37		>10	0.0053	0.0097
AGFb	26.9	33.4	8.37		>10	0.0011	0.027
GTA	32.2	35.0	8.57	12.8	>10	0.0098	0.013
GTB	32.8	32.2	8.47	8.7	>10	0.079	0.013
GTC	35.0	33.8	8.48	9.2	>10	0.0079	0.0049
GTDs	29.0	31.5	7.36	5.5	1.5	0.038	2.0
GTDm			8.35		3	0.059	0.0094
GTDb			8.37		>10	0.077	0.016
GTEs	28.0	33.2	8.38	7.8	>10	0.15	0.061
GTEm	27.5	32.5	8.39	7.6	>10	0.096	0.039
GTEb	27.5	32.5	8.40	7.5	>10	0.081	0.031
GTFs	28.2	34.0	8.39	7.2	>10	0.012	0.0066
GTFm	28.0	34.0	8.39	6.8	>10	0.0041	0.0052
GTFb	27.2	33.9	8.40	6.9	>10	0.0079	0.0055
13 June 1989							
NDA	29.8	34.9	8.50	9.2	>10	0.0063	0.0003
NDB	28.8	28.8	8.46	8.6	>10	0.27	0.0017
NDCs	28.0	35.1	8.48	7.6	>10	0.018	0.0003
NDCm	27.2	35.7	8.28	6.2	>10	0.0029	0.030
NDCb	27.2	16.7	7.42	6.2	3	0.013	1.6
NDDs	27.7	35.8	8.34	6.3	>10	0.0058	0.0074
NDDm	27.3	36.0	8.30	6.2	>10	0.0035	0.0003
NDDb	27.2	36.0	8.30	6.1	>10	0.0024	0.0003
NDEs	27.7	35.9	8.30	6.2	>10	0.0021	0.0003
NDEm	27.4	36.0	8.30	6.4	>10	0.0015	0.0003
NDEb	27.4	35.7	8.10	6.4	>10	0.0012	0.0003
AGA	30.5	36.0	8.46	8.9	>10	0.0037	0.0003

Table 3. Continued.

Station/ Depth ^a	Temp °C	Salinity ‰	pH	O ₂ mg L ⁻¹	Turbidity m	NOx mg L ⁻¹	RP mg L ⁻¹
AGB	30.0	35.6	8.56	10.5	>10	0.0061	0.0013
AGCs	28.5	35.6	8.40	6.2	>10	0.0068	0.0020
AGCm							
AGCb							
AGDs	27.5	35.6	8.29	5.7	>10	0.0019	0.076
AGDm							
AGDb							
AGEs	27.6	36.0	8.30	5.8	>10	0.0066	0.0017
AGEm	27.5	36.1	8.32	5.8	>10	0.0020	0.0010
AGEb	27.5	35.9	8.31	5.9	>10	0.0026	0.0003
AGFs	27.8	35.8	8.29	5.9	>10	0.0051	0.0003
AGFm	27.3	36.1	8.30	5.9	>10	0.0011	0.0003
AGFb	27.3	36.1	8.30	5.9	>10	0.0014	0.012
15 June 1989							
GTA	36.0	33.8	8.47	12.2	>10	0.0021	0.0007
GTB	34.6	34.4	8.50	13.5	>10	0.0049	0.0003
GTC	34.9	34.3	8.52	12.8	>10	0.0023	0.0034
GTDs	27.1	35.6	8.22	7.4	4	0.0054	0.56
GTDm	27.9	36.0	8.22	6.7	>10	0.0054	0.0081
GTDb	27.8	35.6	8.19	6.7	>10	0.0058	0.012
GTEs	28.0	35.6	8.23	7.5	>10	0.0008	0.0030
GTEm	27.8	35.7	8.30	7.3	>10	0.0023	0.0034
GTEb	27.7	35.3	8.30	7.2	>10	0.0021	0.0034
GTFs	28.1	35.6	8.29	7.7	>10	0.0008	0.0010
GTFm	27.8	35.8	8.29	7.2	>10	0.0015	0.0020
GTFb	27.8	34.2	8.29	7.7	>10	0.0030	0.0020
21 September 1989							
NDA	29.5	35.8	8.18	6.7	>10	0.012	0.0070
NDB	29.2	35.6	8.18	6.4	>10	0.013	0.0032
NDCs	29.1	35.8	8.18	6.6	>10	0.044	0.012
NDCm	29.0	35.8	8.17	6.4	>10	0.0082	0.0084
NDCb	28.9	2.2	7.32	6.4	3	0.0000	2.7
NDDs	29.9	35.1	8.25	6.7	>10	0.089	0.0091
NDDm	29.0	36.0	8.18	6.2	>10	0.011	0.010
NDDb	28.9	36.0	8.16	6.2	>10	0.037	0.0028
NDEs	29.0	35.5	8.17	6.2	>10	0.040	0.0011
NDEm	29.0	35.5	8.16	6.1	>10	0.013	0.0011
NDEb	28.8	35.7	8.16	6.1	>10	0.0056	0.0011
AGA	30.8	34.4	8.19	9.4	>10	0.19	0.0018
AGB	30.0	35.9	8.22	8.5	>10	0.034	0.0032
AGCs	30.2	34.9	8.12	7.8	>10	0.17	0.0028
AGCm	30.2	35.6	8.18	7.5	>10	0.083	0.0035
AGCb	30.0	35.8	8.11	7.1	>10	0.049	0.0042
AGDs	29.5	35.4	8.12	6.3	>10	0.011	0.049

Table 3. Continued.

Station/ Depth*	Temp °C	Salinity ‰	pH	O ₂ mg L ⁻¹	Turbidity m	NOx mg L ⁻¹	RP mg L ⁻¹
AGDm	meter	failure	8.14	6.0	>10	0.0066	0.0028
AGDb	29.5	35.9	8.13	6.2	4	0.013	0.028
23 September 1989							
AGEs	29.6	35.6	8.13	6.2	>10	0.013	0.0088
AGEm	29.8	35.7	8.15	6.2	>10	0.0092	0.0021
AGEb	29.8	35.8	8.16	6.2	>10	0.015	0.0028
AGFs	29.2	35.8	8.16	6.1	>10	0.011	0.0011
AGFm	29.1	36.0	8.14	6.1	>10	0.019	0.0035
AGFb	29.0	35.8	8.16	6.1	>10	0.0097	0.0067
GTA	34.0	33.7	8.27	7.8	>10	0.0036	0.0007
GTB	32.0	35.1	8.28	7.1	>10	0.031	0.0053
GTC	34.0	34.6	8.40	8.4	>10	0.011	0.0018
GTDs	31.5	31.3	8.07	5.5	3.5	0.27	0.37
GTDm							
GTDb	29.5	34.7	8.20	6.1	>10	0.36	0.088
GTEs	29.5	35.4	8.21	5.7	>10	0.030	0.0032
GTEm	29.8	35.4	8.22	5.5	>10	0.018	0.0032
GTEb	29.5	35.6	8.24	5.4	>10	0.0061	0.0039
GTFs	29.5	35.7	8.22	5.7	>10	0.012	0.0025
GTFm	29.5	34.9	8.23	5.6	>10	0.0000	0.0018
GTFb	29.9	35.9	8.24	5.5	>10	0.0005	0.0032
12 December 1989							
NDA	30.5	35.0	8.25	6.5	0.6	0.0059	0.0042
NDB	30.8	35.1	8.25	6.7	0.5	0.0047	0.0049
NDCs	30.4	35.1	8.21	6.9	0.35	0.0033	0.025
NDCm	30.4	34.9	8.20	5.9	0.35	0.0025	0.0085
NDCb	30.4	35.3	8.19	5.9	0.38	0.0009	0.011
NDDs	30.8	34.9	8.25	6.3	0.55	0.0068	0.0085
NDDm	30.2	34.6	8.18	5.8	0.28	0.0008	0.0046
NDDb	30.2	33.5	8.18	5.8	0.25	0.0012	0.0046
NDEs	29.5	34.9	8.17	5.8	0.25	0.0009	0.0052
NDEm	29.5	34.6	8.18	5.9	0.2	0.0002	0.0026
NDEb	29.5	33.9	8.18	5.9	0.15	0.0004	0.0036
AGA	34.0	33.9	8.30	8.3	1.5	0.036	0.0062
AGB	32.0	35.1	8.32	8.3	2.1	0.0037	0.0085
AGCs	32.0	34.9	8.29	7.8	1.2	0.0052	0.0078
AGCm	32.0	35.0	8.26	7.6	0.45	0.0043	0.0069
AGCb	32.0	35.0	8.26	7.4	0.7	0.0055	0.0052
AGDs	31.0	34.7	8.18	6.2	1.8	0.0010	0.0685
AGDm	31.0	35.1	8.21	6.0	0.8	0.0003	0.0082
AGDb	31.0	35.0	8.20	5.8	0.6	0.0004	0.020
AGEs	31.0	35.1	8.18	6.2	0.5	0.0042	0.0072
AGEm	31.0	34.9	8.17	6.2	0.48	0.0031	0.022
AGEb	31.0	35.1	8.18	6.2	0.45	0.0025	0.019

Table 3. Continued.

Station/ Depth ^a	Temp °C	Salinity ‰	pH	O ₂ mg L ⁻¹	Turbidity m	NO _x mg L ⁻¹	RP mg L ⁻¹
AGFs	30.5	34.9	8.17	6.0	0.35	0.0010	0.0052
AGFm	30.7	34.7	8.18	5.9	0.35	0.0006	0.0033
AGFb	30.4	34.6	8.20	5.9	0.40	0.0000	0.0078
5 December 1989							
GTA	34.0	35.0	8.35	8.4	0.80	0.0010	0.0084
GTB	33.0	33.1	8.25	7.9	2.20	0.0062	0.014
GTC	33.5	34.3	8.25	7.4	4.20	0.0025	0.0090
GTDs	31.0	29.3	8.06	7.2	4.10	0.023	0.38
GTDm							
GTDb	29.5	34.4	8.24	6.8	0.90	0.0110	0.054
GTEs	29.8	35.1	8.21	6.5	0.40	0.016	0.013
GTEm	29.9	35.3	8.22	6.3	0.45	0.0046	0.010
GTEb	30.1	35.2	8.21	6.2	0.38	0.0032	0.011
GTFs	30.1	35.6	8.23	6.5	0.37	0.0018	0.0077
GTFm	30.1	35.4	8.22	6.3	0.38	0.0003	0.0045
GTFb	30.2	35.3	8.23	6.3	0.35	0.0008	0.011

^a (ND is Northern District, AG is Agana, GT is Agat; s, m, and b are surface, middle (5 m) and bottom (10 m), respectively. NDA is northern district site A. If s, m, or b is not indicated, the site is less than 1 m deep.

Results of linear regression analyses between paired routine monitoring data reveals that the strongest relationship among effluent-associated variables was with reactive phosphate (Table 4), which averages 100 μM (3.1 mg L^{-1}) in the treatment plant effluents. Ammonium content of coastal waters is also strongly related to the presence of effluent, but was less frequently monitored. The equations of the lines describing RP and NH_4^+ as a function of salinity are:

$$\mu\text{M RP} = -2.74 \times \text{Salinity } (\text{‰}) + 92 \quad (R^2 = 0.77, n = 133)$$

$$\mu\text{M NH}_4^+ = -78 \times \text{Salinity } (\text{‰}) + 2500 \quad (R^2 = 0.96, n = 42)$$

Nitrate levels were unrelated to FC densities (Fig. 2a) or to any water quality parameters. Of those parameters required by the EPA to be measured, only phosphate and fecal coliforms were useful in the detection of the effluent in receiving waters, especially once dilution to full-strength seawater occurred.

Because many indicator bacteria are rapidly "inactivated" (i.e. at least rendered unculturable) by UV radiation in surface waters (Borja & Wood 1986), they may become irretrievable from receiving waters via conventional techniques and therefore undetectable prior to the dilution of RP. Nitrate analysis is not useful as an indicator of the presence of effluent on Guam due to the very low levels that commonly occur in effluents and to the essentially ubiquitous flux of nitrate-rich groundwater from either natural seeps or from leaky nearshore water pipes (Matson 1993).

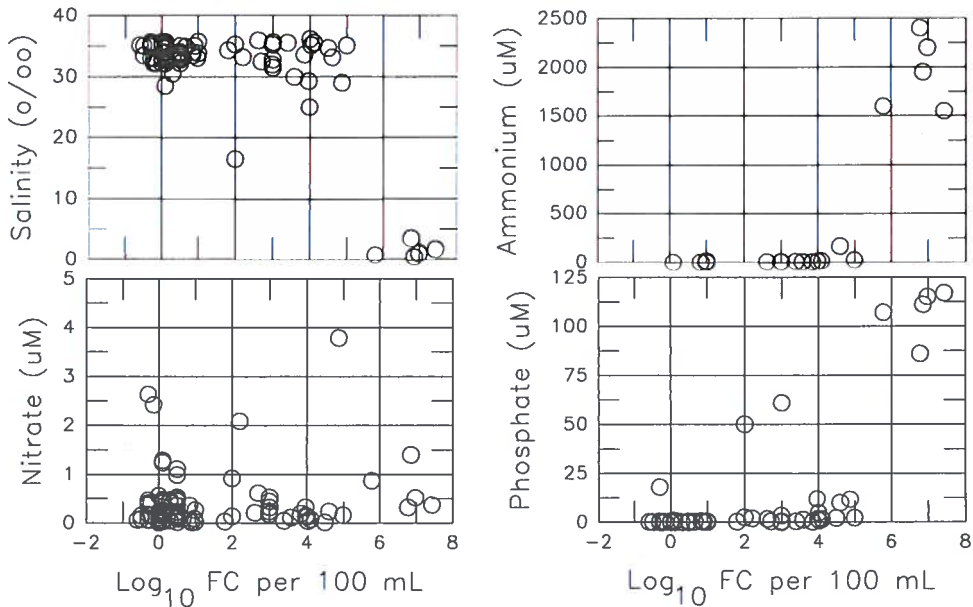


Figure 2. (a) Salinity and Nitrate and (b) Ammonium and Phosphate vs. Log_{10} fecal coliforms per 100 mL.

Table 4. Summary of linear regression results between paired average water quality data.^a

	Salinity	pH	O ₂	RP	NO _x	NTU
Temp	0.28	NS	NS	NS	NS	NS
Salinity	**	0.20	NS	-0.88	NS	0.42
pH		**	0.24	-0.60	NS	NS
O ₂			**	NS	NS	NS
RP				**	NS	0.37
NO _x					**	NS

^a NS = not significant at 0.05. N ranges from 36 (NTU) and 115 (O₂) to 133 (all others).

ORGANIC NITROGEN CONTENT OF ALGAE AND FECES

The TON content of fresh feces in the common sea cucumber *Holothuria atra* (Fig. 3) shows no significant correlation with depth towards the effluent diffusers at the Northern District. The feces of this benthic browser reflects the time-integrated N contents (up to 2 days) of the carbonate sediments within an area of only a few tens of square centimeters. Presumably, if deposition of nutrient-rich particles from the effluent had increased the sedimentary N content, then this would be reflected in the N content of their feces.

Similarly, if the N content of the alga, *Halimeda*, had increased due to local nutrient enrichment, this would be reflected in the ND sediments, which contain substantial amounts of *Halimeda* segments, and upon which the animals feed. However, there was no significant difference between the average TON contents of *Halimeda opuntia* along ca. 30 m transects parallel to and 5, 10, and 14 m from the diffusers at this site (Fig. 4a). Also, there was no change in TON content of the algae associated with decreasing depth and increasing distance from the diffusers (Fig. 4b). Indeed, at this site, divers could be unaware of the presence of effluent unless they actual swam directly over or through the diffuser field. The effluent rises immediately to the surface and apparently has little, if any impact on the benthic community.

In contrast, at Agat the TON contents of the red alga *Galaxaura marginata* on transects 75 (3 m deep) and 100 m (6 m deep) did show a significant change associated with distance from the outfall (Fig. 5). The mean TON contents along a ca. 25 m long transect are significantly different ($P < 0.05$).

Benthic and epiphytic algae are common in these types of shallow, nearshore, turbulent-water communities, and, except at Agana, become abundant in close

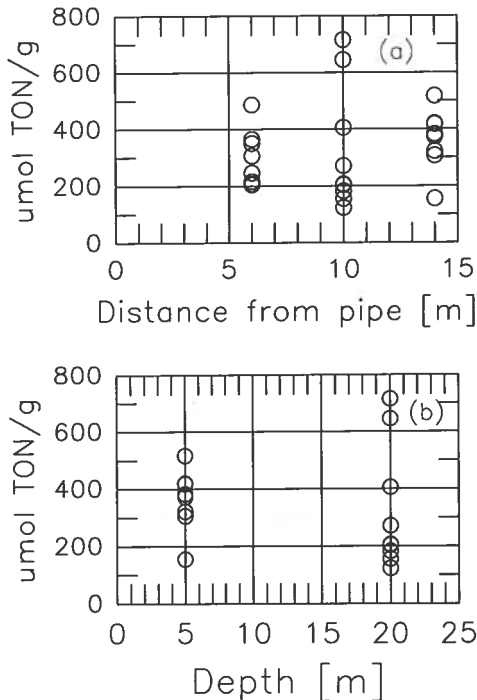
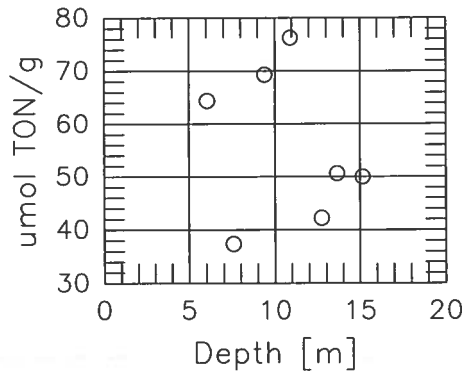


Figure 4. (a) TON in *Halimeda opuntia* over transects of distance and (b) depth towards the diffuser field at the Northern District site.

Figure 3. TON in fresh sea cucumber feces with increasing depth from shore towards the Northern District site.



proximity to the point of discharge of an effluent. This is especially noticeable at Agat where the effluent is diluted in seawater shallower than 3 m (Tsuda & Grosenbaugh, 1977). Adjacent areas north and south of the point of discharge at Agat had fewer algae. Further, this difference at Agat may be reasonably attributed to the fact that the effluent at this site is especially rich in ammonium and phosphate. High ammonium levels in the Agat effluent may be attributed to the routine disposal of pumped septic tank waste in the sewers outside the STP. These wastes are rich in terrestrial sediment that is rich in ammonium (Matson 1989).

In contrast, algae at the Northern District outfall, although generally less abundant, appear more uniformly distributed with distance from the diffusers, based upon qualitative visual observations. At Agana, in the immediate vicinity of the pipe and for about 25 m to either side, the bottom is biologically sparse. In fact, it is often difficult to find any algae except for small tufted forms that are frequently eroded or grazed. This lack of biota may be due to construction of both the sewer island itself, and to recent dredging and burial of the repaired effluent pipe.

From these studies of the TON in biological materials no obvious change can be attributed to the presence of sewage effluents. Except for Agat, perhaps the most long-lasting and significant effects on the communities at these sites was the construction and installation of the effluent pipes. At Agat, the abundance of relic pipes provides a large surface area upon which organisms can grow.

OCCURRENCE OF INDICATORS AND PATHOGENS IN SEDIMENTS

The 1990–1991 surveys of FCs and FSs in coastal sediments revealed the almost ubiquitous presence of both indicator groups (Table 5). Numbers ranged from 0 to 320,000 (FCs) and 10,000 (FSs) per gram of whole wet sediment. The highest numbers and most common occurrences were at the Agana shoreline station A (which was 10m offshore of a street runoff culvert) and in Agana Bay at the Duncas River Delta. The FC/FS ratio (Table 6) was highest in Duncas River delta sediments and lowest at Ypao Beach. High ratios indicate the presence of either predominantly human sewage pollution or of more rapid die-off of fecal streptococci, which thus raises the FC/FS ratio artificially over time. Overall, however, the index shows that human sewage occurs much more commonly at these sites than does fecal material from other warm-blooded animals. Collins (1992) reported FC (19×10^6 100 mL⁻¹) and FS (14×10^6 100 mL⁻¹) levels in six samples of street runoff that had an average ratio of 1.4, which indicates the predominance of non-human fecal pollution. Studies of the potential differential survival of various indicators and of the normal flora of domestic and feral animals on Guam should be conducted so as to evaluate the utility of the FC/FS ratio locally.

Table 5. Coastal sediment data from 1990–1991^a.

Site/date	FC/gram	FS/gram	Depth [cm]	Resuspended to depth		FC/FS
				FC 100 mL ⁻¹	FS 100 mL ⁻¹	
Agana Bay (ASTP at Hotdog stand)						
8/8	0	0	25	0	0	
8/20	260	10	25	1040	40	26

Table 5. Continued.

Site/date	FC/gram	FS/gram	Depth [cm]	Resuspended to depth		FC/FS
				FC 100 mL ⁻¹	FS 100 mL ⁻¹	
9/26	800	460	20	4000	2300	1.7
10/10	750	280	18	4200	1600	2.7
10/24	90	140	12	750	1200	0.6
10/31	13000	30	5	260000	600	430
11/14	720	20	3	24000	670	36
11/21	0	0	2	0	0	
11/28	9040	880	6	150000	15000	10
12/19	2280	310	8	29000	3900	7.4
1/2	120	200	6	2000	3300	0.6
1/30	100	10	8	1300	130	10
2/13	0	10	12	0	83	0.0
2/27	430	20	8	5400	250	22
3/13	210	20	10	2100	200	11
3/27	30	80	10	300	800	0.4
4/17	70	10	18	390	56	7.0
5/8	3700	200	15	25000	1300	19
5/22	20	10	6	330	170	2.0
Dungcas River Delta						
8/08	2000	0	12	17000	0	
8/20	110	0	12	920	0	
9/26	4000	0	20	20000	0	
10/10	4070	220	12	34000	1800	18.5
10/24	300	80	12	2500	670	3.8
10/31	260000	530	10	2600000	5300	490.6
11/14	0	0	5	0	0	
11/28	650	130	7	9300	1900	5.0
12/19	520	50	10	5200	500	10.4
1/02	30	0	12	250	0	
1/30	200	40	10	2000	400	5.0
2/13	140	0	10	1400	0	
2/27	7920	10	15	53000	67	792.0
3/13	350	60	12	2900	500	5.8
3/27	1050	120	12	8800	1000	8.8
4/17	600	20	20	3000	100	30.0
5/8	1110	640	20	5500	3200	1.7
5/22	2400	130	12	20000	1100	19
Ypao Beach (swimming area)						
8/8	10	0	35	29	0	
9/26	300	0	35	860	0	
10/10	10	0	35	29	0	
10/24	0	10	35	0	29	0.0
11/14	0	0	33	0	0	
11/28	10	10	56	18	18	1.0
12/19	0	0	30	0	0	
1/02	0	0	38	0	0	
1/30	0	0	32	0	0	

Acknowledgments

I thank Joanne Collins, Serge Quenga, Sharon Britos, Butch Irish, and Mark Rogers for assistance in the field and laboratory. The willingness of Butch Irish, Tim Rock, and Serge Quenga to dive in sewage should be especially noted. Funding was provided by a grant 14-08-0001-G2-014 from OWRT to the University of Guam Water and Energy Research Institute and a contract between the Public Utilities Agency of Guam and the University of Guam Marine Laboratory. Additional support was provided by the University of Guam Department of Natural Sciences. University of Guam Marine Laboratory Contribution 345.

References

- American Public Health Association, American Water Works Association & Water Pollution Control Administration. 1989. Standard Methods for the Examination of Water and Waste-Water. APHA, AWWA, WPCF. Washington, D.C. 17th Ed.
- Bermudez, M. & T. C. Hazen. 1988. Phenotypic and genotypic comparison of *Escherichia coli* from pristine tropical waters. *Appl. Environ. Microbiol.* 54: 979-983.
- Borja, M. & H. Wood. 1986. Environmental impact of sewage effluent at the marine outfall of the Northern District Sewage Treatment Plant, Guam. Guam Environmental Protection Agency, Tech. Rep.
- Buck, J. D. 1978. Comparison of in situ and in vitro survival of *Candida albicans* in seawater. *Microb. Ecol.* 4: 291-302.
- Collins, J. M. 1992. Transport of fecal bacteria in subsurface water into Tumon Bay, Guam. Proc. 7th Intl. Coral Reef Symposium, Tumon, Guam. (abstr.)
- Curtis, T. P., D. D. Mara & S. A. Silva. 1992. Influence of pH, oxygen, and humic substances on ability of sunlight to damage fecal coliforms in waste stabilization pond water. *Appl. Environ. Microbiol.* 58: 1335-1343.
- Cruz-Cruz, N. E., G. A. Toranzos, G. G. Ahearn & T. C. Hazen. 1988. In situ survival of plasmid-bearing and plasmidless *Pseudomonas aeruginosa* in pristine tropical waters. *Appl. Environ. Microbiol.* 54: 2574-2577.
- Davies, C. M. & L. M. Evison. 1991. Sunlight and the survival of enteric bacteria in natural waters. *J. Appl. Bacteriol.* 70: 265-274.
- Jones, M. N. 1984. Nitrate reduction by shaking with cadmium: Alternative to columns. *Wat. Res.* 18: 643-646.
- Matson, E. A. 1989. Biogeochemistry of Mariana Islands coastal sediments: terrestrial influence on $\delta^{13}\text{C}$, ash, CaCO_3 , Al, Fe, Si and P. *Coral Reefs* 7: 153-160.
- Matson, E. A. 1993. Nutrient flux through soils and aquifers to the coastal zone of Guam (Mariana Islands). *Limnol. Oceanogr.* 38: 361-371.
- Pancorbo, O. C. & H. M. Barnhart. 1991. Microbial pathogens and indicators in estuarine environments and shellfish: critical need for better indicator(s) of human-specific fecal pollution. *J. Environ. Health* 54: 57-63.
- Parsons, T. R., Y. Maita & C. M. Lalli. 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, New York.

- Perez-Rosas, N. & T. C. Hazen. 1988. In situ survival of *Vibrio cholerae* and *Escherichia coli* in tropical coral reefs. *Appl. Environ. Microbiol.* 54: 1-9.
- Rivera, S. C., T. C. Hazen & G. A. Toranzos. 1988. Isolation of fecal coliforms from pristine sites in a tropical rain forest. 1988. *Appl. Environ. Microbiol.* 54: 513-517.
- Stumm, W. & J. J. Morgan. 1981. *Aquatic Chemistry*. 2nd ed. Wiley-Interscience, N.Y.
- Tsuda, R. T. & D. A. Grosenbaugh. 1977. Agat sewage treatment plant: impact of secondary treated effluent on Guam coastal waters. U. Guam Water Resources Research Center Tech. Rep. 3.
- Valdes-Collazo, L., A. J. Schultz & T. C. Hazen. 1987. Survival of *Candida albicans* in tropical marine and fresh waters. *Appl. Environ. Microbiol.* 53: 1762-1767.
- Yoshpe-Purer, Y. & S. Golderman. 1987. Occurrence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in Israeli coastal water. *Appl. Environ. Microbiol.* 53: 1138-1141.
- Zolan, W. J., R. N. Clayshulte, S. J. Winter, J. A. Marsh & R. H. F. Young. 1978a. Urban runoff quality in northern Guam. U. Guam Water Energy Res. Inst. Tech. Rep. 5.
- Zolan, W. J., R. N. Clayshulte & S. J. Winter. 1978b. Urban runoff pollutant adsorption and filtering by selected northern Guam soils and limestone. U. Guam Water Energy Res. Inst. Tech. Rep. 6.

Received 16 Mar. 93, revised 10 July.