APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1995, p. 2235–2241 Vol. 61, No. 6
0099-2240/95/$04.00+0

Occurrence of Fecal Indicator Bacteria in Surface Waters and the Subsurface Aquifer in Key Largo, Florida

JOHN H. PAUL,[1]* JOAN B. ROSE,[1] SUNNY JIANG,[1] CHRIS KELLOGG,[1] AND EUGENE A. SHINN[2]

Department of Marine Science, University of South Florida,[1] and U.S. Geological Survey Center for Coastal Geology,[2] St. Petersburg, Florida 33701

Received 17 January 1995/Accepted 30 March 1995

Sewage waste disposal facilities in the Florida Keys include septic tanks and individual package plants in place of municipal collection facilities in most locations. In Key Largo, both facilities discharge into the extremely porous Key Largo limestone. To determine whether there was potential contamination of the subsurface aquifer and nearby coastal surface waters by such waste disposal practices, we examined the presence of microbial indicators commonly found in sewage (fecal coliforms, *Clostridium perfringens*, and enterococci) and aquatic microbial parameters (viral direct counts, bacterial direct counts, chlorophyll *a*, and marine vibriophage) in injection well effluent, monitoring wells that followed a transect from onshore to offshore, and surface waters above these wells in two separate locations in Key Largo in August 1993 and March 1994. Effluent and waters from onshore shallow monitoring wells (1.8- to 3.7-m depth) contained two or all three of the fecal indicators in all three samples taken, whereas deeper wells (10.7- to 12.2-m depth) at these same sites contained few or none. The presence of fecal indicators was found in two of five nearshore wells (i.e., those that were ≤1.8 miles [≤2.9 km] from shore), whereas offshore wells (≥2.1 to 5.7 miles [≤3.4 to 9.2 km] from shore) showed little sign of contamination. Indicators were also found in surface waters in a canal in Key Largo and in offshore surface waters in March but not in August. Collectively, these results suggest that fecal contamination of the shallow onshore aquifer, parts of the nearshore aquifer, and certain surface waters has occurred. Current sewage waste disposal practices may have contributed to this contamination.

The Florida Keys represent a unique environment in that a relatively high human population density exists adjacent to oligotrophic coral reef communities. Coral reef communities are fragile ecosystems that are particularly susceptible to nutrient loading and to perturbation by anthropogenic activities. There is evidence that increased nutrification of surface waters is linked to accelerating urbanization in South Florida and the Florida Keys specifically (9). A potential source of such nutrient loading and anthropogenic activities may be the waste disposal systems operating in such areas.

With the exception of Key West, the Florida Keys area depends on nonsewered waste disposal systems, including an estimated 30,000 septic tanks (18) and another 619 sewage treatment plant boreholes (25). The sewage treatment boreholes are from package treatment plants, which yield secondary treatment to produce effluents that meet State of Florida standards for disposal into class G-III groundwater (25). Such treatment usually includes activated sludge followed by sand filtration and chlorination prior to injection into boreholes. In the past, there were few guidelines for drilling boreholes, and the depths of existing wells range from less than 1 m to nearly 30 m (25). Current regulations require drilling to 90 ft (27.4 m) and casing to 60 ft (18.3 m), making the latter the effective depth of discharge (25).

Concern for this type of waste disposal practice stems from the very shallow level of the groundwater aquifer in the Keys, the porosity of the subsurface strata, and uncertainty about the holding capacity and migration of materials in such strata. For some time, on-site sewage disposal systems have been known to cause eutrophication of lakes (5). LaPointe et al. (18) correlated nutrient (nitrate, ammonium, and phosphate) levels in canals and subsurface wells with close proximity to septic tanks and compared those levels with the levels in wells in a pristine, control site. The fate of injection well effluent has not been extensively studied.

In addition to nutrient loading of subsurface and surface waters, on-site disposal systems, such as septic tanks and injection wells, are known to be a source of microbial contamination of groundwater (17). Microbial pathogens in sewage include *Salmonella* and *Shigella* spp., *Vibrio cholera*, enterotoxigenic *Escherichia coli*, and over 100 different types of pathogenic viruses. Fifty percent of all waterborne diseases are attributed to contaminated groundwater (17). Outbreaks of hepatitis, gastroenteritis, and Norwalk virus dysentery have been attributed to groundwater contamination from septic tanks (26).

The purpose of this study was to investigate the occurrence of microbial indicators of fecal pollution as well as indicators of total microbial abundance in injection well effluent, in monitoring wells onshore and along a transect offshore into the reef environment, and in surface waters in two locations in Key Largo, Fla. The indicators chosen included fecal coliforms (1), *Clostridium perfringens* (2), and enterococci (19), while the microbial abundance measures included viral direct counts (21), bacterial direct counts (20), determining the amount of chlorophyll *a*, and enumerating marine phages isolated on host 16 (22), a marine bacterium isolated from Tampa Bay.

MATERIALS AND METHODS

Sampling sites. Figure 1 shows the locations of the sampling sites, and Figure 2 is a schematic of the geological setting of the sampling environment and the locations of monitoring wells. A complete description of the monitoring wells appears elsewhere (25). Sampling was done from 16 to 20 August 1993 and 7 to 11 March 1994. In addition to the stations shown in Fig. 1 and 2, secondarily treated sewage effluent was obtained from a plant in the Ocean Reef area. The chlorine residual was neutralized with sodium thiosulfate in sewage effluent samples.

* Corresponding author. Fax: (813) 893-9189.

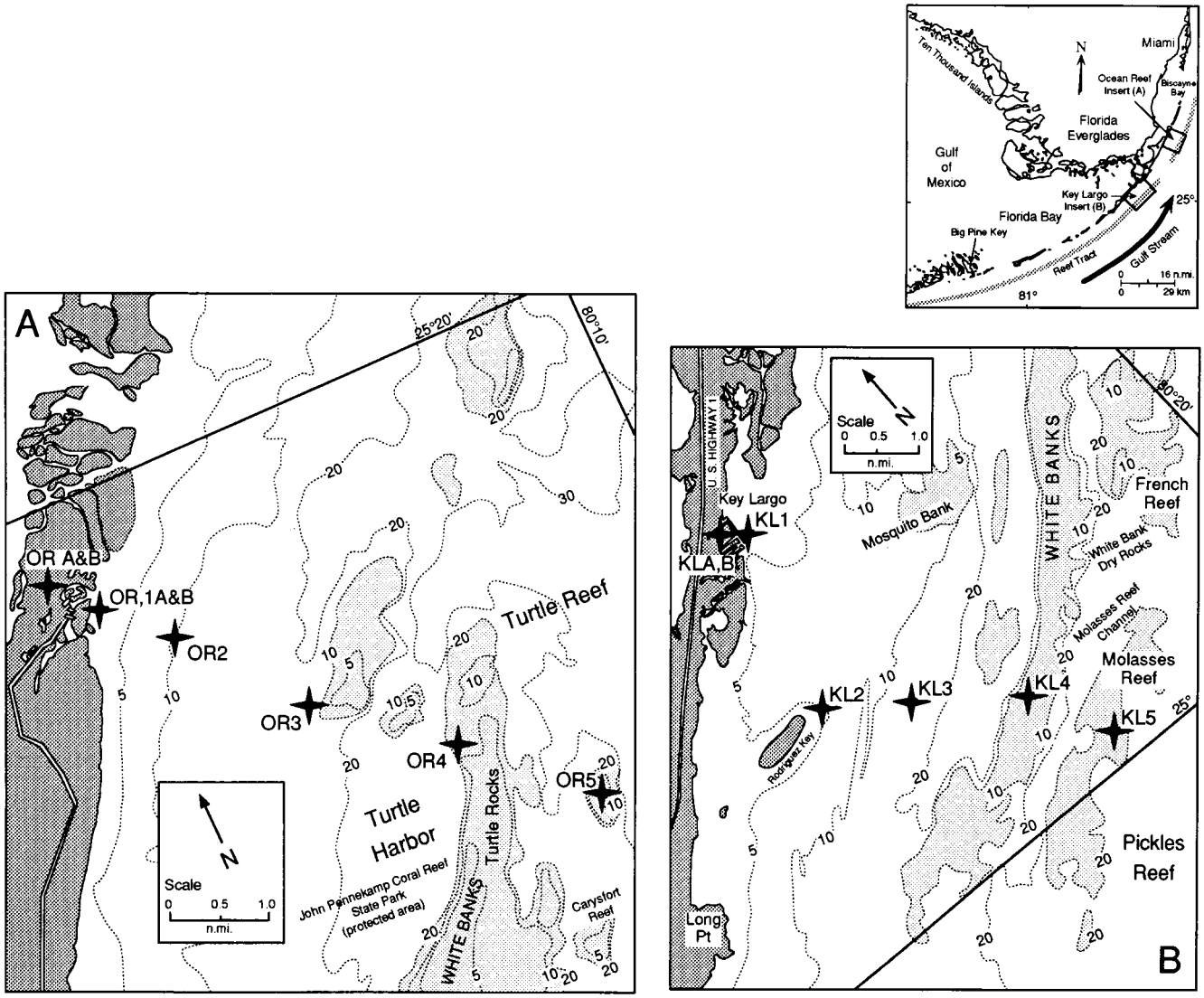

FIG. 1. Locations of monitoring wells and sampling sites in Ocean Reef (A) and Key Largo (B) transects and locations of these transects in South Florida (top).

Sampling. Most offshore samples were taken prior to 10 a.m. (prior to the arrival of recreational divers and pleasure boats) to minimize the chance of fecal contamination of surface waters by boaters. Monitoring wells consisted of polyvinyl chloride pipes (inside diameter, 1 in. [2.54 cm]) (25). To sample the offshore (submerged) monitoring wells, tygon tubing was fitted to a Teel model IP580E impeller battery-operated pump (Dayton Electric, Chicago, Ill.) located on a small boat on the surface. This was then attached to a 1-in. (2.54-cm)-diameter polyvinyl chloride tube cap by scuba divers or skin divers. The wells were pumped for 4 min (approximately 80 liters) to ensure flushing. The water was then collected in 20-liter carboys (for vortex flow filtration), which had been disinfected by sodium hypochlorite treatment (200 ppm for 30 min) followed by the neutralization of hypochlorite with 100 ppm of sodium thiosulfate. For samples not concentrated by vortex flow filtration (i.e., those for direct filtration on membrane filters), samples were collected in sterile 1-liter bottles. For onshore wells (Fig. 1 and 2, KLA, KLB, ORA, and ORB), samples were collected with a Shaklee diaphragm pump with 1-cm (internal diameter) polypropylene tubing. The samples were processed within 4 h of collection. Water samples were used directly (without concentration) for the determination of microbial fecal indicators, the presence of chlorophyll *a*, bacterial direct counts, and salinity. Additionally, samples were concentrated by vortex flow filtration (10 to 20 liters concentrated to 40 to 60 ml) as previously described (21) by using a Membrex Benchmark unit for viral direct counts, phage enumeration on host 16, and the detection of indicators.

Microbial indicators of fecal pollution. Three indicators of fecal pollution were evaluated in the water samples. Although fecal coliforms have classically been the indicator of choice for ambient water quality, current research suggests that *C. perfringens* may be a better indicator of pollution because of the resistant nature of the spore, allowing enhanced survival during water treatment and in environmental waters (2). Enterococci are also known to be more resistant in the environment and may be an indication of the specific portion of the fecal streptococci which are more likely associated with human fecal pollution (19, 24). Water samples were assayed directly from grab samples and from the Membrex concentrate by filtering 0.1, 1.0, 10.0, and/or 100 ml through 47-mm-diameter membrane filters (0.45-μm pore size). The limit of sensitivity for the direct determination was <10/liter, while the assay of the Membrex concentrate increased the sensitivity to approximately <2/liter. For fecal coliforms, membrane filtration for coliforms (MF-C) agar was used, the samples were incubated at 44.5°C for 24 h, and the blue colonies were counted (1). Five percent of the colonies were picked for further verification by Gram staining and plating on eosin-methylene blue agar. *C. perfringens* was enumerated on m-CP medium (2). The medium (catalog no. QC-0284; Difco, Detroit, Mich.) was supplied to us by Mark Sobsey (University of North Carolina). The filters were incubated under anaerobic conditions by using BBL GasPaks for 24 h at 44.5°C. The resulting plates with yellowish colonies were exposed to ammonium hydroxide fumes for 30 s, after which pink-to-red colonies were counted as being *C. perfringens*. Because the method has been shown to possess a 93% specificity with a 2% false-negative rate (2), further verification was not performed. Enterococci were enumerated on mE agar (19). After initial incubation at 41°C for 48 h, the filters were placed on enzyme immunoassay agar for confirmation of enterococci and incubated at 41°C for 20 min. Pink-to-red enterococcus colonies that developed a black or reddish brown precipitate on the underside of the filter were counted.

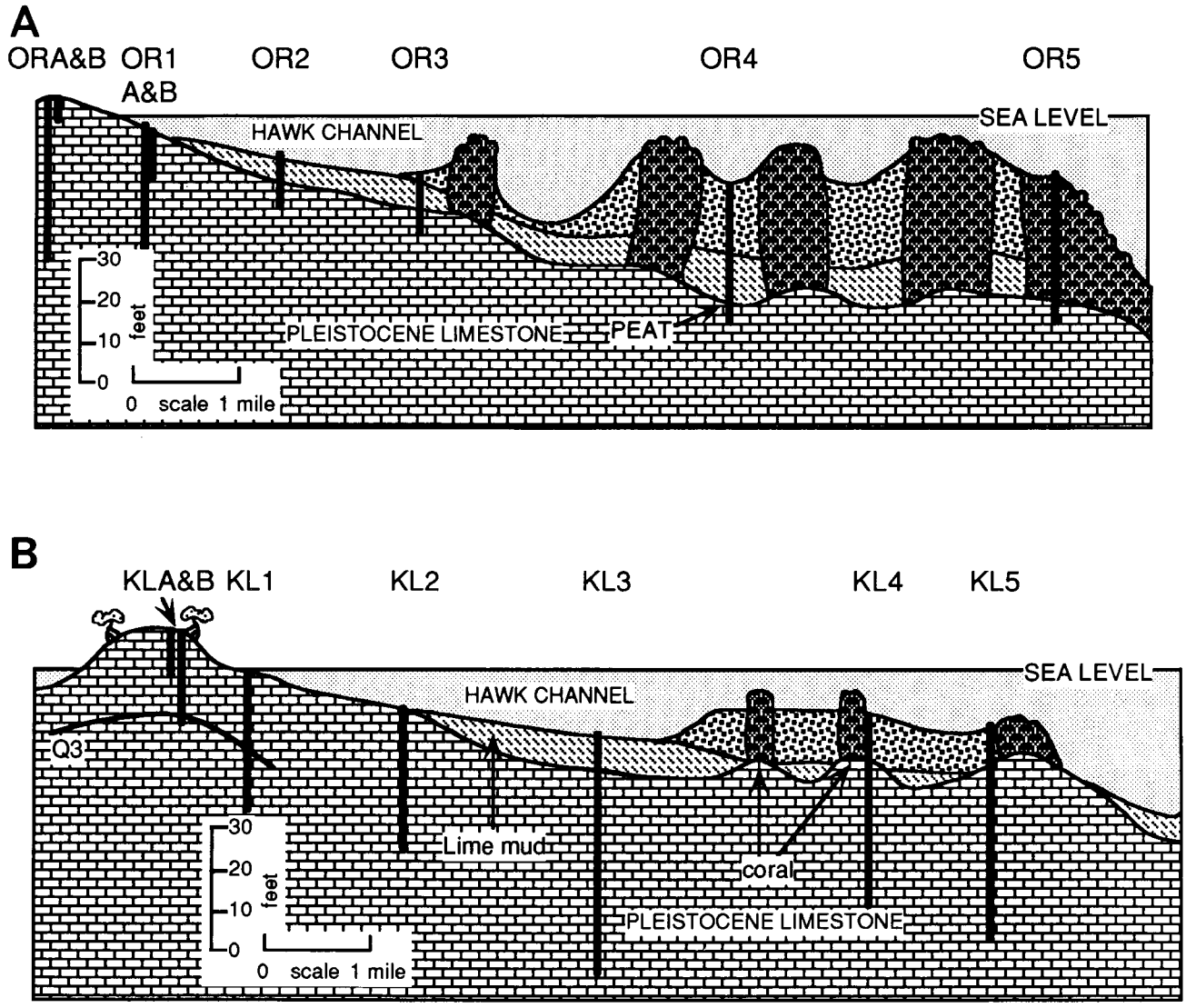

FIG. 2. Geological setting and relative depths of monitoring wells in Ocean Reef (A) and Key Largo (B) transects. The locations of living coral, Key Largo (Pleistocene) limestone, lime mud, and coral rubble sediments are also shown.

This method has been shown to possess a 90% specificity and an ~11% false-negative rate (19).

Aquatic microbial parameters. Viral direct counts were determined in samples concentrated by vortex flow filtration and fixed with glutaraldehyde as previously described (3, 21). Bacterial direct counts were determined by epifluorescence microscopy of 4′,6-diamidino-2-phenylindole (DAPI)-stained samples (20). The amount of chlorophyll *a* was determined fluorometrically in methanol-extracted samples (16). Phages which were infective for the marine bacterium host 16 (tentatively identified as a *Vibrio parahaemolyticus* strain) were enumerated by soft agar overlay as previously described (22). Salinity was determined by refractometry.

RESULTS

Table 1 gives descriptive information on the sampling sites as well as the temperature and salinity of samples collected in August 1993 and March 1994. The OR prefix indicates an Ocean Reef transect well, KL indicates a Key Largo well, and the suffix WC means a water column sample taken above the particular well at the same location. The ORA, ORB, KLA, and KLB wells were located on land, with "A" indicating a deep (>10-m) well and "B" indicating a shallow (<4-m) well, adjacent to each other. The KLA and KLB wells were approximately 20 m from a septic tank drain field and 30 m from a man-made canal that emptied into nearshore coastal waters. The ORA and ORB wells were 457 m from a sewage injection system with 50 wells, which served a large development in northern Key Largo. The ORA and ORB wells could not be located in March 1994 because they were paved over with a crushed stone parking lot. All other wells were located under water on the southeastern side of Key Largo.

Most deep wells (ORA, OR1A, OR4, OR5, and all KL wells) showed a conservation in temperature between the August and March samplings (usually within a degree or 2 of 26°C) and were cooler than the overlying water in August and warmer than the overlying water in March. The salinity of the deep wells was usually equal to or higher than that of the overlying seawater. Only the shallow well ORB had a low salinity indicative of a Ghyben-Herzberg lens (13).

Microbial indicators in the Ocean Reef transect. Figure 3 shows the distribution of microbial indicators of fecal pollution in monitoring wells and sewage effluent in the Ocean Reef

TABLE 1. Stations and wells sampled in this study

Designation	Description	Overlying water depth (m)	Drill depth (m)	Screen interval[a]	August 1993 sample		March 1994 sample	
					Water temp (°C)	Salinity (ppt)	Water temp (°C)	Salinity (ppt)
Ocean Reef area								
ORE	Effluent from sewage treatment plant				33	0	30	1.8
Monitoring wells								
ORA	On land ~300 m from waste injection		12.2	10.7–12.2	30	33.5		
ORB	On land ~300 m from waste injection		12.2	1.8	32.5	4.2		
OR1A	Exposed bedrock	0.61	12.2	11–12.2	26	33.3	25.5	33.8
OR1B	Exposed bedrock	0.61	3.0	1.8–3	29	33.3	25	33.8
OR2	2.4 m of muddy sediment	4.6	4.6	3.4–4.6	26.2	35.1	25	34.9
OR3	2.1 m of muddy sediment	4.9	4.0	2.7–4.0	27	34.9	25.5	35.3
OR4	7.9 m of mud	4.9	10.7	9.4–31.7	27	35.5	26	36.4
OR5	7.9 m of Holocene coral	5.2	10.7	9.4–10.7	27	34.7	26	36.1
Water column samples								
ORWC1	Water above OR1A and -B, 0.2 m	0.61			31.3	34.9	22.5	33.1
ORWC3	Water above OR3, 0.2 m	4.9			31	34.9	22.5	33.1
ORWC5	Water above OR5, 0.2 m	5.2			29.5	33.7	24.0	34.3
Key Largo area								
KLA	On land, next to NURC[b] office		13.7	10.7–11.9	29.0	36.5	24.5	33.6
KLB	On land, next to NURC office		13.7	3.66	33.0	35.3	26.0	36.4
KL1	Drilled in exposed rock	1.1	12.2	11–12.2	26.5	37.1	25.5	35.9
KL2	Exposed bedrock and live corals	1.8	12.2	11–12.2	25.5	35.3	25	34
KL3	Off edge of coral patch	4.3	19.8	19–19.8	26	36	25	34.4
KL4	On edge of coral patch	4.6	15.2	14–15.2	26.5	35.8	21	34.8
KL5	In coral reef	4.9	18.3	17.1–18.3	26.5	36.6	26	35.4
Water column samples								
Canal	Head of Port Largo Canal	5.2			30.5	34.5	25	33.6
KLWC1	Water over KL1, 0.2 m	1.1			31.5	35.4	26	32.4
KLWC3	Water over KL3, 0.2 m	4.3			30	33.4	21.5	34.8
KLWC5	Water over KL5, 0.2 m	4.9			30	33.2	24	34.9

[a] Screen interval is the depth below the benthic interface from which water can be sampled because of holes in the polyvinyl chloride well pipe.
[b] NURC, National Underseas Research Center.

area, and Table 2 shows the distribution of these indicators in the corresponding surface waters. The highest numbers of *C. perfringens* cells, fecal coliforms, and enterococci were found in the effluent from the wastewater treatment plant. All three indicators were also found in the onshore shallow well, ORB, at numbers similar to those in the effluent. This well was the closest to the injection wells of the wastewater treatment plant. The deep onshore well, ORA, had 20 enterococci per liter in August 1993 but no other indicators in any other sampling. No detectable indicators were found at the offshore wells OR1A and OR1B, which were approximately 1 mile (~2 km) from the injection wells. The OR2 station samples contained all three indicators, whereas offshore wells OR3 through OR5 possessed no detectable indicators. A similar trend was observed in the monitoring wells in the March sampling (Fig. 3B), with all four indicators in the effluent and high fecal coliforms at OR2. No indicators were detected in any of the other wells.

In the water column, *C. perfringens* was at the detection limit at ORWC3, and enterococci were detected at ORWC3 and ORWC5 in August 1993. In March, enterococci were detected at ORWC1. Water column levels of indicators were below those detected in the monitoring wells ORB and OR2.

Aquatic microbial parameters in the ocean reef area. In terms of aquatic microbial parameters in monitoring wells, the effluent had the highest viral direct counts and bacterial direct counts and largest amount of chlorophyll *a* (data not shown). The former two parameters are expected in wastewater, but the presence of chlorophyll *a* may be surprising. However, the settling tanks at the package plant in Key Largo are open and exposed to high intensity sunlight (owing to the low latitude), and algal concentrations can be quite high. Microbial parameters in other wells were consistent with oligotrophic environments, except for OR2 and ORB, which had significantly higher values.

Microbial parameters in the water column of the ocean reef area followed a decreasing trend in values from onshore to offshore, as might be expected (Table 2). The highest values for viral direct counts, bacterial direct counts, and phages isolated on host 16 were found at ORWC1, and the lowest were found above the Carysfort coral reef (ORWC5).

Microbial indicators at the Key Largo transect. The distribution of indicators of fecal pollution in the Key Largo monitoring wells is shown in Fig. 4. As with the ocean reef transect, evidence of contamination of the shallow subsurface aquifer was found, as indicated by the presence of all three indicators

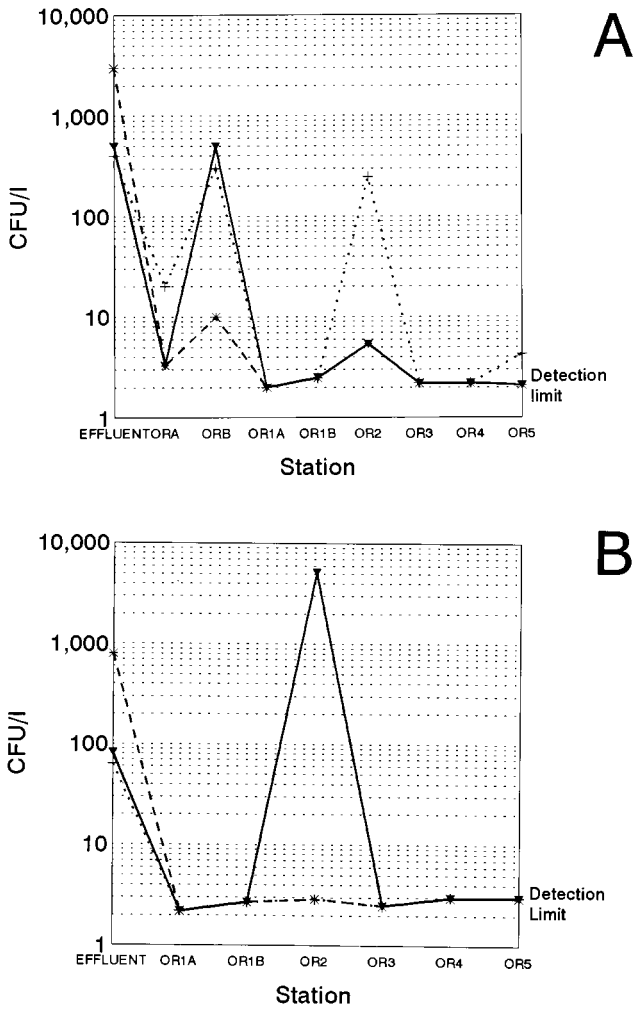

FIG. 3. Microbial indicators of fecal contamination in package plant effluent and monitoring wells in Ocean Reef transect in August 1993 (A) and March 1994 (B). Solid line, fecal coliforms; dashed line, *C. perfringens*; dotted line, enterococci. The values between 2 and 3 CFU/liter represent the detection limit of the methods. The wells ORA and ORB could not be located in March 1994 for sampling.

in the shallow onshore well KLB in August and of two in March but not in the deep well at that site (KLA). Fecal coliforms were detected in the KL1 and KL2 wells in August but not in March, and fecal coliforms were detected at KL5 in March only. Unlike the Ocean Reef transect, the effluent from one of the package plants in the Key Largo area had low levels of the indicators, with the exception of *C. perfringens*, which was detected at 270 CFU/liter (data not shown).

There were few indicators detected in surface waters of the Key Largo transect in August, with the exception of fecal coliforms in the canal, which generally had the poorest water quality of any surface waters sampled (Table 2). In the March sampling, indicators were detected in all water column stations except KLWC5, and values decreased from the canal offshore to the reef environment of KLWC5. The higher levels of the indicators in the surface waters may reflect the higher human population in the Keys in March than in August.

Aquatic microbial parameters in the Key Largo area. Figure 5 shows the microbial parameters at the monitoring wells in Key Largo in August. The highest levels for all parameters were found in the onshore shallow well KLB. The high levels of viruses and bacteria may have been from contact with injection well effluent or septic tank seepage, as suggested by the high levels of indicators in this water. However, the high levels of chlorophyll *a* and marine phages isolated on host 16 suggest that this water freely exchanges with marine surface water. The other wells in this transect had very low levels of viruses, bacteria, and chlorophyll *a*, consistent with subsurface terrestrial aquifers.

The levels of water column microbial parameters were generally higher in the canal than at the other stations, reflecting

TABLE 2. Microbial parameters and indicators in water column samples

Date of sampling	Station	VDC[a] (10^9/liter ± SD)	BDC[b] (10^9/liter ± SD)	Chl *a*[c] (μg/liter ± SD)	φ16[d] (PFU/liter)	CFU/liter		
						Fecal coliforms	Enterococci	*C. perfringens*
August 1993	ORWC1	2.9 ± 1.0	1.7 ± 0.2	0.29 ± 0	156	<2.6	<2.6	<2.6
	ORWC3	2.2 ± 0.3	1.1 ± 0.1	0.18 ± 0.01	1.2	<2.5	24.6	2.5
	ORWC5	1.0 ± 0.2	0.61 ± 0	0.14 ± 0.01	<2.8	<2.8	25	<2.8
March 1994	ORWC1	5.8 ± 2.7	1.2 ± 0.02	0.23 ± 0.003	13.9	2.8	<2.8	10.3
	ORWC3	2.4 ± 1.3	1.3 ± 0.04	0.21 ± 0.01	<2.6	<2.6	<2.6	<2.6
	ORWC5	0.72 ± 0.12	1.0 ± 0.03	0.12 ± 0	<3.1	<3.1	<3.1	<3.1
August 1993	Canal	6.8 ± 2.5	2.0 ± 0.07	3 ± 0.11	103	20	ND[e]	<2.1
	KLWC1	6.4 ± 1.2	1.8 ± 0.03	0.45 ± 0.02	534	<2.0	8.0	2.0
	KLWC3	3.1 ± 0.43	1.1 ± 0.01	0.34 ± 0.02	8.9	<2.5	ND	<2.5
	KLWC5	5.7 ± 1.6	1.0 ± 0.03	0.88 ± 0.4	<2.8	<2.8	<2.8	<2.8
March 1994	Canal	3.9 ± 0.5	2.1 ± 0.25	2.7 ± 0.07	<5.4	2,775	18.3	2.7
	KLWC1	5.2 ± 0.9	1.3 ± 0.07	0.31 ± 0.09	255	25.8	<3.1	13.1
	KLWC3	5.1 ± 1.9	1.5 ± 0.07	0.53 ± 0.02	<2.6	26.3	<2.6	<2.6
	KLWC5	0.4 ± 0.1	0.7 ± 0.02	0.46 ± 0.04	<2.8	<2.8	<2.8	<2.8

[a] VDC, viral direct counts.
[b] BDC, bacterial direct counts.
[c] Chl *a*, chlorophyll *a*.
[d] φ16, plaque titer of phages growing on host 16.
[e] ND, not determined.

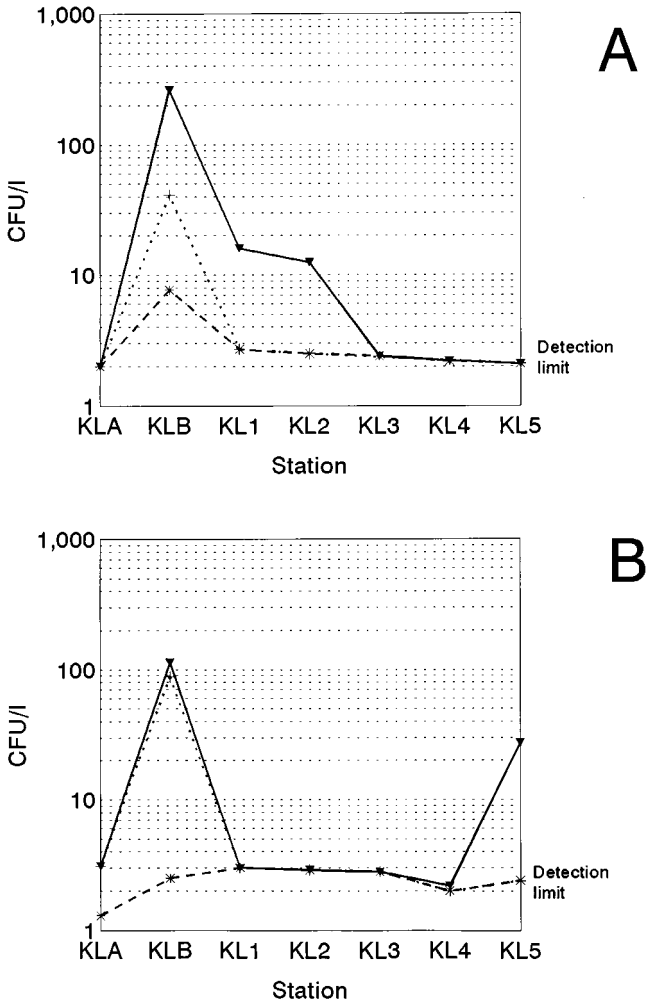

FIG. 4. Microbial indicators of fecal pollution in monitoring wells in Key Largo transect in August 1993 (A) and March 1994 (B). Symbols are as described in the legend to Fig. 3.

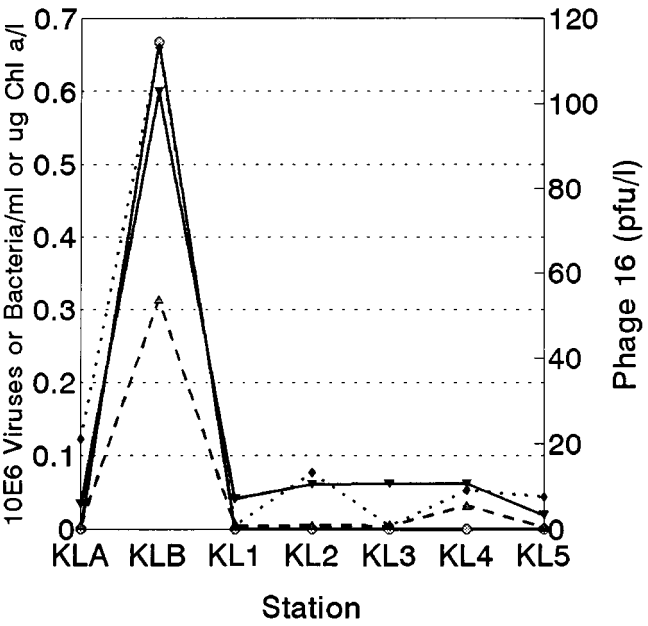

FIG. 5. Aquatic microbial parameters in Key Largo transect monitoring wells in August 1993. Note the extremely high levels of all parameters in the shallow onshore well, KLB. ▼, viral direct counts; ◆, bacterial direct counts; △, chlorophyll *a* (Chl a); ◎, phage 16.

its eutrophic nature (Table 2). A dramatic exception is the phage isolated on host 16, the numbers of which were consistently highest at the KLWC1 station.

DISCUSSION

In this paper we present evidence of fecal contamination of the subsurface aquifer as determined by the presence of bacterial indicators, particularly on the island of Key Largo itself and also at some offshore subsurface locations. Fecal indicators are not native to the subsurface environment and should not survive there long but could be around waste disposal areas, such as septic tank leach fields (4). The shallow wells on the island of Key Largo (ORB and KLB) contained at least two of the three indicators in all samplings. Shinn et al. (25) also found fecal coliforms and fecal streptococci in four of four samplings of the KLB well and once in the ORB well. The former well was just 20 m from a septic tank drain field, and the latter was 450 m from a large (50-well) sewage injection site. The latter services a community which varies in size on weekly and seasonal bases but is not thought to exceed 1,000 residents at its maximal occupancy. Fecal indicators were also found in 4 of 10 samplings of nearshore (<1.2 nautical miles [<2.2 km] from shore) wells (i.e., OR1A and B, OR2, KL1, and KL2). For monitoring wells farther than 1.2 nautical miles (2.2 km) from shore, indicators were found in only 2 of 12 samplings, and one positive result was close to the detection limit for that sample.

The presence of fecal coliforms in the environment may not definitively prove a fecal origin for these bacteria. Fecal coliforms are often present in tropical environments in the absence of any source of fecal contamination (10, 14, 15). For this reason we have used additional fecal indicators, enterococci and *C. perfringens. C. perfringens* has been shown to be a better indicator of human fecal contamination in tropical surface waters (10). Enterococci may be better indicators of human fecal pollution (19, 24), and their levels appear to be better predictors of risk to swimmers of contracting gastrointestinal illness, caused mainly by enteric viruses in sewage-contaminated waters (6, 7). Additionally, enterococci are not affected by salinity levels and have no growth phase in seawater (12).

Besides humans, other potential sources of fecal coliforms are warm-blooded animals, such as raccoons, which are abundant in the Keys. However, a septic tank and sewage injection well field were 20 and 450 m, respectively, from the fecally contaminated KLB and ORB wells, respectively. Although surface waters could conceivably be contaminated by animal feces, it seems unlikely that this material would make it into the subsurface environment in the absence of a mechanism of transport. For example, input into groundwater by seepage after heavy rains could occur during the wet season (August sampling) but this is unlikely to occur during the dry season (March sampling).

In the Ocean Reef transect, contamination does not look like a simple diffusion process from the putative source to the offshore environment, because of the lack of contamination at the OR1A and OR1B sites. In the Key Largo area, the wells were not placed on a linear transect but rather offset between KL1 and KL2 (Fig. 1). Thus, the contamination noted at KL2

may have been caused by processes closer to this well than to the KL1 (Port Largo Canal) area.

The appearance of fecal contamination at some distance from the putative site of origin may be related to the heterogeneity in the Key Largo limestone bedrock. There are areas of extremely high porosity and solution channels such that waste accumulation and accelerated movement can occur. Shinn et al. (25) also found fecal coliforms and fecal streptococci in offshore monitoring wells in the absence of nearshore contamination in the Saddlebunch Keys area. In studies on the subsurface movement of fecal coliforms from septic tank drain fields, high concentrations of fecal coliforms were occasionally found at locations some distance from the drain field. This phenomenon was attributed to sewage movement through cracks, root channels, and fissures (11). Fissures, cracks, and other subsurface features could have resulted in the accumulation of fecal indicators at OR2 and KL2 but not at the more shoreward stations OR1 and KL1.

Another factor which may have affected the detection of the fecal indicators in offshore environments was the distribution of depths of the monitoring wells sampled. In general, contamination was not observed in deep wells (those >10 m deep). Of the 19 samples collected from the deep wells, only 3 gave positive results for a single indicator. Of nine samples taken from shallow wells, five were positive, four of which had more than one indicator. We did not have access to the shallow and deep aquifers at each site (the wells used in this study were not drilled for a systematic examination of the aquifer as a function of depth but rather for the study of the subsurface geology [25]). The lack of contamination at KL1 may be due to the fact that this is a deep well (>11 m) and therefore may have been below the level of contamination in this area. In the Ocean Reef area, OR1B was a shallow well but was not contaminated. OR2 was a relatively shallow well and was contaminated.

There was evidence for only slight contamination of the surface waters sampled in this project. Of 14 surface water marine samples, 8 possessed at least one fecal indicator at a level above background, and only one sample (canal in March) had fecal coliform levels exceeding standards set for Florida surface waters (200 CFU/ml [8]). Standards for acceptable levels of *C. perfringens* and enterococci in Florida surface waters have not yet been established. The canal station, located at the head of the canal, was also relatively close (<500 m) to the shallow monitoring well KLB.

The high volume of exchange that the shallow subsurface aquifer has with the marine surface waters was indicated by the findings at the KLB station, where high levels of wastewater indicators coexisted with marine indicators such as chlorophyll *a* and marine phages (those isolated on host 16). These data suggest rapid mixing of subsurface waters with marine surface waters. This is not surprising because of the proximity of the KLB monitoring well to a canal (about 10 m from the seawall).

We have recently observed the rapid movement of viruses from a septic tank and a simulated injection well through the subsurface environment into marine surface waters in Key Largo (23). Bacteriophages were seeded into the septic tank located near KLB and appeared in the KLB monitoring well in as little as 4 h, in the canal in 10.5 h, and in the outstanding marine waters (KLWC1) within 20.5 h (23). Those studies combined with the results presented here demonstrate that a mechanism exists for the fecal contamination of the subsurface and surface environments by wastewater disposal practices in Key Largo.

ACKNOWLEDGMENTS

This research was supported by the National Underseas Research Center of NOAA and administered by the University of North Carolina at Wilmington and a grant from the National Science Foundation. Special thanks are given to Catherine Liefert for assisting us in locating the monitoring wells.

REFERENCES

1. **American Public Health Association.** 1989. Standard methods for the examination of water and wastewater, 17th ed. American Public Health Association, Washington, D.C.
2. **Bisson, J. W., and V. J. Cabelli.** 1979. Membrane filter enumeration method for *Clostridium perfringens*. Appl. Environ. Microbiol. **37:**55–66.
3. **Boehme, J., M. E. Frischer, S. C. Jiang, C. A. Kellogg, S. Pichard, J. B. Rose, C. Steinway, and J. H. Paul.** 1993. Viruses, bacterioplankton, and phytoplankton in the southeastern Gulf of Mexico: distribution and contribution to oceanic DNA pools. Mar. Ecol. Prog. Ser. **97:**1–10.
4. **Bouwer, H.** 1984. Elements of soil science and groundwater hydrology, p. 9–38. *In* G. Bitton and C. P. Gerba (ed.), Groundwater pollution microbiology. John Wiley & Sons, Inc., New York.
5. **Brezonik, P. L.** 1972. Nitrogen: sources and transformations in natural waters, p. 1–50. *In* H. Allen and J. Kramer (ed.), Nutrients in natural waters. John Wiley & Sons, New York.
6. **Cabelli, V. J.** 1983. Health effects criteria for marine recreational waters. U.S. Environmental Protection Agency report no. EPA-600/1-80-031. U.S. Environmental Protection Agency, Cincinnati, Ohio.
7. **Cabelli, V. J., A. P. Dufour, L. J. McCabe, and M. A. Levin.** 1983. A marine water quality criterion consistent with indicator concepts and risk analysis. J. Water Pollut. Control Fed. **55:**1306–1314.
8. **Dufour, A. P.** 1984. Bacterial indicators of recreational water quality. Can. J. Public Health **75:**49–56.
9. **Environmental Protection Agency.** 1992. Water quality protection program for the Florida Keys National Marine Sanctuary, phase II. Continental Shelf Associates Inc. and Battelle Ocean Sciences, contract no. 68-C8-0105. U.S. Environmental Protection Agency, Washington, D.C.
10. **Fujioka, R. S., and L. K. Shizumura.** 1985. *Clostridium perfringens*, a reliable indicator of stream water quality. J. Water Pollut. Control Fed. **57:**986–992.
11. **Hagedorn, C.** 1984. Microbiological aspects of groundwater pollution due to septic tanks, p. 181–196. *In* G. Bitton and C. P. Gerba (ed.), Groundwater pollution microbiology. John Wiley & Sons, Inc., New York.
12. **Hanes, N. B., and R. Fragala.** 1967. Effects of seawater concentration on survival of indicator bacteria. J. Water Pollut. Control Fed. **39:**97–104.
13. **Hanson, C. E.** 1980. Freshwater resources of Big Pine Key, Florida. Geological Survey open-file report 80-447. Department of the Interior, Washington, D.C.
14. **Hardina, C. M., and R. S. Fujioka.** 1991. Soil: the environmental source of *Escherichia coli* and enterococci in Hawaii's streams. Environ. Toxicol. Water Qual. **6:**185–195.
15. **Hazen, T. C.** 1988. Fecal coliforms as indicators in tropical waters: a review. Toxic. Assess. **3:**461–477.
16. **Holm-Hansen, O., and B. Riemann.** 1978. Chlorophyll *a* determination: improvements in methodology. Oikos **30:**438–448.
17. **Keswick, B. H.** 1984. Sources of groundwater pollution, p. 39–64. *In* G. Bitton and C. P. Gerba (ed.), Groundwater pollution microbiology. John Wiley & Sons, Inc., New York.
18. **Lapointe, B. E., J. D. O'Connell, and G. S. Garrett.** 1990. Nutrient couplings between on-site disposal systems, groundwaters, and nearshore surface waters of the Florida Keys. Biogeochemistry **10:**289–307.
19. **Levin, M. A., J. R. Fischer, and V. J. Cabelli.** 1975. Membrane filtration technique for enumeration of enterococci in marine waters. Appl. Environ. Microbiol. **30:**66–71.
20. **Paul, J. H.** 1982. The use of Hoechst dyes 33258 and 33342 for the enumeration of attached and pelagic bacteria. Appl. Environ. Microbiol. **43:**939–949.
21. **Paul, J. H., S. C. Jiang, and J. B. Rose.** 1991. Concentration of viruses and dissolved DNA from aquatic environments by vortex flow filtration. Appl. Environ. Microbiol. **57:**2197–2204.
22. **Paul, J. H., J. B. Rose, S. C. Jiang, C. A. Kellogg, and L. Dickson.** 1993. Distribution of viral abundance in the reef environment of Key Largo, Florida. Appl. Environ. Microbiol. **59:**718–724.
23. **Paul, J. H., J. B. Rose, J. Brown, E. A. Shinn, S. Miller, and S. R. Farrah.** 1995. Viral tracer studies indicate contamination of marine waters by sewage disposal practices in Key Largo, Florida. Appl. Environ. Microbiol. **61:**2230–2234.
24. **Rice, E. W., T. C. Covert, D. K. Wild, D. Berman, S. A. Johnson, and C. H. Johnson.** 1993. Comparative resistance of *Escherichia coli* and enterococci to chlorination. J. Environ. Sci. Health **A28(1):**89–97.
25. **Shinn, E. A., R. S. Reese, and C. D. Reich.** 1994. Fate and pathways of injection-well effluent in the Florida Keys. U.S. Geological Survey, open file report 94-0000. Department of the Interior, Washington, D.C.
26. **Yates, M. V., and S. R. Yates.** 1988. Modeling microbial fate in the subsurface environment. Crit. Rev. Environ. Control **17:**307–344.