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VARIATION IN COLIFORM BACTERIAL COUNTS ON SAN SALVADOR, BAHAMAS

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ABSTRACT

On islands without sophisticated sewage systems, there is concern that the coastal water used by the local population and tourists may become contaminated by discharge of raw sewage, inadequately treated sewage, or passively leaked sewage. Many people go swimming and snorkeling in the waters along the coast of San Salvador Island, Bahamas; therefore, data were obtained to assess the water safety. The project involved collecting coastal ocean water and analyzing it for elevated levels of coliform bacteria, in particular *Escherichia coli (E. coli),* which could indicate the presence of enteropathogenic organisms and a potential health risk for people snorkeling and swimming in the area.

Because contamination by *E. coli* is related to human population density, water was sampled at eight sites near areas with different population densities. Contamination by *E. coli* was assessed using the MPN (Most Probable Number) table (the index for the most probable number of coliforms present in 100 ml of fluid). Confirmation tests were conducted in the winter of 2000-2001 to distinguish *E. coli* from other coliform bacteria.

Results of our study showed that Bonefish Bay near Club Med, with a MPN of 93, was the only site that came close to approaching the safety limit of 100 MPN, which represents the strictest water safety regulations in the USA. Contamination by *E. coli* was related to local human population density. Bonefish Bay, with the high population density due to Club Med, had the highest MPN values of 93 (as already mentioned) and 23. Graham's Harbor, the second-most population location on the island due to the Gerace Reseach Center, had MPN values of 14, 9, and 0 in the first sampling and 0 the second time. Rice Bay, adjacent to the high school, had an MPN of 9. Fernandez Bay, which has population clusters at either end of the bay, showed 0 MPN. French Bay, which has no population, had MPN values of 0. Even though there was a correlation between population density and MPN, even the maximum was too small to constitute a health hazard for people swimming and snorkeling.

INTRODUCTION

Large metropolitan areas with high populations can afford sophisticated sewage plants. Small islands around the world with minimal population do not have the resources to invest in advanced technology or even "low level" disposal systems. At the lower end of the spectrum are islands that dispose of human waste directly into the ocean. Some sparsely populated islands have leach fields and/or other less-expensive treatment facilities. There is concern on islands that the coastal water used by the local population and tourists may become contaminated by inadequate treatment before being directly released or passively leaked into the adjacent ocean.

Many people go swimming and snorkeling in the ocean waters along the coast of San Salvador; therefore, data were obtained to assess water safety. The project involved collecting seawater from eight sample sites around the island and analyzing them for elevated levels of coliforms, in particular *Escherichia coli* (*E. coli*). Of the eight sample sites chosen, seven of these were located adjacent to sites with varying human densities and one was on an isolated part of the island away from homes (Figure 1).

We hypothesized that the levels of *E. coli* would increase as local human population density

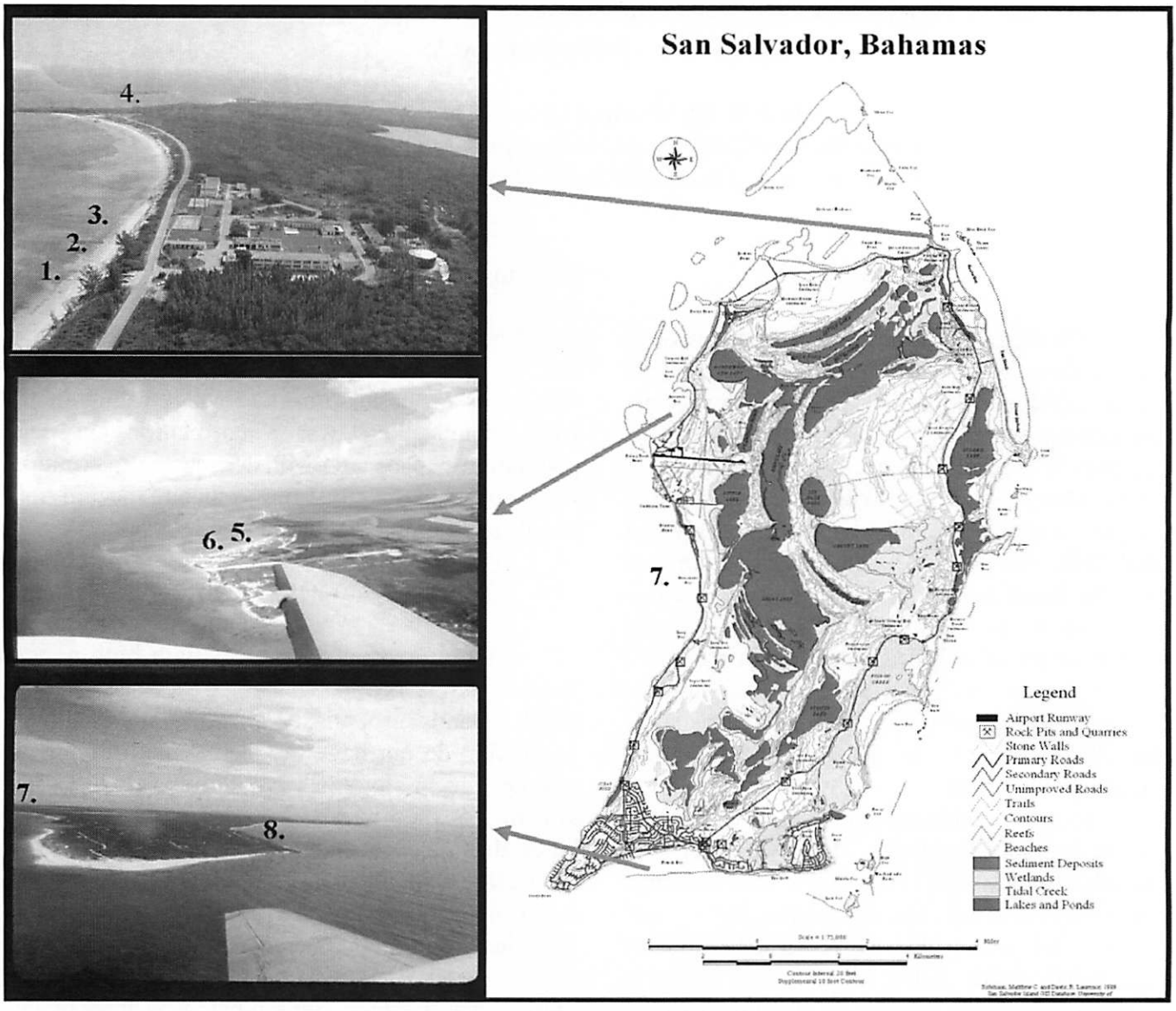

Figure 1. San Salvador Island, Bahamas, showing the location of the ocean water sampling sites. These locations were chosen to ascertain the presence of safe vs. contaminated water.

increased near the ocean. In a previous study, Balcerzak *et al.* (1990) found that contamination of ground water in wells on San Salvador was correlated with population density. We also asked whether contamination levels would be high enough to pose a health risk in the coastal seawater. If the sewage is disposed into the seawater in great amounts, this could increase the presence of an enteropathogenic organism and be a potential health risk for people snorkeling and swimming (Atlas & Bartha, 1987).

MATERIALS AND METHODS

Eight sites were sampled. These included, from the highest population density to the least, Bonefish Bay (sites 5 and 6), Graham's Harbor (sites 1, 2, 3), Rice Bay (site 4), Fernandez Bay (site 7), and French Bay (site 8) (Figure 1). The specific sampling sites were: 1) Graham's Harbor - Field Station Ramp; 2) Graham's Harbor- Pipe (where the GRC has a discharge pipe); 3) Graham's Harbor - Water Tower; 4) Rice Bay; 5) Bonefish Bay - Sewer Pipe; 6) Bonefish Bay -

Property Fence, 7) Fernandez Bay, and 8) French Bay. The three sites in Graham's Harbor were sampled twice to detect a change in coliform levels after the Gerace Research Center had been populated for a few days.

Five people wearing rubber gloves collected the seawater. They were placed in the seawater about 3-6 m from the shore in a cross-shaped pattern. At a given signal, everyone opened their sterile bag (100 ml) and filled it with seawater. Each bag was sealed and brought into the shore, where each bag of seawater was poured into one larger (500 ml) sterile plastic bag. At the same time as the seawater collection took place, another person on the team recorded the water temperature, the wet air temperature, and the dry air temperature.

The seawater samples were brought back to the laboratory for further analysis. Phenol Red Lactose (PRL) broth (Sigma) and EMB (Eosin Methyl Blue) agar (Difco) plates were made according to the instructions on the package in the microbiology laboratory at Mississippi State University (MSU). Two batches of PRL broth were made: one double strength and one normal strength. The broth was transferred into screw top test tubes of two different sizes (16x150 mm and 20x150 mm) that contained a Durham tube. The large test tubes were used for the double strength broth and the small ones for the normal strength broth. The test tubes were autoclaved at 121°C/15 pounds/squares inch for 15 min.

Samples of seawater were transferred into test tubes containing different volumes of lactose broth. All transfers were made using sterile pipettes and aseptic technique. We transferred 10 ml of the seawater from each sample site into each of three test tubes with lactose broth (10 ml, double strength). We transfered 1 ml of the seawater into three test tubes containing 9 ml of lactose broth (normal strength). Finally, 0.1 ml of seawater was transferred into three test tubes containing 9.9 ml of lactose broth (normal strength). A control tube was made by inoculating bacteria known to be *E. coli* into a test tube containing 9.9 ml of lactose broth (normal strength). The reason for triplicate samples was to ensure the 95% confidence limits of the predicted index of the most probable number of enterics, which is the standard procedure (Greenberg *et al.*, 1965).

Table 1. This table was used to assess E. coli *levels in ocean water. The Most Probable Number (MPN) Index and 95% Confidence Limits for various combinations of positive and negative results are shown when three 10 ml portions, three 1 ml portions, and three 0.1 ml portions were used.*

Number of Tubes Giving Positive Reactions Out of			MPN Index per 100ml	95% Confidence Limits	
3 of 10 ml each	3 of 1 ml each	3 of 0.1ml each		Lower	Upper
0	0	1	3	<0.5	9
0	1	0	3	<0.5	13
1	0	0	4	<0.5	20
1	0	1	7	1	21
1	1	0	7	1	23
1	1	1	11	3	36
1	2	0	11	3	36
2	0	0	9	1	36
2	0	1	14	3	37
2	1	0	15	3	44
2	1	1	20	7	89
2	2	0	21	4	47
2	2	1	28	10	150
3	0	0	23	4	120
3	0	1	39	7	130
3	0	2	64	15	380
3	1	0	43	7	210
3	1	1	75	14	230
3	1	2	120	30	380
3	2	0	93	15	380
3	2	1	150	30	440
3	2	2	210	35	470
3	3	0	240	36	1300
3	3	1	460	71	2400
3	3	2	1100	150	4800

The test tubes were incubated at 35°C ± 0.5°C for 48 hr and checked for color change, gas production, and turbidity at 24 and 48 hr. The test tubes that showed gas production and a yellow color change were streaked onto EMB agar and incubated for 24 hr at 35°C ± 0.5°C to serve as a confirmation test for *E. coli*. The number of posi-

tive plates (plates with *E. coli* growth) was compared with the Most Probable Number (MPN) table (Table 1) to obtain the index for the MPN of coliforms present in the seawater (Greenberg *et al.* 1965).

RESULTS

The coliform tests using PRL broth showed a yellow color change and gas production in most of the test tubes for most sample sites (Table 2). When the cultures were grown on EMB agar, which was the confirmation test for *E. coli*, the number found to be *E. coli* colonies was far fewer than the number of test tubes with yellow color change and gas production. Based on the MPN Table (Table 1), there were few sites where *E. coli* was present (Table 2).

When *E. coli* was present, the MPN values were usually low (<15). However, the two Bonefish Bay locations had much higher values: 23 and 93 (Table 2).

DISCUSSION

Water quality restrictions vary from state to state in the USA. In California, officials will close public beaches if the coliform count surpasses 100 coliforms/100 ml water, whereas in New York City the limit is 3000/100 ml (Burrows, 1968). In our study, Bonefish Bay (Club Med North Side), with an MPN of 93, was the only location that came close to approaching the strictest regulations in the USA. Its index, 93, still falls within the safety limits.

There was an apparent correlation between the places where people live and the occurrence of *E. coli*. For instance, Bonefish Bay, where Club Med is located, had MPN values of 23 and 93 coliforms present in 100 ml of seawater. Graham's Harbor, the second-most populated place on the island, had MPN values of 14, 9, and 0 for the first sampling of its three sites. With the second sampling, the MPN dropped to 0 for all three sites. This was surprising since the GRC upon the second sampling contained an additional 70 students. Rice Bay, adjacent to the high school, had an MPN of 9, which is an order of magnitude below the "danger" limit. Fernandez Bay, which has population clusters at either end of the bay, showed 0 MPN. This site is frequented by divers and snorkelers, so it is comforting to know that the water is safe. Additionally, French Bay, which has no population, had 0 coliforms in 100 ml of seawater.

Although there was a correlation between populated sites adjacent to the coast and the MPN, the MPN values were too small to constitute a health hazard for people swimming and snorkeling during the winter of 2000-2001. When *E. coli* was present, the MPN values were so low it would be improbable that the contamination

Table 2. Results of the triplicate samples taken at different sample sites on San Salvador Island, Bahamas. The Most Probable Number of coliforms was ascertained by using Table 1.

Site	Gas yellow	Confirmation Test	Most Probable Number	Water temp. F°	Air temp. dry. F°	Air temp. wet. F°	Time
Graham's Harbor, Out of Field Station Ramp 01/02/01							
1a	3/3	2/3	14	69	71	66	8:15a.m.
1b	3/3	0/3		69	71	66	8:15a.m.
1c	3/3	1/3		69	71	66	8:15a.m.
Graham's Harbor, Pipe 01/02/01							
2a	4/4	2/4	9	69.5	70	66	8:25a.m.
2b	3/3	0/3		69.5	70	66	8:25a.m.
2c	0/3	0/3		69.5	70	66	8:25a.m.
Graham's Harbor, Old Water Tower 01/02/01							
3a	3/3	0/3	0	69	70	66	8:31a.m.
3b	3/3	0/3		69	70	66	8:31a.m.
3c	0/3	0/3		69	70	66	8:31a.m.
Rice Bay 01/01/01							
4a	3/3	2/3	9	71	70	63.5	4:45p.m.
4b	1/3	0/3		71	70	63.5	4:45p.m.
4c	0/3	0/3		71	70	63.5	4:45p.m.
Bonefish Bay, Club Med, Sewer Pipe 01/01/01							
5a	3/3	3/3	23	72	70	65	4:15p.m.
5b	1/3	0/3		72	70	65	4:15p.m.
5c	0/3	0/3		72	70	65	4:15p.m.
Bonefish Bay, Club Med, North Side by Property Fence 01/01/01							
6a	3/3	3/3	93	70	70	65	3:31p.m.
6b	2/3	2/3		70	70	65	3:31p.m.
6c	0/3	0/3		70	70	65	3:31p.m.
Fernandez Bay 01/02/01							
7a	1/3	0/3	0	74	72	64	3:58p.m.
7b	2/3	0/3		74	72	64	3:58p.m.
7c	0/3	0/3		74	72	64	3:58p.m.
French Bay 01/03/01							
8a	3/3	0/3	0	68	68	63	3:56p.m.
8b	0/3	0/3		68	68	63	3:56p.m.
8c	0/3	0/3		68	68	63	3:56p.m.
Graham's Harbor, Out of Field Station Ramp 01/04/01							
9a	3/3	0/3	0	69.5	68	65	11:00a.m.
9b	3/3	0/3		69.5	68	65	11:00a.m.
9c	0/3	0/3		69.5	68	65	11:00a.m.
Graham's Harbor, Pipe 01/04/01							
10a	3/3	0/3	0	67	68	64	11:08a.m.
10b	3/3	0/3		67	68	64	11:08a.m.
10c	0/3	0/3		67	68	64	11:08a.m.
Graham's Harbor, Old Water Tower 01/04/01							
11a	3/3	0/3	0	70	68	64	11:13a.m.
11b	0/3	0/3		70	68	64	11:13a.m.
11c	1/3	0/3		70	68	64	11:13a.m.

would constitute a health hazard for people swimming and snorkeling in the coastal seawater.

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