S. Kotelnikova, Rakesh Patel, "Ecological Survival Properties of Pelagic & Benthic Indicator Microorganisms from the St. John's River Outflow in Grenada": GSP-SRGI-06003 - 20 April 2006- April 2007.

West Indies is a region with developed tourism. Clean and healthy atmosphere, aquatic and terrestrial entities is a pre-requisite for the existence of contemporary tourist industries in Caribbean. Close inspection of the marine environment will mediate the recognition of Grenada as a country ratifying the Kyoto agreement in Climate Change. Therefore, monitoring of the drinking and fresh recreational water for pollutions and coliforms contributing to the health risks for the population is of great importance for public health. Therefore we have established Environmental Testing Unit based at the Department of Microbiology, serving SGU and funded by SGU for drinking water and coastal water regular testing and quality assurance. This service is also useful for disaster management and preparedness. Currently, I supervise a team of 5 people, which is serving as a great resource not only for SGU community but also for graduate research in Microbiology and Public Health (WINDREF Annual Report 2004).

Extensive agricultural and tourist exploitation of the Winwards Islands along with growing human population may lead to irreversible pollution and eutrophication. The fact of the recent fish-kill and coral reef death is an indication of on-going environmental disturbance in the region. The long-term monitoring is crucial for understanding anthropogenic and climatic trends and their potential impact on aquatic environments. Ten coastal water sites have been monitored for 9 months at monthly or weekly basis for physical parameters (pH, 8.0-8.2 temperature, 29°C; oxygen, 2.95-6.67 mg/l, BOD5,0.9-6.0 mg/l; salinity, 34-38 ppt) and to evaluate indicators of faecal pollutions (CFU, MPN of coliforms, faecal coliforms, and faecal enterococci). Sixty three strains of gram-negative bacteria were isolated from marine and fresh water and saved as culture collection. Fifty percent of them were identified as potential pathogens (*Enterobacter, E.coli, Serratia marcescens, Klebsiella ozonae, Proteus, Shigella flexnery, Pantea, Cedeca, Pasteurella haemolitica*), and twenty percent as non-pathogenic (*Cryseobacteria sp, Pseudomonas, Citrobacter fruendii, Ochrobacterium*).

Viable counts of lactose-fermenting gram-negative bacteria were found at numbers exceeding health risk levels in 80% of tests between May and October of 2003 and in 50% of tests between October and March. Faecal Enterococcus were found in 75% of samples. Frequency of pollution decreased during the dry season but did not cease indicating the sewage run-off, and recreational yachts discharge being potential sources of water pollution (Kotelnikova et al., 2005).

The dilution rates of indicators Enterococcus faecalis and Escherichia coli were studied in the St. John's River estuary in Grenada, West Indies. The health risk zones were established based on the levels and frequency of bacteriological pollution. In accordance with the World Health Organization (WHO) health risk guidelines, the E. faecalis Most Probable Number (MPN) values were in the range of <1% Gastrointestinal (GI) ; <0.3% Acute Febrile Respiratory Illness (AFRI) to a 1-5% GI; 0.9-1.9% AFRI health risk within 100 m from the St. John's River outflow site in St. George's Bay. These values were the result of river water dilution, where the MPN levels for both indicator organisms from the river water showed a AFRI health risk of >3.9% along with a GI risk being >10% . The distance intervals further than 100 m showed fluctuating values in MPN levels and corresponding health risks. E. faecalis and E. coli strains isolated were resistant to 35.7% and 42.9% of the antibiotics tested, respectively.

The development of antibiotic resistant pathogens is of growing concern medically in recent years therefore, determining the presence of such pathogens in the environment is of great

importance. Antibiotics are readily available in local pharmacies in Grenada, and if used with some degree of frequency, can produce the potential for a growing population of antibiotic resistant strains. With this in consideration, it might be expected that indicators of enteric origin in St. George's Bay would display some form of antibiotic resistance.

According to the results, both indicators displayed antibiotic resistance to different antibiotic agents. In a study conducted by Arvanitidou et al. (2001), enterococcal resistance to erythromycin was commonest (57.3 %), while high-level resistance to kanamycin and streptomycin were 31.3 and 9.8% of the isolates respectively. Also high-level resistance ampicillin, vancomycin, or gentamicin was not detected in any of the isolates examined (Arvanitidou, et al., 2001). The results from this project showed susceptibility to erythromycin instead of resistance, while a high-level resistance to streptomycin was observed and reflected here. Also E. faecalis susceptibility to ampicillin, gentamicin, and vancomycin from the results from this project coincided with the findings from Arvantidou et al. (2001).

Our discovery of antibiotic resistant populations of the indicator organisms E.coli and E. fecalis is not new as antibiotic resistant microorganisms have been detected earlier in the sea water of Prickley Bay (Warner et al., 1998). However the resistance have never been associated with the indicators in Grenadian seawater. We observed number of similarities in the patterns of antibiotic resistance between the unidentified marine bacteria that have been isolated from Prickley Bay, Grenada in the previous study of Warner et al., 1998 that showed resistance to penicillin (40% of isolates), streptomycin (20%), oxacillin (40%) , erythromycin (10%), sulfomethoxazole x trimethoprim (40%) and gentamycin (50%). The spread of the resistance through sewage sludge contaminated waters has been well documented earlier (Grabow et al., 1974, 1975; Qureshi and Qureshi, 1992). It has been noted that the antibiotic resistant E.coli showed better survival in the marine water (Qureshi and Qureshi, 1992). Supposedly it may be a consequence of correlation of the amount of genome allocated to the conjugative plasmids providing genome flexibility which in turn allows the organism to adapt to excessive UV exposure and high salinity in the tropical marine water.

St. George's Bay can effectively dilute and remove the majority of the incoming pathogen load from the St. John's River effluent. However, a health risk of <1% GI; <0.3% AFRI to 1-5% GI; 0.9-1.9% AFRI with a lowest probability of 1 case of gastroenteritis in 100 exposures and a negligible AFRI illness rate to a highest probability of 1 in 20 of gastroenteritis for a single exposure and approximately 1 in 50 AFRI risk, was present according to WHO guidelines.

There was an indication of hypoxic conditions throughout the bay, with the lowest values in the 95% confidence interval within the hypoxic range in all distance intervals. This supports the assumption that hypoxic conditions would be found in the bay. However, since a greater percentage of dissolved oxygen values were above the chronic criterion for growth at most distance intervals, it can be concluded that non-hypoxic conditions are observed most of the time.

The antibiotic resistance of the indicators E. faecalis and E. coli supports the hypothesis of finding such organisms in St. George's Bay. However, both indicators from St. George's Bay were susceptible to a greater percentage of the antibiotics tested, and therefore would not warrant an immediate health concern with possible infection by these pathogens. St. George's Bay can be labeled as a source of antibiotic resistant pathogens, but most likely not of significant medical concern, since a greater percentage of the antibiotics tested were efficient in limiting the growth of the indicators isolated from the bay.