EPA GRO Summer Internship Final Report

**Quantification of Enterococci and Identification of Fecal Pollution Sources
in Beaches of the Eastern Region of Puerto Rico
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This summer I had the opportunity of working in the Ecosystems Research Division (ERD) of the National Exposure Research Laboratory (NERL) in Athens, GA. Under the guidance of Dr. Marirosa Molina, research microbiologist at the ERD/NERL in Athens, I immersed myself in the molecular aspects of my research project titled, "Quantification of Enterococci and Identification of Fecal Pollution Sources in Beaches of the Eastern Region of Puerto Rico."

In Dr. Molina's lab, the main interest lies in water quality, especially water quality of recreational waters. I was very fortunate to be in a place where my passion was the main focus. That being said, the purpose of my project was to measure the quality of recreational waters in four beaches located in the Eastern region of Puerto Rico. One of the beaches is the Seven Seas, located in Fajardo. This beach is next to a preserve. The second beach is the "Balneario La Monserrate", which is located in Luquillo, close to approximately 50 food establishments. "El Escambrón" in San Juan is a beach with a great deal of urban impact. The fourth beach is located in a small town with an agriculture-based economy, the "Balneario de Patillas" in Patillas. Water samples were collected every two weeks from early February throughout May. The water samples were plated on mEI agar using a membrane filter method, filtrated onto polycarbonate filters, and stored to conduct molecular assays at the EPA in Athens.

The molecular assays performed at the EPA were Quantitative Polymerase Chain Reaction (qPCR) and Microbial Source Tracking (MST). The first technique, qPCR, allows the quantification of Enterococci sequences using specific detection chemistry. This quantification is not source specific. Nevertheless, identifying the source of fecal contamination can help to implement the proper preventive measures to avoid future contamination. MST allowed us to determine the source of fecal pollution in the beaches studied. Due to the difference between intestinal microorganisms of different animal groups we were able to determine the main source of fecal pollution in each of the beaches using primer sets that discriminate between human, ruminant, and bird fecal pollution. These primers target the 16S rDNA of fecal Bacteroides.

The overall results show that during the months of February and March, three of the studied beaches had better microbial quality than in the months of April and May. These three beaches were: the Seven Seas, the Balneario La Monserrate, and El Escambrón. Nevertheless, for the first two months, the Balneario de Patillas showed the highest enterococci density reported by colony counts and qPCR. On the other hand, this last beach showed the lowest microbial density (<1 CFU / 100mL) and no enterococci target sequence copies (TSC) for the months of April and May. In April and May, the Seven Seas, La Monserrate and El Escambrón had their highest enterococci density as reported by colony counts and qPCR. Among the four beaches analyzed, Seven Seas showed the best microbial water quality with entorococci counts below eight CFUs throughout the four months of the study. On the other hand, MST showed that the main sources of fecal pollution in the Seven Seas and El Balneario de Patillas are human and ruminant feces. For the Balneario La Monserrate and El Escambrón, the main source of fecal contamination was reported to be ruminant feces.

Besides the molecular work I did in the lab, I was also exposed to three intense weeks of fieldwork. I participated in rainfall simulation experiments in the field with a team of scientists and engineers. The goal of these experiments was to evaluate the release and overland

transport of pathogens, indicators, nutrients, and total suspended solids from areas affected by animal wastes, water infiltration and runoff. I was also introduced to statistical analyses, to which I had never been exposed. I believe the fieldwork and statistics experience will come in handy with future research projects.

The most challenging part of this summer was to balance schoolwork with my research project. The majority of the students at my university went on strike last semester. After the strike ended in July, classes recommenced. Accordingly, after a long day at the lab or in the field I had schoolwork waiting for me. However, this unexpected circumstance helped me improve my time management skills.

Since the first day of my summer internship I had to apply skills I acquired from previous internship experiences and the work I do at my campus. Mastering basic skills such as pipetting is crucial when doing molecular work. The simplest mistake can ruin a reaction! Similarly, my computer and software skills proved very useful when it came to analyzing statistical data. As with every type of research, knowledge comes with every single thing on which you experiment. The skills I thought I had mastered were refined by this experience.

I have been longing to intern at the EPA ever since my first year in college. Even though I have attended scientific conferences, I have never been exposed to so many scientists working on environmental issues. Everyone at the EPA is environmentally conscious, which gave me a sense of belonging. For instance, while at the lab, I was amazed by the honest desire of its employees to recycle.

Throughout my internship I learned that I love to work on beaches, I am passionate about water microbiology, and that I find molecular work amazing. I also learned that fieldwork is an acquired taste. Moreover, I learned that I have great time management skills and that I am a good autodidact. This experience has made me realize that I enjoy learning and producing knowledge.

That being said, this semester I will be applying to graduate school. This internship experience has solidified my desire to pursue a PhD in environmental microbiology. After my PhD, I wish to work for an agency, such as the EPA, whose main interest is our safety and the preservation of the environment.

I came to the EPA eager to learn more about the environment and water microbiology. Needless to say, I left having learned so much more than I expected... I came home with a refueled passion for the environment and the knowledge that there are people who still care about it. My advice to next year's GRO fellows would be: ENJOY! Get involved in as much as you can. This will not only enrich your research interests but it will open a window to a plethora of ideas.