

Conservation International Research: Rapid Monitoring of Coral Reef Microbes
Forest Rohwer (frohwer@sciences.sdsu.edu)
San Diego State University
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Overall status: We have completed a first generation model of the fluorometer and have conducted testing in the lab and on three field excursions. A second generation model using an avalanche photo detector (APD) instead of a photomultiplier tube has been designed, but not field tested.

Achievements: A generation 1 model of fluorometer has been designed, built, lab and field tested.

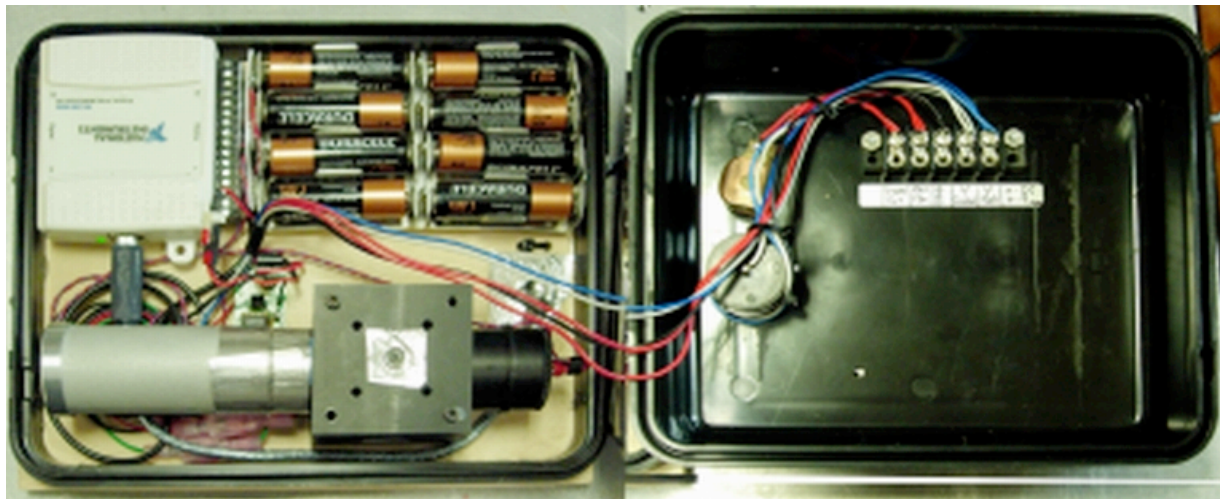


Figure 1: Here is the 1st generation device in its package

Lab experiments:

Initial experiments with version 1 of the fluorometer shows that seawater at a concentration of 1.0×10^5 cells/ml can be distinguished from the control sample.

The background/control is made up of 10 mls lab aquarium water, 0.02 micron filtered, 10 microliters SYBR Gold and 10 microliters DNase 1 (10 mg/ml)

The experimental fraction is made up of lab aquarium water, 0.45 micron filtered, 10 microliters SYBR Gold and 10 microliters DNase 1 (10 mg/ml)

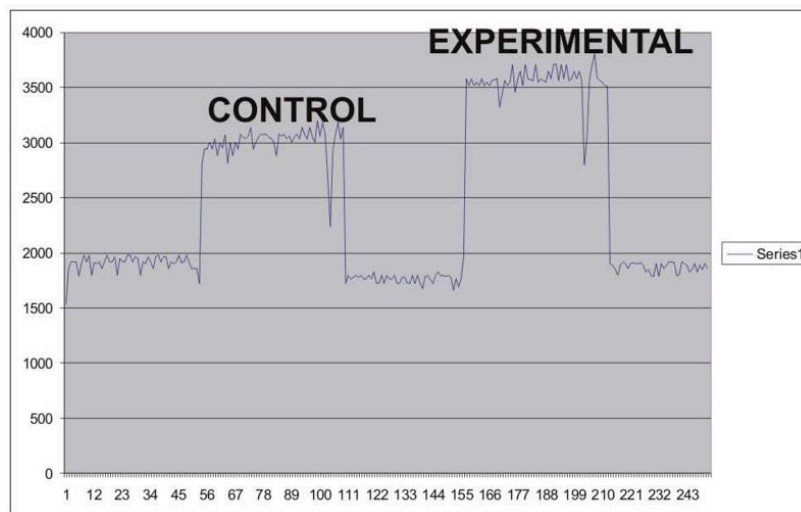


Figure 2: Preliminary experiments show that microbes at a concentration of 10^5 cells/ml have a significantly larger signal than the control

Field Experiments:

The generation 1 model bulk fluorometer has been tested on coral reef waters from the Main Hawaiian Islands, the Caribbean, and the Phoenix Islands.

Table 1: Information regarding the field testing

Field Testing Location	Date / Field biologist	Sites Measured with Fluorometer
Main Hawaiian Islands, NOAA RAMP cruise	Tracey McDole	six
Caribbean, Puerto San Garo, Brazil	Mark Hatay	eight
Phoenix Islands	Stuart Sandin	eleven

Limitations: Though the device has shown to be convenient, robust, and consistent, we are not obtaining the sensitivity that is necessary for detecting moderate changes in microbial activity on coral reefs. One problem that we have observed in the first generation model is a drift in light intensity over time. The photo-multiplier tube (PMT) is extremely sensitive and as a result, it is influenced by the illumination of the light source and producing unreliable readings.

Next steps: We have designed a second generation model that uses an avalanche photo detector (APD) instead of a photomultiplier tube (PMT). APDs are theoretically much more robust than PMTs and should help eliminate some of the drift that has been observed with generation 1 model. The second generation model needs to be lab and field tested.

Science Impacts: Important products from these proposed activities will be the development of methods to detect changes in the microbial community by non-microbiologists. These methods will be incorporated into a Personal Data Assistant (PDA) that includes the appropriate sensors and organizes the collection of the data.

The development of such tools will enable resource managers and technicians to incorporate the monitoring of microbes as they analyze the ecological condition of their respective marine management areas (MMAs) and the effectiveness of their management activities. The use of microbial profiles as one of the three components of a composite health index for tropical nearshore marine habitats, in combination with macro-benthic and fish community censusing, will be an important new advance in ecological monitoring and may provide early warning signs of the potential for a disease outbreak and /or the degree of threat poised by some new anthropogenic activity.

Conservation Impacts: Continued investigations in the field of microbial communities on coral reefs will produce information that further clarifies the role microbes play in coral reef community health – both inside and outside of MMAs. Key to accomplishing this will be improved characterization of the microbial community around coral reefs, determining how this community changes when faced with human-induced impacts, and identifying what stress is imposed on coral reefs by increases and changes in the microbial community. A better understanding of the role of microbes will encourage more research in this topic leading to efforts in how to minimize disease threats posed by them to coral reefs, hopefully in advance of disease outbreaks. Newly acquired information, techniques and tools will make it more practical for technical and management staff of MMAs to monitor the state of microbial communities and coral reef health.

1) The level of success you had in meeting the original hypotheses and 2) The level of success in producing the stated deliverables.

The purpose of the research was to design and build a device that was small, portable, easy to use, and could detect differences in microbial abundances in aquatic ecosystems. The monitoring device was built and tested successfully. A lab view interface designed to run the device was also completed. When tested in the lab, the monitoring device measured clear differences between incremented microbial abundances.

During the field testing, it was observed that the photo-multiplier tube (PMT) was subject to drift over time, resulting in unreliable measurements at later time points. We designed and purchased the supplies to build a second generation monitoring device which uses a photo-avalanche detector instead of a PMT. The second generation device has yet to be tested.

3) Why the original plan of working with Fairoz did not work out?

At the time we submitted the proposal M.F.M. Fairoz was in a good position to test the monitoring device in Sri Lanka. Fairoz had been monitoring reefs in Sri Lanka influenced by different stressors by enumerating microbial abundances directly and by determining the reef water chemistry. During the preliminary stages of the research, Fairoz continued to monitor the Sri Lankan reefs by counting manually. Fortunately for Fairoz, he was accepted to a Ph.D. program in Japan. However, he was no longer in Sri Lanka when the instrument was ready to be tested. The monitoring device was tested on three field expeditions as described in the final report.

4) The plans for testing and future use of the version #2 microbe monitor (and what is still needed to make it a successful tool and one that can be distributed for use in a handful of field sites).

Currently, we do not have immediate plans to test the second generation device because of the lack of funding for this project. When opportunities arise, we will bring the instrument on field expeditions. As there is still no commercially viable solution, we would like to continue our efforts in building a real time monitoring device for counting microbes.