I. Report Title: Characterization of human pathogens and fecal bacterial load on Maui

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II. Abstract

We are proceeding essentially as outlined in the proposal with regard to the water sampling, water chemistry testing, Enterolert culture tests and PCR-based screening of target microbes. Many of the initially proposed targets are being dropped (as expected) from the analyses, but we have now confirmed that we are able to detect a range of both general and human-specific indicators of fecal contamination in tropical marine waters. In itself, this is an accomplishment and will result in publication of the protocols for future studies. In general, the bacterial load detected using the EPA standard Enterolert assay in the nearshore waters of Maui is quite low, with only 10 of the sites exceeding background detection limits. In contrast, the PCR-based detection approach reveals up to 77% of sites are positive for general Bacteriodales, which is considered a general fecal indicator bacterium. Despite that, only 4 of the 148 samples tested to date show positive for the presence of human-specific indicators, and these are being re-run and sequenced to confirm that they are indeed from a human source. In addition, we will be field-testing a newly proposed EPA standard protocol for realtime PCR quantification of general Bacteriodales and Enterococcus spp. in order to compare the results with that of the Enterolert assay. Paired water quality samples for each microbial contamination sample have been sent for processing, and the data will be compared with the fecal contamination results as the study progresses.

III. Executive Summary

a) Water sampling, water chemistry testing, Enterolert culture tests and PCR-based screening of target microbes is proceeding as planned. We have now confirmed that we are able to detect a range of general and human-specific fecal contamination targets.

b) Technique optimization is still underway for human pathogenic bacteria such as Clostridium, but the fecal contamination protocol is now relatively well established and data collection is completed for our first full sampling event (March 8-12, 2010) across the four sites: Honolua, Kahekili, Kihei and Ahii Kinau.

c) Overall detection of Enterococcus spp. bacteria using the EPA standard Enterolert assay in the nearshore waters of Maui is quite low, with only 10 of the sites exceeding background detection limits of 10cfu/100ml. These ten samples are split among Kihei (2/10), Kahekili (4/10), and Ahii Kinau (4/10).
d) Detection of general fecal bacterial contamination from filtered water samples using PCR screening is variable among sites, but found in up to 77% of the water samples (Kihei). In contrast, human-specific indicators of fecal contamination have been detected in only 4 of the 148 samples processed to date (2 hits on Human Polyoma Virus and 2 hits on *Methanobrevibacter smithii*). These samples are now being re-run and sequenced to confirm that they are indeed from a human source.

IV. Purpose

The overarching goal of this project is to determine if freshwater seeping onto the reefs of Kahekili and Kihei, Maui is a source of human fecal bacteria and/or human pathogens. Specifically, we are working to elucidate and evaluate the spatio-temporal abundance pattern of selected human pathogens and human and non-human fecal-associated bacterial species in coastal waters on Maui. Additionally, we seek to evaluate correlations between salinity, turbidity, water quality, algal cover, bacterial concentrations, and proximity to pore water seeps in these areas relative to control sites in which there are dense human populations but no known seeps, and freshwater seeps without human population (Honolua and Ahii Kinau). Together these data will allow us to infer whether waste-water influx, via reef pore water seeps, and associated nutrients and/or pathogens may be affecting the coastal reef biota, and whether there is a correlation between pathogen concentration and disease incidence amongst swimmers and other utilizers of coastal waters on Maui.

V. Approach

We are just beginning our regular monthly water sampling surveys (modified slightly depending on weather) at Kahekili, Kihei, Honolua and Ahihi Kinau. After several months of protocol development and ground-truthing of the techniques, a test run of sampling was performed March 1-3, and the first complete sampling event took place March 8-12, 2010. We successfully extracted DNA from all samples collected as demonstrated by our ability to amplify bacterial 16S RNA gene from all 148 samples, without any amplification in the negative control tests. Through consultation with microbial ecologist Jody Harwood, we chose the Bacteriodales as a primary indicator for non-human (general) fecal bacterial contamination. Roughly 70% of the samples (97/138) proved to be positive for Bacteriodales. Interestingly, Kihei showed slightly higher bacterial contamination load than Kahekili (77% vs 60%) in this first sampling event. Negatives were tested for PCR inhibition with control DNA spikes (extracted sewage at $10^{-4}$ dilution), and all amplified the expected product (i.e., the results showed that all negatives were true negatives and not the result of an error or PCR artifact).

We also tested for the presence of human fecal microbes in the water samples with three human specific primer sets. Human specific *Bacteriodes* PCR with H183F and Bac708R primers turned out negative for all samples. Only two samples were positive for the human polyoma virus (HPyV, Fig. 1) and two other samples were positive for the archaeabacterium *Methanobrevibacter smithii*. Clearly, these results are preliminary,
and these samples are now being re-run and sequenced to confirm the finding. Protocol development and ground-truthing experiments are still underway to detect *Clostridium coccoides*, *Clostridium perfringens* and *Enterococcus faecalis*.

Finally, we are just about to begin testing of a newly proposed EPA standard qPCR assay for general Bacteriodales and *Enterococcus* spp. in order to compare the results with that of the standard Enterolert assay.

VI. **Findings**

Results thus far are based on a single sampling event of each site, and are therefore preliminary and to be treated with caution, but show promise to meet the goals and objectives we set out to address. Overall detection of *Enterococcus* spp. bacteria using the EPA standard Enterolert assay in the nearshore waters of Maui is quite low, with only 10 of the sites exceeding background detection limits of 10cfu/100ml. These ten samples are split among Kihei (2/10), Kahekili (4/10), and Ahii Kinau (4/10). In comparison, detection of general fecal bacterial contamination using the filtered water and PCR screening is variable among sites, and found in up to 77% of the water samples tested in this first sampling period. It is important to note that the presence of general Bacteriodales in the samples is not unexpected in near-shore waters, since most animals carry high levels of that group; this is the exact reason it was selected as a general indicator of fecal bacteria in the environment. Although non-human sources of fecal contamination are relatively ubiquitous (as expected) human-specific indicators of fecal contamination have been detected in only 4 of the 148 samples processed to date (2 hits on Human Polyoma Virus and 2 hits on *Methanobrevibacter smithii*). These samples are now being re-run and sequenced to confirm that they are indeed from a human source.

VII. **Evaluation**

Progress to date is as expected. We are proceeding essentially as outlined in the proposal with regard to the water sampling, water chemistry testing, Enterolert culture tests and PCR-based screening of target microbes. Some protocol development is still underway, and many of the initially proposed targets have been dropped from the analysis because they are either not found in the environment or because technical limitations prevent unambiguous interpretation of the findings. Regardless, we have now confirmed that we are able to detect a range of general and human-specific fecal contamination targets, and are working on the ground-truthing of the human pathogenic bacteria to complete the microbial target protocols. The water samples have been sent to the lab and the results of the water quality testing are starting to come in. The timeline to complete this ambitious project was always tight, but we expect to complete the project on time.

VIII. **Recommendations to managers**

Will be provided upon completion of this study

IX. **Dissemination of Project results:**

Thus far, results have been presented at the HCRI quarterly meetings, and Robin Knox has been actively involved with disseminating the findings on Maui.