Final Report for the Hawaii Invasive Species Council FY2021

Investigating microbial linkages within holdfast filaments of the invasive leather mudweed, *Avrainvillea lacerata*, in coastal Oahu

Project Start Date 1/1/2021- Project End Date 12/31/2021

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Deliverable 1: Map with *Avrainvillea lacerata* and native species microbial study design delimited

*Figure 1: Six square plots of 10*10 m^2 were delineated in collaboration with Mālama Maunalua on the relevant reef region (red circle) at Kuli'ou'ou, to test the efficacy of H_2O_2 concentration in various assemblages unique as compared to the previous funded project at Paikō. Site selection was limited as we were limited in the number of people that could participate due to COVID-19. We kept the field team to a reduced number of students to maintain physical distancing of at least six ft apart.*

Deliverable 2: # plots (100 m^2 areas each) treated for *Avrainvillea lacerata*

The delineation of six plots was made in collaboration with Mālama Maunalua, who had approximate estimates of average percent cover of *Avrainvillea* plants over time throughout the Kuliʻouʻou Reef region on the east side of Maunalua Bay. The site we chose to work in was considerably more distant from shore than the previous HISC funded site in 2020 at Paikō, which was about 300 meters from the shoreline at Kuliʻouʻou Beach Park. The substrate was similar to the substrate at Paikō with a mix of rocky and sandy substrate, albeit more sand was found at the Kuliʻouʻou site. Additionally, there was higher percentage of cover of benthic native algal taxa at this site, which made it a good candidate for this year's study as we could see if the treatments could be used to help in restoration sites with abundant native flora.

Twenty plants in each plot (80 plants total) were chosen out of a total area of 400 m^2. Individual plants were chosen at random points along a transect within the plots for the purposes of the scientific experiment. 30mL of 3% and 10% H_2O_2 were administered to ten plants in each plot to test the efficacy of the treatment on individual plants. The plants that were selected at random were first identified to species using the Hawaiian Reef Plants field guide and were marked with a small and diminutive marking system (Huisman et al., 2007). A small PVC ring was labeled and attached to the plants using a zip tie and a lead weight was added for stability. The lead weights were attached to the zip tie with fishing line to prevent the marks from falling off due to tidal influxes or increased wave action.

The treatments were considerably less effective in this site as compared to the Paikō reef region, with no significant difference being found between the treatment and control. We think there are many reasons why this might have happened in this site. For instance, there is considerably higher water flow in Kuliʻouʻou as the site is in close proximity to the channel for boat traffic. This water flow causes a greater dilution of the treatment over time than in lower motion water flow, causing the time to reach reaction equilibrium to decrease. Additionally, there may have been error involved in the specific Diving Pulse Amplitude Modulated Fluorometer unit we were using in 2021 as we sent our two other operational units that we used in 2020 for factory repair, calibration, and pressurization testing for the housing to insure use at depth.

Although there were errors in our measurements of photosynthesis, we observed discoloration in the individuals that were treated with both concentrations of hydrogen peroxide, in congruence to our observations at Paikō. The surrounding macroscopic biota was observed to be unaffected by the targeted treatments to *Avrainvillea lacerata* in this site. No distinct mortality was observed as the individuals were highly embedded in the substrate and benthic flora and as such were more cryptic and harder to make distinct observations on.

Deliverable 3: # plots (100 m^2 areas each) with seagrass and native algae transplants

Three plots were identified as having above typical abundances of seagrass blade density and were southwest of the treatment plots. These plots were identified for ideal locations for seagrass propagule source and reciprocal transplantation. However, we were limited to the amount and expertise of volunteers in this year of experimentation due to COVID-19. As there were limitations in our field team capabilities to undergo a proper transplantation effort, we decided to take an alternative approach to assessing microbial inhibition in the field setting using molecular tools. We submitted biological samples from our original field site for microbial sequencing analysis at Mānoa‘s new Microbial Genomics and Analytical Lab (MGAL; https://icemhh.pbrc.hawaii.edu/mgal), to better understand the internal microbial community. This is important to study as there may be inhibitory effects from these microbes that may prevent species turnover in the sediment where these invasive species grow.

Ten samples of *Avrainvillea lacerata* were collected from the west end of the Paikō reef flat. Because we were interested in interactions with the substrate, we separated the holdfast (roots) from the blade and stipes (leaves) of the individuals for a paired complementary microbial analysis. We hypothesized that due to substrate dynamics (i.e. influence by the rhizosphere/phycosphere) that there would be different microbial assemblages between the bottom half and top half of the organism. The blades and stipes were separated as one unit as they were both growing above the substrate in the water column, potentially allowing for a different assemblage to inhabit the interwoven, sponge-like thallus. Fungal isolation methods and media for standard marine samples were used for culture.

The summary of the microbial analysis was that there was a considerable difference (at least three fungal species) that were found in the blades and stipes of the organism that were not found in the holdfast. However, there were other fungal species in the holdfast that were not able to be amplified or identified and may represent a new taxon. Seeing that this was the first attempt at trying to describe the microbial community in this organism, and the likelihood of novel and undescribed species level identifications to be found, a more specialized approach may be needed to better refine the differences the differences between the tissue types that were analyzed. A few of the genera that were able to be cultured and amplified in the blades and stipes and not the holdfast of the organisms was *Fusarium* and *Penicillium.*

Deliverable 4: # of control plots (100 m^2 areas each)

Two of the six plots (200 m^2) were set aside for control measurements at Kuli‘ou‘ou and were separated from treatment plots to test if there were plot wide effects from selected treatment plots. The results from these control plots were comparable to previous results from controls that were measured at the Paikō reef region the year before. It was possible that the use of the Diving Pulse with red pulsed light might have been measuring different tissues than compared to the one with blue light used the year before. For instance, the red light could have had a higher affinity to measuring more of the internal photosynthetic symbionts such as cyanobacteria than does the one with blue light. However, our results show that the controlled organisms were comparable regardless of the type of light source used.

Deliverable 5: Results and recommendations

Although the results from the photosynthesis measurements showed to insignificant, the results from this project based on observations are informative in that there could be additional treatments developed for higher water motion areas. From the work at Paikō we know that oxidative damage can and will impact the growth and the alga if applied for long enough duration and intensity before the reaction reaches equilibrium. If an oxidative agent using the hydrogen peroxide as an active ingredient could be developed into a substance that can be held in place on the organism, then a treatment could be developed for areas with higher water and could additionally help prevent the dissolution of the treatment into non target areas via water motion.

Further chemistry is required to better adhere the aqueous solution to targeted phospholipid bilayers. The use of surfactants is common practice in the application of biocides to many organisms. However, surfactants may not provide the stickiness that is required to create a targeted application in the aqueous environment of highly abundant reef systems. Although surfactants may not be miscible in water, they do not prevent the movement and emulsification within the water column. Further research is required in the development of a caustic paste or semisolid that can be applied to targeted organisms in areas with consistent water flow and wave motion to prevent the dissolution of treatment. Containment systems may be another option.

Additional research could also investigate ways to catalyze the reaction more efficiently. In areas of the ocean with less sunlight available, catalyzers may be needed to allow the aqueous solution of hydrogen peroxide to effectively break down to water and oxygen. Some of these catalysts may include inorganic constituents such as carbonates of sodium (soda ash or baking soda) or compounds including iron. Whether new chemical formulations are created, the careful study, application of treatments, and monitoring of reef communities will be necessary in continuing to control the widespread nature of this and other marine invasive algal species.

Deliverable 6: At least one peer-reviewed publication

A publication is being proposed for the algal-fungal associations that were discovered in the process of this project with the development of more specialized approaches, however the journal has yet to be determined for this work. Further work to substantiate this publication will be continued to sample the sediment itself including both the surrounding sand and mud that the target organism is found to be growing in. These substrates and tissue types are to resampled to further substantiate the results from the microbial analysis and will be slated for publication if the results are found to be impactful. From this work, there is potential to describe novel species that have not yet been described to science.

Literature Cited

Huisman, J. M., I. A. Abbott, and C. M. Smith. 2007. Hawaiian reef plants. *University of Hawaii Sea Grant College Program.*