USDI-FWS Award F13AC00200 Final Report

The ecology of Rhodactis howesii at Palmyra Atoll: determining mechanisms of invasion and patterns of succession

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Motivation: Invasive species represent a persistent form of biotic pollution across multiple aquatic and terrestrial ecosystems. These invaders put pressure on native species and can cause local or regional extinctions as native species are outcompeted for valuable resources. Invasive species are often introduced to an ecosystem through anthropogenic vectors (transfer mechanisms); including but not limited to ship-ballast water, agricultural trade, and in some instances, deliberate introduction. Although many invasions are the result of the introduction of a non-native species, there are cases in which disturbances in an ecosystem may cause a species that was native but present in low abundance to proliferate and spread in a manner similar to an invasive non-native species. The mechanism of the invasion of the corallimorph *Rhodactis howesii* at Palmyra Atoll has been a matter of debate since the species was first observed in the 1990s. The goals of our research were to examine possible mechanisms that may influence the invasion of the corallimorph across the atoll as well as to monitor spread, and investigate potential control options. Specifically, we examined whether there was evidence to suggest that iron released from the *Hui Feng* Longliner shipwreck might have provided a limiting nutrient that allowed the corallimorph to bloom. Further, we established a number of permanent monitoring plots across the atoll to monitor change in abundance over time. In addition, we conducted *in situ* dose response experiments to identify the potential of a variety of chemicals to kill the corallimorph in small plots. Finally, using the most effective treatments identified in the dose response experiments we conducted a large restoration experiment to determine the potential for corallimorph control and habitat restoration at sites with the highest density of corallimorph on Palmyra Atoll, adjacent to the former Hui Feng wreck site.

1. Lab Experiment/Iron Enrichment:

Thierry Work and his colleagues proposed the hypothesis that iron leaching from the Hui Feng Longliner wreck on the Western terrace of Palmyra Atoll may be providing a limiting nutrient to the corallimorph that was the catalyst for invasion. To test this hypothesis, we collected corallimorph samples from the reef terrace site Penguin Spit Inner (5m depth) and conducted an enrichment experiment at the field station on Palmyra. Corallimorph samples were placed in a

water table with airstones and fresh seawater collected from the western terrace and allowed to acclimate for two days. A piece of the shipwreck was also collected and sliced into 12 equally sized pieces (approximately $3cm^2$ each). After acclimation, the corallimorph samples were placed into 1-liter growth chambers, 12 contained 1 piece of shipwreck metal and 12 were used as controls. The growth chambers were placed into a water table with circulating seawater to regulate temperature. Each chamber was provided with airstones to prevent hypoxia and to allow for "flow" within the chambers. Airstones were kept consistent in specific chambers throughout the experiment to prevent any cross contamination of metals. The seawater used in the experiments was collected just outside the channel on the Western terrace. 50% of the water was changed every other day throughout the 2-week experiment. *Rhodactis* samples were buoyant weighed and photographed at the beginning and end of the experiment. All specimens were frozen and taken back to Scripps Institution of Oceanography for trace metal analysis after the experiment was complete. We were specifically interested in examining if something leaching from the metal contained in the shipwreck was enhancing corallimorph growth or overall health.

Enrichment Results: Throughout the course of the two-week experiment 5 of the 12 treated corallimorph samples dissolved within their growth chambers resulting in 100% loss of tissue. In comparison, the control corallimorphs experienced no loss in tissue, although they didn't show significant growth over the two-week period. Statistically, the corallimorph samples exposed to the shipwreck metal demonstrated a significant *loss* in tissue when compared to the controls (T-test: p-value = 0.0012*). These results suggest that the wreck metal contains something potentially toxic to the corallimorph (at least in the concentrations that our samples were exposed to in our experiment). Mean trace metal concentrations of *Rhodactis* tissue showed high levels of iron in both the control and metal-treated corallimorphs, although the wreck treatment samples were slightly elevated (Figure 1).

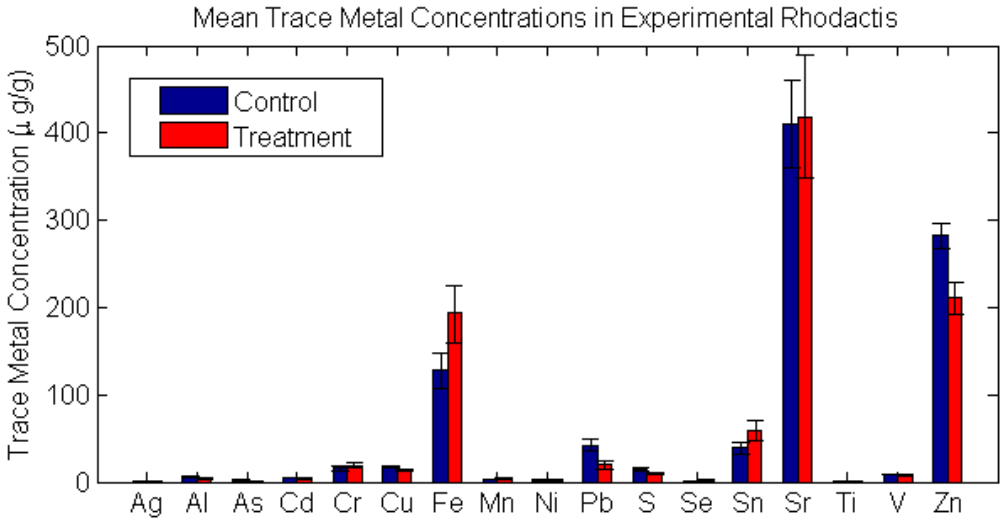

Figure 1: Mean trace metal tissue concentrations from control and metal-treated *Rhodactis* following a laboratory exposure experiment. Bars represent 1 standard error.

These results were somewhat surprising but still provide us with interesting data regarding the impact of the shipwreck on benthic organisms on the reef. Tin (Sn) was also seen in the corallimorph tissue and may suggest the presence of Tributyltin on the wreck, a chemical commonly found in antifouling paints that has been made illegal due to its extreme toxicity to animals. Given the wreck's age, it is quite possible that the paint contained Tributyltin which has been slowly leaching into the water and negatively impacting benthic organisms at the Longliner wreck site on the reef terrace. Because of the lack of control on metal concentrations from our experiment (due to using actual pieces of the wreck rather than stock solutions) we are not able to conclusively determine the influence of Iron specifically on corallimorph growth and/or abundance.

2. Change in Abundance over Time

A) Long Term Monitoring Data across the Atoll 2009-2015

Our team established a series of permanent monitoring plots on Palmyra Atoll in 2009 including 4 forereef sites (10 m depth), 4 reef terrace sites (5 m depth) and 1 backreef site (5 m depth; established in 2010). At each site one 50 m transect line was established along the depth contour and 10 0.63 m^2 plots were marked with stainless steel eyebolts in 2 opposing corners. Every time our team was present on Palmyra we photographed these plots to determine percent cover of all benthic organisms. While this long-term monitoring study was not initially established to track corallimorph invasion it has been very helpful in doing so.

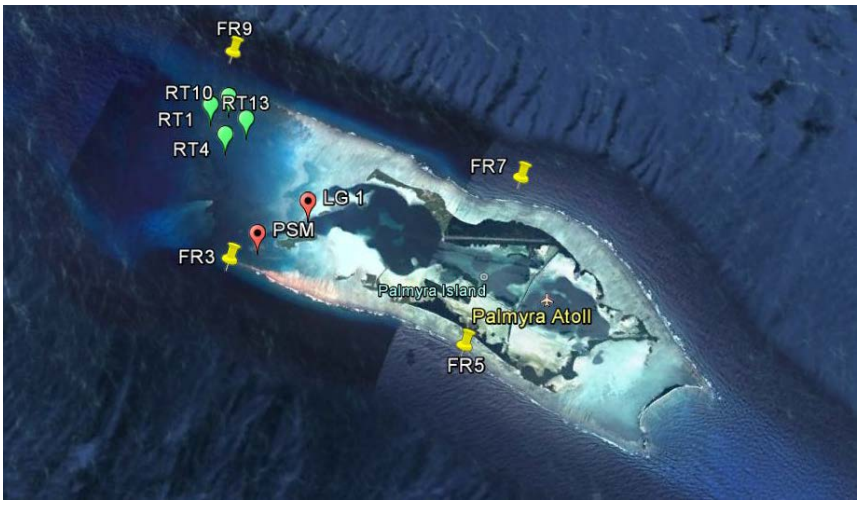

Figure 2. Map of Palmyra Atoll showing the location of permanent monitoring locations. All aside from LG1 have been monitored from 2009-2015.

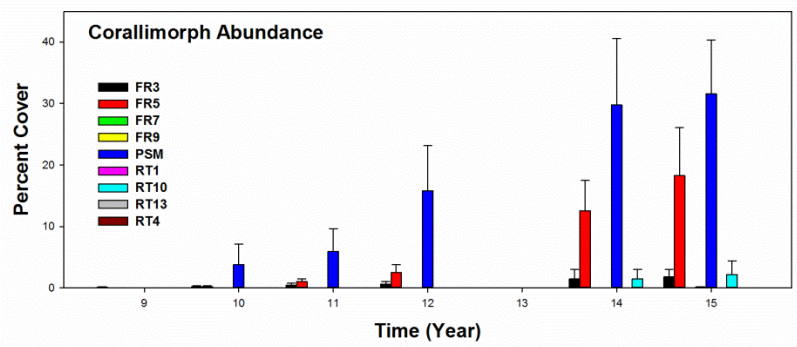

Figure 3. Average corallimorph cover at the site level across all monitoring plots on Palmyra atoll from 2009-2015 (no data for 2013). Data are means (n=10) +/- 1 SE for each site.

Results: In 2009, none of 90 permanent plots (9 sites, 10 plots) had corallimorph present. However, now the corallimorph is present in varying numbers (18 total, 20% of plots) of plots at 4 out of the 9 sites (2 forereef, 1 terrace and 1 backreef site). In some of the long-term monitoring plots the corallimorph has reached 100% cover and has remained so for more than 1 year. In particular Penguin Spit Middle has been overrun with corallimorph where it now occurs in all 10 plots and continues to increase in percent cover over time. In addition to our plots, the corallimorph has spread extensively around the mooring at PSM and has moved broadly through the site over the last 5 years. Other locations where it is particularly abundant include FR5 on the south shore forereef where it is now present in several plots. While it is present at both FR3 (3 plots) and RT10 (1 plot) it is not highly abundant and is as of yet only present in a few of the plots. An important side note is that the corallimorph has been observed at nearly all of our sites despite the fact that it is not present in all of our long-term monitoring plots. As the corallimorph becomes more abundant it seems to be replacing habitat that was formerly occupied by various types of benthic organisms including reef building corals and crustose coralline algae as well as filamentous turf algae. It is unclear what the future of these sites will be without management intervention especially at locations such as PSM where the corallimorph is now one of the single most abundant organisms on the reef.

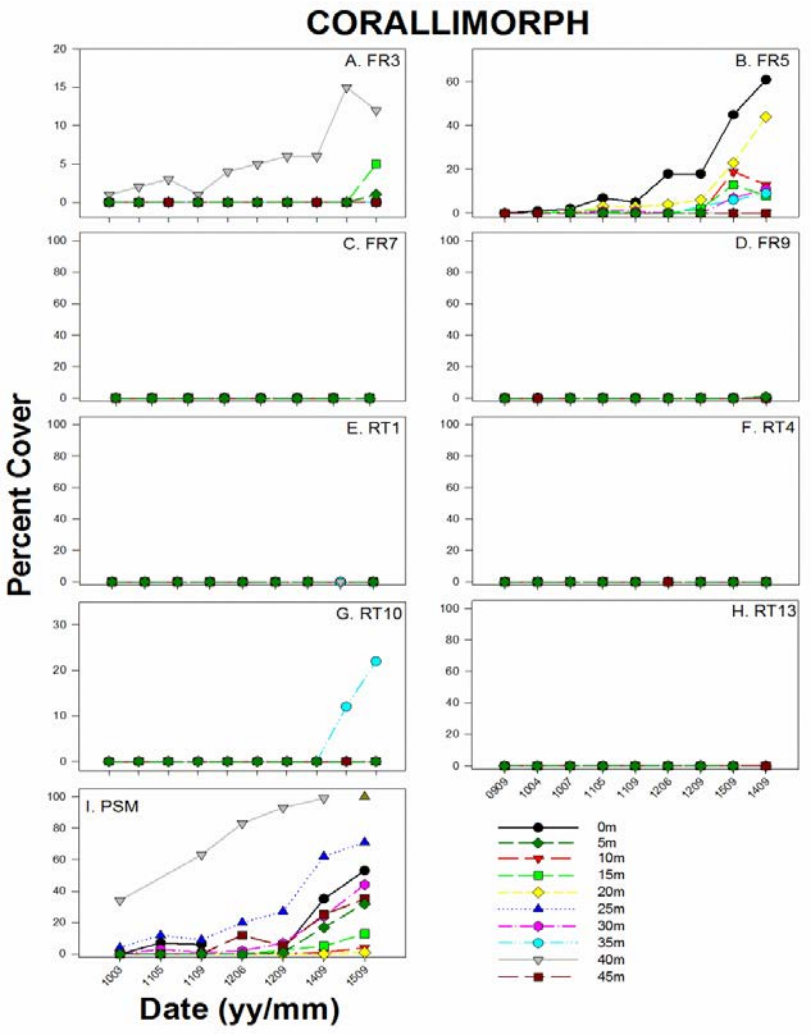

Figure 4. Corallimorph abundance at the individual plot level highlighting invasion rates and abundance within specific sites.

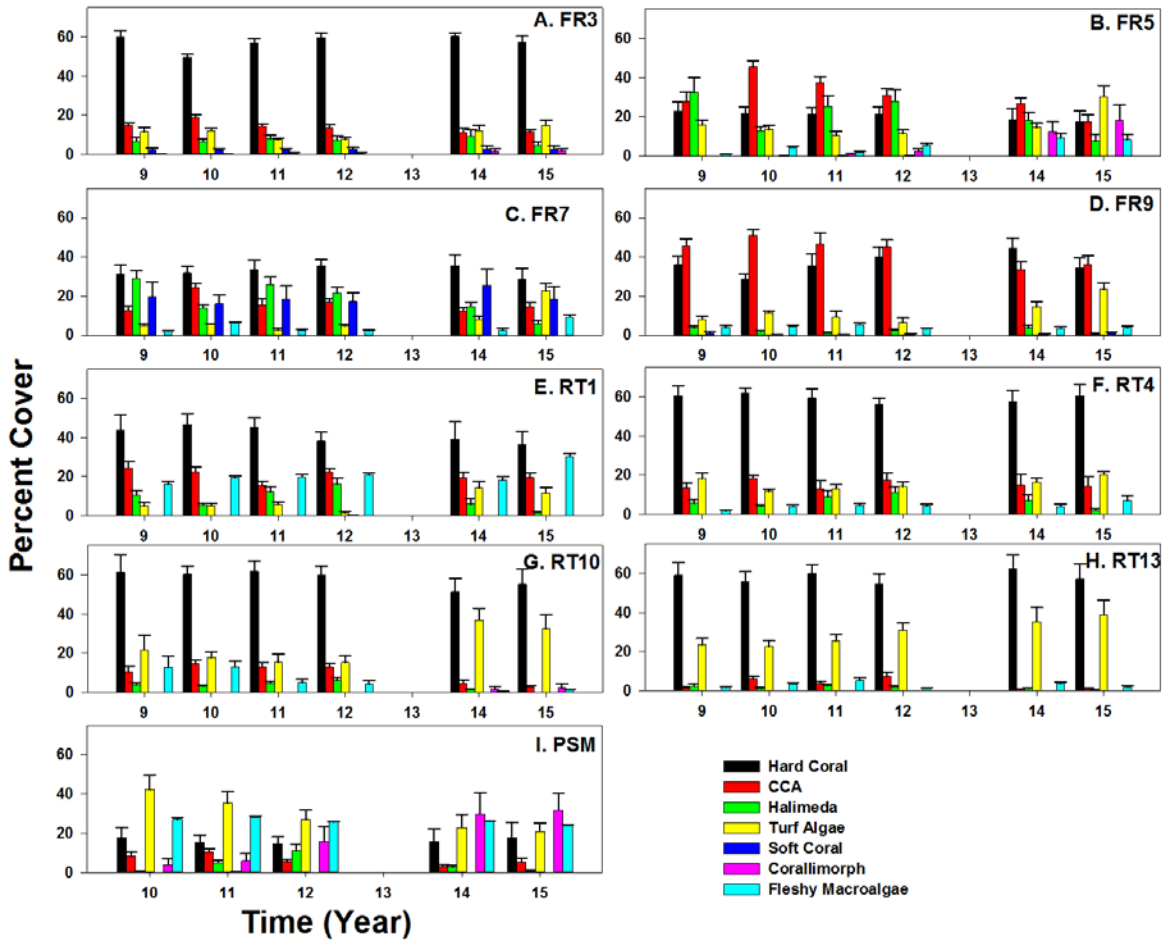

Figure 5. Long term monitoring data from Palmyra Atoll showing mean percent cover (n-10 quadrats) of dominant benthic functional groups +/- 1 SE between 2009-2015 (no data for 2013). Corallimorph is shown in pink.

B) Change over Time at the Longliner Wreck Site-New Phototransects Before & After Wreck Removal

Four additional permanent photo-transects were established in 2013 to compliment the other long-term monitoring data that our team performs around the atoll. These four transects were established in the cardinal directions (North, South, East, and West) radiating out from the former wreck site with the specific goal of tracking how the corallimorph changed in abundance following wreck removal. All transects were photographed in Sept of 2013, 2014, and 2015 and photoquadrats were used to determine the rate of spread or retraction of the corallimorph and the changes in benthic community composition before and after the wreck was removed. The recent completion of these analyses shows a **clear and consistent decrease in the corallimorph cover**

at the Longliner site in all 4 cardinal directions (Figure 4). Initially between 2013-2014, it still appeared to be increasing in cover in the northerly direction but by 2014-2015 all transects showed a general decrease in abundance. These data are particularly exciting since the Longliner wreck was removed in 2014. These data suggest that either the corallimorph reached its population density carrying capacity and has since began declining in abundance at these sites or perhaps the physical removal of the wreck itself has had a positive influence on habitat restoration at this particular location. It is important to note that the corallimorph was particularly abundant at this site (more so than any other site on the atoll).

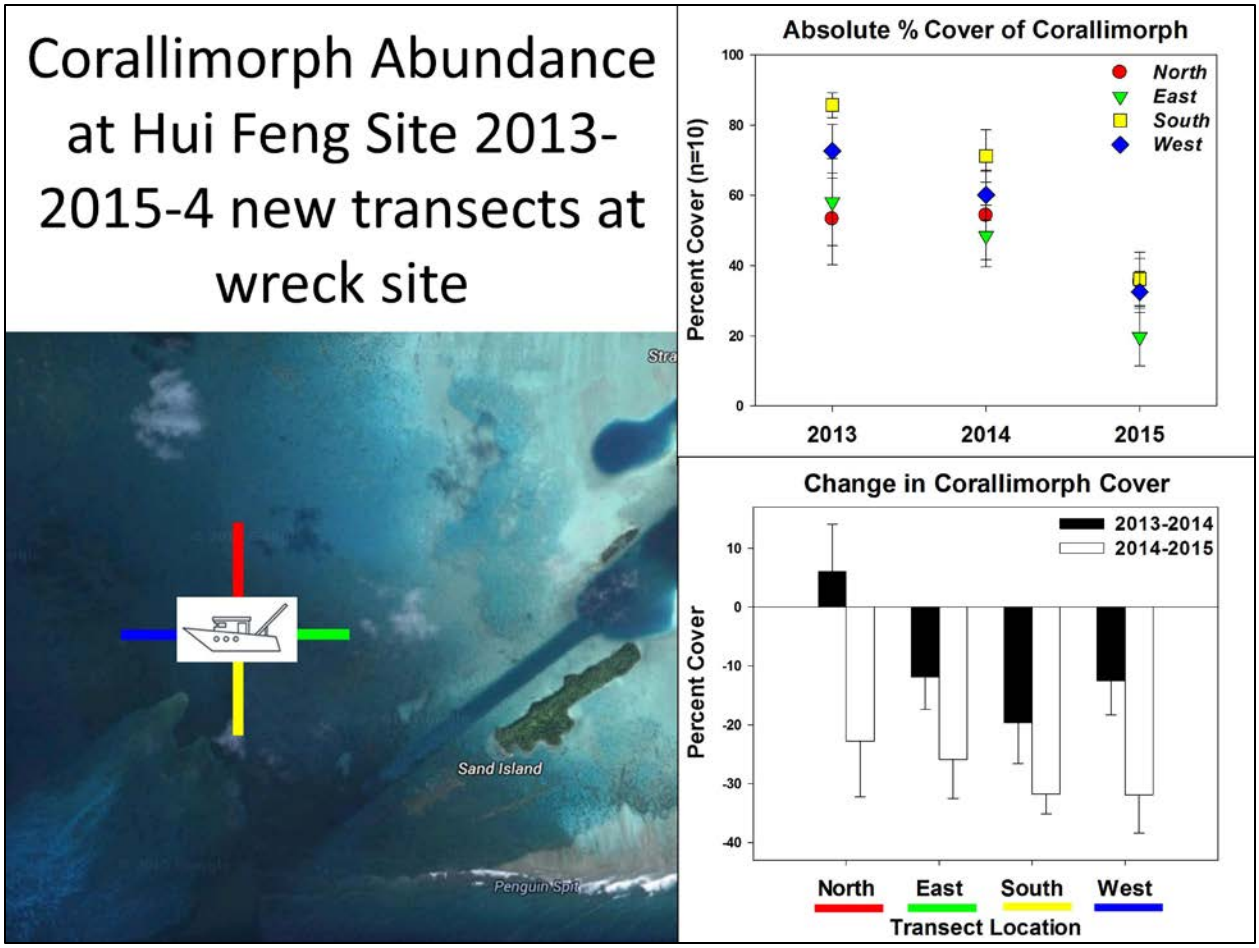

Figure 6. Location of the 4 cardinal transects radiating out from Longliner wreck installed in August of 2013. The plot in the upper right shows absolute corallimorph cover over time while the plot in the lower right shows the change in corallimorph cover between 2013-2014, and 2014-2015. Over the last year, all plots have shown a decline in corallimorph cover following wreck removal.

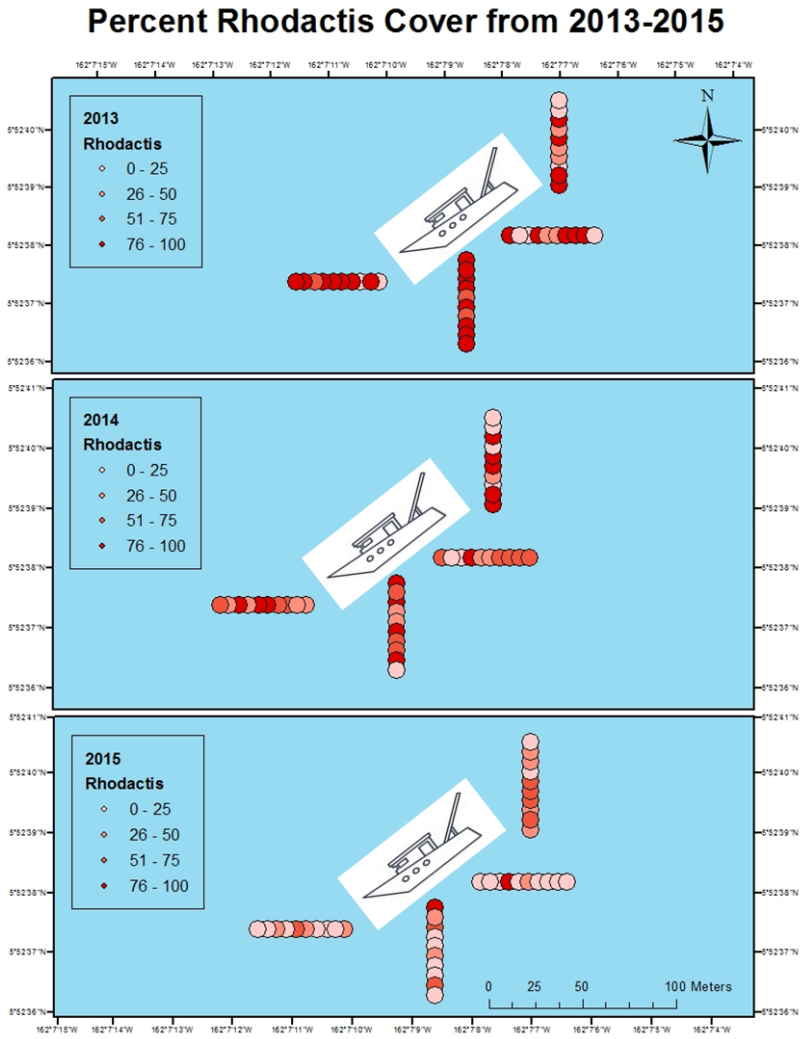

Figure 7. Graph showing the absolute percent cover of the corallimorph at the Longliner sites along the North, East, South, and West transects from 2013-2015. Data are presented at the individual quadrat level and color indicates progressively greater abundance. These data show that in 2013 the majority of the plots ad >50% cover of corallimorph but that by 2015 most plots has < 50% corallimorph cover. It is not entirely clear what is causing a reduction in population density but the change corresponds with the removal of the wreck from this site.

C. Change over Time at the Longliner Wreck Site: 200 m^2 Photomosaic Plots at the Longliner

In 2013 a 10 x 20 m (200 m2) photomosaic plot was established at the Longliner site where 1000s of pictures were taken of the reef benthos in consecutive sequence. Images were later stitched together to make one large photomosaic. In the laboratory, the image was digitized by outlining all of the areas that was occupied by corallimorph. These photomosaics were collected each summer between 2012-2014 and the variation in corallimorph abundance was determined each year. The data suggest that coralolimorph abundance continued to increase between 2012 and 2013 but then began decreasing between 2013 and 2014 following the removal of the wreck. These patterns can be seen in Figure X where the white area indicates corallimorph while the black area indicates “other” benthic substrates. In 2014 it is clear that some of the dense patches of corallimorph are beginning to break up and become less continuous.

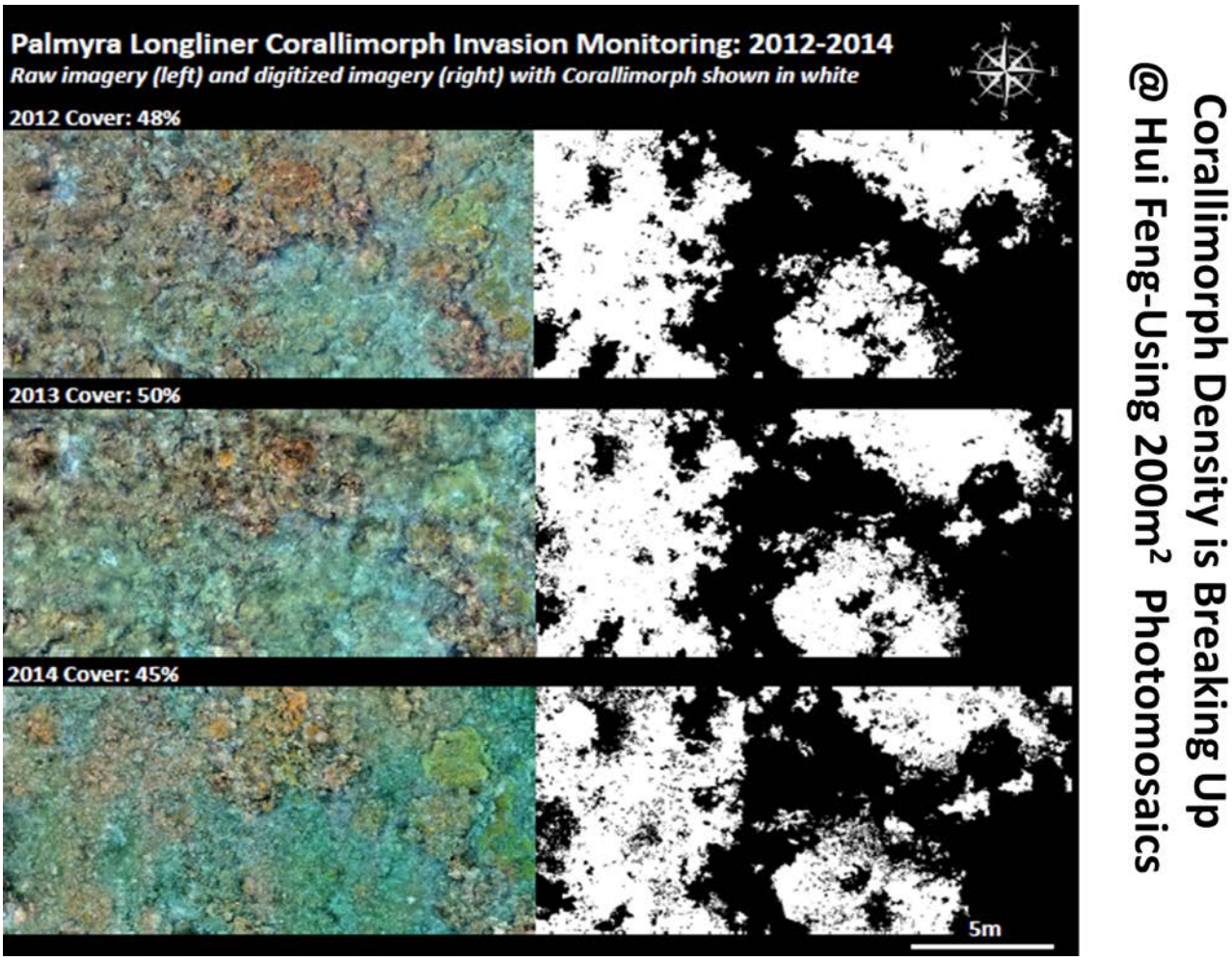

Figure 8. Sequence of the photomosaics at the Longliner site between 2012-2014. The black and white images represent the digitized photomosaics with white representing corallimorph and black representing other benthic substrate types. In the 2014 digitized image it is clear that the large patches of corallimorph are starting to break up and become less dense.

3. Dose Response Experiments to Control Corallimorph *in situ*

Three different chemicals were tested for corallimorph removal efficacy before the larger clearing plots (described below) were established. In order to do this, three transects were laid north of the Longliner wreck site in areas of 100% corallimorph coverage. Eleven 0.25m^2 plots were laid out every 5 meters and marked using small bolts and tags in two corners of the frame. Photographs were taken to provide initial corallimorph coverage. The first plot was left as a control (no tarp, no treatment), while the second plot was established as a "tarp control." Plastic sheeting was laid out over the plot and weighted down with sandbags along the edges to prevent movement of the tarp and minimize water flow. The next 9 plots were "tarped" and treated with

varying concentrations of the associated chemical treatment (granulated Citric Acid, Acetic Acid, or Chlorine). Treatments were left on the plots for 24 hours and then the tarp was removed and the plots were re-photographed. A third time point photograph was also taken 3 days later to examine final corallimorph coverage after exposure and recovery from the treatments.

Visually, citric acid appeared to be the most effective means of removal in the smaller scale dose response experiments. Chlorine was also extremely effective, although it appeared to require a higher concentration than citric acid when the tarps were first removed. Acetic acid had little to no effect on the corallimorphs and the small irritation that was initially apparent after the tarp was removed was gone by the third time point. Interestingly, the tarp control for the vinegar had a negative impact on *R. howesii* demonstrating the importance of water movement and light for their growth and physiology. A larger plot of citric acid was attempted but, unfortunately, the results seen in the dose response experiment did not seem to scale up in size to tlarger plot sizes likely due to rapid rate in which this chemical dissolved in seawater in comparison to chlorine.

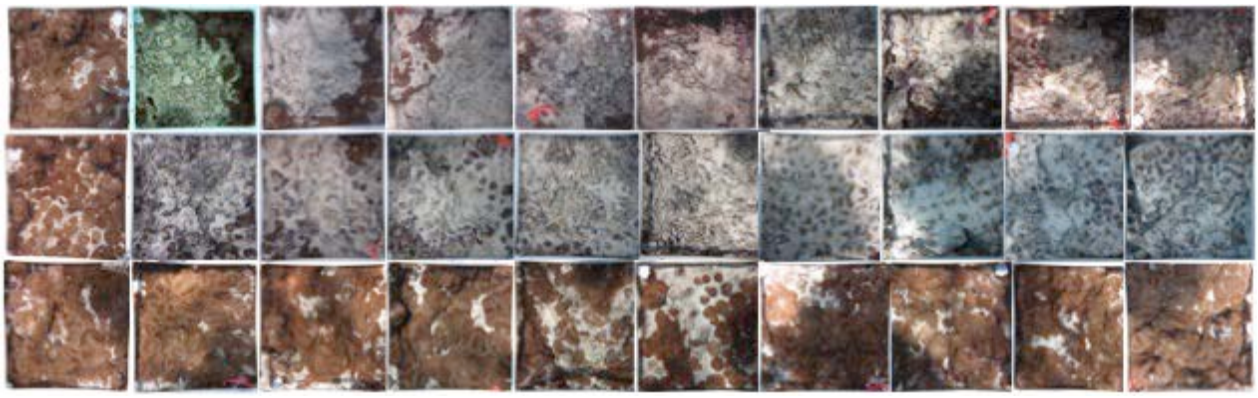

Figure 9. Final time point photoquadrats of the *in situ* dose response experiment in order from top to bottom: Citric Acid, Chlorine, and Acetic Acid with increasing concentration from left to right.

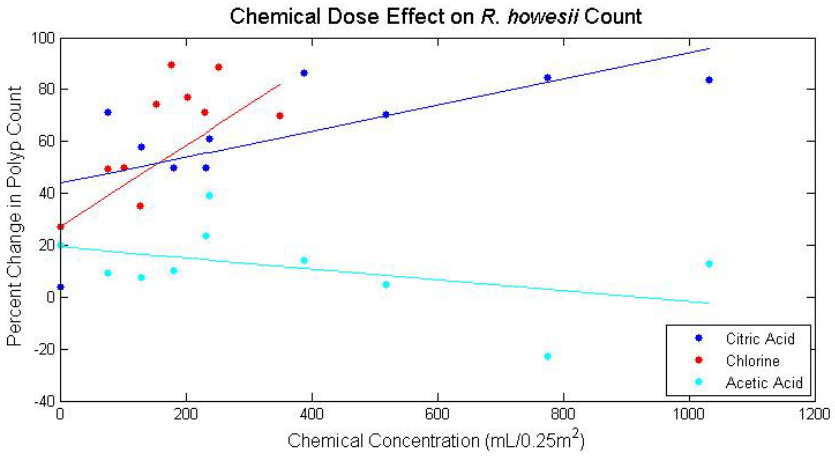

Figure 10. Percent decrease in corallimorph cover following exposure to increasing concentration of each of three chemical treatments. Both citric acid and chlorine showed nearly 100% decrease in polyp density following exposure to high concentrations of the respective treatments.

4. Large-scale Corallimorph Removal and Restoration Experiment

In order to better inform mitigation efforts underway at Palmyra, a series of large clearings were established around the Longliner wreck site to investigate the recovery and succession of the reef after removal of the corallimorph. The Smith lab established fifteen 12x12 ft plots in areas of close to 100% corallimorph coverage around the former Longliner wreck site. 3 replicates of 5 different treatments were installed in the plots as follows:

Treatment 1: No corallimorph removal (Control)

Treatment 2: Corallimorph Removal, no transplant (Removal Control)

Treatment 3: Corallimorph Removal, Random Transplantation (methodology described below)

Treatment 4: Corallimorph Removal, Same Species Aggregations (see below)

Treatment 5: Corallimorph Removal, Species Cross Aggregations (see below)

12 of the large plots were covered and treated with chlorine at a concentration of 6.9 liters of granulated chlorine/plot (3 plots were left as controls). Plots were left covered with tarps and chlorine for 48 hours. Remnant corallimorph was removed by hand so that each plot was completely clear of any *Rhodactis*. Photographs were taken prior to clearing and the following corallimorph removal to provide initial benthic cover data. In 9 of the plots, 3 species of coral were transplanted into the plots (*Pocillopora damicornis*, *Acropora acuminata*, and *Montipora capitata*) as well as crustose coralline algae (CCA, *Lithophyllum* sp.) and calcification accretion units (settlement tiles, CAUs). The random transplant treatment was divided into sixteen squares and each square was randomly assigned a coral species, CAU, or CCA (3 of each per plot, 15 squares total, 1 blank). The Same Species Aggregation

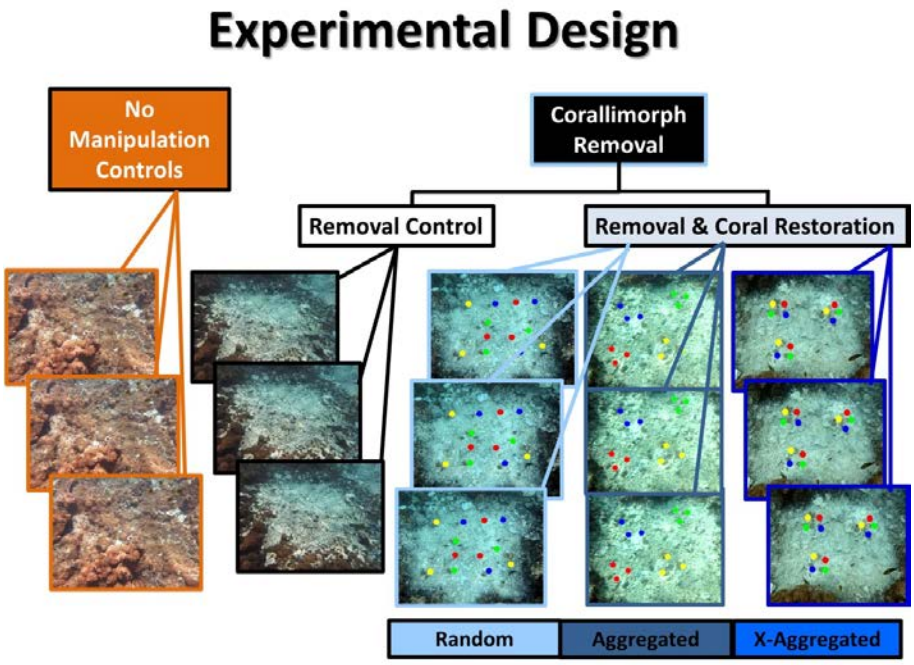

Figure 11. Experimental design for the corallimorph restoration experiment.

plots were divided into quadrats and 3 fragments of each of the 4 species were transplanted into their respective quadrant. The Cross Aggregations followed the same methodology but each quadrant received one of each species. The plots were then photographed again after the transplants were completed.

In 2015 the plots were maintained by removing any corallimorph that had re-invaded the plots and replacement of any loose or damaged coral transplants within the plots. Each plot was then 3D imaged to create a "mosaic" of the plot before and after maintenance that can be used for data analysis. The photo "mosaic" compilation of the plots is currently underway so that we can begin to examine rates of growth, succession, and recruitment in all plots over time. It is our hope that the transplant plots will provide valuable restoration data and assist in determining the most effective way of helping the reef recover to a community dominated by corals and coralline algae.

Although quantitative data is still being generated from the plots, visual observations from the plot showed minimal corallimorph re-invasion and in some, coral recruitment. Corals that were transplanted into the plots had shown considerable growth, although some had bleached and died during the warming event and required replacement. These visual results suggest that the clearings may provide us with a valid form of mitigation to assist with reef recovery after the invasion. If the plots had minimal corallimorph regrowth after a year, despite being in high corallimorph coverage, we can most likely expect even better results when spot clearing smaller satellite populations of *Rhodactis* around the atoll.

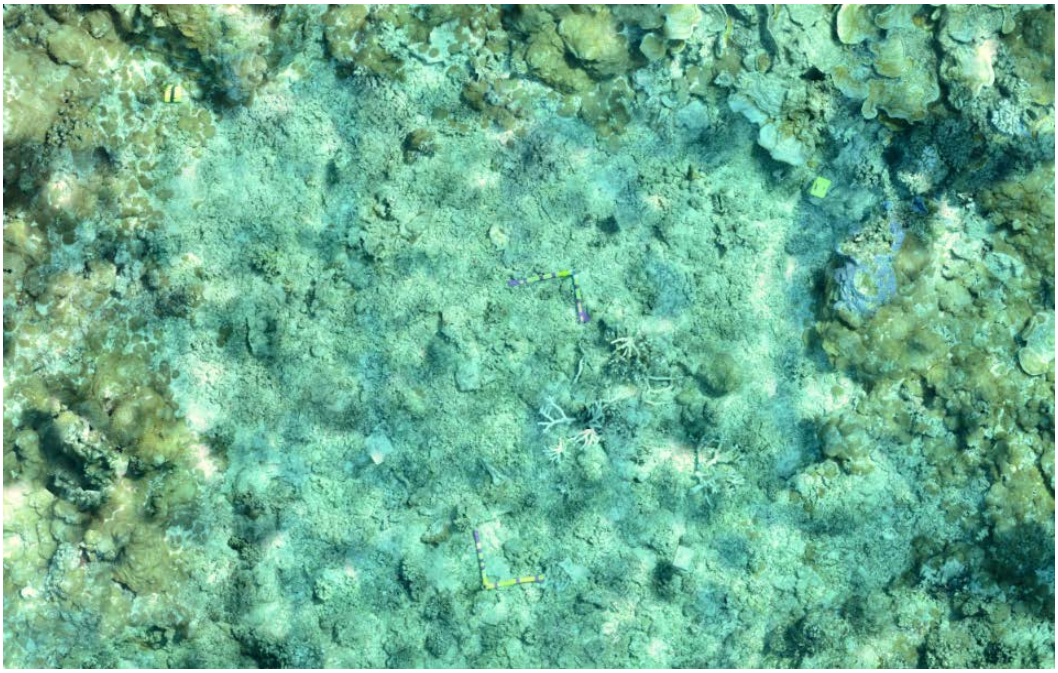

Figure 12. Image mosaic of a cleared corallimorph "mega-plot" one year after the removal and establishment of the plots.

Conclusions: The past three years of work have been made possible by the Jim Maragos Grant from the U.S. Fish and Wildlife Service and have gone a long way towards furthering our understanding of the corallimorph invasion at Palmyra Atoll. Although the enrichment experiment did not provide us with definitive results regarding the impact of the iron from the shipwreck, it did demonstrate the negative effects of the wreck on the reef benthos. However, the data collected this past year is encouraging from a management standpoint. The decrease in the corallimorph cover around the Longliner site suggests that removal of the wreck has been beneficial to the recovery of the reef. Additionally, the minimal re-invasion of the corallimorph that was seen in the plot clearings is encouraging, suggesting that clearing efforts at Palmyra may prove successful and assist in reef rehabilitation. This work has provided us with much needed basic growth rates for the corallimorph along with a potential mitigation and rehabilitation strategy that can be implemented around the atoll. Additional clearing plots, especially at smaller, satellite populations, may help curb the spread of the corallimorph around Palmyra. The recovery of the reef at the Longliner site provides further encouragement that the benthic habitat will begin to recover as the corallimorph dies off and is removed from sites. It is our hope that these data will help better inform the management strategies underway at Palmyra today, and that they will provide managers with some basic tools to help in recovery efforts.

A manuscript describing the spatial and temporal invasion of the corallimorph at Palmyra Atoll is currently in process and will be submitted to a peer-reviewed journal for publication by June of 2016. Details of the paper and publication will be sent to the U.S. Fish and Wildlife Service when available.