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Invasion Mechanisms of the corallimorph, Rhodactis howesii, at Palmyra Atoll /

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Invasion Mechanisms of the corallimorph, Rhodactis howesii, at Palmyra Atoll

A thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

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2014

The Thesis of Amanda Lockett Carter is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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University of California, San Diego

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This thesis, in full, is currently being prepared for submission for publication of the material. Carter AL, Deheyn DD, Lewis LS, and Smith JE. The dissertation author was the primary investigator and author of this material.

ABSTRACT OF THE THESIS

Invasion Mechanisms of the corallimorph, Rhodactis howesii, at Palmyra Atoll

by

Amanda Lockett Carter

Master of Science in Biology

University of California, San Diego, 2014

Professor Jennifer E. Smith, Chair
Professor Elsa E. Cleland, Co-Chair

As coral cover declines globally, management of remaining reefs is increasingly important. Even remote and ‘pristine’ reefs are not free from anthropogenic impacts. Biological invasions have been observed in the Pacific Remote Islands Marine National Monument on Palmyra Atoll, a National Wildlife Refuge, and on Kingman Reef. A corallimorph, *Rhodactis howesii*, was observed on the reefs and is causing phase-shifts on coral dominated habitat. Phase-shifts can lead to loss of ecosystem services by altering the physical structure and characteristics of the reef. The invasions have been linked to shipwrecks at Palmyra Atoll and Kingman Reef. In 1991, a vessel grounded on the Palmyra reef terrace and subsequent growth of *R. howesii* was observed. The corallimorph now covers ~2km^2 of the reef. *Rhodactis howesii* has also been observed around the wreck at Kingman Reef and the invasion mechanisms are largely unknown. It has been hypothesized that shipwreck iron has added a limiting nutrient to the waters, facilitating corallimorph growth. Population genetics, trace metal analysis, enrichment experiments, and *in situ* monitoring of the corallimorph were used to test this hypothesis. Results show increasing populations of the corallimorph at both proximal and distal sites to the wreck but a lack of correlation between iron content and proximity to the wreck. Additionally, exposure to shipwreck metal resulted in decreased growth and mortality of the corallimorph, suggesting that metal levels had a toxic effect. These results further an understanding of this invasive species and assist in advancing management strategies on reefs impacted by similar invasions.

Introduction

Coral reefs are among the most diverse and productive ecosystems on the planet, yet they are under significant pressure from both natural and anthropogenic stressors. Reefs are exposed to a myriad of different threats including but not limited to: ocean warming and acidification, nutrient pollution, overfishing, algal blooms, and phase-shifts (Hughes et al., 2003; Bellwood et al., 2004; Lapointe, 1997; Done, 1992). As coral cover declines globally, the few relatively 'pristine' reefs left become critically important. They serve as both a resource and a baseline to help understand how reef systems respond to isolated stressors without the added input of local human impacts seen on populated islands. The Pacific Remote Islands Marine National Monument (PRIMNM) was established in 2009 to help protect and conserve some of these remaining reefs. Included in the PRIMNM are Kingman Reef and Palmyra Atoll, two of the healthiest reefs (Sandin et al., 2008; Williams et al., 2013) that are largely isolated from anthropogenic stressors. However, despite their protected status, both atolls have experienced significant anthropogenic impacts due to shipwrecks.

Shipwrecks on coral reefs can have contrasting effects on the associated reef community depending on the context. First, the wreck itself may cause a large or acute form of physical disturbance that causes damage to the reef structure during grounding. Second, in many locations shipwrecks are used intentionally as "artificial reefs" that can provide habitat and shelter for a variety of marine organisms (Perkol-Finkel et al., 2006). However, when vessels run aground on low-lying atolls in oligotrophic waters, they cause not only physical damage but can result in nutrient fueled algal blooms as well. The term 'black reef' has been coined to describe the cyanobacterial blooms that are often seen around shipwrecks after their groundings (Wegley Kelly et al., 2012; Schroeder et al., 2008). Waters around many low-lying atolls, such as Palmyra and Kingman, tend to be highly oligotrophic due to the oceanographic context and lack of land-based nutrient input (Wegley Kelly et al., 2012). Studies have suggested that the sudden availability of iron from shipwrecks may alleviate nutrient limitation and thus cause cyanobacterial or algal blooms. The name 'black reef' comes from the murky, dark appearance of the reef and water column surrounding the wrecks. Algal blooms have also been linked to enhancing microbial activity that can cause coral mortality on the reef (Smith et al., 2006). These blooms can cause permanent phase-shifts on sections of the reef and in some instances, have been linked to subsequent species invasions (Norstrom et al., 2009). Phase shifts can involve both indigenous and non-indigenous species of algae or other organisms (such as soft corals, sponges, or invertebrates) (Smith et al., 2002; Ward-Paige et al., 2005; Stobart et al., 2005). Phase shifts can result in mass coral mortality and ultimately, in loss of ecosystem function and services (Smith et al., 2005; Folke, 2004). The most commonly studied phase shifts are that involve a switch from hard coral dominated reefs to macroalgae-dominated communities. However, invertebrate invasions, such as corallimorphs, represent a serious threat to coral reefs and their mechanisms are largely unstudied (Norstrom et al., 2009). Corallimorphs (Cnidarian: Anthozoan: Hexacorallia: Corallimorpharian) are highly competitive on reef habitats due to their ability to rapidly reproduce and spread using both asexual reproduction (fission, budding, fragmentation) and sexual reproduction (Kuguru et al. 2007). They possess large, stinging nematocyst cells that cause coral tissue degradation and necrosis (Work et al. 2008). Given these competitive physical characteristics, corallimorphs have strong invasive potential.

Invasive Species: The Breakdown

Invasive species represent a persistent form of biotic pollution across multiple aquatic and terrestrial ecosystems. Impacts associated with species invasions have been estimated to cause approximately $97 billion in damages from 1906-1991 in the United States alone (OTA, 1993). In addition to their economic impact, invasive species can be fierce competitors and may cause extinction as the native species are outcompeted. Invasive species are often introduced to an ecosystem through anthropogenic vectors (transfer mechanisms) such as ship-ballast water, agricultural trade, and in some instances, deliberate introduction. There is a wide-range of terminology used to describe invasive species, and it is important to understand some of the distinctions associated with these terms. “Invasive” species is often used to describe a species that has spread and had a significant impact on an ecosystem, regardless of its origin. “Invasive Alien Species” or IAS is used to refer solely to species that are non-indigenous to the ecosystem where they have proliferated (Ruiz and Carlton, 2003).

Although many invasions occur because of an introduction of a non-native species, there are cases in which disturbance in an ecosystem allows a species that was native, but present in low abundance, to proliferate and spread in a manner similar to an IAS. It is important to examine both types of invasions in order to better understand what factors contribute to an ecosystem’s risk for invasion. There are numerous hypotheses regarding the vulnerability of an ecosystem to invasion, ranging from the amount of anthropogenic disturbance to the level of biodiversity present (Folke, 2004). Contrasting views in the ecological literature suggest that ecosystem stability may be strongly influenced by diversity. Charles Elton was a strong proponent of this diversity-stability hypothesis, stating that simple, less-complex ecosystems were at higher risk of invasion when compared to richer, more dynamic systems. This hypothesis (biodiversity-stability-invasibility) may explain why coral reefs are in general less known for biological invasions in contrast to other ecosystem types. Nonetheless, biological invasions have been observed on healthy, intact reef ecosystems after ‘black reef’ blooms and shipwrecks (Wegley Kelly et al., 2012).

Introduction to the Problem:

Palmyra Atoll and Kingman Reef have both experienced cyanobacterial blooms and species invasions near shipwrecks on their reefs. A Taiwanese long-lining vessel was deliberately grounded on the reef terrace at Palmyra Atoll in 1991. Men aboard the vessel believed that they could attain U.S. citizenship by grounding on the reef. Their careless act caused considerable physical destruction at the atoll when the vessel impacted a hard-coral dominated section of the reef terrace. At the time of the grounding, Palmyra was privately owned by the Fullard-Leo family and was unoccupied. Because of this, there are few accounts or observations regarding what occurred in the vicinity of the wreck in the years following its grounding. 9 years after the wreck, The Nature Conservancy (TNC) purchased Palmyra and scientific monitoring and research began on the atoll. Observational reports state that a cyanobacterial bloom was seen around the shipwreck. By 2004, low densities of the fleshy corallimorph *Rhodactis howesii* were seen. There are contrasting opinions regarding the indigenous status of *Rhodactis howesii* to Palmyra Atoll. *R. howesii* is a native Indo-pacific species but there are no documented sightings of it around Palmyra Atoll before the shipwreck in 2004. It is possible that the shipwreck served as a vector for introduction of the highly competitive species of corallimorph. *R. howesii* apparently expanded across the atoll and by 2008, covered around $2km^2$ of the reef terrace (Work et al., 2008). The corallimorph proved to be a fierce competitor on the reef, causing tissue necrosis in corals likely because of its large poisonous stinging cells (nematocysts). Work et al. hypothesized that iron or some other nutrient leaching from the shipwreck was enhancing corallimorph growth.

During this time period, a similar shipwreck was observed on Kingman Reef, an atoll about 70km north of Palmyra. This vessel was teak-hulled and first noticed on the reef crest in 2007. By 2010 the wreck had been pushed across the reef and it’s debris was observed on both the fore reef and back reef of Kingman (Wegley Kelly et al., 2012). Cyanobacterial and algal blooms were observed around the shipwreck and subsequent invasion of a corallimorph was seen shortly thereafter. Observations made by visiting scientists and the refuge manager suggest that the corallimorph has continued to spread at Kingman Reef, although it hasn’t reached the densities observed at Palmyra Atoll.

The hypothesis that iron or some other metal leaching from the wreck has enhanced the growth of the corallimorph has now led to removal of the shipwrecks from both Palmyra and Kingman through a grant awarded to the U.S. Fish and Wildlife Service. The wrecks were removed in October-December of 2013, a few months after the completion of the study discussed here.

Given the paucity of scientific data on the invasion of *R. howesii* across Palmyra and Kingman Atolls, my Master's thesis focused on several aspects the biology and ecology of this invasive species. The overall goal of the study was to investigate what role the long-lining vessel has played in the corallimorphs' expansion across the atoll. Our specific aims were 1) to determine whether or not the shipwreck served as a vector for introduction of a non-native species and, 2) to determine the effect that the presence of the shipwreck metal has had on the growth and distribution of the corallimorph.

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Methods & Materials

The Shipwreck as a Vector for the Invasion of Rhodactis howesii

Population Genetics

Population genetics of the corallimorph around Palmyra Atoll were assessed for two reasons: 1) to accurately identify the species of corallimorph present around the atoll and, 2) to determine if there was any evidence that this was a non-native species or population. To do this, samples of the corallimorph were collected on SCUBA at 5 sites around Palmyra Atoll: the Longliner Wreck, Reef Terrace 10 (RT10), Penguin Spit Fore Reef (FR3), Tanagers South Shore (FR5), and North Beach (Fore Reef 7). An additional collection of corallimorph tissue was taken from the wreck at Kingman Reef, about 70km North of Palmyra, to provide a genetic comparison. Sites were generally selected to cover a wide geographic area at different distances from the potential vector—the shipwreck. The collection sites around Palmyra are shown in Figure 1 and their collection depths and distances from the wreck are listed in Table 1. Samples collected around Palmyra were immediately preserved in ethanol and then transported back to Scripps Institution of Oceanography (SIO) for processing. The samples collected at Kingman Reef were frozen in an -80°C freezer and then shipped back to SIO on ice. DNA was extracted from the corallimorph samples using a *Qiagen* DNeasy Blood and Tissue Kit and then compared to the sequence for *Rhodactis howesii* as described in Chen et al. 1996. PCR primers were developed using the conserved 18S and 28S regions to isolate and amplify the ribosomal ITS1 and 5.8S regions following Chen et al. 1996. The primers were ordered from *Bioneer Corporation* in Alameda, California and are shown below:

BD1f 5’-GTCGTAACAAGGTTTCCGTA-3'

BD2r 5’- ACCCGCTGAATTTAAGCATAT-3'

ISf 5' -GGTACCCTITGTACACACCGCCCGTCGCT-3'

2SS r 5' -GCTTTGGGCTGCAGTCCCAAGCAACCCGACTC-3'

Amplification was performed using the following protocol: 38 cycles consisting of 94˚C (3 min), 94˚C (30 sec), 50˚C (45 sec), 72˚C (1 min), and 72˚C (7 min). Amplified DNA was then sent out for analysis at *Retrogen*, Inc. in San Diego, California. The resulting sequences were analyzed using *Sequencher* (version 5.1) software to look for mutations in the corallimorphs genome. We predicted that if the population were clonal, isolation of the ITS1 region would show little to no genetic variation. This would suggest that the *Rhodactis* around Palmyra Atoll stemmed from one initial invader that was introduced to the reef, with the shipwreck serving as a potential vector.

Testing the Iron Enrichment Hypothesis

Trace Metal Analysis of Corallimorph Tissue

In order to examine whether a limiting nutrient from the wreck (such as iron) was influencing the spread and abundance of the corallimorph, I compared tissue metal content at varying distances from the wreck. We postulated that heavy metals (including iron) in corallimorph tissue near the wreck would be elevated compared to tissue collected at distal sites. To test this, samples were collected on SCUBA at sites ranging from 0 to 13km from the wreck and at depths ranging from 5m on the reef terrace to 20m on the fore reef. Samples were collected at 8 sites total, including 1 next to the shipwreck at Kingman Reef. Sample locations are shown on Figure 1 and collection site, depth, and replication are shown in Table 2. Samples collected around Palmyra Atoll were preserved in formalin and then shipped back to SIO for analysis. The sample set collected at Kingman Reef was frozen at -80°C and then shipped back to SIO on dry ice.

All samples were analyzed for trace metal content using the following methodology: approximately 500 mg of corallimorph tissue were dissected from each sample, weighed, and then dried for more than 24 hours (or until all moisture had evaporated). Samples were then re-weighed and 500µl of Trace Metal Grade Nitric Acid ($HNO_{3,}$ Fisher Scientific) were added and weights were recorded again. Samples were run through a microwave for complete digestion of corallimorph tissue. 500µl of the resulting liquid was then added to 2ml of purified water and run through an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) located at SIO to attain levels of trace metals present in corallimorph tissue (µg/g dry weight). Sample preparations were done under controlled conditions to minimize any trace metal contamination and are described fully in Deheyn et al. 2005. The trace metals analyzed around the atoll include: Silver (Ag), Aluminum (Al), Arsenic (As), Cadmium (Cd), Cromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Nickel (Ni), Lead (Pb), Selenium (Se), Tin (Sn), Strontium (Sr), Titatium (Ti), Vanadium (V), and Zinc (Zn). Tin concentrations were not analyzed at all sites so they were removed from atoll wide analyses. Outliers further than 3 standard deviations away from the mean were removed from the data set before analysis as this likely indicated contamination of the sample. Due to the large variability in metal values, the data set was normalized using a Log (X+1) data transformation. To assist in visualization of this multivariate data set, I calculated a resemblance matrix using Euclidian distance in Primer 6 (version 6). In order to determine how trace metal tissue concentrations varied as a whole across Palmyra and Kingman, a one-factor permutational multivariate analysis of variance (PERMANOVA) was run on the resemblance matrix with “site” as the factor. *Post hoc* contrasts were used to compare relative differences across sites.

Effects of metal from the shipwreck on the growth of R. howesii

To investigate the effects of the metal from the shipwreck on corallimorph growth rates, an enrichment experiment was conducted in individual mesocosms using corallimorphs collected on SCUBA from the reef terrace site Penguin Spit Inner (Figure 1, 5m depth). 30 loosely attached corallimorphs were collected using latex gloves and plastic bags filled with seawater to prevent any tissue damage or metal contamination. The specimens were then placed in a water table with a bubbler and fresh seawater and allowed to acclimate for two days. A piece of the shipwreck just below the waterline was pried off using a wrench while on snorkel and cut into 12 equally sized pieces (approximately 3cm^2 each). 24 individual *R. howesii* specimens were selected after acclimation and placed in 1-liter growth chambers, 12 contained 1 piece of shipwreck metal each and 12 were grown without the addition of any metal. The growth chambers were placed in a water table filled with circulating seawater that served as a water bath to regulate temperature. Temperature readings were taken in the morning and evening using a HACH temperature sensor (mean=28.4°C ± 0.27). Each chamber was provided with aeration using a pump and an air-stone that was kept consistent throughout the duration of the experiment to prevent any cross-contamination of metals. The seawater used in the experiments was collected offshore from the Western terrace of Palmyra Atoll. 50% of the water was changed every other day throughout the 2-week experiment. Control and treatment chambers were rotated within the water bath during each water change to prevent edge effects associated with variability in lighting and temperature within the water table. *Rhodactis* samples were buoyant weighed at the beginning and end of the experiment to the nearest 0.001g. No intermediate buoyant weights were taken because of the stress associated with weighing for the specimens.

We predicted that exposure to shipwreck metal would result in elevated growth rate of the corallimorph, *Rhodactis howesii.* To test this, I examined the change in the buoyant weight of all corallimorph specimens (n=24) over time. Because the data were not normally distributed, we used a non-parametric Wilcoxon/Kruskal-Wallis (Mann-Whitney) test with the statistical software JMP (version 11). Trace metal levels in the corallimorph tissue were analyzed in the control and treated groups following the same procedures listed above (Deheyn et al. 2005). Outliers were calculated at greater than 3 standard deviations away from the mean and removed from the data set. The multivariate data set was analyzed using a one-factor PERMANOVA following the procedure described above.

Variability in the Abundance of Rhodactis howesii across Space

2010 Baseline Data

In order to examine the change in abundance of *Rhodactis* howesii at Palmyra Atoll a baseline estimate of corallimorph cover was needed. It was predicted that if metals leaching from the shipwreck were influencing the invasion, we would see higher cover (abundance) of the corallimorph at the shipwreck with a gradual decrease in abundance with distance. In order to test this, a photographic data set from a monitoring trip in 2010 was utilized. During the 2010 monitoring expedition, a number of permanent benthic monitoring sites and temporary transects around Palmyra were established. These included fore reef, reef terrace, and back reef sites. All permanent fore reef sites were established at 10m depth and permanent back reef and reef terrace sites were at 5m depths. A number of temporary monitoring transects were laid out at 5, 10, and 20 meter depths on the fore reef, however these sites were photographed in 2010 only. All sites (both permanent and temporary) are listed in Table 3 with their associated depths, habitat type, and distance from the shipwreck. At each site 10 – 20 individual photographs were taken every 5m along a 50m transect line using a Canon G9 camera attached to a 0.72m^2, PVC "photoquadrat." Permanent sites were marked with stainless steel eyebolts hammered into the reef at opposing corners and then cemented using the marine epoxy Z-SPAR. The photoquadrat frame is snapped onto the two eyebolts in the same orientation each time to create a spatially consistent photographic time series.

Photographs taken at the temporary and permanent sites around Palmyra Atoll were analyzed using the image analysis program Photogrid (version 1.0) in order to determine how mean benthic cover varied spatially across all sites in 2010. 100 stratified, random points were identified to the finest level of taxonomic resolution possible, which in most cases was at the genus or species level. For this study, photos were analyzed using genus level resolution when possible (aside from turf algae and crustose coralline algae which were identified at the functional group level) and data were subsequently sorted into functional groups. *Rhodactis*, however, was kept separate from other fleshy invertebrates. After all photographs were analyzed, benthic cover per sampling unit (quadrat) was plotted across all sites at the atoll starting at the Longliner wreck and radiating outward. Percent cover within a site was used for the initial baseline data to prevent loss of single data points with corallimorph in mean calculations. A logistic regression was then used to test the relationship between proximity to the shipwreck and percent cover of *Rhodactis.* SYSTAT (version 13) software was used to run the logistic regression and Sigma Plot (version 11) was used to visualize the results. We postulated that if the shipwreck were driving the invasion across the atoll, there would be a positive correlation between distance from the Longliner and corallimorph cover.

Spatial and Temporal Change in Rhodactis Cover from 2010-2013

Change in *Rhodactis* cover from 2010-2013 was analyzed by processing photographs from 7 permanently established benthic monitoring sites: the Longliner Wreck, Penguin Spit Middle, Penguin Spit Inner, Penguin Spit Outer, Reef Terrace 30P, Fore Reef 3, and Fore Reef 5. At each site a total of 10 individual 0.72 m^2 plots were established every 5m along a 50m transect line and marked with two stainless steel eyebolts as described above. The PVC photoquadrat was used to take all photographs in the spring of 2010 and then in the summer of 2013. All photographs were cropped and edited in Adobe Photoshop and analyzed for benthic cover using Photogrid (methods described above). Data were once again sorted into functional groups with *Rhodactis* kept separate in order to examine the relationship between the changes in corallimorph cover with proximity to the shipwreck. Percent change in *Rhodactis* cover between the initial sampling in 2010 and the most recent sampling in

2013 was calculated to determine if corallimorph abundance were increasing, decreasing, or remaining the same over time.

The data were plotted against distance from the wreck to see if percent change varied by site and proximity to the Longliner. We examined the effect of initial cover on percent change from 2010 – 2013 and predicted that we would see increased change in cover at sites closer to the wreck if leaching metals were stimulating corallimorph growth.

These data were also used to examine the colonization rate of *Rhodactis* within invaded sites from 2010 – 2013. We predicted that sites with higher invasion levels (measured by plots containing corallimorph) would show higher colonization rates of *Rhodactis* during the time series. This relationship was examined by recording the number of plots with corallimorph in them per site, per year. The count of invaded plots in 2010 was subtracted from the 2013 count and then divided by 3 years giving us an average colonization rate in plots/year.

Growth Rate and Density of Rhodactis within Invaded Sites from 2010-2013

In order to calculate the population growth rate of the corallimorph, I measured areal expansion of *Rhodactis in situ* from 2010-2013 using the permanent photographs taken at Penguin Spit Middle, Fore Reef 3, and Fore Reef 5 (the only sites with continuous plot data covering all three years). The photographs were analyzed using the JAVA image analysis software Image J. Photographs were uploaded into the program and calibrated to a set length to allow for surface area calculations. The calibration for the photographs was set using the 90cm length of the PVC photoquadrat frame. Individual polyps and clusters of *R. howesii* were traced by hand using a Wacom Bamboo™ tablet and stylus and given unique identifiers that allowed them to be traced through all time points. Surface area of each individual or cluster was calculated using the calibrated scale of each photo, providing total areal coverage of corallimorph per photo (plot) in cm^2.

Corallimorph areal coverage per year (growth rate) was calculated for each site and plot that had continuous data for all three years (FR3 40m plot, FR5 0 and 25m plots, PSM 25m, 30m, and 40m plots). We predicted that if proximity to the wreck and initial cover influence growth rate, elevated growth rates would be observed at Penguin Spit Middle (the site closest to the wreck) compared to the two fore reef sites. Growth rates were estimated using linear regressions fit to the change in areal coverage from 2010 – 2013. The 7 slopes from the linear regressions were used to calculate mean growth rate (cm^2) of corallimorph cover per year.

Surface area measurements were used to calculate mean polyp and cluster size. Changes in density per plot were calculated by dividing total areal coverage by average polyp size from 2010-2013.

This thesis, in full, is currently being prepared for submission for publication of the material. Carter AL, Deheyn DD, Lewis LS, and Smith JE. The dissertation author was the primary investigator and author of this material.

Results

Understanding the Invasion Ecology of Rhodactis Howesii

Population Genetics

DNA was successfully extracted from all samples collected around Palmyra Atoll (n=25). The resulting sequences were compared to that given for *Rhodactis howesii* in Chen et al. 1996 and resulted in a positive identification. Unfortunately, the samples from Kingman were partially defrosted during transit and thus, the DNA was too degraded for successful extraction and sequencing.

Amplification of the ITS1 and 5.8S regions was successful and resulted in sequences for all samples collected around Palmyra Atoll (n=25). The ITS1 and 5.8S regions showed minimal variation between individuals at all sites around Palmyra.

Testing the Iron Enrichment Hypothesis

Trace Metal Analysis of Corallimorph Tissue

Results of the one-way PERMANOVA on the similarity of all metals in corallimorph tissues (using Euclidean distance) across sites showed significant variation with a p-value of 0.001* (Table 4). A pair-wise test was used to determine if and how the Longliner wreck compared to the additional sites around Palmyra and Kingman. Results are presented in Table 4 but are not corrected for multiple comparisons. A canonical analysis of principle coordinates (CAP) was run *post hoc* to show distance-based correlations in tissue metal content between sites (Figure 2). Figure 3 shows the trace metal vectors from the CAP. A *post hoc* SIMPER was run to examine which metals contributed to the significant difference seen between the similarities of all metals in the corallimorph tissues (Table 5).

Growth Experiment

Throughout the course of the 2-week experiment 5 of the 12 metal treated *Rhodactis* dissolved in their growth chambers resulting in 100% loss of buoyant weight. Seawater was drained off and the dissolved remnants were stored in 50ml Falcon tubes, preserved in ethanol, and then sealed and brought back to SIO for trace metal analysis. Change in buoyant weight of all corallimorph specimens (n=24) was analyzed using a non-parametric Wilcoxon/Kruskal-Wallis (Mann-Whitney) test with JMP (version 11). A non-parametric test was used so that the 100% loss of buoyant weights could be included in the analysis given that they appeared to be a clear result of treatment (presence of wreck metal). The test showed a significant difference between treatment and control with a p value = 0.0036* (Table 6).

ICP-OES trace metal analysis values falling outside of 3 standard deviations from the mean were labeled as outliers and removed from the data set prior to creating a resemblance matrix using Euclidian distance on the normalized data. The results of the one-factor PERMANOVA showed a significant difference of p= 0.0006 (Table 7). A *post hoc* SIMPER was run to determine which metals contributed to the significant difference seen between the treated and control *Rhodactis* (Table 8).

Changes in Benthic Cover of Rhodactis howesii

2010 Baseline Data

Figure 3 shows the mean benthic cover of all functional groups and *Rhodactis* around Palmyra Atoll in 2010 starting at the Longliner Wreck and radiating outwards. The logistic regression run in SYSTAT showed a non-significant relationship between percent corallimorph cover and proximity to the shipwreck with a p-value of 0.159 (Figure 4).

Spatial and Temporal Change in Rhodactis Cover from 2010-2013

Percent change of *Rhodatis* was calculated from 2010 – 2013 for the 7 sites that were photographed in both years (LLW, PSM, PSI, RT30, FR3, FR5). Positive change was seen for all sites with the exception of the Longliner Wreck, where a decrease in corallimorph cover was observed (Figure 5). Figure 6 shows the effect of initial corallimorph cover on percent change for the aforementioned 7 sites. The Longliner Wreck (LLW) shows a high initial cover of corallimorph in 2010 and a loss of cover by 17.1% by 2013 (Figure 6). Removal of the Longliner site’s negative trend from the data set resulted in a positive growth trend for all other sites (Figure 7).

Colonization rates from 2010 – 2013 for the sites are shown in Table 9. All sites with the potential for growth (those with non-invaded plots) showed increased spread of corallimorph across plots. No decrease in spread was observed; plots were either static or showed an increase in cover over time.

Growth Rate and Density of Rhodactis within Invaded Sites from 2010-2013

Data collected using the Image J photo analysis program showed positive growth at all sites from 2010 – 2013 (PSM, FR3, FR5). Corallimorph areal expansion (Figure 8) was plotted and linear regressions were fit to calculate the average growth per site per year. These slopes were then averaged to estimate the find the mean growth rate of the population to be 351.47cm^2/year (± 105.25cm^2).

Mean polyp and patch size of *Rhodactis* at Palmyra from 2010 – 2013 was calculated using the unique identifier data within the Image J software ($8.38cm^2$ and $267.51cm^2$ respectively). The mean polyp size was used to calculate the polyp densities per plot throughout the time series and show a general trend of increasing polyp density (Figure 9) over time. The Penguin Spit Middle 40meter (PSM_40M) plot was the only one to show a slight decrease in polyp density from 2012 to 2013 but corallimorph cover in this plot was nearly 100% by 2012.

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Discussion

Most phase shift studies to date have focused on shifts from coral to macroalgae-dominated communities (Done, 1992; Hughes, 1994; Bruno, 2009). There is limited data regarding phase-shifts from hard coral dominance to dominance by a fleshy invertebrate, especially those dominated by corallimorphs. Corallimorph invasions have been documented in the Red Sea and Zanzibar after major disturbances on the reef, such as oil pollution or bleaching events (Chadwick-Furman & Spiegel, 2000; Loya, 2004). In comparison, this is one of the first studies to examine a corallimorph invasion on a relatively 'pristine' reef and compliments the work previously done by Work and colleagues on Palmyra Atoll (Work et al., 2008).

This study is also the first to genetically confirm that the corallimorph around Palmyra Atoll is *Rhodactis howesii*. However, investigation into the population genetics of the *Rhodactis* around Palmyra was less definitive. Based on our results there is either 1) no variation in the Internal Transcribed Spacer of the rRNA transcription unit (ITS1) and 5.8S regions chosen or, 2) no variation in the *Rhodactis* genome around Palmyra Atoll. Chen et al. 1996 found high conservation of the ITS1 region in *Rhodactis indosinensis,* a closely related species, suggesting that another region of the genome may serve better for looking at population level variation within the corallimorph at Palmyra Atoll. Alternatively, the lack of variation seen in the isolated regions could suggest that the population is largely clonal, or familial, which would support the hypothesis that *Rhodactis howesii* was brought to Palmyra Atoll on the shipwreck. Unfortunately, due to the lack of geographically distinct genetic material from Kingman (or another *R. howesii* population) for comparison, we cannot state that the lack of variation seen is due to clonal relationships. In order to resolve this, additional samples would be needed. If possible, this future direction could provide the comparison needed to draw conclusions regarding the population genetics and ultimately the invasion history of the corallimorph around Palmyra Atoll and at Kingman Reef.

Iron levels around Palmyra atoll and at Kingman Reef, as determined by trace metal analysis in corallimorph tissue, show high variability. Surprisingly, the Penguin Spit Fore Reef (FR3) and Kingman sites showed the highest levels of iron, not the Longliner wreck as was expected (Figure 10). Iron levels were, on average across sites, higher than any other metal signatures measured, with a mean concentration of 1122.72µg/g dry weight ± 158.9. For reference, the second highest metal concentration seen was that of aluminum at 270.05µg/g dry weight ± 42. The iron levels seen at Kingman and at FR3 show a maximum of around 2500µg/g dry weight that is 2x greater than the average across all sites. These levels may be a result of contamination, although Tanager South Shore (FR5) also shows high levels of iron and is close to FR3 (Figure 1). It is possible that these elevated levels are due to water flushing out of the lagoon and leaching onto the fore reef. The lagoon at Palmyra contains large quantities of military debris and sunken shrapnel and could represent a significant source of pollution at sites close to the channel. Other studies examining iron loads in invertebrates have found maximum levels of around 480µg/g dry weight in contaminated streams, suggesting that the levels seen at Palmyra and Kingman are extremely elevated (Winterbourn, 2000). Some of the difference may be the result of iron-containing, photosynthetic zooxanthellae that are present in the corallimorphs tissues.

Copper was also elevated at FR3 (Figure 11) with high levels of variation that might suggest an issue with the sampling at this site. Contamination of the sample during collection, transit, or processing may have occurred. Copper (Cu) was of particular interest since it is commonly used as an anti-fouling agent on ship hulls and is known to hinder fertilization in reef-building corals (Reichelt-Brushett, 1999). However, even if the levels of copper seen at FR3 were included, the maximum concentration was still around 5µg/g (Figure 11), which is far lower than the 15-40µg/L that was found to have negative effects on coral fertilization rates (Reichelt-Brushett 2005). However, the copper concentrations used in the Reichelt-Brushett experiment were attained in seawater that the coral gametes were grown in, not in tissue concentrations, which makes comparison difficult. Trace metal analysis of the seawater at varying sites around the atoll would compliment this work and previous studies as well.

Trace metal analysis of corallimorph tissue at sites around Palmyra and at Kingman Reef showed significant differences between suites of metals (Table 4). The pair-wise tests showed that the Longliner site significantly differed from the Fore Reef 7, Fore Reef 3, Fore Reef 5, and Kingman sites (Table 4). Interestingly, the other reef terrace sites tested did not have significantly different metal suites when compared to the Longliner site (RT1, RT10; Table 4). This suggests that trace metals on the reef terrace stay largely localized when compared to those on the fore reef. The canonical analysis of principle coordinates (Figure 2) shows clear spread between the Longliner site and all others. Interestingly, it also shows that the Longliner Wreck metal suite and Kingman Wreck metal suite are highly separated. Elevated levels of iron at the Fore Reef sites and Kingman site appear drive the significant difference observed as was discussed above (Figure 10). These data do not appear to support the hypothesis that elevated iron around the shipwreck is driving the invasion (Work et al. 2008). Rather, they show that iron levels appear to be elevated at sites distal to the shipwreck suggesting that the iron enrichment seen is the result of another nutrient input. As discussed before, flushing from the lagoon could play a role in the iron levels seen at Penguin Spit Fore Reef (FR3). The levels seen at Kingman reef are more difficult to explain given that the wreck there is primarily teak. However, it is a newer shipwreck and metals leaching from its hull may be higher than those currently leaching out of the Longliner Wreck on Palmyra. Given these data, it was necessary to find another means of testing the effect that iron, or shipwreck metal, was having on the growth of the corallimorph.

The enrichment experiment provided us with a method to further test the effect that shipwreck metal may be having on the growth of the corallimorph around Palmyra. The results of the experiment clearly do not support the hypothesis that metal is facilitating corallimorph growth, showing a significant loss in buoyant weight ($p = 0.0036$) (Table 6) for the corallimorphs grown within the treatment chambers. Interestingly, we saw complete mortality in 5 of the treated *Rhodactis* samples, suggesting a toxic effect of metal in the experiment. Trace metal analysis of the specimens from the experiment did show significantly higher levels of iron in the treated corallimorphs (Figure 12). Zinc and lead were also significantly higher, but within the control specimens rather than those exposed to shipwreck metal. A one-factor PERMANOVA was run to further examine which metals might be driving the results seen from the growth experiment. The PERMANOVA showed a significant difference ($p = 0.0006$, Table 7) between the metals in the control and treated

Rhodactis. A *post hoc* SIMPER revealed that lead, arsenic, and tin were the top three contributing metals to the significant difference seen between the control and treatment. Interestingly, the high levels of lead were seen in the control specimens, not the treated ones (Figure 12), suggesting that lead was not responsible for the negative response of the corallimorphs to treatment. Uncontaminated marine sediments carry around 5 – 40µg/g dry weight of arsenic (Neff, 2009). The concentrations of arsenic within corallimorph tissue in the experiment were well within this range or lower suggesting that it did not reach toxic levels. Tributyltin (TBT) was a commonly used anti-fouling agent painted on ship hulls through the 1980s before it was banned due to its high levels of toxicity. The tin signature seen in the experiment is not specific enough to say if it was TBT, however the possibility that it was could explain the mortality seen in 5 of the 12 treated *Rhodactis.*

It should be noted that the experiment was limited in its lack of flow-through seawater within each chamber. We attempted to combat this with 50% water changes every other day but despite this, conditions did not accurately mimic the amount of water movement and flushing that would occur around the wreck. The lack of water flow meant that any metal leaching from the pieces of wreck stayed in close contact with the *Rhodactis* specimens throughout the duration of the experiment. Additionally, due to the limited time available on the island, collected specimens were not able to acclimate for more than 2 days. An attempt was made to ensure healthy *Rhodactis* at the start of the experiment by collecting a large number of polyps and selecting the most robust specimens after the acclimation period. However, it would have been preferable to use polyps that had acclimated for at least a week before being placed into the experiment.

Given the limitations of the growth experiment, *in situ* growth rates and benthic cover of the corallimorph were examined around the atoll. Analysis of the benthic photographs from 2010 showed close to 100% cover of *Rhodactis* at the Longliner wreck site but 0% cover at the adjacent site, Reef Terrace 23 (Figure 3). Rather than seeing a linear relationship between corallimorph cover and proximity to the shipwreck, satellite populations of the corallimorph are apparent around the atoll. These data suggest that distance from the wreck did not directly affect percent corallimorph cover in 2010. They could suggest that the corallimorph has radiated out from the central point of the wreck and then distributed to satellite sites via sexual reproduction or transference of polyps. New, starting populations would also explain the lower densities of *Rhodactis* at distal sites.

Change in percent cover of corallimorph around the atoll from 2010 – 2013 did not show a clear trend, although the only site that experienced a negative change in percent cover was the Longliner Wreck (LLW) itself (Figure 5). In order to further examine this we looked at the effect of the initial percent cover of *Rhodactis* on the change in percent cover observed (Figure 6). Initially a negative correlation between starting *Rhodactis* cover and change in percent cover from 2010 to 2013 was observed. However, once the Longliner site is removed from the data set, a positive correlation between initial cover and change in cover becomes apparent (Figure 7). Because of the high initial coverage of the corallimorph at the Longliner site, one wouldn’t expect to see significant growth since cover cannot exceed 100%. The most plausible change would be decrease in percent cover which we see at the LLW site of -17.1%.

Areal expansion from 2010 – 2013 showed increasing growth rates at all sites measured (Fore Reef 3, Fore Reef 5, and Penguin Spit Middle). All linear regressions showed a positive slope and were averaged to provide a mean *Rhodactis* growth rate of 357.41cm^2/year (Figure 8). Because of the lack of data from the Longliner Wreck site itself, we cannot draw conclusions regarding these growth rates to that of the *Rhodactis* next to the wreck itself. A longer, more complete time series of photos near the wreck and at additional sites would make this data set more robust. However, these data do provide the first numerical growth rate of *R. howesii* and, when combined with the colonization rates (Table 9) and the polyp density changes (Figure 9), the corallimorphs expasion across Palmyra could be modeled for future years.

Colonization of the corallimorph in plots per year from 2010 – 2013 is shown in Table 9. The Longliner site exhibited no colonization because all of the plots present at the site already contained corallimorph in 2010. Penguin Spit inner, middle, and outer all showed increased colonization over the time period, as did Fore Reef 5. Although the colonization data is interesting, it is important to note that these are only *within* the photographs. Changes in density of the corallimoph across the same sites were also all positive with the exception of the Penguin Spit Middle 40m plot (Figure 9). The PSM_40M plot likely showed a minor decrease in density because it was close to maximum coverage in 2012 as shown in Figure 9.

Arguably, the data set used for these Image J analyses was not ideal since it lacked continuous data near the wreck. However, these data do not show any significant relationship between corallimorph cover and proximity to the shipwreck. Although some of the highest levels of corallimorph are present at the Longliner Wreck, the other sites with high *Rhodactis* cover (such as Penguin Spit Middle) are up to a kilometer away from the wreck, with minimal to no *Rhodactis* in between. This suggests that the invasion of the corallimorph is far more complex than the initial iron enrichment hypothesis suggested (Work et al. 2008). Rather, these data suggest that corallimorph growth is more dependent upon initial starting cover of the corallimorph, with increased *Rhodactis* coverage leading to faster growth rates. This suggests that the corallimorph is primarily producing via asexual budding which would explain the lack of variation seen in its genome. Transference of individuals to more distal sites could be occuring via researchers movement around the atoll (corallimorph fragments attached to dive gear, wet suits, etc).

There does appear to be a saturation limit as is seen at Penguin Spit Middle. However, since the plots represent a bounded space, it is possible that expansion has continued at those sites but outside of the plots. Our inability to measure continued spread is simply a limitation of the permanent transect methodology. This limitation means that although these data provide a reasonable idea of the expansion of the corallimorph, it is not a complete picture.

Conclusions

This suite of experiments does not show a significant relationship between corallimorph growth and cover with proximity to the wreck. Additionally, the lack of genetic variation prevents us from drawing conclusions regarding the native or non-native status of the corallimorph at Palmyra Atoll. However, despite these results, the corallimorph does appear to be associated with the wreck in some manner given its initial bloom after and around the grounding. The 2010 benthic data (Figure 3) suggests that transference of the corallimorph around the atoll may be occurring, resulting in the smaller, distal populations observed. Management policies requiring sanitation of gear when moving from the Longliner Wreck or Penguin Spit sites to others might help prevent additional spread of the corallimorph to further locations. However, this is purely speculation and would require further population genetics analysis for confirmation. It seems apparent that the actual cause of the corallimorph invasion is far more complex than we were able to determine here. However, this study is an important first step in assessing the damage caused by the wreck and the subsequent corallimorph invasion.

We are in a unique situation to study the recovery of the reef now that the shipwreck has been removed. Further experimentation on Palmyra Atoll will include large plot clearings at varying distances from the wreck site to study benthic succession. These future data will help inform the restoration process underway at Palmyra and Kingman by providing valuable insight regarding where corallimorph removal efforts should be focused. The role that the wreck has played in the invasion may become clearer as we watch the trajectory of the corallimorph population after wreck removal. This case study strongly supports the belief that we need more immediate and intensive management strategies in place to help mitigate, or possibly even prevent, phase-shifts and invasions on our remaining “intact” coral reefs (Bellwood 2004, Hughes 2003). Understanding the role that shipwrecks play on low-lying atolls, such as Palmyra and Kingman, will assist us in better management of reefs and more immediate response to ship groundings in the future.

This thesis, in full, is currently being prepared for submission for publication of the material. Carter AL, Deheyn DD, Lewis LS, and Smith JE. The dissertation author was the primary investigator and author of this material.

Figures & Tables

Figure 1. Corallimorph collection sites around Palmyra Atoll in 2010.

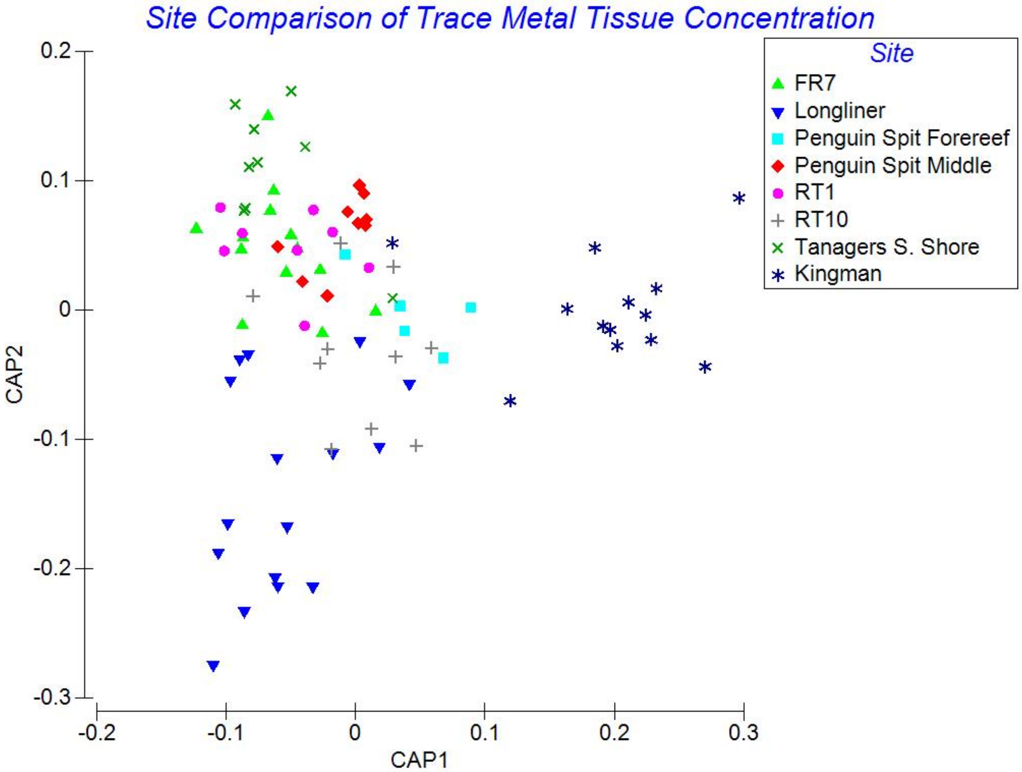

Figure 2. Canonical analysis of principle coordinates (CAP) showing distance based correlations between metal suites at sites around Palmyra Atoll and Kingman Reef.

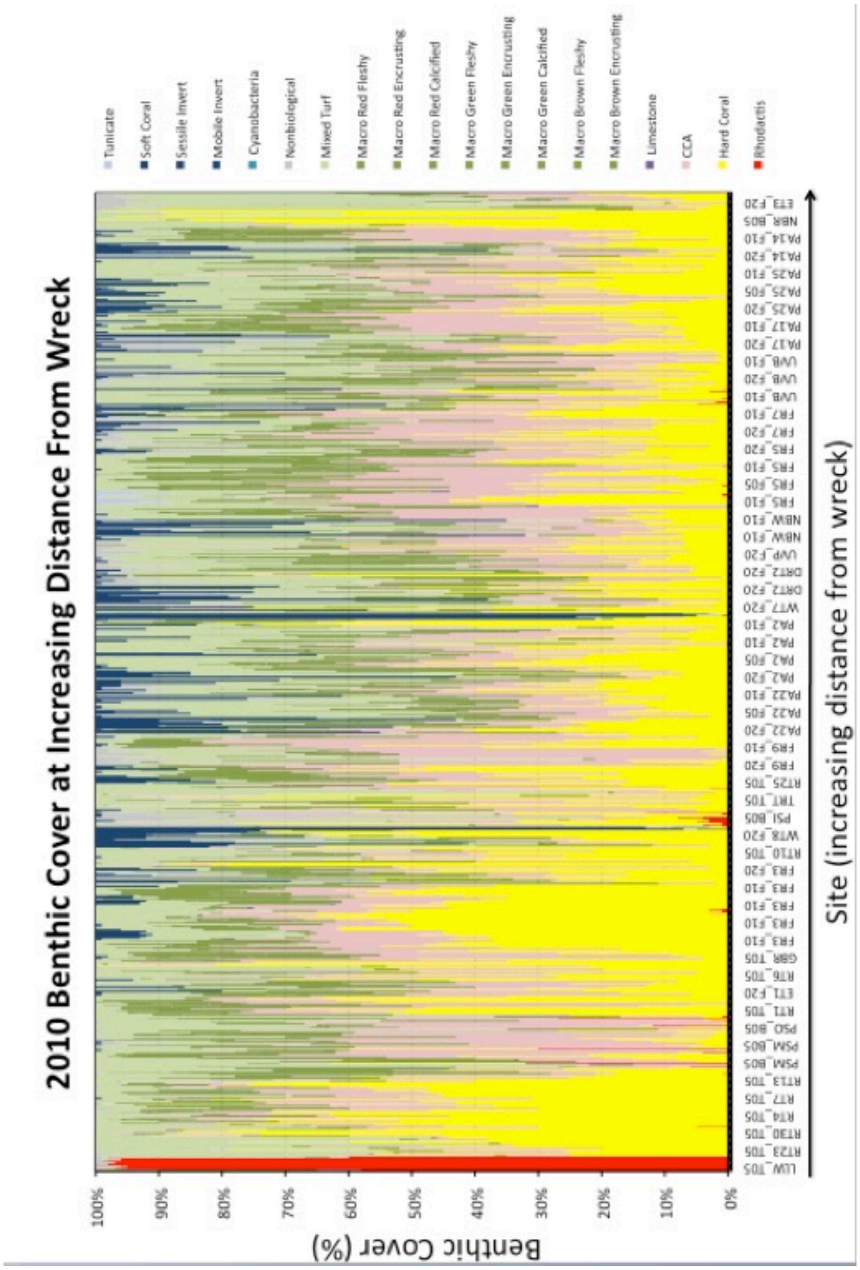

Figure 3. Mean benthic cover per transect of functional groups and *Rhodactis* across all sites at Palmyra Atoll in 2010 (n=50 sites).

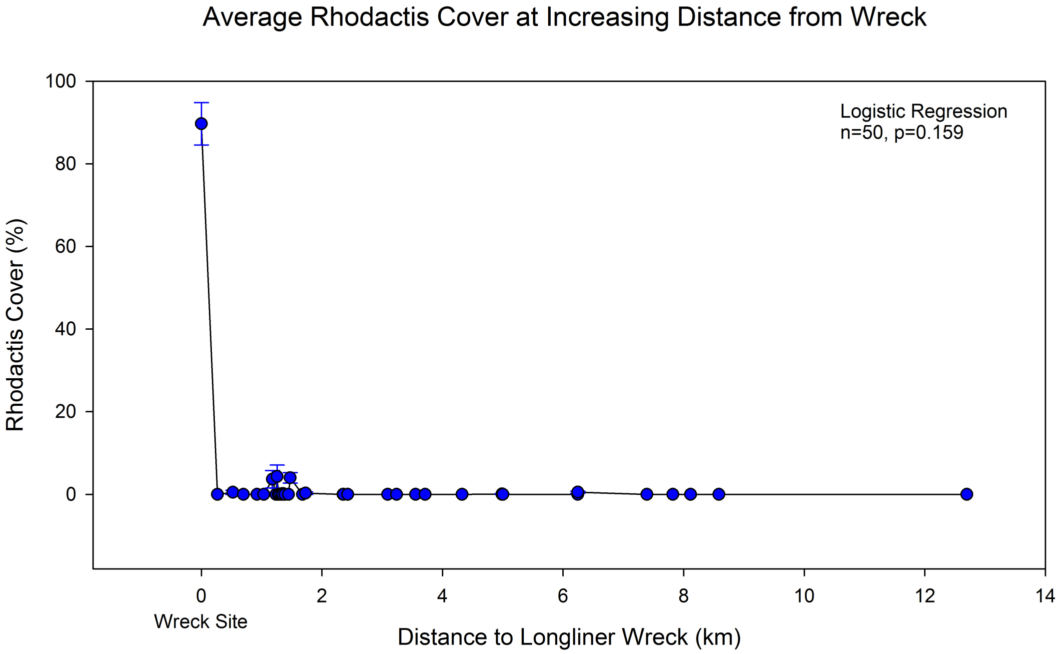

Figure 4. The results of a logistic regression showing the lack of a statistically significant relationship (p=0.159 with an n=50) between percent *Rhodactis* cover and distance to the Longliner shipwreck on Palmyra Atoll.

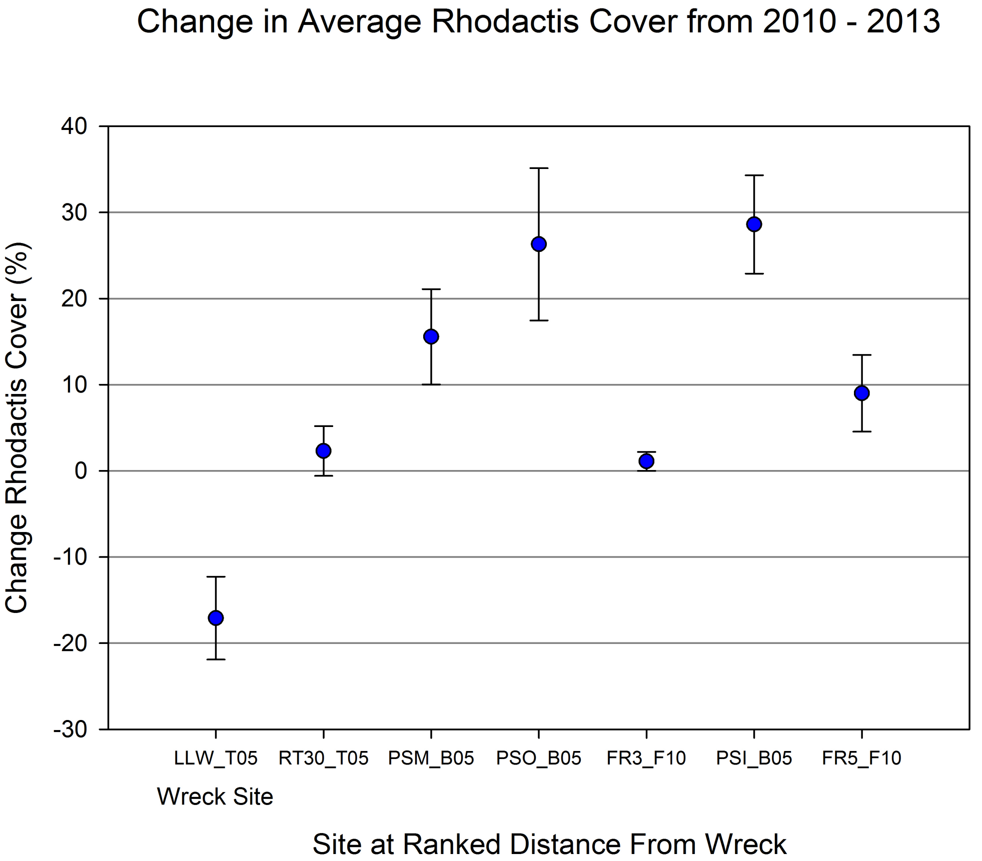

Figure 5. Changes in benthic cover of *Rhodactis* per site with standard error from 2010 – 2013 starting at the Longliner site and radiating out at increasing distance from the wreck.

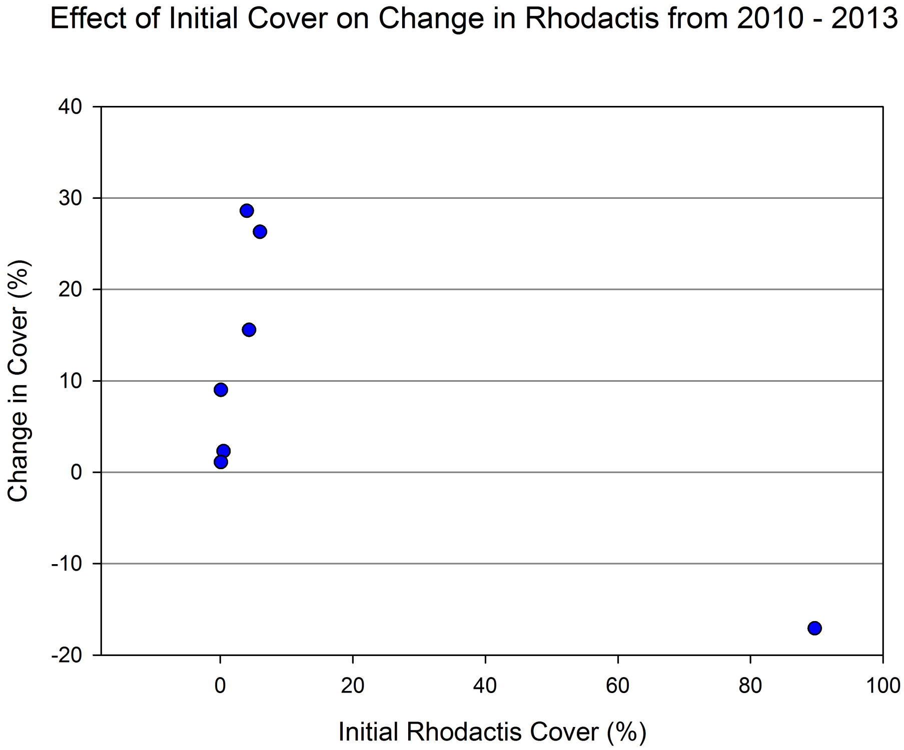

Figure 6. The relationship between starting *Rhodactis* cover and percent change in cover from 2010 – 2013. Data set includes the Longliner Wreck site with an initial starting cover of 89.7%. High initial cover resulted in a 17.1% decrease for the site over the time series.

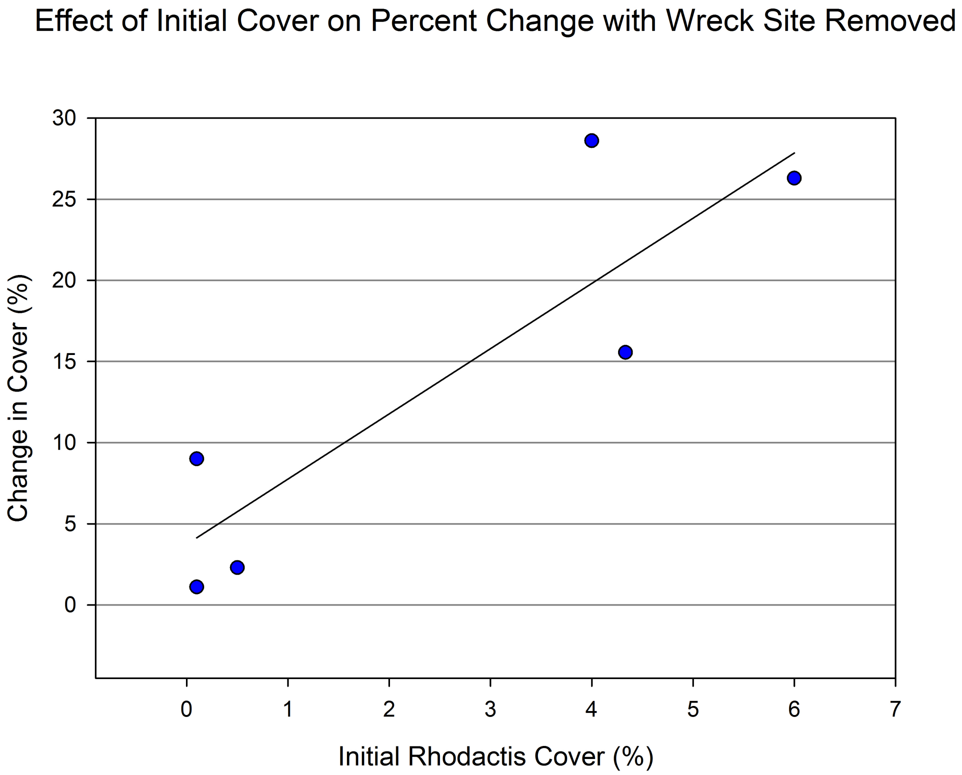

Figure 7. The effect of starting *Rhodactis* cover on percent change from 2010 – 2013 with the Longliner site removed to show that all other sites increased.

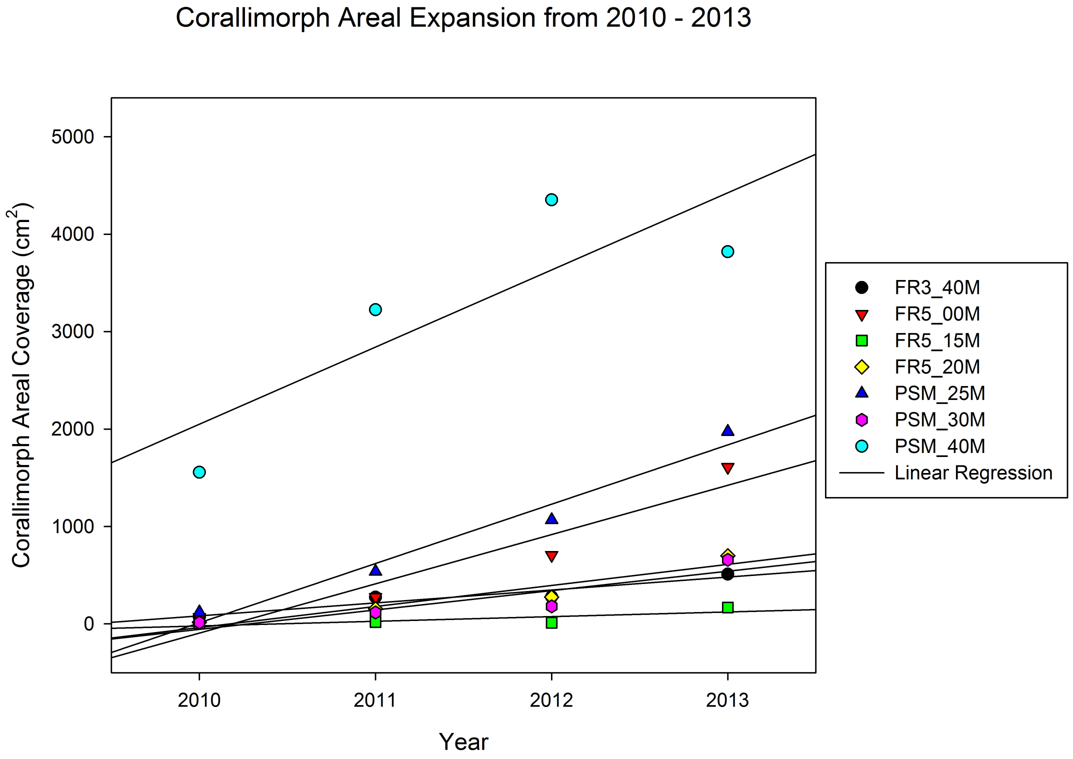

Figure 8. Areal expansion of the corallimorph across plots with continuous data from 2010 – 2013. Average growth rate was **357.41cm^2/year** (se = 105.25cm^2). Maximum plot area was 5400cm^2 and is the y-maximum on the graph.

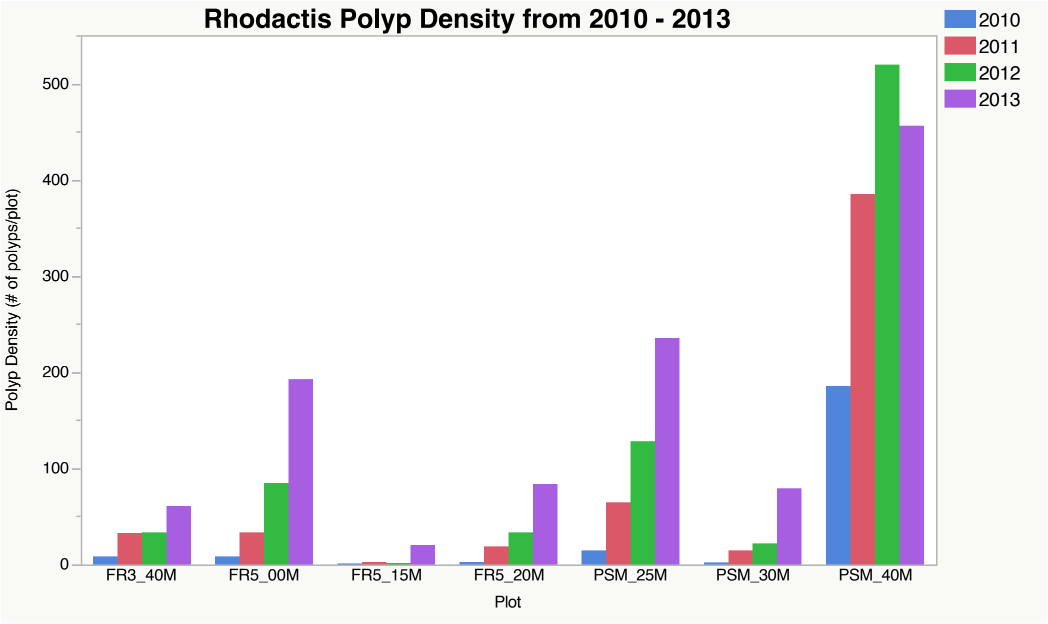

Figure 9: Changes in polyp density per plot from 2010 – 2013.

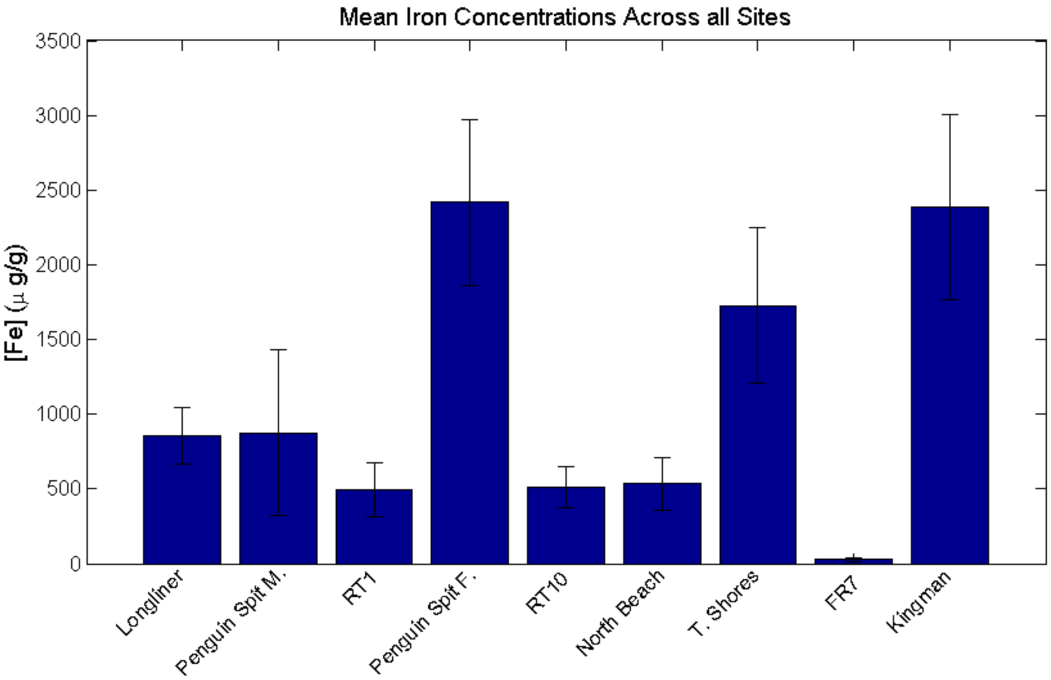

Figure 10: Mean iron concentrations of corallimorph tissue for all sites around Palmyra and at Kingman reef. Bars reflect standard error.

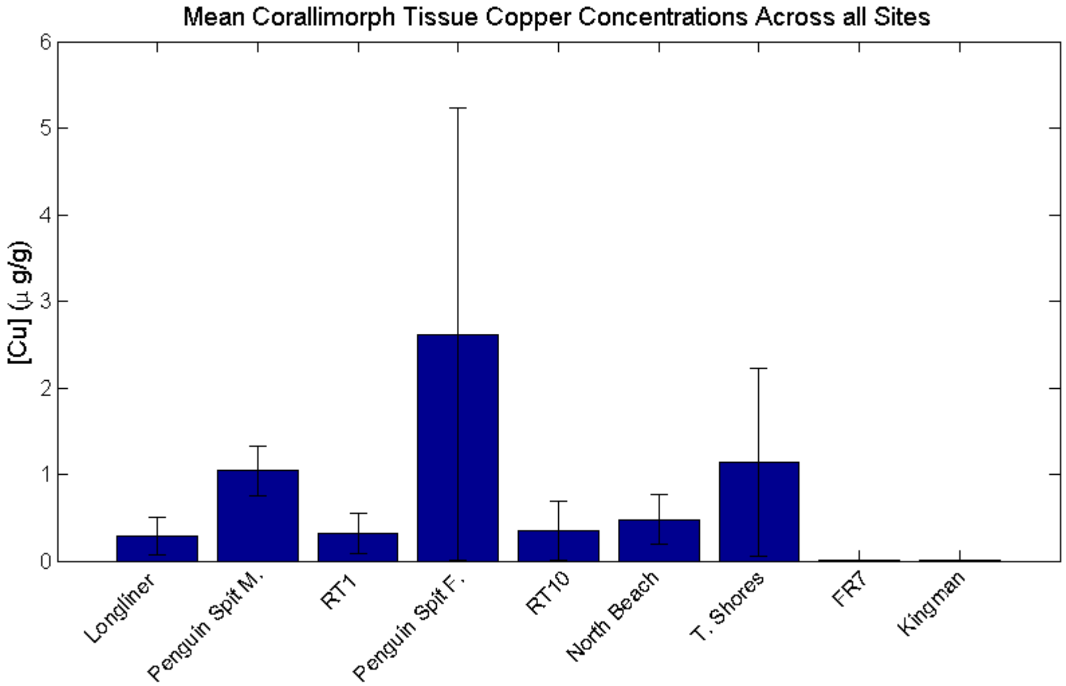

Figure 11: Mean copper concentrations from corallimorph tissue for all sites around Palmyra and at Kingman Reef. Bars reflect standard error.

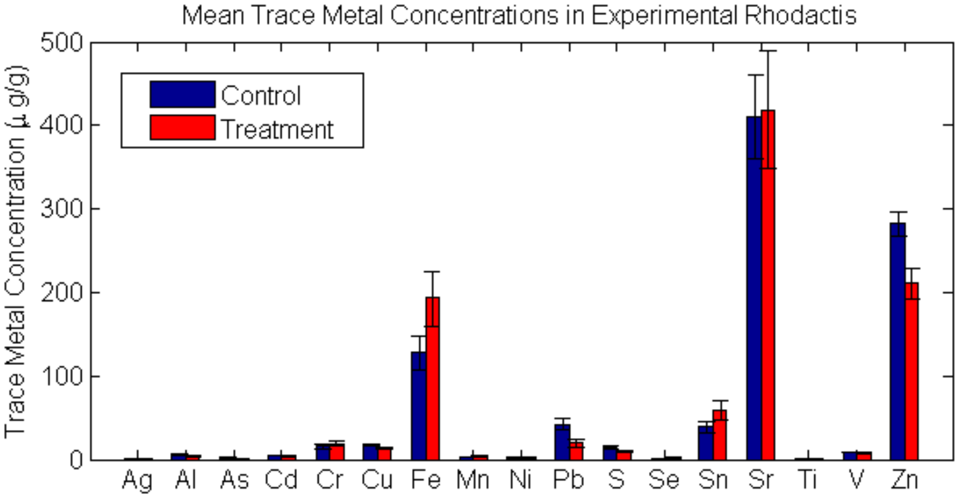

Figure 12: Mean trace metal tissue concentrations from control and treated *Rhodactis* in growth experiment. Bars represent standard error.

Table 1. Corallimorph collection sites for population genetics with associated distances from the Longliner wreck.

Site Name	Site Code	Depth (m)	Distance from Wreck (km)
Longliner Wreck	LLW	5	0
Reef Terrace 10	RT10	5	1.39
Penguin Spit Forereef	FR3	10	1.35
North Beach	FR7	10	5.00
Tanager South Shore	FR5	10	4.99
Kingman Reef	KR	5	70

Table 2. *Rhodactis howesii* tissue collection site data for trace metal analysis.

Site Name	Site Code	Depth (m)	Distance from Wreck (km)	Reps
Longliner Wreck	LLW	5	0	19
Reef Terrace 10	RT10	5	1.39	11
Reef Terrace 1	RT1	5	1.276	9
Penguin Spit Middle	PSM	5	1.18	10
Penguin Spit Forereef	FR3	10	1.35	5
Tanager South Shore	FR5	10	4.99	10
North Beach	FR7	10	5.00	16
Kingman Reef	KR	5	70	15

Table 3. Permanent and temporary site data from 2010 monitoring trip. "Dist" represents distance in meters from the shipwreck and depth code represents "F" for fore reef, "T" for reef terrace, or "B" for back reef with the associated meter depths.

SiteCode	Site	Type	Depth Code	DIST
DRT1	PANWR_Drift1	Monitoring	F20	3238.54
DRT2	PANWR_Drift2	Monitoring	F20	3552.96
ET1	Eastern Tip 1	Monitoring	F20	12869.5
ET3	Eastern Tip 3	Monitoring	F20	12697.25
FR3	SIO Fore Reef 3	Monitoring	F20	1349.09
FR3	SIO Fore Reef 3	Permanent	F10	1349.09
FR5	SIO Fore Reef 5	Monitoring	F20	4986.52
FR5	SIO Fore Reef 5	Permanent	F10	4986.52
FR7	Fore Reef 7	Monitoring	F20	5000.25
FR7	Fore Reef 7	Permanent	F10	5000.25
FR9	Fore Reef 9	Monitoring	F20	2348.86
FR9	Fore Reef 9	Permanent	F10	2348.86
GBR	G-Banger	Monitoring	T05	1339.88
LLW	Longliner	Permanent	T05	0
FR5	SIO Fore Reef 5	Monitoring	F05	4986.52
NBR	North Barren	Monitoring	B05	8583.79
NBW	NBeachW	Monitoring	F10	4325.16
PA14	PALF14	Monitoring	F20	8114.73
PA14	PALF14	Monitoring	F10	8114.73
PA17	PALF17	Monitoring	F10	7389.58
PA17	PALF17	Monitoring	F20	7389.58
PA2	PALF2	Monitoring	F05	2426.92
PA2	PALF2	Monitoring	F20	2426.92
PA2	PALF2	Monitoring	F10	2426.92
PA22	PALF22	Monitoring	F05	2356.2
PA22	PALF22	Monitoring	F20	2356.2
PA22	PALF22	Monitoring	F10	2356.2
PA25	PALF25	Monitoring	F05	7820.76
PA25	PALF25	Monitoring	F20	7820.76
PA25	PALF25	Monitoring	F10	7820.76
PSI	Penguin Spit Inner	Permanent	B05	1472.07
PSM	Penguin Spit Middle	Permanent	B05	1177.76

Table 3. Continued

SiteCode	Site	Type	Depth Code	DIST
PSO	Penguin Spit Outer	Permanent	B05	1256.14
RT1	Reef Terrace 1	Monitoring	T05	1275.84
RT10	Reef Terrace 10	Monitoring	T05	1386.29
RT13	Reef Terrace 13	Permanent	T05	1034.18
RT22	Reef Terrace 22	Permanent	T05	1237.71
RT23	Reef Terrace 23	Monitoring	T05	265.35
RT25	Reef Terrace 25	Monitoring	T05	1728.62
RT30	PAL-30-P-B	Permanent	T05	522.04
RT4	Reef Terrace 4	Permanent	T05	696.24
RT6	Reef Terrace 6	Monitoring	T05	1317.66
RT7	Reef Terrace 7	Monitoring	T05	921.18
TRT	Tortugonas	Permanent	T05	1677.24
UVB	Uvic_Bird	Monitoring	F20	6243.75
UVB	Uvic_Bird	Permanent	F10	6243.75
UVP	Uvic_Paradise	Monitoring	F20	3712.59
UVP	Uvic_Paradise	Permanent	F10	3712.59
WT7	Western Tip 7	Monitoring	F20	3089.83
WT8	Western Tip 8	Monitoring	F20	1443.36

Table 4. PRIMER 6+ PERMANOVA site-wide analysis summary statistics.

<table>
<tr><th colspan="12">Resemblance Matrix</th></tr>
<tr><td colspan="2">Source; df</td><td colspan="2">SS</td><td colspan="2">MS</td><td colspan="2">Pseudo-F</td><td colspan="2">P(perm)</td><td colspan="2">Unique perms</td></tr>
<tr><td colspan="2">Site; 8</td><td colspan="2">741.59</td><td colspan="2">92.699</td><td colspan="2">6.83555</td><td colspan="2">0.001*</td><td colspan="2">997</td></tr>
<tr><th colspan="12">Pair-wise Tests</th></tr>
<tr><td colspan="3">Groups</td><td colspan="3">t</td><td colspan="3">P(perm)</td><td colspan="3">Unique perms</td></tr>
<tr><td colspan="3">Longliner, FR7</td><td colspan="3">2.9879</td><td colspan="3">0.0012*</td><td colspan="3">9319</td></tr>
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<tr><td colspan="3">Longliner, RT1</td><td colspan="3">1.726</td><td colspan="3">0.052</td><td colspan="3">9879</td></tr>
<tr><td colspan="3">Longliner, RT10</td><td colspan="3">0.98566</td><td colspan="3">0.3368</td><td colspan="3">9928</td></tr>
<tr><td colspan="3">Longliner, FR5</td><td colspan="3">2.4341</td><td colspan="3">0.0019*</td><td colspan="3">9918</td></tr>
<tr><td colspan="3">Longliner, Kingman</td><td colspan="3">3.6068</td><td colspan="3">0.0002*</td><td colspan="3">9941</td></tr>
</table>

Table 5: *Post hoc* SIMPER results from site-wide trace metal analysis. Metals that showed no significant contribution levels were removed. The top three contributing metals for the Longliner signature are highlighted in grey.

Site Comparison	**Metal Contributions (%)**								
	Al	**As**	**Cd**	**Cr**	**Fe**	**Mn**	**Sr**	**V**	**Zn**
Longliner vs. Fore Reef 7	21.56			21.16	22.09	.46		6.86	15.27
Longliner vs. Penguin Spit Fore Reef	17.02	8.34		37.84	6.36	5.63		7.62	7.19
Longlinver vs, Penguin Spit Middle	17.14	2.77		23.87	21.87	5.41		6.90	13.30
Longliner vs. RT1	26.92			21.86	14.76	3.56		6.9	16.53
Longliner vs. RT10	29.56			23.51	13.5	4.8		6.2	12.7
Longliner vs. Tanager S. Shore	17.29		2.88	28.0	3.8	4.12		6.06	30.6
Longliner vs. Kingman	23.43			27.99	5.10	7.36	11.95	5.59	9.32

Table 6. Statistical results examining differences in growth rates within *Rhodactis howesii* to experimental and control treatments.

Wilcoxon/Kruskal-Wallis Test			
1-way Test	X^2	df	p value
Treatment	8.4621	1	0.0036*

Table 7. PRIMER 6 + PERMANOVA growth experiment statistics.

Resemblance Matrix			
Groups	t	P (perm)	Unique perms
Control, Treatment	1.8896	0.0006	9557

Table 8: *Post hoc* SIMPER results of growth experiment PERMANOVA showing relative contributions of each metal. Highlighted metals represent the top three contributors to the Longliners metal signature.

Variable	Group Control	Group Treatment	Metal Contribution (%)	Cumulative (%)
Lead (Pb)	3.62	2.72	19.95	19.95
Arsenic (As)	0.715	0.561	11.33	31.27
Tin (Sn)	3.51	3.98	9.16	40.43
Selenium (Se)	0.268	0.992	8.59	49.02
Iron (Fe)	4.72	5.17	7.83	56.85
Croumium (Cr)	2.65	2.9	6.50	71.14
Aluminum (Al)	1.84	1.4	5.35	76.48
Strontium (Sr)	5.93	5.92	4.90	81.38
Sulfur (S)	2.73	2.35	3.06	84.44
Manganese (Mn)	1.4	1.45	2.61	87.05
Cadmium (Cd)	1.67	1.43	2.46	89.52
Titanium (Ti)	0.332	0.119	2.38	91.90
Zinc (Zn)	5.63	5.32	2.21	94.11
Copper (Cu)	2.91	2.6	1.85	95.56
Silver (Ag)	0.92	0.765	1.81	97.77
Nickel (Ni)	1.21	1.09	1.38	99.15
Vanadium (V)	2.31	2.18	0.85	100.00

Table 9: Total number of plots that contained *Rhodactis* across sites from 2010 – 2013.

Site	Count of 2010	Count of 2013	Colonization (plots/year)
FR3_F10	1	1	0
FR5_F10	1	7	2
LLW_T05	10	10	0
PSI_B05	9	10	0.33
PSM_B05	3	7	1.33
PSO_B05	4	6	0.67
RT30_T05	1	1	0

References

Bellwood, D. R., Hughes, T. P., Folke, C., and M. Nyström, (2004), Confronting the coral reef crisis, Nature, v 429, p 827-833.

Bruno, J. F., Sweatman, H., Precht, W. F., Selig, E. R., and V. G. W. Schutte, (2009), Assessing evidence of phase shifts from coral to macroalgal dominance on coral reefs, Ecology, v 90:6, p 1478-1484.

Chadwick-Furman, N. E., and Spiegel, M., (2000), Abundance and clonal replication in the tropical corallimorpharian Rhodactis rhodostoma, Invertebrate Biology, v 119(4), p 351 – 360.

Chen, C. A., Willis, B. L., and D. J. Miller, (1996), Systematic relationships between tropical corallimorpharians (Cnidaria: Anthozoa: Corallimorpharia): Utility of the 5.8S and Internal Transcribed Spacer (ITS) regions of the rRNA transcription unit, Bulletin of Marine Sceince, v 59(1), p 196 – 208.

Conklin, E. J., and J. E. Smith, (2005), Abundance and spread of the invasive red algae, Kappaphycus spp., in Kane'ohe Bay, Hawai'i, and an experimental assessment of management options, Biological Invasions, v 7(6), p 1029 – 1039.

Deheyn, D. D., Gendreau, P., Baldwin, R. J., and M. I. Latz, (2005), Evidence for enhanced bioavailability of trace elements in a marine ecosystem of Deception Island, a volcano in Antartica, Marine Environmental Research, v 60, p 1-33.

Done, T. J., (1992), Phase shifts in coral reef communities and their ecological significance, Hydrobiologia, v 247, p 121 – 132.

Folke, C., Carpenter, S., Walker, B., Scheffer, M., Elmqvist, T., Gunderson, L., and C. S. Holling, (2004), Regime shifts, Resilience, Biodiversity in Ecosystem Management, Annual Review of Ecology, Evolution, and Systematics, v 35, p 557 – 581.

Hughes, T. P., (1994), Catastrophes, Phase Shifts, and Large-Scale Degradation of a Caribbean Coral Reef, Science, v 265(5178), p 1547 -1551.

Hughes, T. P., Baird, A. H., Bellwood, D. R., Card, M., Connolly, S. R., Folke, C., Grosberg, R., Hoegh-Guldberg, O., Jackson, J. B. C., Kleypas, J., Lough, J. M., Marshall, P., Nyström, M., Palumbi, S. R., Pandolfi, J. M., Rosen, B., and J. Roughgarden, (2003), Climate Change, Human Impacts, and the Resilience of Coral Reefs, Science, v 301, p 929 – 933.

JMP®, Version 10. SAS Institute Inc., Cary, NC, 1989-2007.

Kuguru, B., Winter, G., Beer, S., Santos, S. R., and N. E. Chadwick, (2007), Adaptation strategies of the corallimorpharian *Rhodactis rhodostoma* to irradiance and temperature, Marine Biology, v 151, p 1287 -1298.

Lapointe, B., (1997), Nutrient Rhresholds for Bottom-up Control on Macroalgal Blooms on Coral Reefs in Jamaica and Southeast Florida, Limnology and Oceanography, v 42(5), p 1119 – 1131.

Loya, Y., Lubinevsky, H., Rosenfeld, M., and E. Kramarsky-Winter, (2004), Nutrient enrichment caused by in situ fish farms at Eilat, Red Sea is detrimental to coral reproduction, Marine Pollution Bulletin, vol 49(4), p 344 -353.

Neff, J. M., (1997), Ecotoxicology of arsenic in the marine environment, Environmental Toxicology and Chemistry, v 16(5), p 917-927.

Norström, A. V., Nyström, M., Lokrantz, J., C. Folke, (2009), Alternative states on coral reefs: beyond coral-macroalgal phase shifts, Marine Ecology Progress Series, v 376, p 295-306.

OTA, (1993), Harmful Non-Indigenous Species in the United States, Office of Technology Assessment, United States Congress, Washington, DC.

Perkol-Finkel, S., Shashar, N., and Y. Benayahu, (2006), Can artificial reefs mimic natural reef communities? The roles of structural features and age, Marine Environmental Research, v 61, p 121 – 135.

Reichelt-Bruschett, A. J., and P. L. Harrison, (1999), The Effect of Copper, Zinc, and Cadmium on Fertilization Success of Gametes from Scleractinian Reef Corals, Marine Pollution Bulletin, v 38(3), p 182-187.

Reichelt-Brushett, A. J., and P. L. Harrison, (2005), The effect of selected trace metals on the fertilization success of several scleractinian coral species, Coral Reefs, v 24, p 524-534.

Ruiz, G. M., and J. T. Carlton, (2003), Invasive Species: Vectors and Management Strategies, Nature, Island Press.

Sandin, S. A., Smith, J. E., DeMartini, E. E., Dinsdale, E. A., Donner, S. D., Friedlander, A. M., Konotchick, T., Malay, M., Maragos, J. E., Obura, D., Pantos, O., Paulay, G., Richie, M., Rohwer, F., Schroeder, R. E., Walsh, S., Jackson, J. B. C., Knowlton, N., and E. Sala, (2008), Baselines and Degradation of Coral Reefs in the Northern Line Islands, PLoS ONE, v 3(2): e1548. doi:10.1371/journal.pone.0001548.

Schroeder, R. E., Green, A. L., DeMartini, E. E., and J. C. Kenyon, (2008), Long-term effects of a ship-grounding on coral reef fish assemblages at Rose Atoll, American Samoa, Bulletin of Marine Science, v 82(3), p 245 -264.

SigmaPlot version 11.0, from Systat Software, Inc., San Jose California USA, www.sigmaplot.com.

Smith, J. E., Hunter, C. L., C. M. Smith, (2002), Distribution and reproductive characteristics of nonindigenous and invasive marine algae in the Hawaiian islands, Pacific Science, v 56(3), p 299-315.

Smith, J. E., Shaw, M., Edwards, R. A., Obura, D., Pantos, O., Sala, E., Sandin, S. A., Smriga, S., Hatay, M., and F. L. Rohwer, (2006), Indirect effects of algae on coral: algae-mediated, microbe-induced coral mortality, Ecology Letters, v 9, p 835-845.

Stobart, B., Teleki, K., Buckley, R., Downing, N., and M. Callow, (2005), Coral recovery at Aldabra Atoll, Seychelles: five years after the 1998 bleaching event, Philos Trans R Soc A, v 363, p 251 – 255.

Ward-Paige, C. A., Risk, M. J., Sherwood, O. A., and W. C. Jaap, (2005), Clionid sponge surveys on the Florida reef tract suggest land-based nutrient inputs, Marine Pollution Bulletin, v 51, p 570-579.

Wegley Kelly, L., Barott, K. L., Dinsdale, E., Friedlander, A. M., Nosrat, B., Obura, D., Sala, E., Sandin, S. A., Smith, J. E., Vermeij, M. J. A., Williams, G. J., Wilner, D., and F. Rohwer, (2012), Black reefs: Iron-induced phase shifts on coral reefs, The International Society for Microbial Ecology, v 6, p 638 – 649.

Williams, G. J., Smith, J. E., Conklin, E. J., Gove, J. M., Sala, E., and S. A. Sandin, (2013), Benthic communities at two remote Pacific coral reefs: effects of reef habitat, depth, and wave energy gradients on spatial patters, PeerJ, v 1, p e81.

Winterbourn, M. J., McDiffett, W. F., and S. J. Eppley, (2000), Aluminum and iron burdens of aquatic biota in New Zealand streams contaminated by acid mine drainage: effects of trophic level, The Science of the Total Environment, v 254, p 45 – 54.

Work, T.M, Aeby, G. S., and J. E. Maragos, (2008), Phase Shift from a Coral to a Corallimorph-Dominated Reef Associated with a Shipwreck on Palmyra Atoll, PLoS ONE, v 3(8), e 2989.