Final report. Submitted by Dr. Greta Aeby. 7/30/13

A. **Award Number**: NA11NOS4820018

B. **Project Title:** Pathogenesis, associated morbidity and potential control of *Acropora* growth anomalies in American Samoa

C. **Total Amount of Award:** $124,396.00

Federal funds= $62,008.00; Matching funds = $62,388.00

D. **Federal Funds received to date:** $62,008.00

E. **Award Period:** 7/01/2011-6/30/13 (no cost 6 month extension)

F. **Period Covered by this Report:** 7/01/2011-6/30/2013

G. **Summary of Progress and Expenditures to Date:**

Introduction

Coral disease has emerged as a serious problem worldwide and models of climate change predict increases in coral bleaching and disease. Resources managers are in the difficult position of having to plan for damage from increases in diseases and development of strategies to maintain coral reef resources. This requires a good working knowledge of which coral diseases pose a serious threat to reefs and the development of tools to mitigate damage from disease. Within American Samoa, the coral reef ecosystem has supplied its people with food, shoreline protection and other goods and services. Yet now, coral reefs are being degraded by both human induced impacts (land-based pollution and overfishing) as well as impacts from climate change (coral bleaching and disease) (Fenner et al. 2008). Concern for the future status of American Samoa's reefs, in the face of global climate change, have led local managers to identify coral bleaching and disease as major threats and a priority focus for research and management (Fenner et al. 2008). Already numerous coral diseases have been documented on the reefs of American Samoa but at a low prevalence (Aeby et al. 2009, Vargas-Angel and Wheeler 2009) leaving reef managers well positioned to develop proactive strategies to minimize future damage from diseases. However, of particular concern to managers is the lack of information as to which diseases are creating the greatest impacts and what can be done to manage them. Development of management strategies requires an understanding of basic disease processes (etiology, pathogenesis, mode of transmission, etc.). Within American Samoa, acroporid corals are especially vulnerable to diseases including *Acropora* growth anomalies (AGAs) (Fig.

1)(Aeby et al. 2009, Vargas-Angel and Wheeler 2009). Although the causes of GAs in corals are unknown, they are associated with reduced colony growth (Cheney 1975, Bak 1983), partial colony mortality (Peters et al. 1986, Work et al. 2008), and decreased reproduction (Yamashiro et al. 2000, Work et al. 2008b) and therefore could negatively impact the fitness of host populations. Acroporids worldwide, appear to be highly susceptible to GAs and have been recorded in over 17 species from the Florida Keys and across the Indo-Pacific (Coles & Seapy 1998, Peters et al. 1986, Sutherland et al. 2004, Work et al. 2008, Williams et al. 2008). Prior work in American Samoa found AGAs to a common disease affecting four species of *Acropora* (*Acropora cytherea, A. hyacinthus, A. clathrata, A. abroitanoides*) (Aeby et al. 2009, Aeby et al., unpub. data), yet little is known of the pathogenesis, etiology or virulence of this disease. Our objectives were to investigate the epizootiology of AGAs including disease prevalence, incidence, etiology, progression (pathogenesis) and associated morbidity from the disease in terms of changes in colony growth, reproduction or mortality.

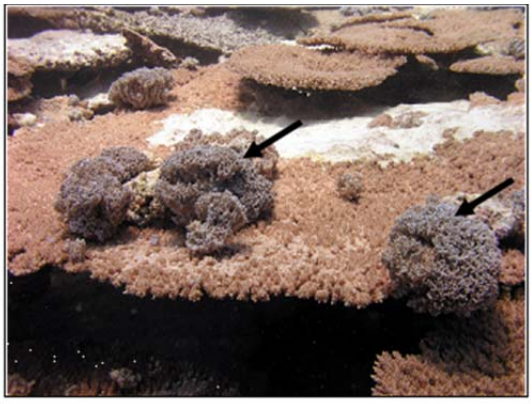

Figure 1. *Acropora cytherea* with growth anomalies (black arrows).

One strategy for promoting coral reef resilience in the face of global climate change is to reduce or eliminate other stressors (Marshall & Schuttenberg 2006). Hence, we also tested a mechanism of disease management. Coral disease management has successfully been used for black band disease in the Florida Keys and *Turbinaria* white syndrome in Australia. Hudson (2000) successfully treated black band disease (70% effective) by removing the pathogen by suction and covering the affected area with modeling clay. In Australia, Dalton et al. (2010) found that mechanical removal of the advancing disease margin for *Turbinaria* colonies affected by a tissue loss disease ("white syndrome") was successful at halting the disease in 80% of the colonies. Although information regarding the treatment of coral diseases is still limited, these two studies show that disease management can be accomplished in the marine environment.

Project objectives

1. Conduct quantitative surveys to determine the prevalence (proportion of colonies affected) and incidence (change in disease through time) of *Acropora* growth anomalies in American Samoa**.**

2. Determine the effect of growth anomalies on the health (growth, mortality, reproduction) of *Acropora* colonies.
3. Determine the rate of disease progression of *Acropora* growth anomalies.
4. Determine whether surgical removal can be used to manage *Acropora* growth anomalies on reefs.
5. Examine the microscopic morphology of lesions (*Acropora* growth anomalies) at the light and electron microscopy levels

Methods

Our team traveled to Tutuila, American Samoa in September 2011 to pick a study site and initiate the work. Originally, we wanted to conduct the study in Vatia but a hurricane came through that demolished the reefs. We conducted towed diver surveys throughout Vatia but there were few intact *Acropora* table corals remaining. A quick towed-diver survey of the reefs within Fagatele Bay found little evidence of hurricane damage with abundant *Acropora* table corals. Hence, we set up our study within Fagatele Bay National Marine Sanctuary (FBNMS).

Within FBNMS ($S14^0$ 21.991 $W170^0$ 45.754), three belt transect were laid out and marked with cow tags at 5 meter intervals. All *Acropora* table corals, (*A. cytherea, A. hyacinthus, A. clathrata*), with growth anomalies within the belt transects were tagged, mapped, and photographed (n=17). An equal number of healthy *Acropora* table corals were also tagged, mapped and photographed (n=17). Growth anomalies were removed from 5 of the mapped colonies with the remaining colonies left untreated. Samples were collected from the 5 treated colonies as well as 5 control colonies for laboratory analyses of reproductive output and scanning for viruses using electron microscopy (EM).

Results and discussion

Objective 1. Conduct quantitative surveys to determine the prevalence (proportion of colonies affected) and incidence (change in disease through time) of *Acropora* growth anomalies in American Samoa.

Surveys were conducted in Fagatele Bay in 2011 and again in 2012. We found coral cover to be stable (56.3% in 2011 and 65.6% in 2012) with no significant change in prevalence of *Acropora* growth anomalies (AGA) (1.9% in 2011 and 1.5% in 2012). Interestingly, we found a reduction in crustose coralline algae (CCA) cover (39.3% in 2011 and 29.4% in 2012). An opportunistic survey of CCA disease at this site (coralline lethal orange disease (Littler and Littler 1995) and black fungal disease (Littler and Littler 1998) showed high levels of these diseases and an increase in disease from 2011 to

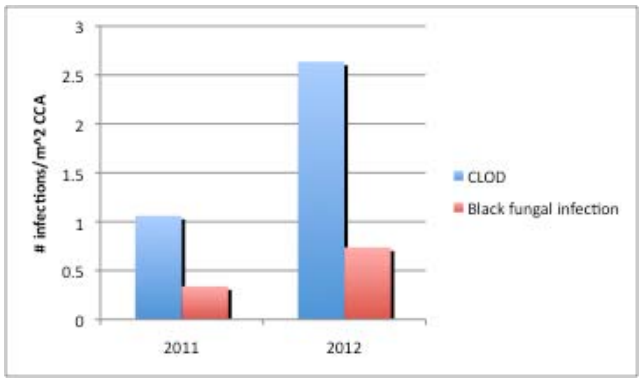

Fig. 2. Change in levels of crustose coralline algae diseases at one site in Fagatele Bay.

2012 (Fig. 2). These CCA disease levels are higher than previously reported from American Samoa (Aeby et al. 2008).

Objective 2. Determine the effect of growth anomalies on the health (growth, mortality, reproduction) of *Acropora* colonies.

To determine the effect of growth anomalies on the health of colonies, 12 *Acropora hyacinthus* colonies with growth anomalies (GAs) were tagged and left intact, 5 colonies with GAs were tagged and their GAs removed and 16 healthy *A. hyacinthus* colonies were tagged as controls. All colonies were located and photographed a year later and digital analysis used to determine overall colony growth. Colony sizes (perimeters) of GA affected ones ranged from 101.2 cm to 871.4cm and healthy control colonies ranged from 165.6cm to 894.8cm. Growth anomalies ranged in size from 0.5cm^2 to 72cm^2. All *A. hyacinthus* with GAs (removed or not) showed a reduction in colony size whereas most healthy colonies showed an increase in colony size (Fig. 3). Most of the reduction in colony size appeared to be from breakage with portions of the table completely missing as opposed to the coral skeleton being in place but dead. It may be that the chronic energy drain, created by GAs, is leaving colonies less resilient to withstand the usual stressors such as strong water flow commonly found at that site. GA colonies also had a higher occurrence of partial colony mortality as compared to healthy corals (Fig. 4). The case fatality rate was much lower for AGA (8.3%) as compared to *Acropora* white syndrome with case fatality rates usually around 50% (Aeby et al. 2011).

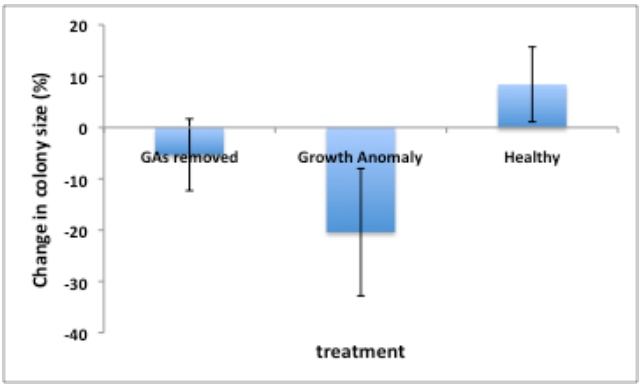

Fig. 3. Effect of GAs on growth of *A. hyacinthus* colonies.

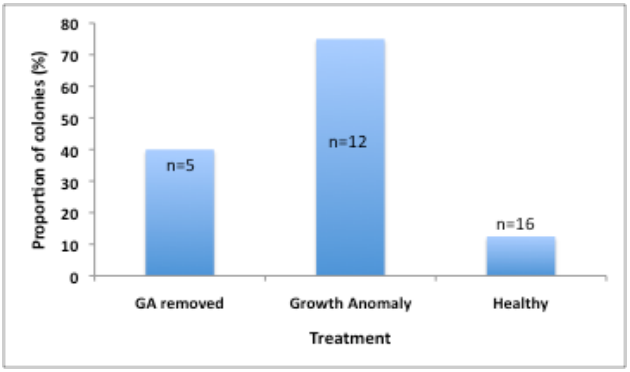

Fig. 4. Proportion of tagged colonies with partial to total mortality occurring between 2011 and 2012.

Reproductive output (avg. # eggs per polyp) was compared between healthy control colonies, healthy areas of GA-affected colonies and the GAs. No significant difference in number of eggs was found between the healthy controls and the healthy portion of affected colonies (Kruskal-Wallis, P>0.5). Healthy control colonies had an average of 5.72 eggs/polyp (SD±0.92) compared to healthy portion of affected colonies with an average of 5.88 eggs/polyp (SD±1.1). However, there was a significant reduction in egg number in the growth anomalies (Wilcoxon sign rank, $p<0.0001$). Only one GA out of five had any evidence of reproductive development. Hence, reduction in colony reproductive output, due to disease, would be proportional to the number and size of growth anomalies on the colony. The average proportion of the surface area of tagged colonies affected by GAs and, thus area of reduced reproductive output, ranged from 0.071 to 6.73% with a mean of 2.16% reduction in fecundity per colony.

Objective 3. Determine the rate of disease progression of *Acropora* growth anomalies.

AGA is a progressive disease with 10 out of 12 tagged colonies (83%) having an increase in number of growth anomalies between 2011 and 2012, and one of the colonies that showed no increase in number of GAs did so because it suffered 100% mortality. Increases in number of GAs per colony ranged from 1 to 8 (avg. 3.3 new GAs/colony/year) but the increase was not always straightforward as in several cases (2 of 12) the GAs from 2011 had grown into a single larger GA in 2012 (Fig 5). Partial mortality of GAs was also found in 6 of the 12 colonies (50%). It has been suggested that the GAs are areas of fast growth lacking the usual complement of zooxanthellae and so do not have the nutritional resources to continue to survive (Work et al. 2008).

Fig. 5. Picture on left shows 3 GAs on colony 16 in 2011. Picture on right shows same area with the 3 GAs growing into a single GA in 2012.

Objective 4. Determine whether surgical removal can be used to manage *Acropora* growth anomalies on reefs.

Five *A. hyacinthus* colonies had their GAs removed in 2011 to determine if surgical removal could be a method of treatment. The lesions created by GA removal healed very well in all cases but GAs regrew in 4 of the 5 colonies (Fig. 6). Some of the regrowth occurred along the margins of the original removal site, which was not surprising as it was noted at the time of removal that residual GA tissue remained along the margins. However, there was also growth of new GAs at sites away from the original removal. The five colonies with GAs removed appeared more resilient than the colonies with GAs left in place showing less partial mortality and breakage (Figs. 3 & 4). However, the sample size was small and so this treatment method would need further study to determine its effectiveness.

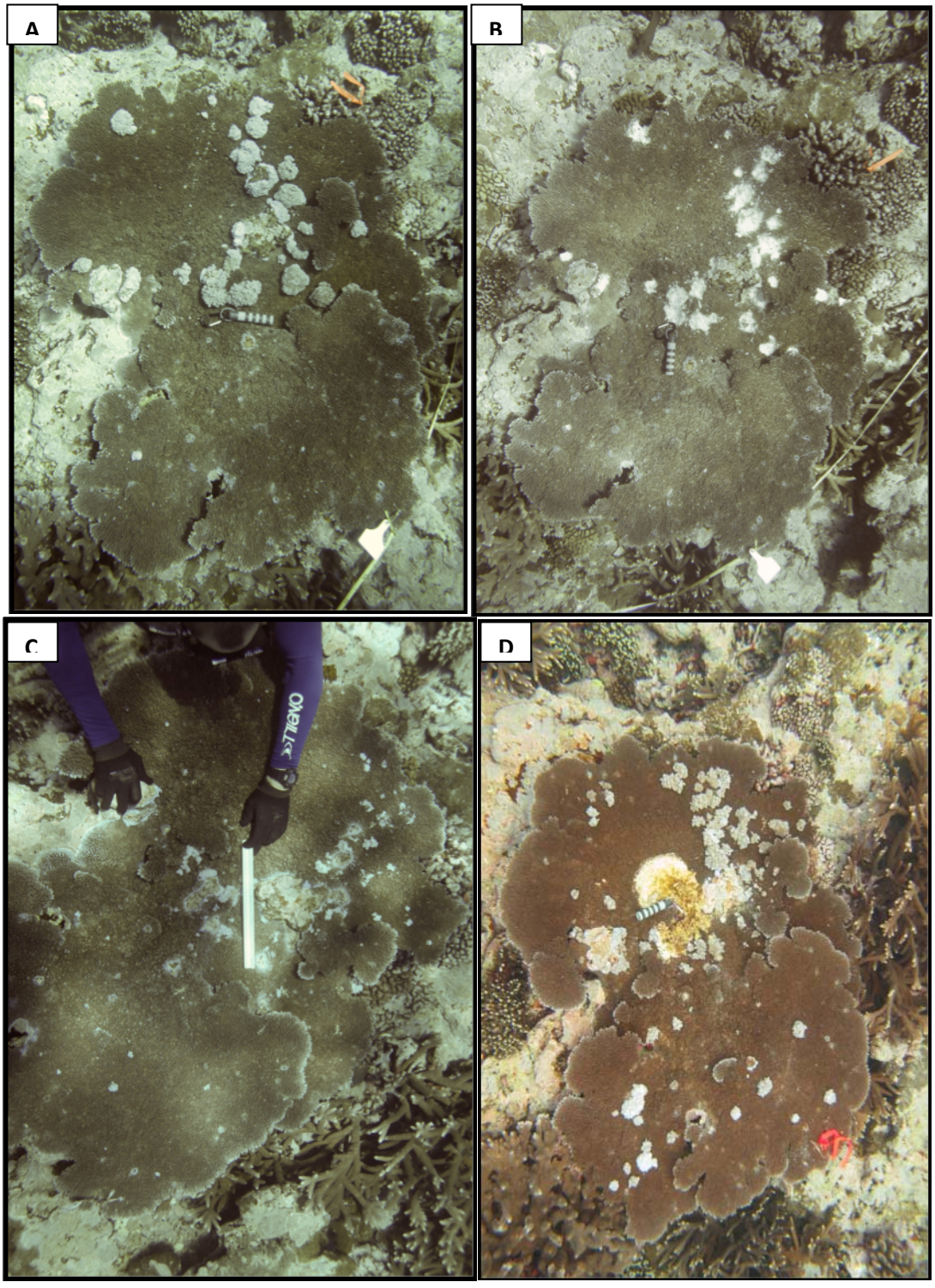

Fig. 6. Colony #10 before GA removal (A), after GA removal (B), after 3 months (C) and after 1 year (D).

Objective 5. Examine the microscopic morphology of lesions (*Acropora* growth anomalies) at the light and electron microscopy levels

Samples were collected from 5 colonies with growth anomalies as well as 5 control colonies to examine tissue using transmission electron microscopy (TEM). All of the samples were embedded in resin and were scanned for cellular changes associated with growth anomalies and evidence of viral particles. Normal tissues revealed a uniform epidermis, mesoglea, and gastrodermis with intact zooxanthellae (Figs. 7 & 8). Tumored tissues revealed fragmented nerve plexus, absence of pigment cell, tortuous and fibriated calicodermal margins, and degenerating zooxanthellae. Cells of gastrodermis, epidermis, and calicodermis were characterized by cytoplasmic vacuolation, sparse chromatin, thin stringy cytoplasm, and detaching desmoid processes (Figs. 9 & 10). No viral-like particles were observed in any of the samples. Many sections of tissue, both tumored and healthy, displayed signs of cellular abnormalities that could be indicative of environmental contaminants or stress and this should be investigated further.

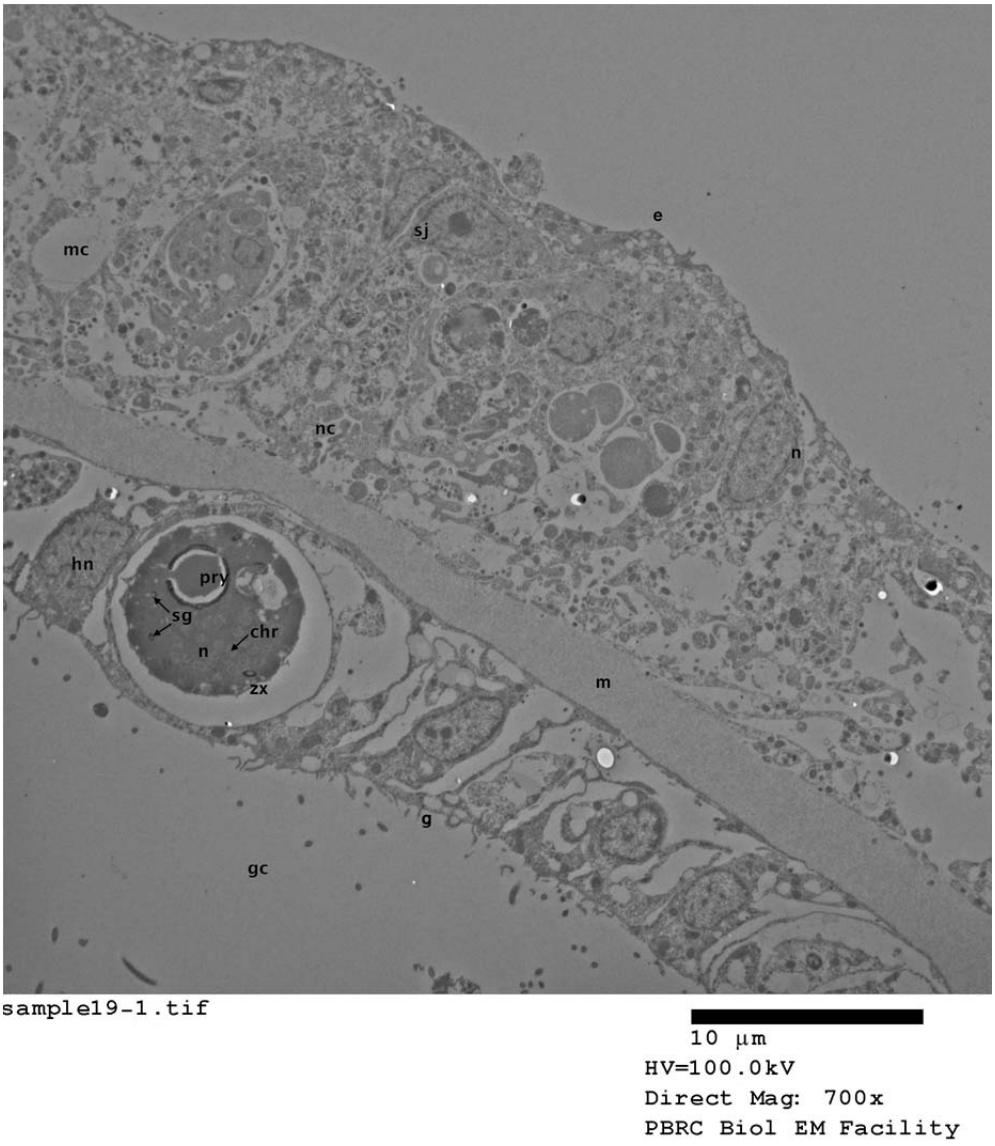

Fig. 7. TEM (700x magnification) of the epidermis, mesoglea and gastrodermis of healthy *A. hyacinthus* tissue. The gastroderm is highly vesiculated with secretory cells and symbionts within host cells with accompanying nuclei. The zooxanthellae are intact, separated from host cell by intercellular space, and contain chlorplasts (staked thalykoids), pryenoid, nucleus (with chromosmes), starch granules, and mitochondria. The lining of the gastrovasular canal is covered with microvilli. The mesoglea is well defined and borders to gastrodermis and epidermis by a smooth margin. In this section, epidermal cells have fragmented nuclei and cytoplasm with generall loss of cellular architecture. N:nucleus, zn: zooxanthellae, m:mesoglea**,** mc: mucous cell, chr: chromosome, e: epiderm, g: gastroderm, hn: host cell nucleus contained within the host cell, sj: septae junction, gc: gastrovascular canal, sg: starch granule, nc: nerve cells, pry: pryenoid

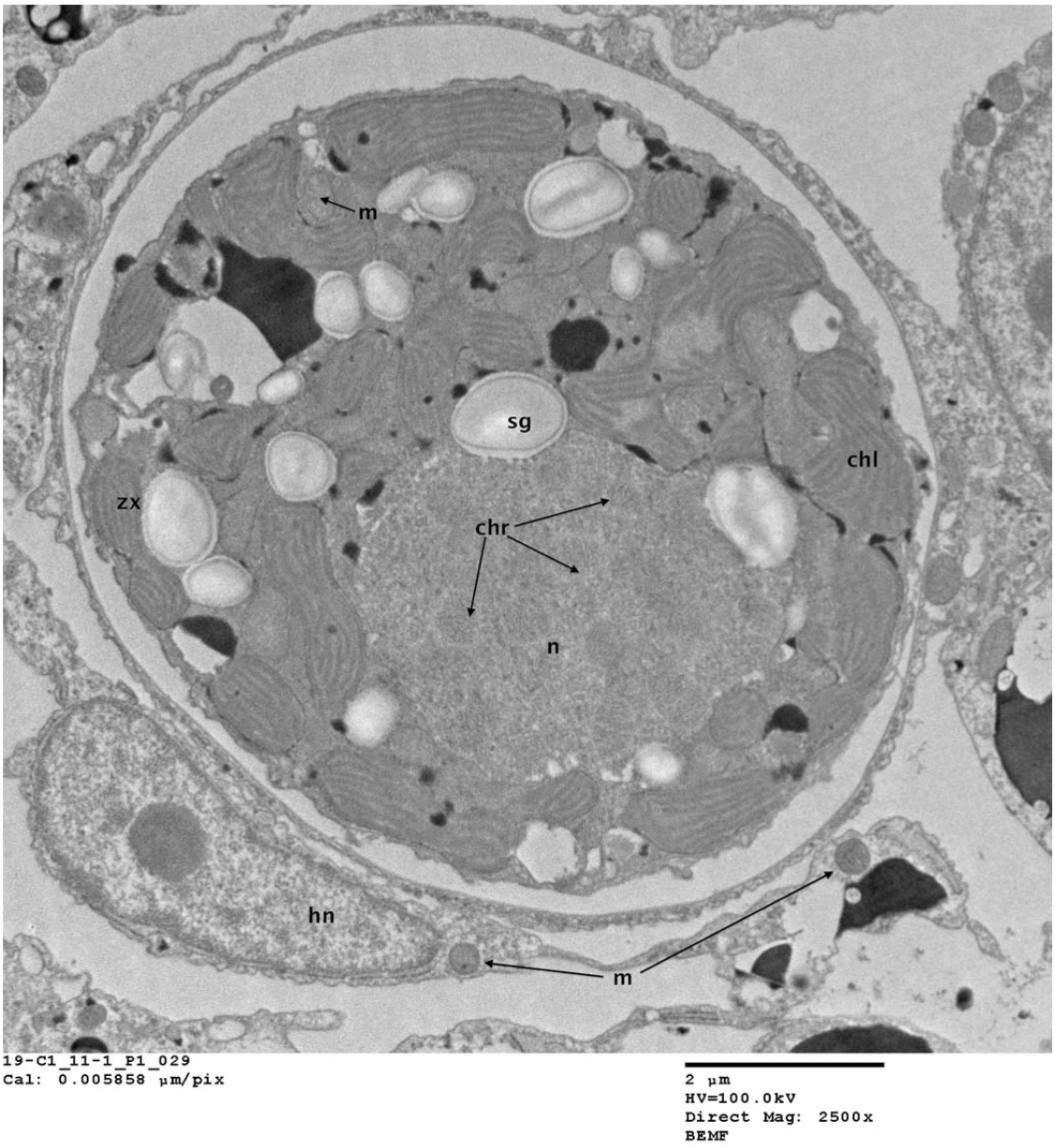

Fig. 8. TEM of a zooxanthellae located in healthy tissue from *A. hyacinthus* at 2500x magnification. The chlorplasts are lamellae comprised of closely appressed thylakoids. Starch granules have a distinct halo and a rounded appearance. The nucleus is apparent with condensed chromosomes visible. Mitochondria have well-defined membranes with cristae present. The host cell's nucleus is visible as well. N: nucleus, chl: chloroplast, sg: starch granule, m: mitochondria, chr: chromosome, hn: host cell nucleus contained within the host cell.

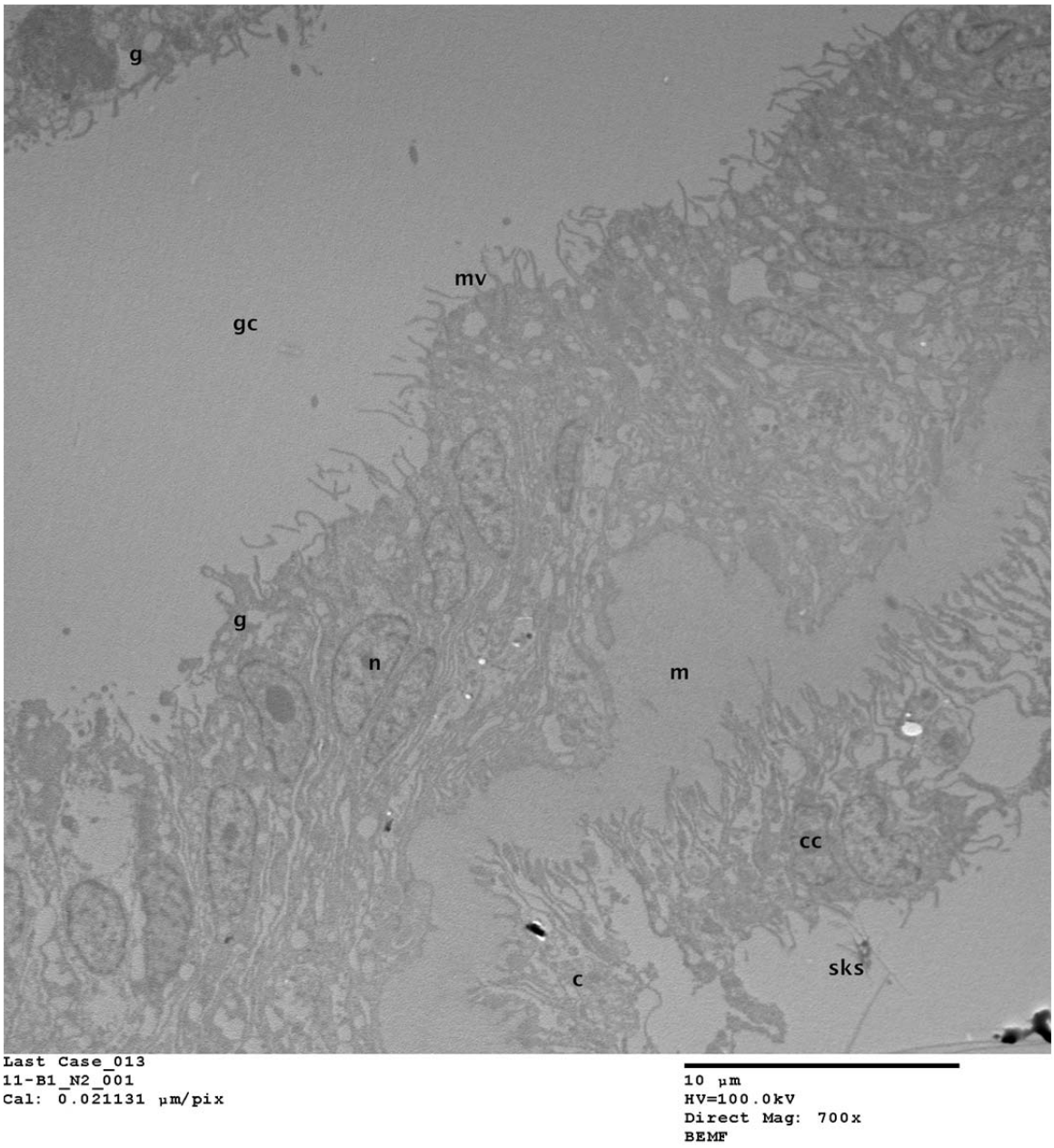

Fig 9. TEM section (700x magnification) of the gastrodermis, gastrovascular canal, the mesoglea, the calicoderm and the skeletal space of growth anomaly tissues. The gastroderm adopts a more ciliated columnar appearance, is devoid of zooxanthellae, and highly vesiculated with elongate nuclei. The mesoglea is apparent with a sinuous to undulating border with prominent dendriditic projections on from the calicodermis., on the calicodermal side, projects. The calicodermis adopts a more cuboidal and vacuolated appearance. N:nucleus, m:mesoglea, gc: gastrovascular canal, g: gastroderm, mv: microvilli, sks: skeletal space, cc: calicocyte, c: calicoderm.

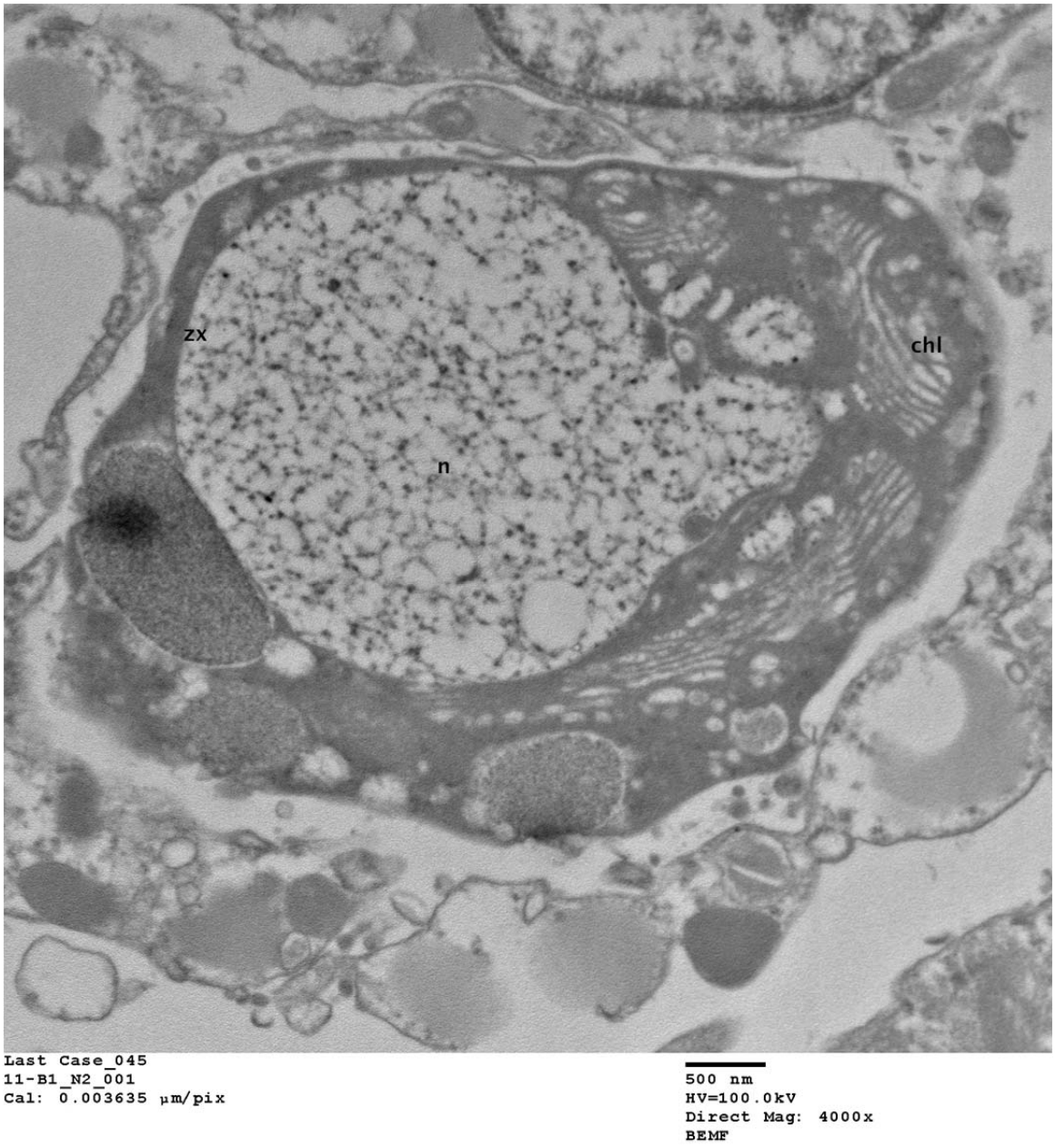

Fig 10. TEM of zooxanthellae within tumor tissue from *A. hyacinthus* at 4000x magnification. Zooxanthella is characterized by a large central amorphous vacuole with clumps of debris forming a filagree and occupying ca. 80% of the cell. Thylakoids appear disorganized and there is absence of mitochondria, distinct pyrenoid and starch granules. N: nucleus, chl: chloroplast, zx: zooxanthellae.

Project summary

- Prevalence of growth anomalies on table *Acropora* at the study site in Fagatele Bay was 1.9% in 2011 and 1.5% in 2012
- Coral cover was stable at 56.3% in 2011 and 65.6% in 2012
- Crustose coralline algae (CCA) cover declined from 39.3% in 2011 to 29.4% in 2012
- Two CCA diseases (coralline lethal orange disease and black fungal disease increased from 2011 (CLOD=1.1 lesions/m^2 CCA: BFD=0.34 lesions/m^2 CCA) to 2012 (CLOD=2.6 lesions/m^2 CCA: BFD=0.74 lesions/m^2 CCA)
- It is recommended that further CCA disease surveys be conducted and followed through time to determine if the disease increase we found was suggestive of a consistent temporal trend or simply an anomalous pattern
- Colonies with GAs had reduced growth and resilience and increased partial colony mortality as compared to healthy colonies
- Case fatality rate (total colony mortality) after one year was 8.3%
- Reproductive output of the healthy portion of GA colonies was not affected but GAs showed little to no eggs or spermeries
- Effect of GAs on colony reproduction would be proportional to the number and size of growth anomalies on the colony.
- The average proportion of the surface area of tagged colonies affected by GAs and, thus area of reduced reproductive output, ranged from 0.071 to 6.73% with a mean of 2.16% reduction in fecundity per colony.
- AGA is a progressive disease with 10 out of 12 tagged colonies (83%) having an increased number of growth anomalies after one year
- Increases in number of GAs per colony ranged from 1 to 8 (avg. 3.3 new GAs/colony/year)
- Partial mortality of GAs occured in 6 of the 12 colonies (50%).
- Removal of GAs appeared to increase colony resilience but GAs regrew in most of the treated colonies
- It is recommended that the study be repeated with a larger sample size and colonies followed further through time to determine ultimate treatment effectiveness
- No evidence of viral particles were found in GAs
- Cellular abnormalities in both healthy and GA affected tissue from colonies within Fagatele Bay were observed which could be indicative of environmental contaminants or stress and this should be investigated further

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