Final Report for Endangered Species Research Permit (NOAA) STX026-12

Title:

Staghorn survey and bioassays to test for improved condition in thickets to guide restoration efforts.

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Background:

While the degradation of Caribbean coral reefs has ignited international efforts to restore corals to denuded reefs, current methods for outplanting corals are largely lacking scientifically-based management protocols. In recent years, over 32 restoration efforts in nine Caribbean countries have been established in attempt to repopulate reefs with nursery-reared corals (Young-Lahiff et al 2012). These nurseries are focusing their restoration efforts on the staghorn coral, *Acropora cervicornis*, a "critically endangered" species on the global IUCN Red List. National Oceanic and Atmospheric Association (NOAA) is also required to formulate an *A. cervicornis* recovery plan under the Endangered Species Act, yet progress has been hindered by knowledge gaps on where and how to out-plant nursery-reared colonies to maximize restoration success. To align with the stated needs of the NOAA *Acropora* recovery plan, this research aims to develop and disseminate scientifically-informed guidance for staghorn coral outplanting to enhance the habitat and ecosystem function (NOAA 2009).

An opportunity to uncover the role of this coral species in reef ecosystem function is offered studying the handful of extant and/or newly rebounding populations of *A. cervicornis* in the Caribbean. This research is aimed to track and monitor these naturally occurring *A. cervicornis* populations of various densities to refine targets of recovery and health for this keystone species. Specifically, we aim to improve restoration efforts by determining optimal colony density, size, and configuration for outplanting colonies. Studying naturally-recovering populations as a template, this study can direct coral restoration to mimic the success of these natural populations and thereby improve restoration success.

Based on our 2012 monitoring of several extant populations of *A. cervicornis* in the Caribbean (Huntington and Miller in prep), we hypothesized that *A. cervicornis* growth rates and tissue condition are enhanced when very dense spatial configurations (i.e. "thickets") of coral are achieved. Our research from 2013 focused on observational studies and an experimental bioassay to discern a mechanism to account for this greater coral condition, which we hypothesized was from the indirect enrichment of the coral from organic nutrients excreted form fish sheltering within the thicket. To test this hypothesis, we used an experimental bioassay study in two Caribbean locations: Dry Tortugas National Park (DRTO), Florida and St. Thomas, United States Virgin Islands (USVI) to determine if coral condition was enhanced when transplanted to a thicket site versus a low density *A. cervicornis* site. While DRTO contains both high density spatial configurations of *A. cervicornis* and high abundances of schooling reef fishes, schooling fishes are rarer in the *A. cervicornis* populations in St. Thomas. By performing the same bioassay experiment in each location we can isolate the influence of reef fishes to facilitate higher condition in *A. cervicornis.*

St. Thomas Bioassay Experiment Sites:

Location	Treatment	Latitude	Longitude
Botany Bay (BB2)	Sparse	N 18.35546°	W 65.03509°
Botany Bay (BB3)	Sparse	N 18.35490°	W 65.03508°
Flat Cay (FC1)	Dense	N 18.31647°	W 64.98766°
Flat Cay (FC2)	Dense	N 18.31643°	W 64.98799°

Table 1. Locations, site names, approximate coral densities (treatment), and coordinates of the study sites.

Research Activities:

A. Co-located coral and fish surveys

Four 10 x 2m transects were conducted at each of the sites listed above. The transects were initiated at a colony of *Acropora cervicornis* and oriented non-randomly along the bottom to include the greatest amount of *A. cervicornis* as possible. The start of each transect was permanently marked with an 8" metal stake driven into non-living substrate to facilitate temporal sampling of coral growth. A fish surveyor swam the length of the transect at a constant speed over five minutes, recording each species within 1m of the transect tape. The fork length of each fish was estimated and counted in the appropriate size bin (0-5cm, 6-10cm, 11-20cm, 21-30cm, 31-40cm, >40cm).

The coral surveyor followed behind the fish surveyor, measuring every *A. cervicornis* colony along the transect whose centroid fell within 1m of the transect tape. Maximum length, width (perpendicular to maximum length), and height of each colony was measured with the aid of meter-stick with centimeter increments. If colonies were growing closely together where the

individual could not be distinguished, the group of colonies was marked as a “thicket,” and the dimensions of the cluster were recorded. Live fragments that were not cemented to the substrate were included in the survey, but the colony was noted as a unattached “fragment.” Partial mortality, paling/bleaching tissue, disease, snail predation, fireworm predation, and damselfish damage were all visually estimated for each colony as a percentage.

Total linear extension (TLE) of the branches in each *A. cervicornis* colony was estimated from colony dimensions using the regression relationship described in Huntington and Miller (2013). Estimated total TLE (including non-living skeleton) and live TLE (TLE corrected for live tissue based on visually estimated percent live tissue cover for that colony) were calculated. Live TLE from all colonies was summed to give an estimate of live staghorn density whereas total TLE, also summed from all colonies, provides an estimate of structure (including both live and dead) created by *A. cervicornis*.

B. *In-situ* apical tip growth

Apical tip growth was measured on several resident colonies at each site. Typically, one colony with more than eight apical tips was tagged at the beginning of each transect near the aforementioned metal stake (to facilitate relocation). To increase sample size, we tagged two additional colonies at Flat Cay and five additional colonies at Botany Bay. “Tagging” consisted of attaching a colored cable tie to a branch of the colony exactly 3cm from the end of a single apical tip. This was repeated eight times on each colony with different colored cable ties. All of the colonies were tagged either on 7/4/13 or 7/5/13. Dimensions of each colony (maximum length, perpendicular width, and maximum height) were also measured.

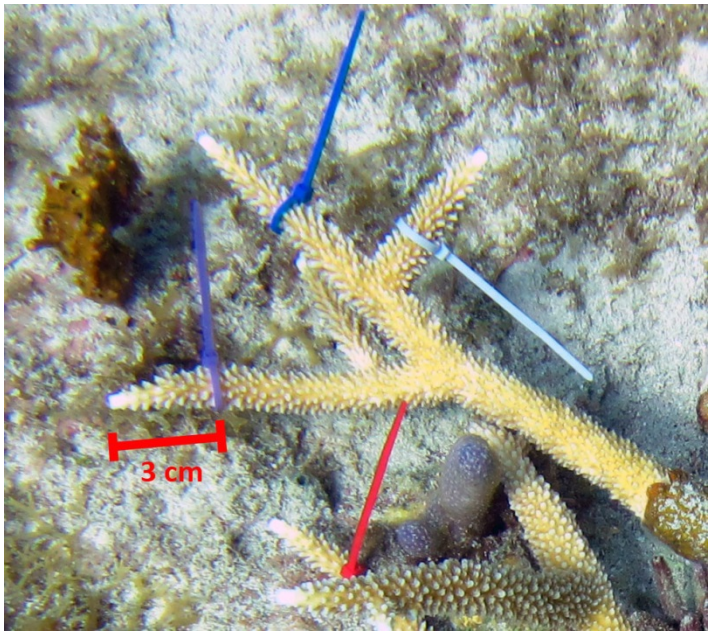

Figure 1. Single-apical tips were tagged with different colored zip ties exactly 3cm from the end of the growing tip.

After 11 weeks, we returned to measure growth. Length of each apical was measured using Vernier calipers to the closest millimeter, from the top of the cable tie to the tip. In some cases, branches exhibited partial mortality, fragmented, or were damaged. These were excluded from analyses. Growth was then calculated by subtracting the starting length (3cm) from the final length, and dividing by the number of days the tips were allowed to grow. The growth data is presented in cm per year.

C. Bioassay experiment

The objective with this experiment was to test differential growth of donor nursery coral "nubbins" in sparse versus dense assemblages (at different sites) of *A. cervicornis*. Coral nubbins were collected from coral nursery at to the west of Flat Cay, maintained by The Nature Conservancy. First, coral tips were stained with Alizarin Red dye while still on the "trees" in the nursery. The corals absorb the dye into the skeleton as they grow, leaving a pinkish layer in the skeleton. *A. cervicornis* requires about six hours of growing time, in decent sunlight, in order to absorb enough dye into the skeleton to be detectable. We attached plastic bags filled with the dye to 40 tips in the nursery, and let them sit for approximately six hours on 7/4/13. The stained colonies represented three different clones, which were evenly distributed amongst the study sites to limit variation of genetic responses on growth.

Figure 2. Left: coral nubbins being stained with Alizarin Red dye at the Flat Cay coral nursery. Right: stained nubbins being attached to polypro lines (n=10 per line) to outplant on the reef.

The stained nubbins were then removed from the donor fragment and their maximum length and basal width were measured. While underwater, stained nubbins were then affixed to four independent 5m polypro lines with cable ties, approximately 50cm apart from each other on the line, so that each line had 10 nubbins. The polypro lines were then deployed, one at each site (FC1, FC2, BB2, BB3). The lines were attached to nonliving substrate securely with cable ties to reduce effects of current and swell. In order to reduce stress on the corals when transporting them to their respective sites, the polypro lines with nubbins were placed in a cooler with wet towels.

Figure 3. Nubbins on polypro lines deployed at dense site Flat Cay (left) and sparse site Botany Bay (right)

The nubbins were allowed to grow for 66 days before being collected on 9/18/13. Survivoriship of the transplanted nubbins was high; only two of the 40 outplanted nubbins died over the course of the experiment, both at Botany Bay. After retrieving the polypro lines from the bottom, the nubbins were carefully removed from the line, individually wrapped in labelled aluminum foil packages, and immediately stowed on ice. Upon returning to the dock, they were placed in a -80°C freezer for a day before being sent to the NOAA/SEFSC office in Miami on dry ice, where they were stored in a -80°C freezer until further analysis.

Upon removal from the -80°C freezer, we measured their post-growth maximum length and width with Vernier calipers to the nearest millimeter. Tissue was then removed from the nubbins using an airbrush with deionized water. The tissue was collected in a bag to be analyzed for zooxanthellae or nutrients (see below). After thoroughly rinsing in deionized water, the nubbin skeletons were dried in a drying oven for three days at 50°C.

Nubbin Growth

After the nubbin skeletons were thoroughly dried, their mass was recorded to the nearest milligram. The volume of each nubbins was calculated by placing each nubbin in a graduated cylinder partially filled with deionized water. The volume of displaced water was then calculated to the nearest tenth of a milliliter.

After the post-growth weights and volumes were calculated, the nubbins were dried again for three days at 50°C. The new growth (whatever was not stained pink from the Alizarin) was then removed with sand paper until the only "initial" nubbin remained. The pre-growth nubbins were then weighted and volume calculated, following the aforementioned procedure. This allowed us to calculate growth in multiple ways, including the changes in length, weight, volume, and density (weight/volume).

Zooxanthellae (Symbiodinium spp.)

The coral tissue and deionized water were collected in a small plastic bag as the tissue was being removed with an airbrush. The sample was then homogenized and volume recorded. Then, 2mL of each tissue sample was centrifuged at 10,000 g for 10 minutes and supernatant discarded. The remaining sample was rinsed with deionized water, placed on the vortex mixer for approximately 10 seconds. This process was repeated two additional times to ensure the *Symbiodinium spp.* sample was adequately rinsed. The sample was fixed with 50 μL of Lugols solution to preserve the *Symbiodinium* cells for counting. Cell counts were made using a hemocytometer by loading 0.1 mL of the sample on each side of the hemocytometer and counting the number of cells in five 0.1 x 0.1mm squares on each side for a total of 10 squares counted (Fig. 4).

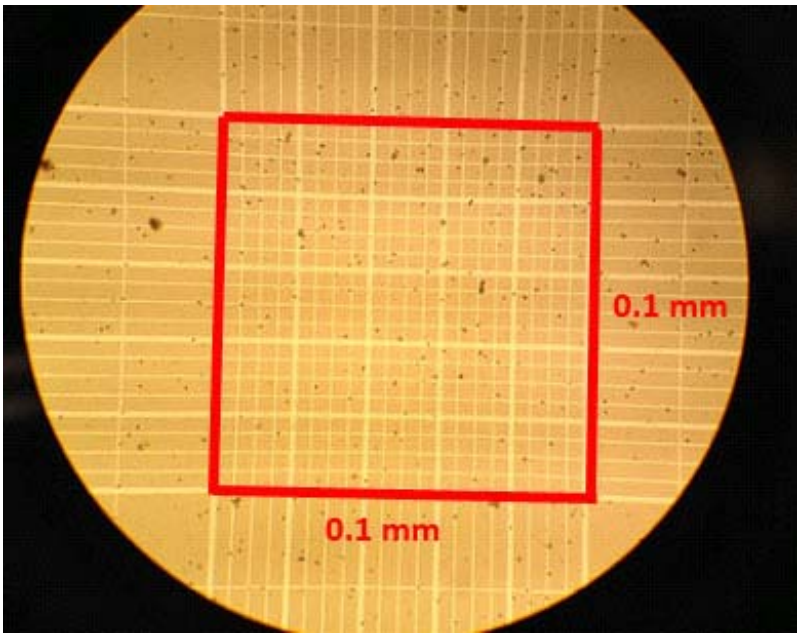

Figure 4. View of *Symbiodinium spp.* cells under the hemocytomter. Each small brown dot is a single cell, while the larger brown dots are clumps of cells or debris. The red square represents the area of one of ten replicate counts for each nubbin.

From these measurements, the mean number of cells per 0.1 μL was calculated and extrapolated to calculate the number of cells/cm^2. The surface area of the coral sample was estimated by treating the nubbin as a cylinder, with the surface area as the side of the cylinder, taking the final length measurement and middle width measurement for use in the calculation.

Nutrients

The remainder of the coral tissue sample was dried for three days at 50°C until all moisture was removed. The samples were then ground into a powder to be analyzed for carbon and nitrogen content. Phosphorus content would have also been analyzed, but there was not enough coral tissue material to conduct an accurate analysis. Additionally, the coral tissue from the Flat Cay samples (n=20) was removed with dnaB instead of deionized water, which would have confounded the nutrient analyses. Therefore, only the samples from Botany Bay sites (n=17) were analyzed for carbon and nitrogen.

Results

It is important to note that these results from St. Thomas are preliminary as they are part of a larger, NOAA Coral Reef Conservation Program grant that will be completed at the end of 2014. Hence the results presented here will likely have more meaning within the greater context of data collected over the entire course of the study (2012-2014) in other locales (Dry Tortugas, Puerto Rico, Belize, South Florida). Data collection and analysis is still underway at other sampling locales and copies of additional products/publications will be provided in due course. Some of the results provided below include comparative data from the Dry Tortugas.

A. Co-located coral and fish surveys

Acropora cervicornis abundance

The abundance of *A. cervicornis* varies substantially on a regional basis. Of the most abundant areas surveyed in both regions, the populations found on Pulaski Shoal in the Dry Tortugas are almost 8 times as dense as those found in St. Thomas, USVI (Fig. 5. As such, the binning of transects by *A. cervicornis* abundance was conducted separately for each sampling region so “sparse” and “dense” represent different abundances appropriate for each locality.

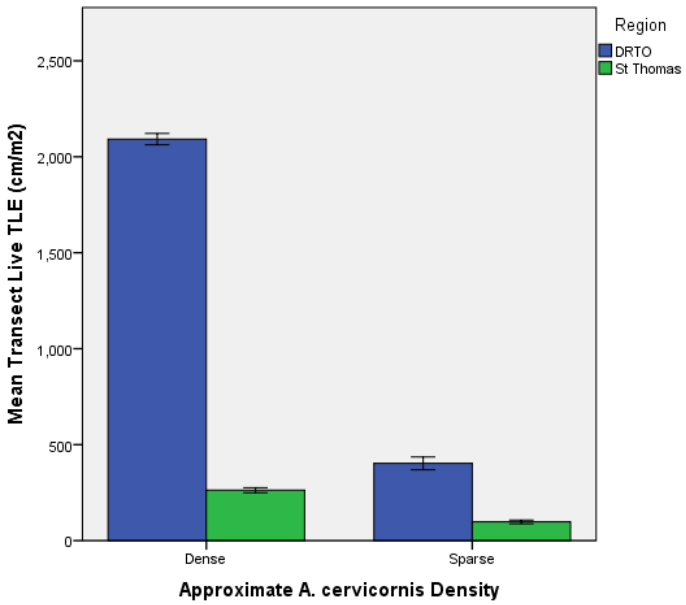

Figure 5. Mean live TLE of *A. cervicornis* per transect for densely and sparsely populated areas in DRTO and St. Thomas from 2013 sampling. The terms "dense" and "sparse" are relative for each region.

The live total linear extension (TLE) of *A. cervicornis* was over 2.5 times greater at the dense populations at Flat Cay than the sparse assemblages at Botany Bay ($t=19.896$, $p<0.001$). The colonies at dense sites displayed significantly greater partial mortality (52.4 ± 1.5%) than those at sparse sites (25.0 ± 2.3%).

None of the sparse areas in St. Thomas experienced damage from damselfish. However, colonies in dense areas had an average of 3.6 ± 0.8% of damaged coral tissue from damselfish. No predation by the corallivorous fireworm, *Hermodice carunculata*, was recorded at any of the sites in St. Thomas (in contrast to our observations in DRTO). Snail predation by *Coralliophila abbreviata* was minimal, affecting only 6 of the 199 colonies surveyed and was not significantly different between dense and sparse areas. A total of 21.8% of the colonies in dense areas exhibited some form of disease whereas only 6.1% of colonies in sparse areas showed evidence of disease. Overall, the mean percentage of the colony that was affected by disease (i.e. level of observed recent mortality) was low (1.8 ± 0.3%), and not significantly different between sparsely and densely populated areas of *A. cervicornis*.

In both the DRTO and St. Thomas sites, the prevalence of unattached fragments was more common on transects with sparse populations of *A. cervicornis* than their dense counterparts (Table 2). In St. Thomas, twice as many fragments were found on sparse transects, despite dense transects having three times as much coral from which fragments could generate.

			% of Total		
		N	Colony	Fragment	Thicket
DRTO	Dense	429	74	14	12
	Sparse	210	77	23	0
St. Thomas	Dense	132	72	4	24
	Sparse	66	85	15	0

Table 2. Prevalence of individual colonies, fragments, and thickets binned by *A. cervicornis* abundance.

Fish community structure

There was no significant relationship found between species richness and *A. cervicornis* abundance across all sites in St. Thomas ($p>0.05$). In DRTO, however, a significant positive relationship is clear ($R^2=0.390$, $F=16.611$, $p<0.001$). It appears that in DRTO, the trend only becomes apparent when higher coral abundances are present (live TLE > 1,250 cm/m^2). It is possible that a relationship between species richness and *A. cervicornis* abundance was not seen in St. Thomas because the live TLE did not exceed 400cm/m^2.

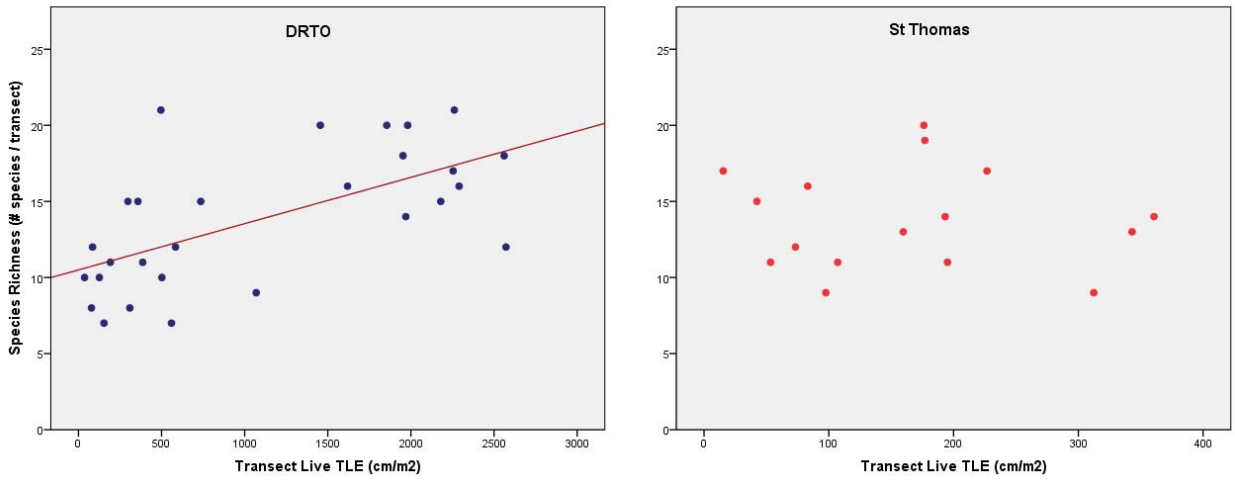

Figure 6. A significant positive relationship exists between fish species richness and *A. cervicornis* abundance in DRTO. This relationship does not appear to exist in St. Thomas. Note that scales for live TLE are not the same in the two graphs.

Following a similar trend to species richness, the dense sites in DRTO maintained the highest fish abundance and biomass. The difference was particularly apparent with small (<15cm) reef fish, where the dense areas retained 223% greater abundance and 613% greater biomass ($p<0.001$, $p<0.001$ respectively; see Fig. 7) than sparse sites. The trend held with fish abundance in St. Thomas, though the difference was much less marked (121% greater, $p<0.05$). Although

fish biomass also trended greater (173%) in dense areas, the difference was not significant (p=0.064).

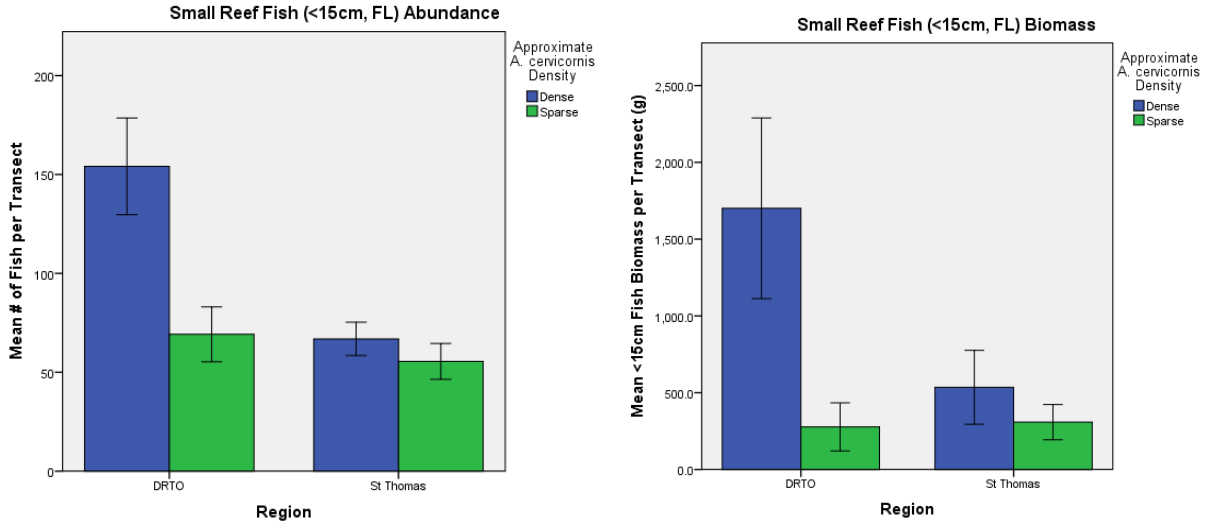

Figure 7. Abundance (left) and biomass (right) of small reef fish (<15cm, FL) across transects of densely and sparsely populated *A. cervicornis*. FL = fork length

Upon closer inspection, it appears that the grunt (Haemulidae) family tends to be the primary driver of the greater reef fish abundance and biomass seen in the dense sites in DRTO. Grunts comprise up to 70.6 ± 8.1% of the biomass seen on dense transects and 23.4 ± 7.7% on sparse transects. In St. Thomas, grunts were virtually absent across all sites comprising only 0.39 ± 0.19% of the biomass in the dense areas and none recorded in the sparse areas.

In St. Thomas, there is also a significant positive relationship between live TLE and damselfish abundance (R^2=0.664, F=27.636, p<0.001) as well as damselfish biomass (R^2=0.648, F=25.760, p<0.001). A similar response may be expected in DRTO, where the range of live TLE is exaggerated, however, no significant relationship exists between live TLE and damselfish abundance (p>0.05) nor biomass (p>0.05).

B. *In-situ* apical tip growth

No significant difference (p>0.05) was found among tagged branch tip growth between the dense (Flat Cay) and sparse (Botany Bay) populations of *A. cervicornis*, nor did a significant regression exist between branch tip growth and transect live TLE (p>0.05). However, the data from DRTO suggests that a significant positive relationship between branch tip growth and transect live TLE (R^2=0.093, F=18.260, p<0.001).

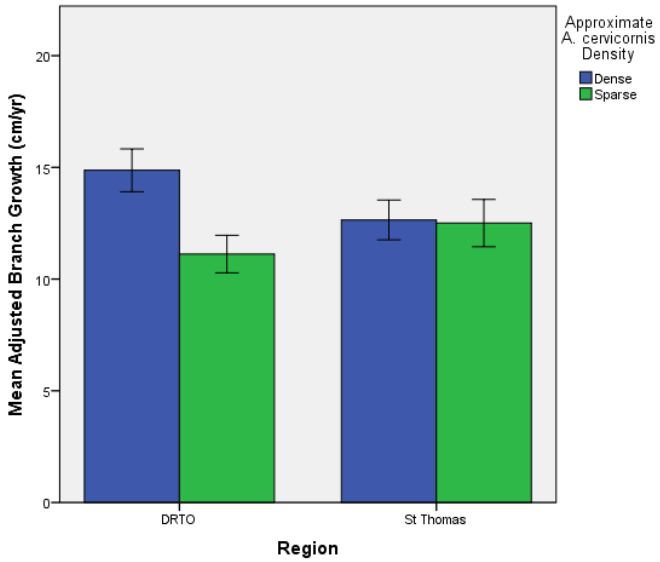

Figure 8. Branch tip growth comparison between sparse and dense assemblages of *A. cervicornis* in DRTO and St. Thomas. The growth seen in apical tips in dense assemblages is 3.8cm per year greater than those in sparse assemblages in DRTO ($t=5.852$, $p<0.001$), but not in St. Thomas ($p>0.05$).

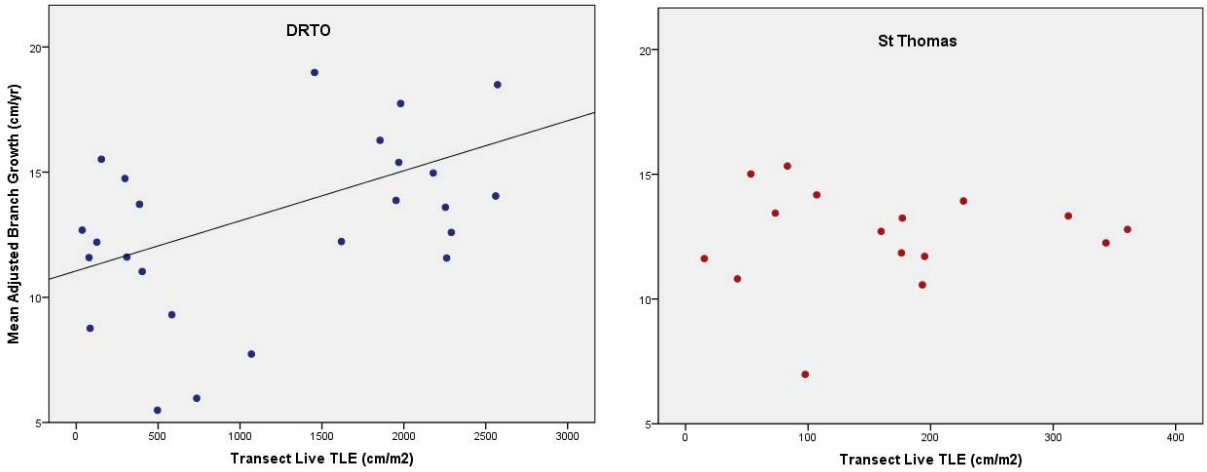

Figure 9. A significant positive relationship exists between apical tip growth (cm/year) and transect live TLE (cm/m^2) in DRTO ($R^2=0.093$, $F=18.260$, $p<0.001$). No significant relationship was detected in St Thomas ($p>0.05$). Note that scales for live TLE are not the same in the two graphs.

C. Bioassay Experiment

Nubbin growth

In St. Thomas, there was no statistically significant difference between nubbin growth (as length) in the dense versus sparse areas ($p>0.05$). Additionally, there was no significant difference of change and volume nor weight between the sparse and dense assemblages ($p>0.05$).

We believe that these differences seen here may not be ecological in nature, but an artifact of the methodology used. Over the course of the last year, we have learned ways to improve upon the methods used for the outplant experiment. We seek to repeat an outplant experiment with refined methods and in different locales and focus on growth length as a parameter rather than volume, weight, and density.

Zooxanthellae (Symbiodinium spp.) counts

A t-test revealed that zooxanthellae densities were greater ($t=9.422$, $p<0.001$) in outplanted nubbins growing in the dense assemblages at Flat Cay than at the sparser at Botany Bay. It is interesting to note however, that the opposite trend is seen in the Dry Tortugas, where nubbins outplanted in sparse populations of *A. cervicornis* tended to have greater densities of *Symbiodinium spp.* than the nubbins in dense assemblages ($t=-3.509$, $p<0.01$).

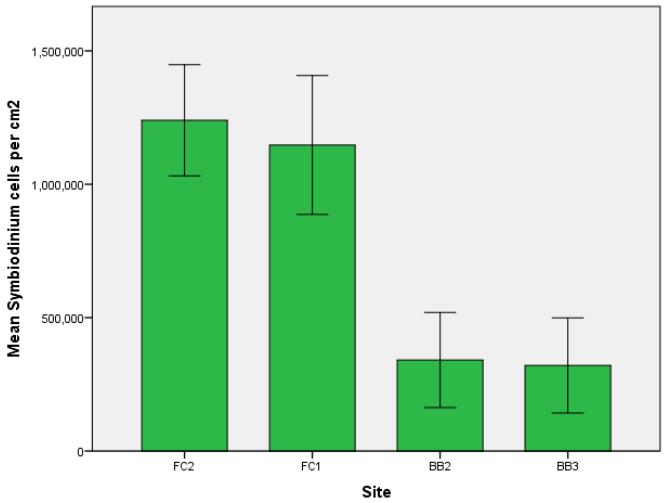

Figure 10. Graph showing mean number of *Symbiodinium spp.* cells per cm^2 tissue area in the outplanted nubbins.

Nutrients

Due to a laboratory error, the tissue on the nubbins from FC (the dense areas) was removed with a confounding buffer (dnaB) instead of deionized water. Thus, only coral nubbins from the sparse areas in St. Thomas were analyzed for carbon and nitrogen content. All of the nubbins from DRTO were analyzed for carbon and nitrogen. There were no significant differences of the nitrogen content in the nubbins between St. Thomas and DRTO. There was also no significant

difference found in nitrogen content between the nubbins growing in sparse and dense sites in DRTO.

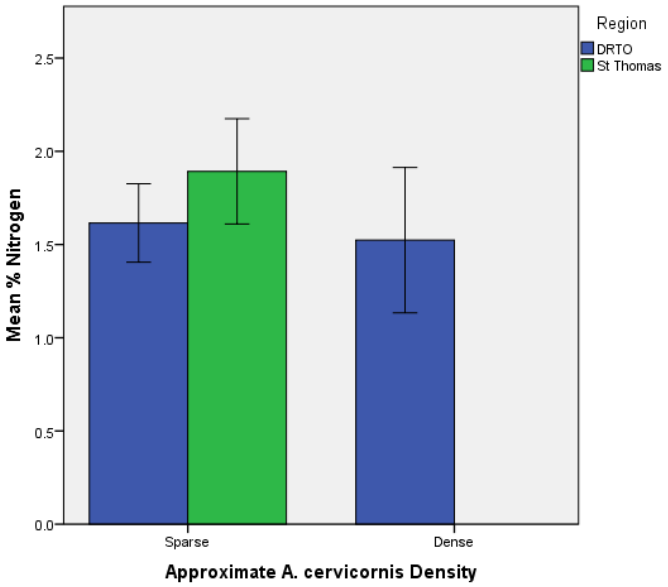

Figure 11. Percent nitrogen composition of the outplanted nubbin samples in the dense and sparse areas of St. Thomas and DRTO.

Implications for management

Trends seen in the super-dense stands of *Acropora cervicornis* in the Dry Tortugas are substantial and ecologically significant. These super-dense thickets support greater species richness, biomass, and abundance of fishes, particularly grunts. The in-situ branch growth of *A. cervicornis* colonies is greater than their sparse counterparts on Pulaski shoal. These trends were lacking in the St. Thomas sites examined. We suspect that a threshold of colony density exists, where the ecological benefits of *A. cervicornis* thickets become far more evident. As such, restoration of *A. cervicornis* may benefit when colonies are outplanted in greater densities at the cost of covering a greater area with a sparse configuration of colonies. However, this management suggestion is preliminary: we plan to continue research over the next year in different locales to expand our dataset.

Anticipated products of project:

Conservation efforts to improve Caribbean reef function depend on informed restoration projects. We can improve the success of *Acropora* restoration projects ongoing across the Caribbean by understanding both the attributes of the coral colonies and coral-reef fish

interactions that favor successful *Acropora* condition. Moreover, if reef fish favor the complex, 3-dimensional reef habitats created by *A. cervicornis,* this points to enhanced ecosystem functions for coral restoration efforts to proceed in tandem with marine reserve protection targeting reef fish recovery. By performing this same study component in St. Thomas and the Dry Tortugas National Park, this research will directly explore the potential for positive facilitation between reef fish communities and the endangered staghorn coral. Our findings will contribute to the growing knowledge base of ecological dynamics within these coral reef ecosystems that can be used to directly influence ongoing staghorn restoration efforts in the Caribbean. Based on the results of this experimental approach and continued monitoring of these extant populations, we will generate scientifically-informed restoration "best practices" as well as peer-reviewed publications to guide restoration efforts to mimic naturally recovering populations and establish the role of this recovery in restoring reef community function.

Acknowledgements

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