Population Dynamics and Genotypic Richness of the Threatened *Acropora* spp. and their Hybrid in the U.S. Virgin Islands Annual Report 2019

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NOAA CORAL REEF CONSERVATION PROGRAM

NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
NOAA
U.S. DEPARTMENT OF COMMERCE

Field Schedule and Samples

September 10

- No-Name Bay Transects
- Lovango Transects (no coral colonies)

September 11

- Newfound Bay (001-008, P001-P004)
- Nursery with Alex
- Processed all samples at hotel

September 12

- Ramshead (P005-P008)
- Yawzi Point (P009-P012)
- Reef Bay (P013-P016)
- Snorkeled Great Salt Pond
- Processed *A. palmata* samples at hotel

September 13

- Great Salt Pond (009-017)
- Reef Bay (018-021)
- Nursery
- Processed all samples at hotel

September 14

- Thatch Transects (P017-P018)
- Snorkeled Hans Lollik (P020-P023) and Kevin's Reef
- Processed *A. palmata* samples at hotel

September 15

- Kevin's Reef (022-029, P024-P027)
- Hans Lollick (046-053)
- Snorkeled Botany Bay
- Nursery
- Processed all samples at hotel

Sampling and Site Information

Colony location, depth and size

Site demographics and distance

The goals of the research are:

- Map the distribution of acroporid colonies; elkhorn (*Acropora palmata*), staghorn (*A. cervicornis*), and fused staghorn (the hybrid, *A. prolifera*), by genotype to better understand the spatial relationships and extent of distinct genotypes.

- Establish fragments of *A. cervicornis* and *A. prolifera* in the University of the Virgin Islands Coral Nursery near Flay Cay.

- Determine relative growth rates and general health of the fragments in the coral nursery and subsequent out-planting success between the parental species and the hybrid.

In September 2019, we collected 63 colonies from reefs surrounding St. Thomas and St. John as a continuation of a multifaceted approach to understand the ecology and population dynamics of the threatened Acropora taxa. Starting in 2009, three long-term transect sites were established at Thatch Cay, Lovango and No-Name Bay around eastern St. Thomas and St. John. These sites have been used to quantify changes in *A. cervicornis* cover and determine the relative health of the colonies on the transect. At No-Name Bay, the hybrid between *A. cervicornis* and *A. palmata* was discovered in quantities higher than that of the parental species, and it appears to be expanding.

The first attempt to quantify this shift at No-Name Bay from habitat previously shared with *A. cervicornis* to solely *A. prolifera* was completed in 2018 (Nylander-Asplin, 2018– manuscript in edits). Data collected in 2019 was added to this long-term data set and will provide insight regarding population fluctuations post-hurricane and disturbance events. Along with quantifying abundances of *A. cervicornis* and *A. prolifera* at these sites, colonies sampled in 2019 were genotyped using microsatellites to determine genotypic richness and thus the main method of propagation for each taxa and site. High genotypic richness values are indicative of sexual reproduction and recruitment, while low richness values are found in populations that spread via asexual fragmentation and propagation.

In addition to quantifying shifts in coral cover, the colonies collected for genotypic analysis were also fragmented and hung in the University of the Virgin Islands (UVI) coral nursery near Flat Cay on the south side of St. Thomas. Historically, *A. cervicornis* has been used as the main coral species in acroporid out-planting initiatives. Here, we are investigating the comparative growth rates and general health of *A. cervicornis* and *A. prolifera* in a nursery setting. By using parental and hybrid colonies from different sites, colonies that are naturally more resilient to thermal stress, disease and other disturbance events can be identified and further studied with the main goal of propagating and out-planting these colonies back onto the reef.

Once colonies are out-planted, we can observe the health and growth rates of *A. cervicornis* and *A. prolifera* in situ to identify differences in health, growth and survival. The success of Caribbean acroporids lies in the ability of these corals to propagate and persist on the reef. Using naturally well-adapted colonies in out-planting initiative can increase the likelihood of long-term success of the colonies.

Nylander-Asplin, Hannah F. "Population Dynamics and Genotypic Richness of the Threatened Acropora spp. and their Hybrid in the US Virgin Islands." (2018).

Tissue Sampling and Genotypic Analysis Methods

In September, 2019, 1 cm tissue samples were collected from the apical tips of *A. cervicornis* (n=16), *A. palmata* (n=27), and *A. prolifera* (n=20) at several site throughout St. Thomas and St. John. Samples were collected haphazardly at all sites due to variations in population size. Tissue clippings were placed in pre-labeled baggies and transported in a temperature controlled cooler prior to processing. Tissue samples were preserved in 96% molecular grade ethanol and stored at -20°C until extraction.

Samples were genotyped using microsatellites developed by Baums et al. (2009), and protocols slightly modified by Fogarty et al. (2012) and performed in Nylander-Asplin (2018). Tissue samples are transferred to CHAOS (4M guanidine thicyanate 0.1% N-lauroyl sarcosine sodium, 23 mM Tris pH 8, 0.1M 2-mercaptoethanol, ultra-pure water) for tissue digestion for 3-5 days prior to extraction. DNA is then extracted using a SprintPrep DNA Purification kit, magnetic bead-based protocol (Beckman Coulter Genomics/Agencourt Bioscience Corporation). For each sample, 50 µl of tissue is mixed with10 µl of Agencourt AMPure XP (magnetic beads), and 80 µl of 100% isopropyl. After mixing, the deep well plate is affixed to a magnetic plate for 10 minutes, and drained by inverting. Once drained, a sequence of 5 rinses is performed using 200 µl of cold 70% ethanol and dried for 1 hour. When the beads are observed to be dried and cracked, 50 µl of 1X TE buffer was added to each sample and placed on a shaker plate for 60 minutes, rotating 90 degrees each 15 minutes. Finally, the supernatant is pipetted from each well after an additional 15 minutes on the magnetic plate. DNA was quantified using a microplate spectrophotometer (Nanodrop- ThermoFischer Scientific).

The extracted DNA is PCR amplified using 5 microsatellite primers [loci 166, 181, 187, 182, 207 (Baums et al. 2009)]. Per modified protocols in Fogarty (2010) and Fogarty (2012), each microsatellite primer is PCR amplified separately using 5X PCR buffer, 2.75 mM of $MgCl_2$, 0.8 mM of dNTPs and 0.5 µl of Taq polymerase. The annealing temperature is loci-specific, with an initial denaturation step of 94°C for 3 minutes, followed by 35 cycles of 94°C for 20 sec, either 55°C (for primer 207), 56°C (for primer 182) or 59°C (for primer 166, 181 and 187) for 20 seconds, and 72°C for 30 seconds, followed by a final extension of 72°C for 30 minutes.

PCR products are then multiplexed in two combinations with primers 166,181, and 187 in a single multiplex, and primers 182 and 207 in another. The multiplex was completed using 12.5µl HiDI Foramide (1:12) and 0.5µl of an internal size standard, Rox 400x (Applied Biosystems, Foster City, CA). Samples were sent to Florida State University Sequencing Facility for fragment analysis. Any samples that were not successfully amplified were re-run individually. Samples were then binned and analyzed using GeneMapper5 software. Finally, Microchecker 2.3.3 was used to isolate stutter peaks, allele dropout and null alleles, if present. Genotypic richness (the total number of unique genotypes divided by the total number of samples) was calculated for each site.

Baums, IB, MK Devlin-Durante, L Brown, and JH Pinzón. 2009. "Nine novel, polymorphic microsatellite markers for the study of threatened Caribbean acroporid corals." *Molecular Ecology Resources* 9 (4):1155-1158.

Fogarty, Nicole D. 2012. "Caribbean acroporid coral hybrids are viable across life history stages." *Marine Ecology Progress Series* 446:145-159.

Nylander-Asplin, Hannah F. "Population Dynamics and Genotypic Richness of the Threatened Acropora spp. and their Hybrid in the US Virgin Islands." (2018).

Sample and Site Information

Wpt#	Species	Tag #	Depth (ft)	Site
1	H	001	4	Newfound Bay
2	C	002	5	
3	C	003	5	
4	C	004	5	
5	C	004	12	
6	H	006	5	
7	H	007	5	
8	H	008	5	
9	H	009	3	Great Salt Pond
10	H	010	3	
11	C	011	3	
12	C	012	7	
13	C	013	9	
14	C	014	8	
15	H	015	4	
17	H	017	7	
18	H	018	2	Reef Bay
19	H	019	2	
20	H	020	1	
21	H	021	1	
22	C	022	14	Kevin's Reef
23	C	023	14	
24	C	024	10	
25	H	025	5	
26	H	026	5	
27	H	027	5	
28	H	028	5	
29	C	029	5	
46	C	046	7	Hans Lollik
47	C	047	8	
48	C	048	10	
49	C	049	13	
50	H	050	5	
51	H	051	5	
52	H	052	5	
53	H	053	5	

Wpt#	Species	Tag #	Depth (ft)	Site
P001	P	001*	3	Newfound Bay
P002	P	002*	4	
P003	P	003*	3	
P004	P	004*	2	
P005	P	005*	5	Ramshead
P006	P	006*	4	
P007	P	007*	6	
P008	P	008*	4	
P009	P	009*	8	Yawzi Point
P010	P	010*	5	
P011	P	011*	4	
P012	P	012*	4	
P013	P	013*	4	Reef Bay
P014	P	014*	3	
P015	P	015*	3	
P016	P	016*	5	
P017	P	017*	1	Thatch Cay
P018	P	018*	2	
P020	P	020*	6	Hans Lollik
P021	P	021*	6	
P022	P	022*	5	
P023	P	023*	5	
P024	P	024*	6	Kevin's Reef
P025	P	025*	6	
P026	P	026*	5	
P027	P	027*	5	

A. cervicornis	*A. prolifera*	*A. palmata*
		

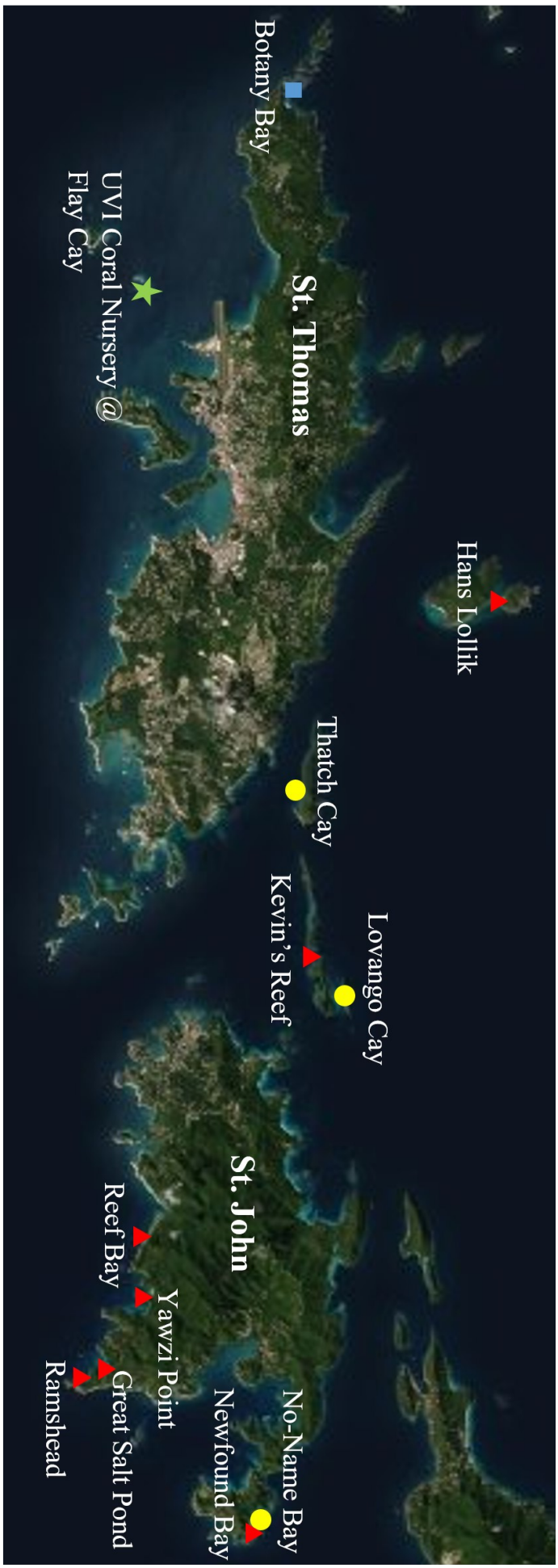
Site Demographics

Long-term monitoring site previously sampled (2017-2018)

Surveyed, and sampled

Surveyed, not sampled

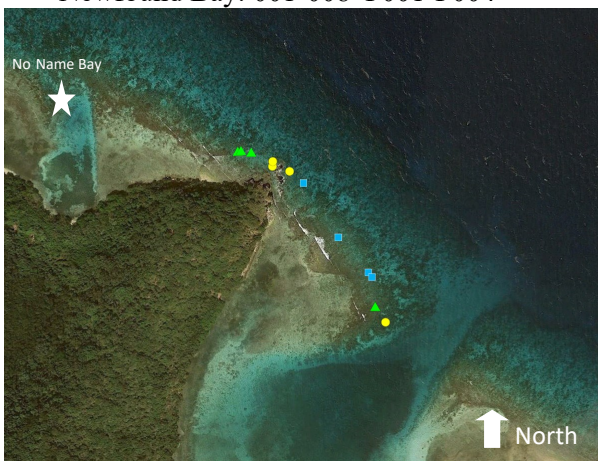
UVI Coral nursery @ Flat Cay



2019 Sample Sites

A. cervicornis
A. prolifera
A. palmata

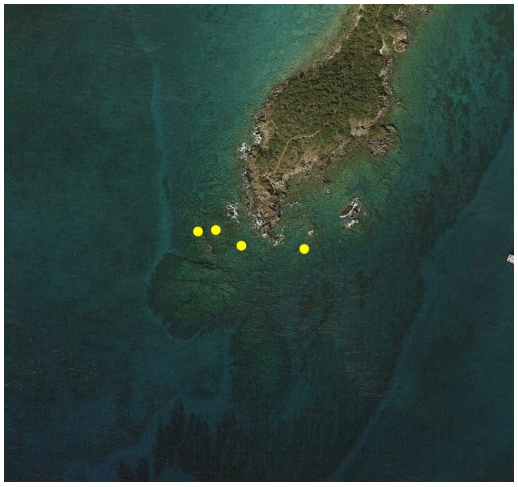
Newfound Bay: 001-008 P001-P004



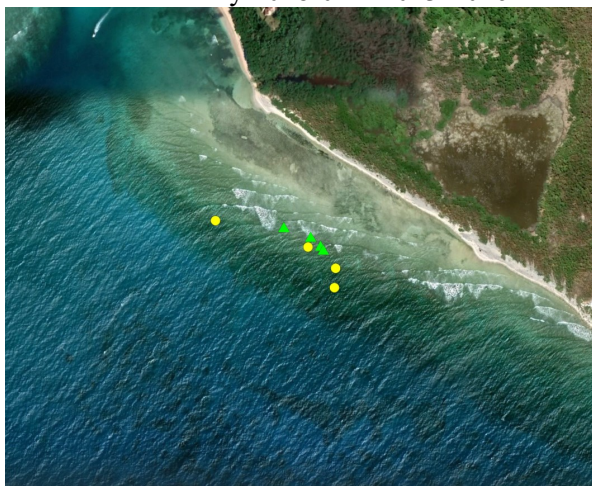
Ramshead: P005-P008

Great Salt Pond: 009-017

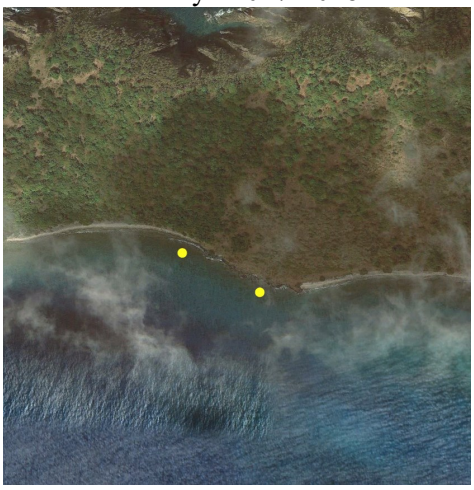
Yawzi Point: P009-P012



Reef Bay: 018-021 P013-P016



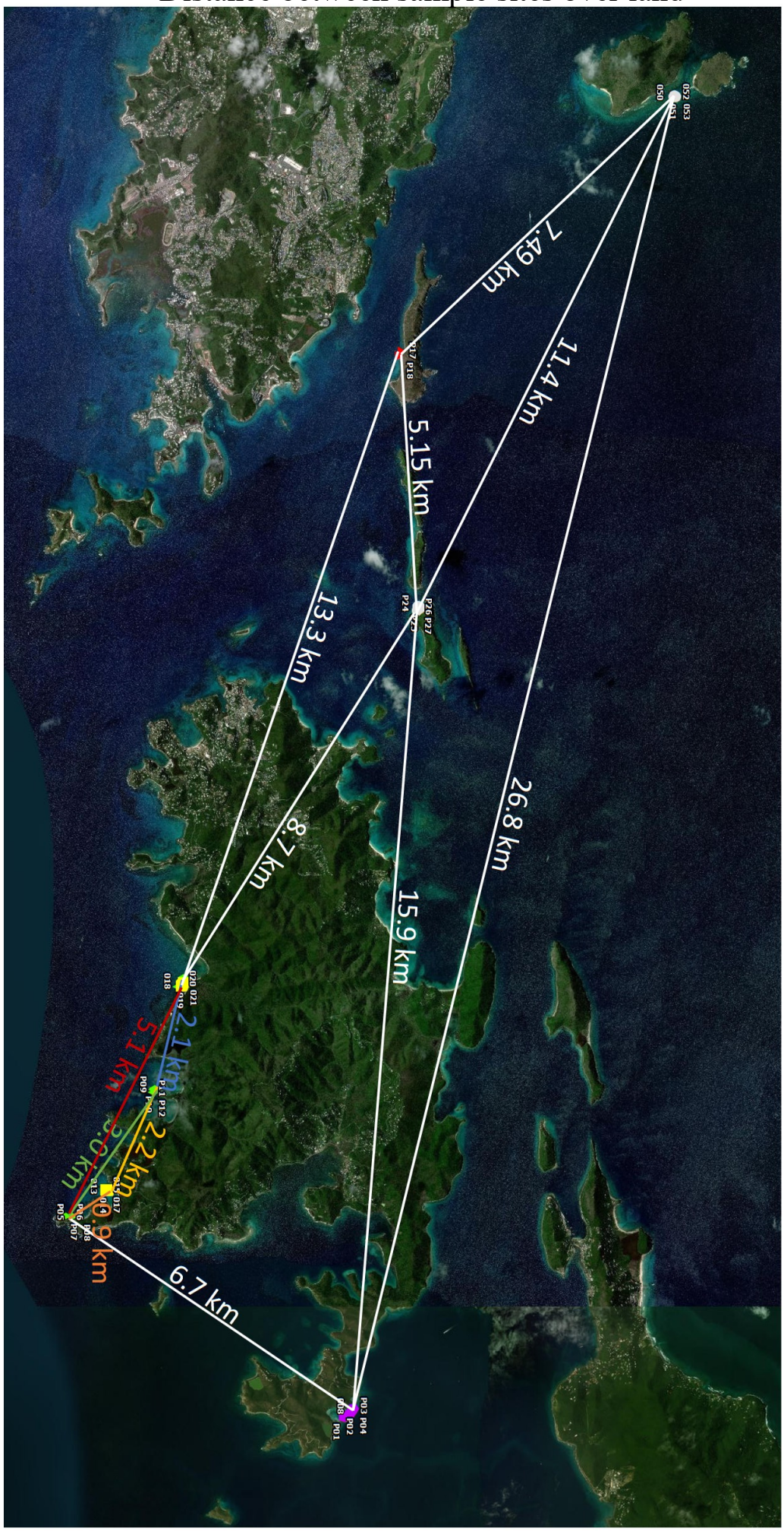
Thatch Cay: P017-P018



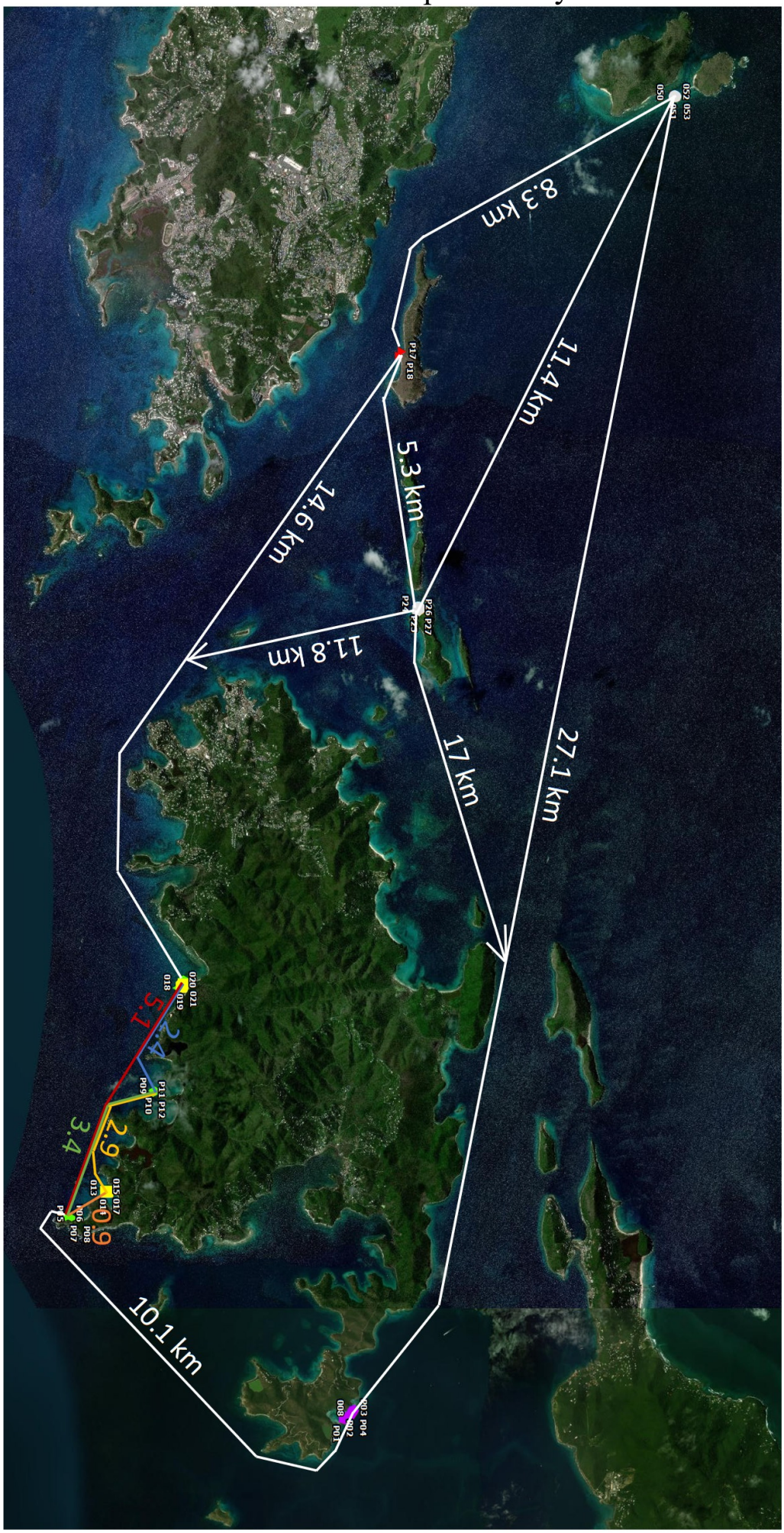
Kevin's Reef: 022-029 P024-P027

Hans Lollik: 046-053 P020-P023

Distance between sample sites over land



Distance between sample sites by boat



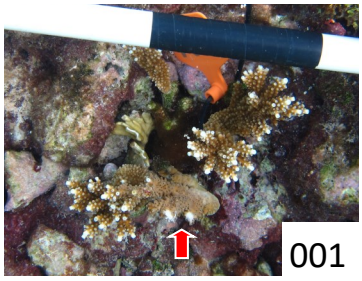
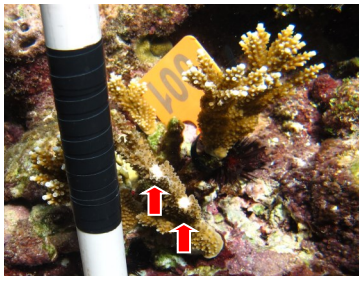
Coral Key

Colony ID photos for each genotypic sample

Newfound Bay

Tags 001-008 **P001-P004**

tag clipping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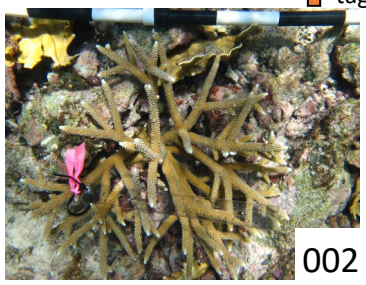

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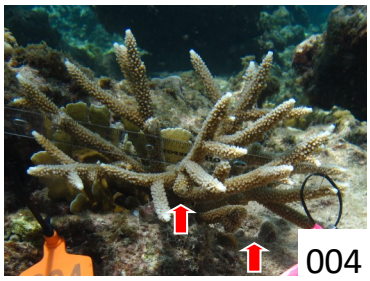
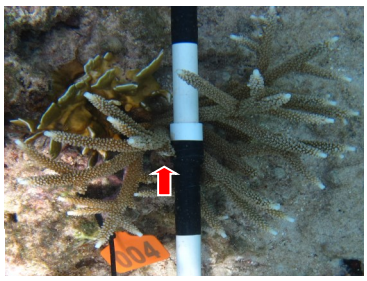
003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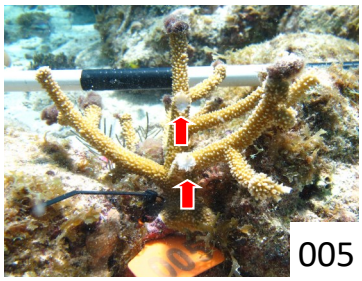
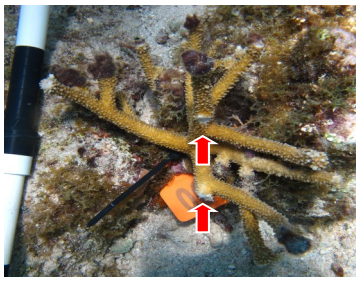
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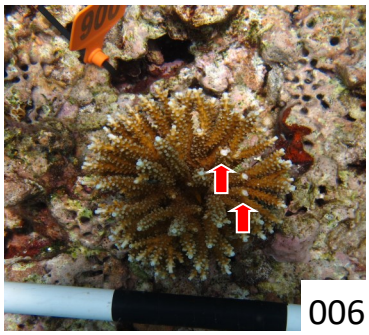

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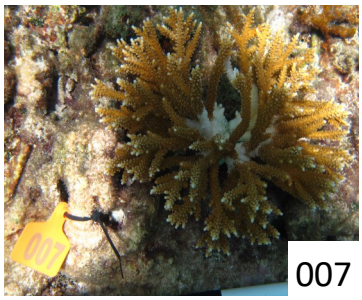

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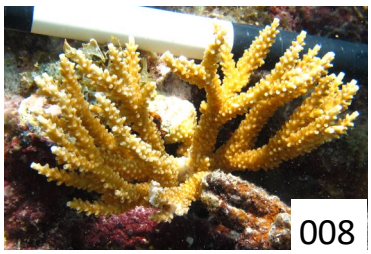

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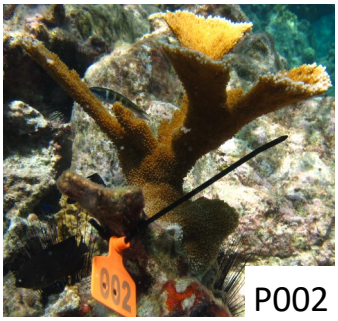
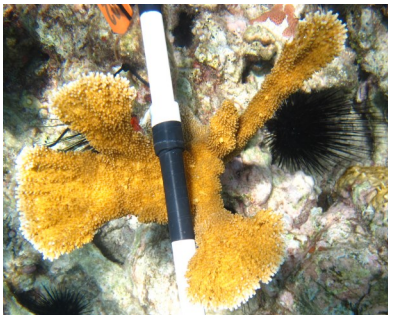
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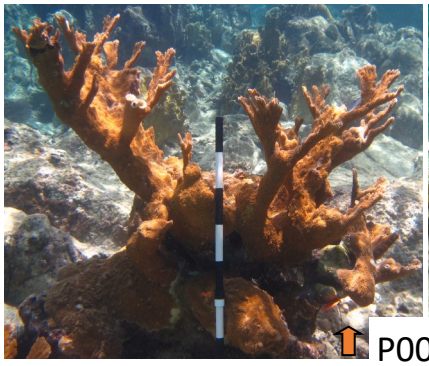
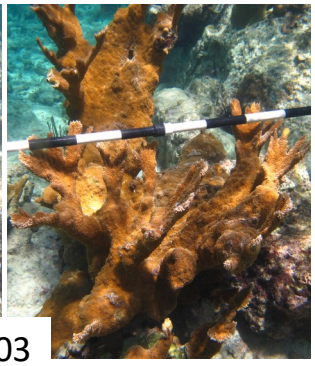
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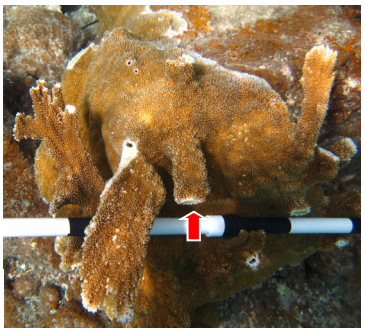
P004

Ramshead

Tags P005-P008

tag clipping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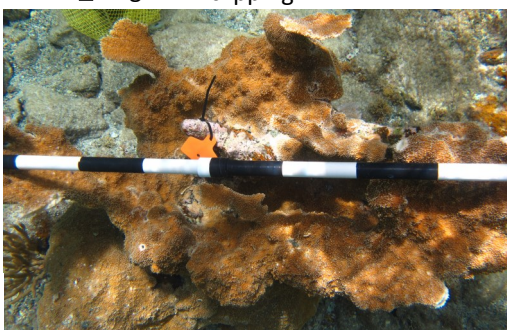

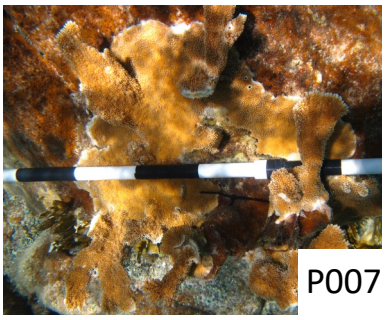
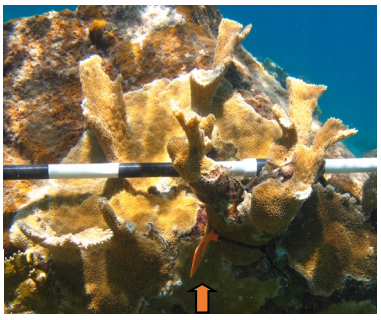
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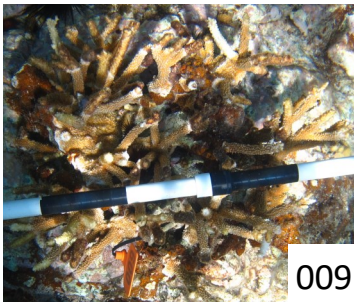
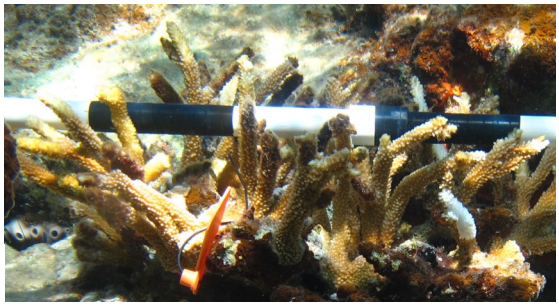
P008

Great Salt Pond

Tags 009-017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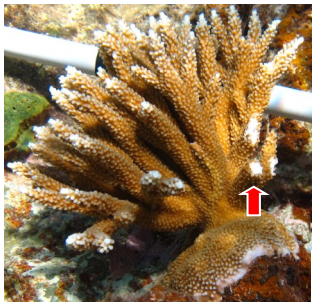
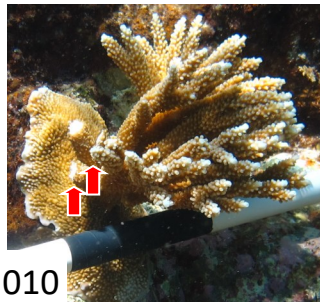
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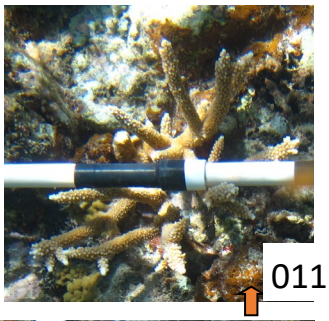
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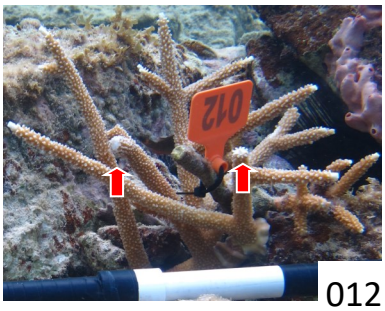

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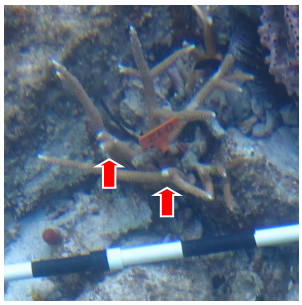

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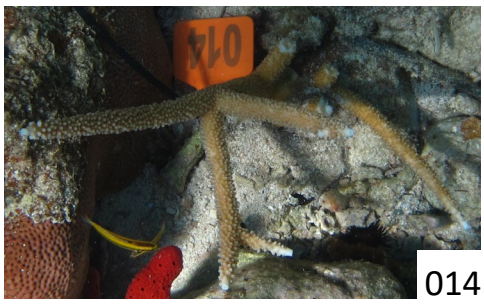

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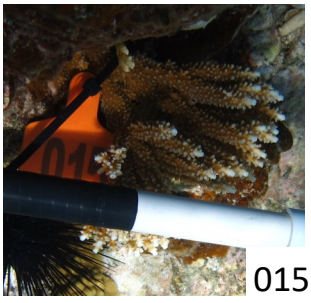
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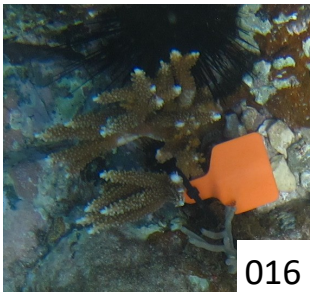
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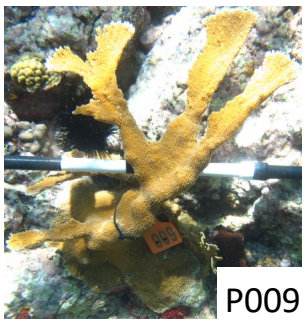
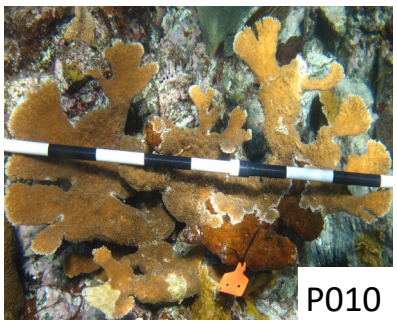
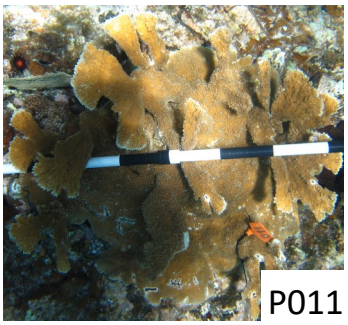
Yawzi Point

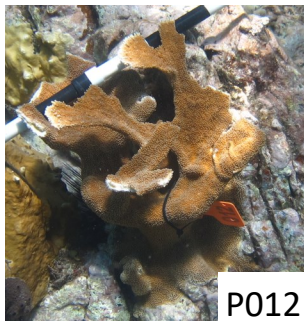
Tags P009-P012

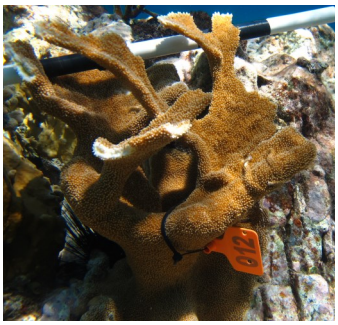
tag clipping











Reef Bay

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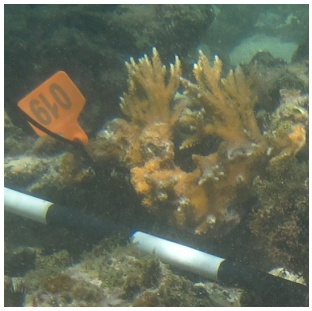
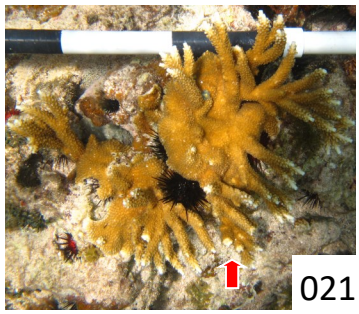
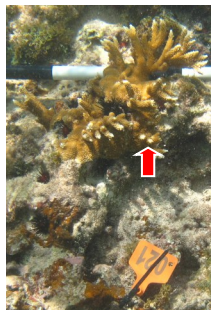
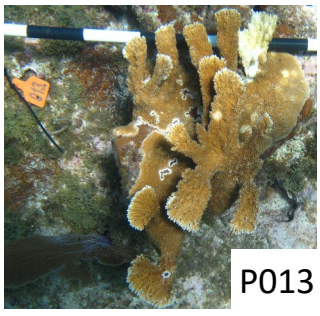
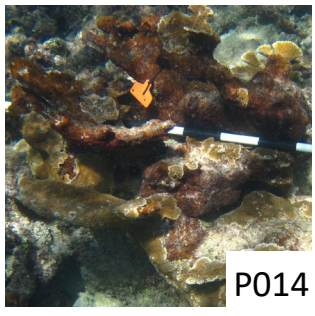
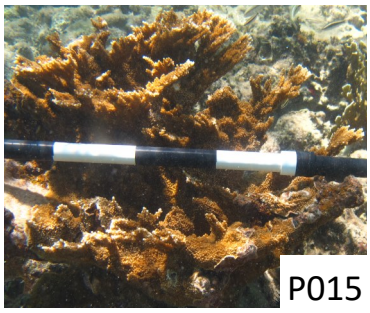


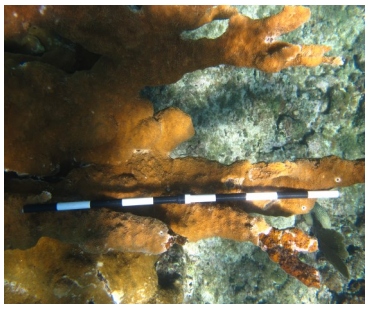








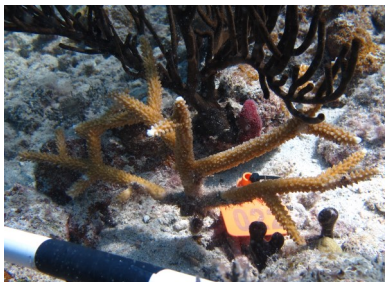




Kevin's Reef

Tags 022-029 P024-P027

⇧ tag ⇑ clipping



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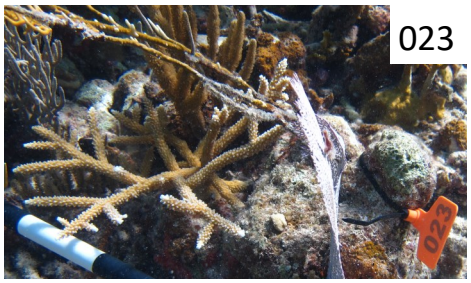

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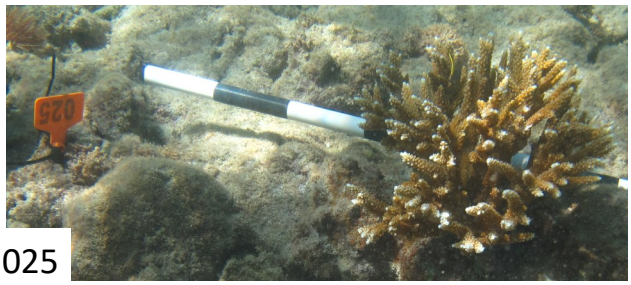

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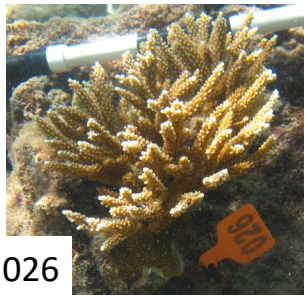

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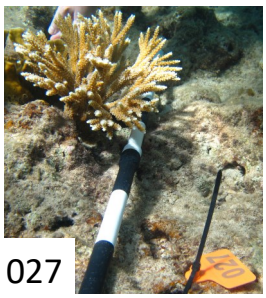

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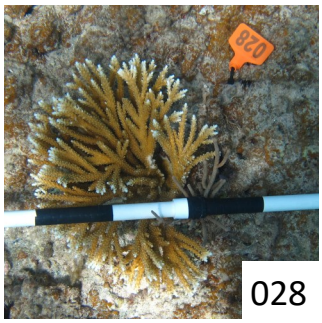

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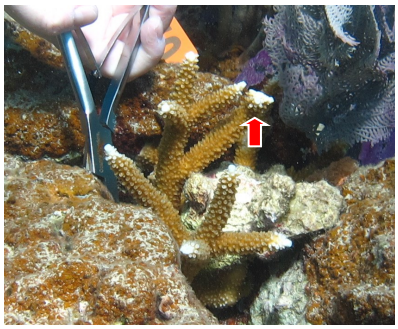

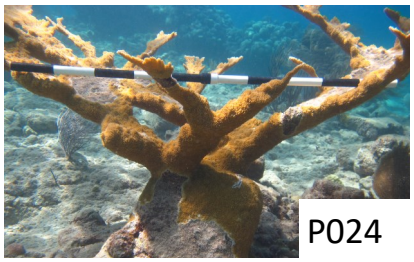

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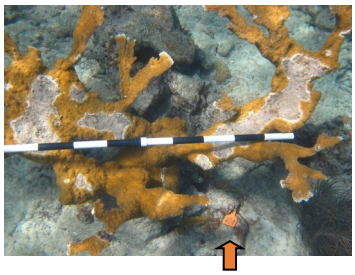

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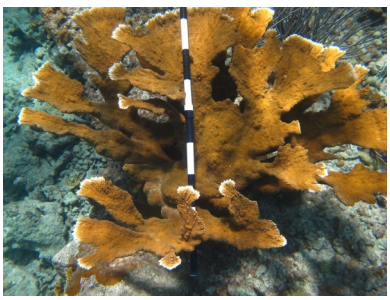

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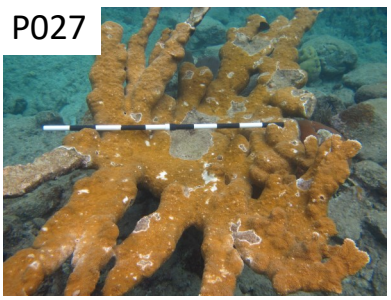
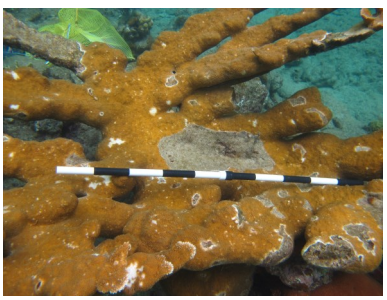
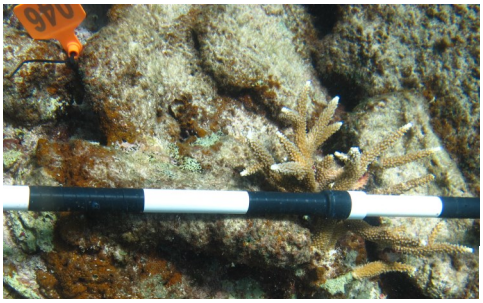
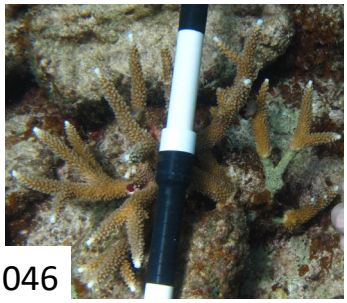




Hans Lollik

Tags 046-053 P020-P023

tag clipping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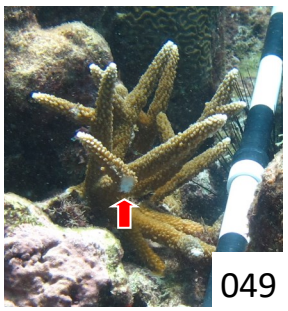
047



048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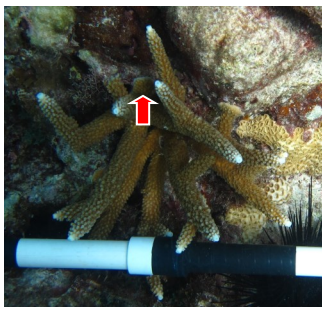



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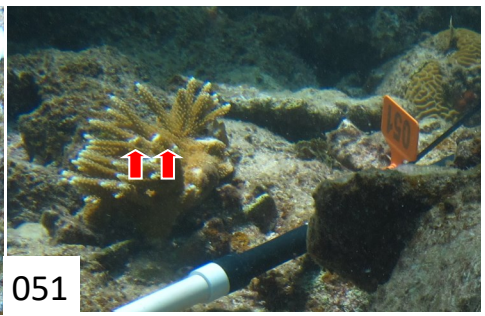

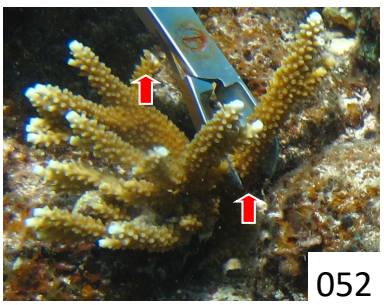
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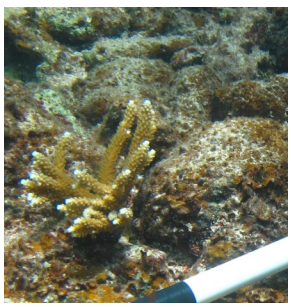


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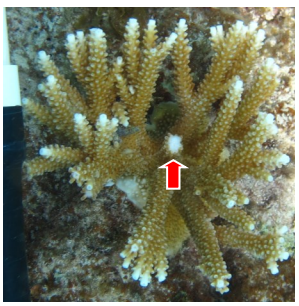

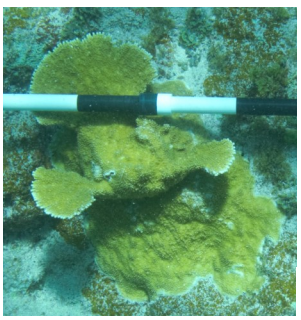

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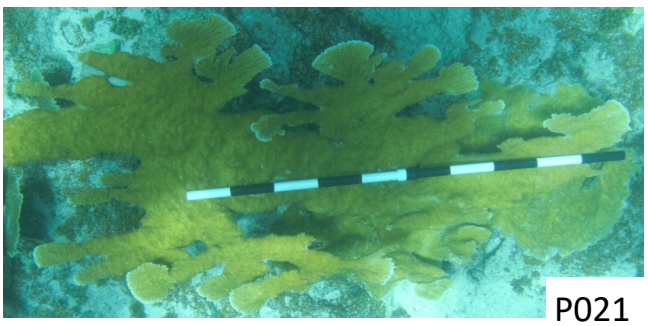
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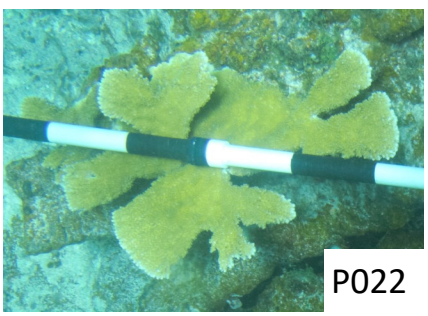
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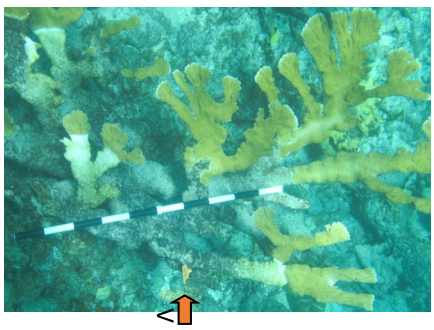
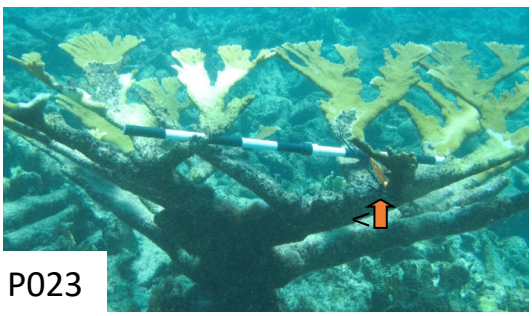


P023

UVI Coral Nursery

Schematics and location of each colony and tree

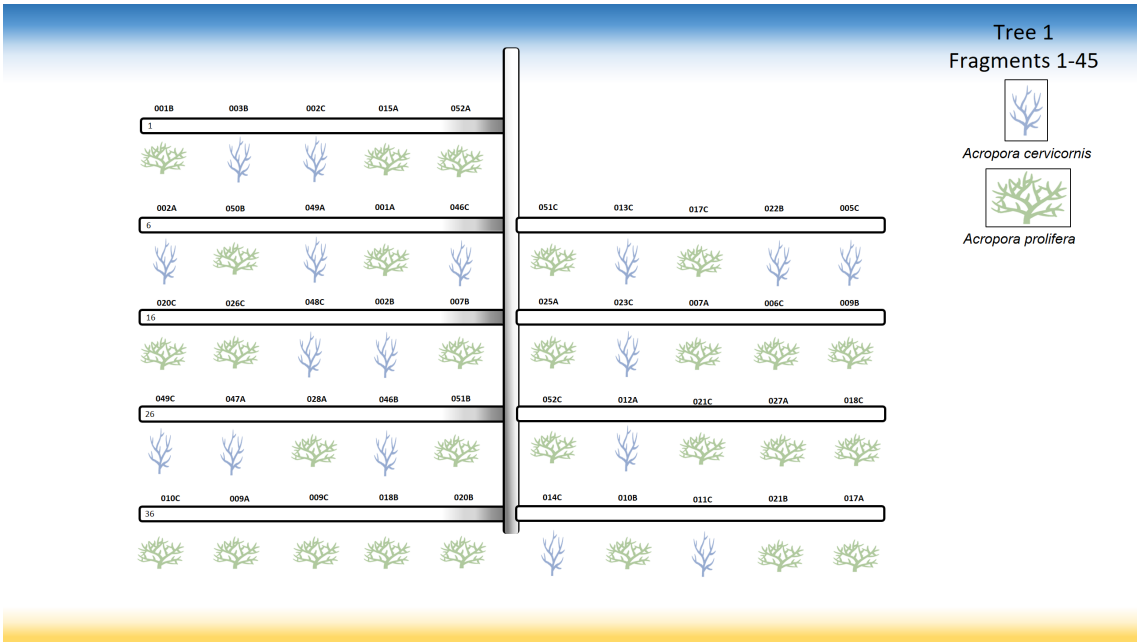
UVI Coral Nursery Methods

The same *A. cervicornis* and *A. prolifera* colonies that were tagged and clipped for genotypic analysis were placed in the UVI coral nursery at Flay Cay on the south side of St. Thomas. *Acropora palmata* colonies were not included in the nursery. Two trees were constructed with PVC tubing, an anchor and float. Holes were drilled vertically along each branch and monofilament line (Stren 30lb .022 inch diameter) was strung through each hole and secured on the top of the tube with an aluminum sleeve (Hi-Seas GS-F-500 size F). At the bottom of each monofilament line, a loop was made and an aluminum sleeve was crimped on one side to allow the loop to be loosened and tightened around the colony underwater.

Colonies were clipped (n=36, 3cm) and placed in pre-labeled baggies with tags indicating the fragment number and pre-determined location on the nursery trees. Each colony had three replicates that were placed randomly on either tree (n=103 fragments). Prior to placing in the nursery, each fragment was measured to provide a baseline for growth analysis. Any paling or white on the fragments was noted prior to being placed in the nursery. Fragments were transported in individual baggies to reduce rubbing and friction and placed in a temperature controlled cooler. Water temperature was carefully monitored to reduce the likelihood for thermal shock during transport. The time between removing the fragment to placing it in the coral nursery was reduced as much as possible.

Two divers were deployed (one to each tree) with fragments pre-determined for that tree. For each colony, the tags were removed from the baggies and zip-tied to the top of the PVC tube to the monofilament line. The fragment was then placed in the monofilament loop at the bottom of the line and the monofilament was tightened via the aluminum sleeve to secure the fragment. The location of each replicate was mapped for each tree. A diver from UVI will monitor the colonies one or twice per month as they grow. Each fragment will be re-measured and a general health code will be assigned (Healthy, Pale, White and Alive, White and Dead, Algae Covered, Diseased, Removed). The number of apical polyps and any growth morphologies will also be noted. Once fragments reach an appropriate size to be out-planted, they will be re-measured once more prior to removal from the nursery.

USVI Coral Nursery Schematics

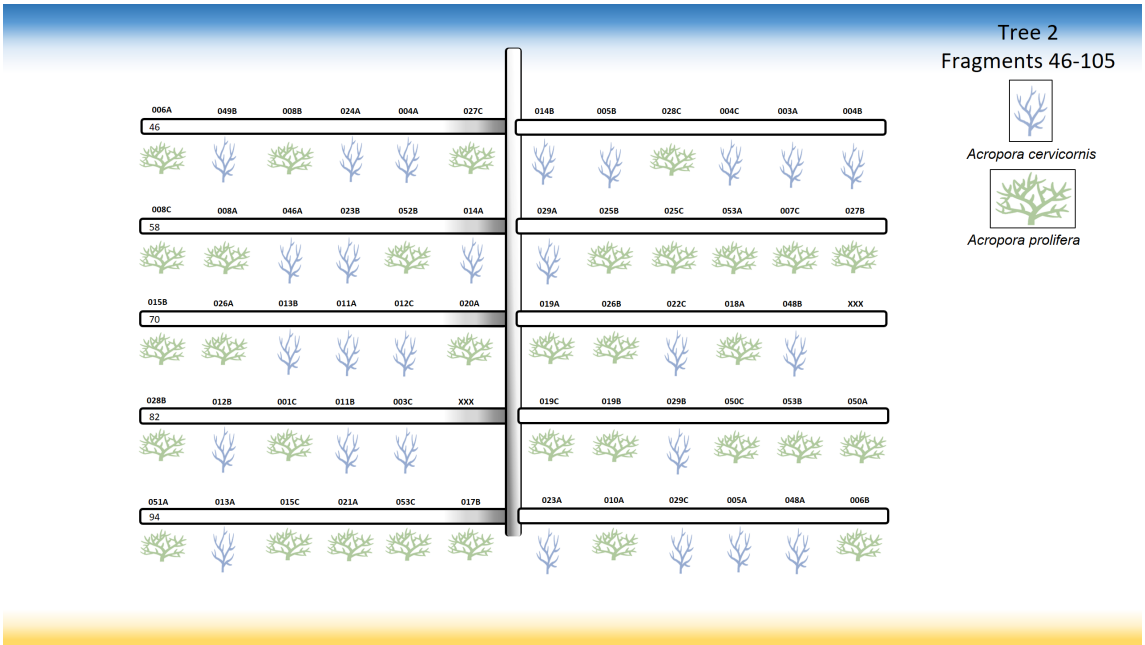


Tree 1:

45 Fragments

28 *A. prolifera*

17 *A. Cervicornis*



Tree 2:

58 fragments

32 *A. prolifera*

26 *A. cervicornis*

USVI 2019 Nursery Map- Species

#	A	B	C
1	1	1	2
2	1	1	1
3	2	1	2
4	2	2	2
5	2	2	1
6	2	2	1
7	1	1	2
8	2	2	2
9	1	1	1
10	2	1	1
11	2	2	1
12	1	2	2
13	2	2	1
14	2	2	1
15	1	2	2
17	1	2	1
18	2	1	1
19	2	2	2
20	2	1	1

#	A	B	C
21	2	1	1
22		1	2
23	2	2	1
24	2		
25	1	2	2
26	2	2	1
27	1	2	2
28	1	2	2
29	2	2	2
46	2	1	1
47	1		
48	2	2	1
49	1	2	1
50	2	1	2
51	2	1	1
52	1	2	1
53	2	2	2

A. cervicornis

No Fragment

A. prolifera

Number indicated tree

USVI 2019 Nursery Map- Tree

#	A	B	C
1	H	H	H
2	C	C	C
3	C	C	C
4	C	C	C
5	C	C	C
6	H	H	H
7	H	H	H
8	H	H	H
9	H	H	H
10	H	H	H
11	C	C	C
12	C	C	C
13	C	C	C
14	C	C	C
15	H	H	H
17	H	H	H
18	H	H	H
19	H	H	H
20	H	H	H

#	A	B	C
21	H	H	H
22		C	C
23	C	C	C
24	C		
25	H	H	H
26	H	H	H
27	H	H	H
28	H	H	H
29	C	C	C
46	C	C	C
47	C		
48	C	C	C
49	C	C	C
50	H	H	H
51	H	H	H
52	H	H	H
53	H	H	H

Tree 1

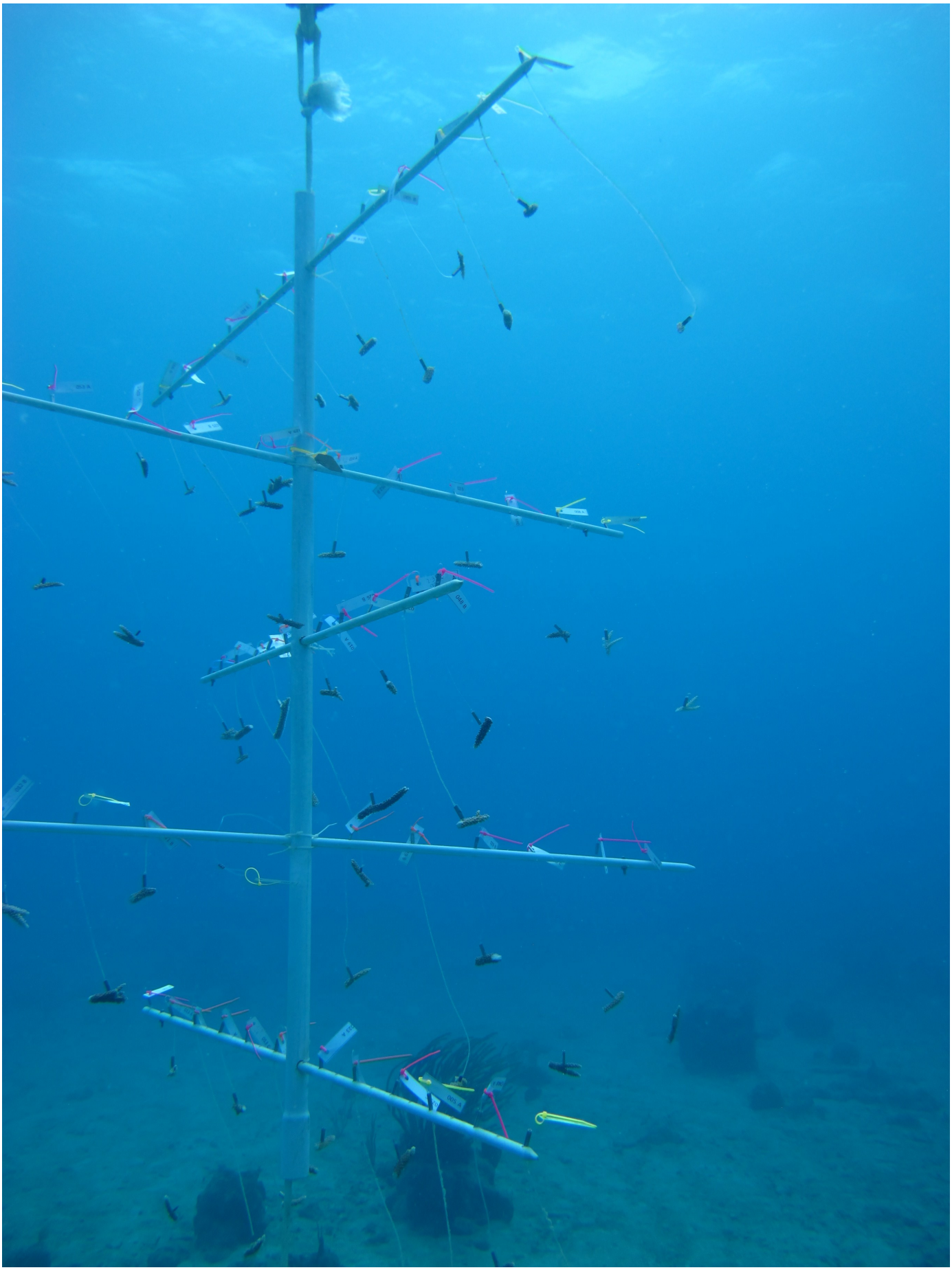
No Fragment

Tree 2

H= Hybrid

C= Cervicornis

Tree 1 in the UVI Coral Nursery with colonies hanging from the braches. White tags attached via colored zip ties can also be seen.



Transect Data

2009-2019 transects data at:

Thatch Cay (1-4)

Lovango Cay (5-6)

No-Name Bay (7-9)

Long-term Transect Analysis Methods

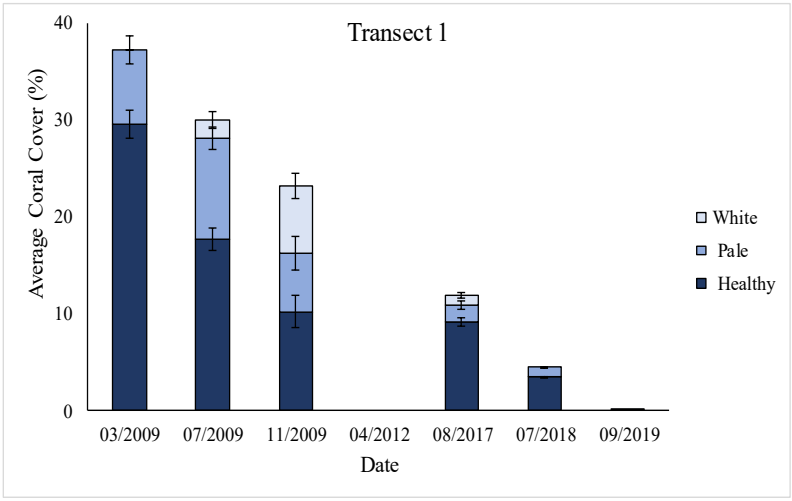
Long-term transects established by the National Oceanic and Atmospheric Administration, National Marine Fisheries Service (NOAA NMFS) have been used to document *A. cervicornis* rich habitats 1-3 times annually at Thatch Cay, St. Thomas and Lovango Cay, and No-Name Bay in St. John, U.S. Virgin Islands. A total of nine permanent transects (10 x 2m) were established near *A. cervicornis* thickets using permanent steel posts. During each survey, photographs were taken every 1m along both sides of the transect. Therefore, each transect was comprised of 20 photographs. A 1-m PVC stick placed perpendicularly to the transect tape was used to provide a known length to aid in photo analysis.

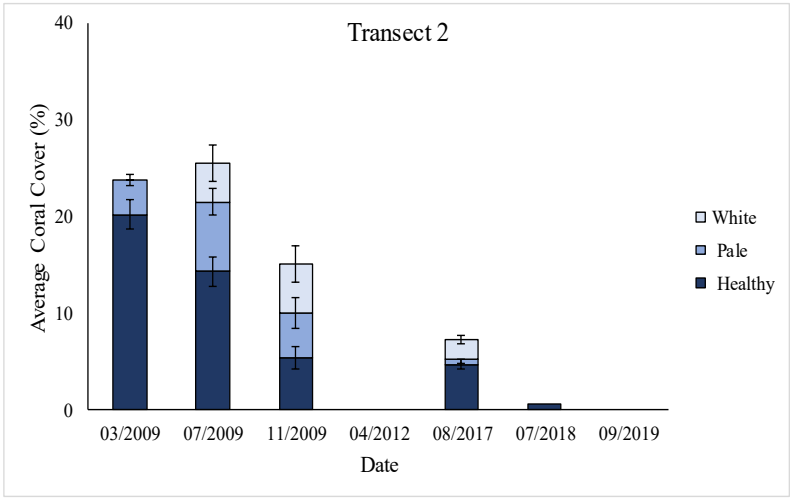
Photographs (n=1140) were analyzed from surveys conducted in 2009 (March, July, November), 2012 (April), 2017 (August), 2018 (July), and 2019 (September). Analysis methods using Matlab and Adobe Illustrator were used to quantify the percent cover of each square meter along either side of each transect. Each transect photo was scaled and rectified using MatLab R2017b. Live tissue (healthy, pale, or white in color) was traced using a brush tool (size 3, black, transparency 0%) using Adobe Illustrator 2017 software. The traced image was then overlaid on a white background to isolate live tissue from skeleton, algae, and other benthic cover. The composite black and white image was analyzed using MatLab R2017b to quantify the percentage of total coral tissue cover. The data were analyzed with a particular concentration on variations within and between sites to determine intra- and inter-site variation. Similarly, the differences in live coral cover were compared between the sampling periods. In 2009, transect surveys were conducted three times, thus providing inter-annual variation data as well.

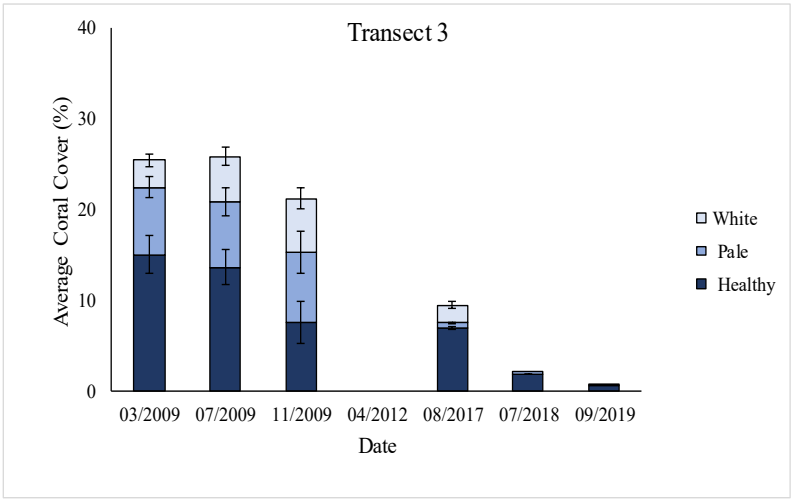
The condition of the tissue on each colony was analyzed similar to percent cover. Using post-standardized photos from the initial analysis, areas of coral colonies that were pale or white were isolated and quantified separately. Tissue that was not dark relative to other colonies was identified as pale. To avoid bias in identifying pale or healthy tissue, the same individual quantified all photographs for the tissue analysis. It was impossible to determine the cause of the white areas (i.e., bleached or denuded skeleton) from the photographs, as they could have been caused by disease, bleaching, or predation events. Therefore, any tissue identified as ‘white’ was excluded from the total percent cover analysis. Once the tissue condition was isolated, it was quantified using a MatLab script to determine the percent cover of each tissue type (i.e., healthy, pale, white).

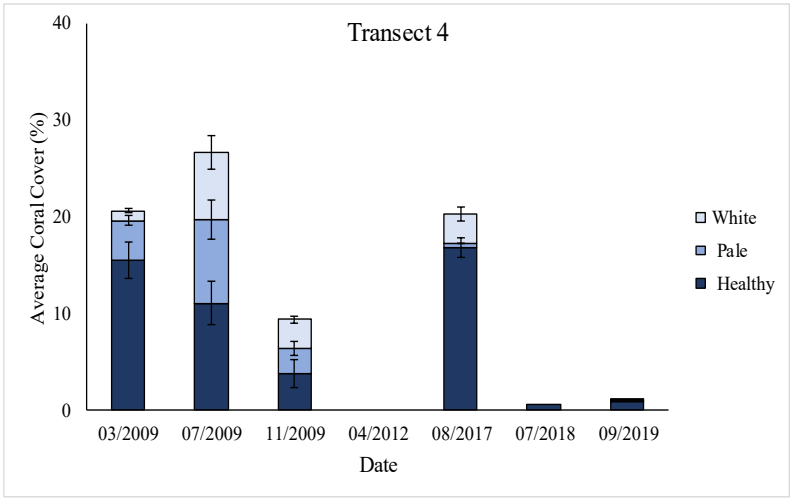
Thatch Cay

Transect 1-4



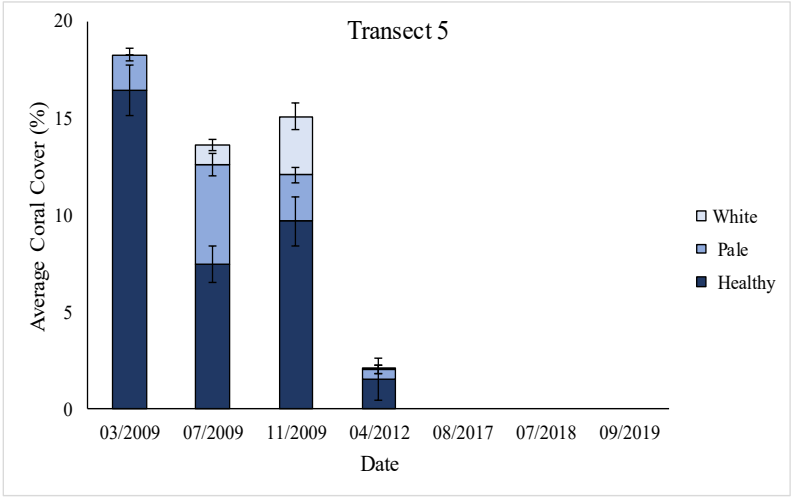


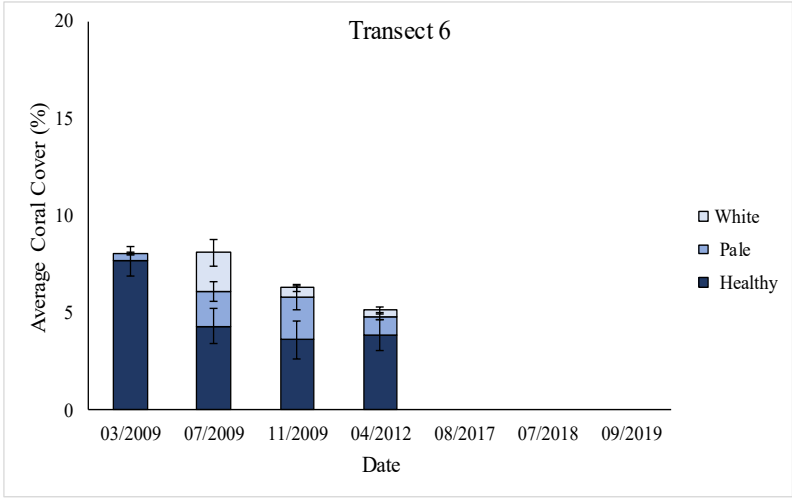




Lovango

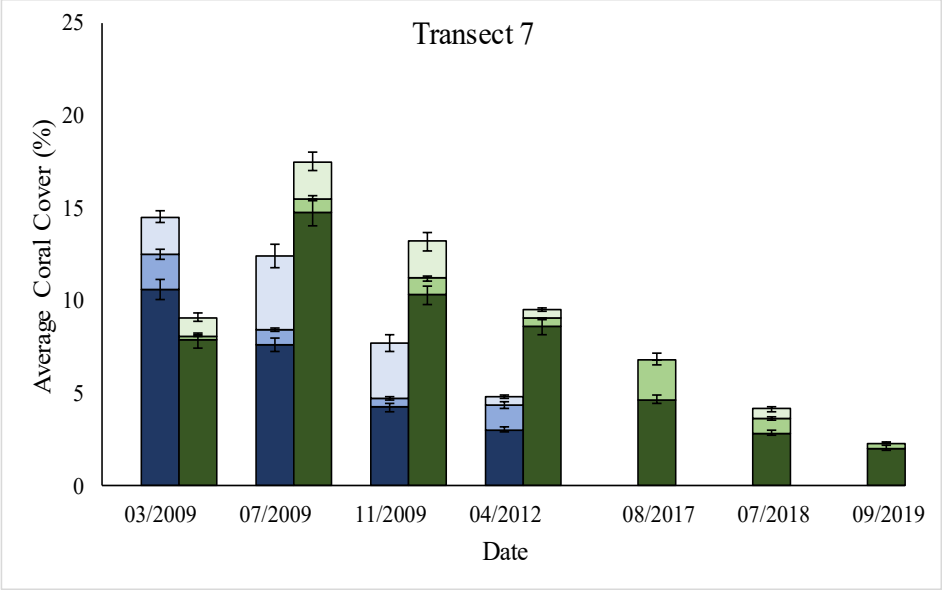
Transect 5-6

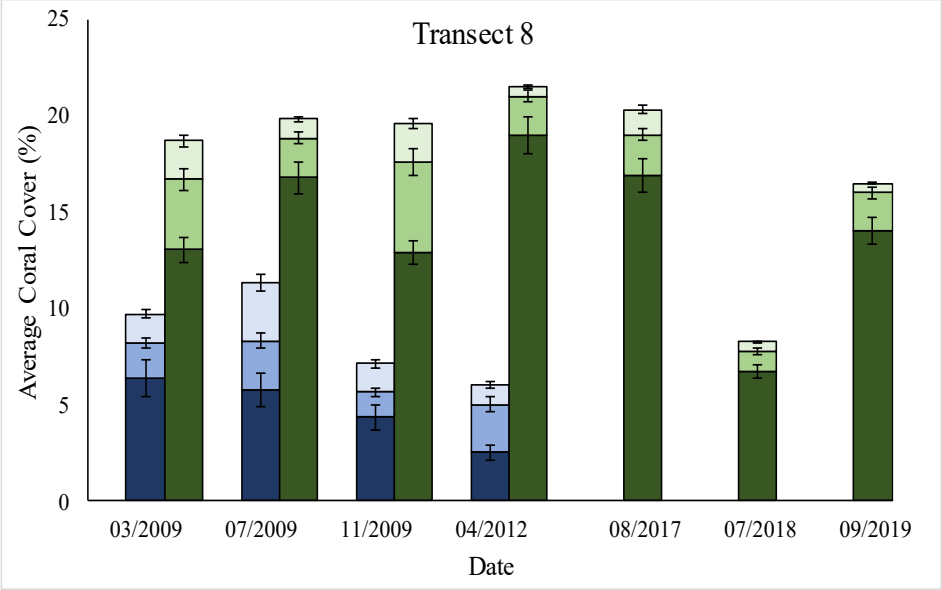


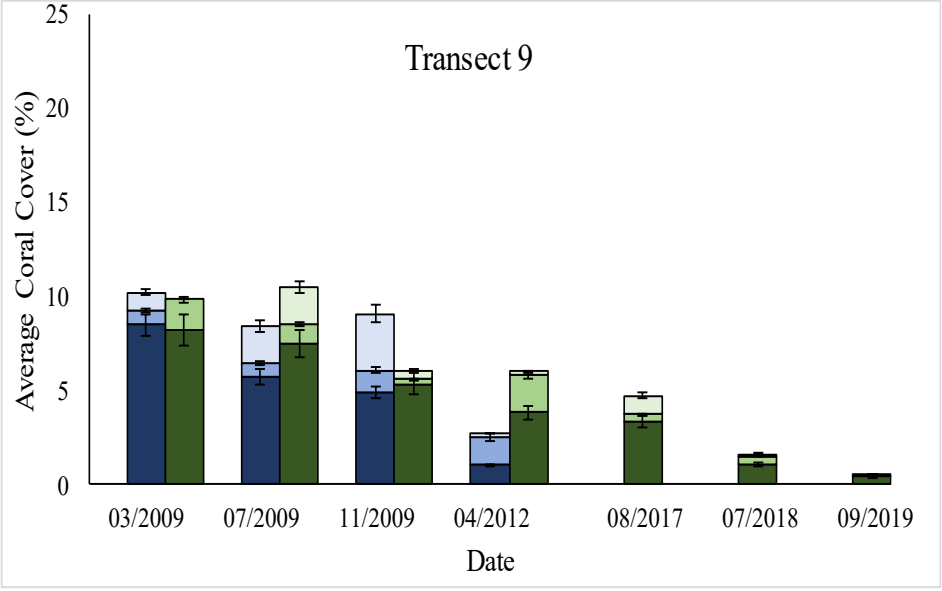


No-Name Bay

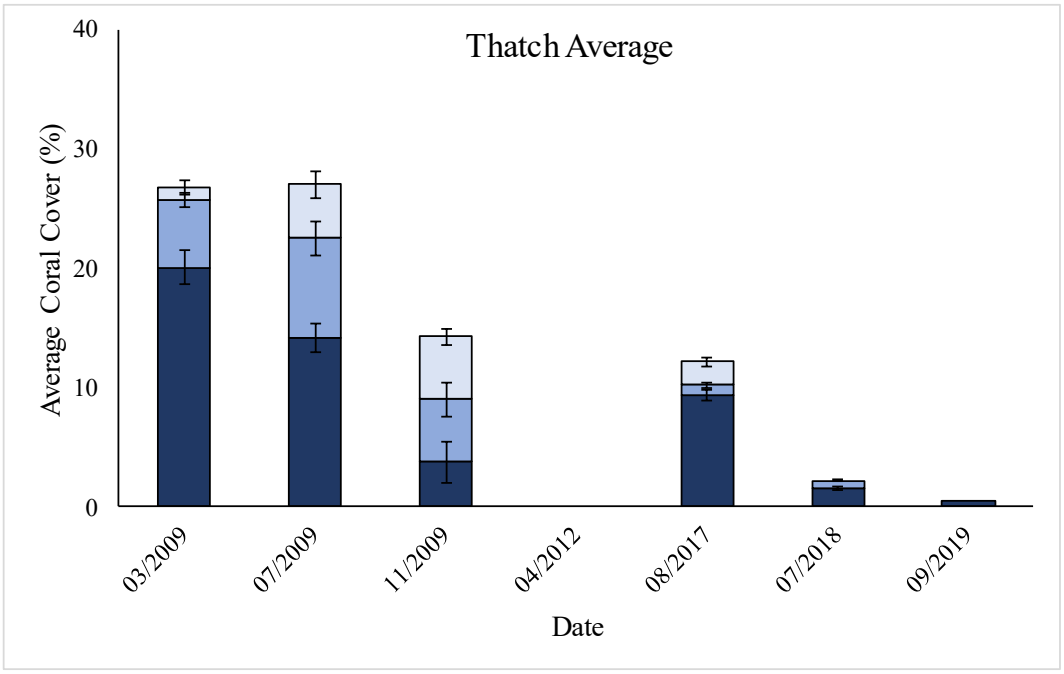
Transect 7-9

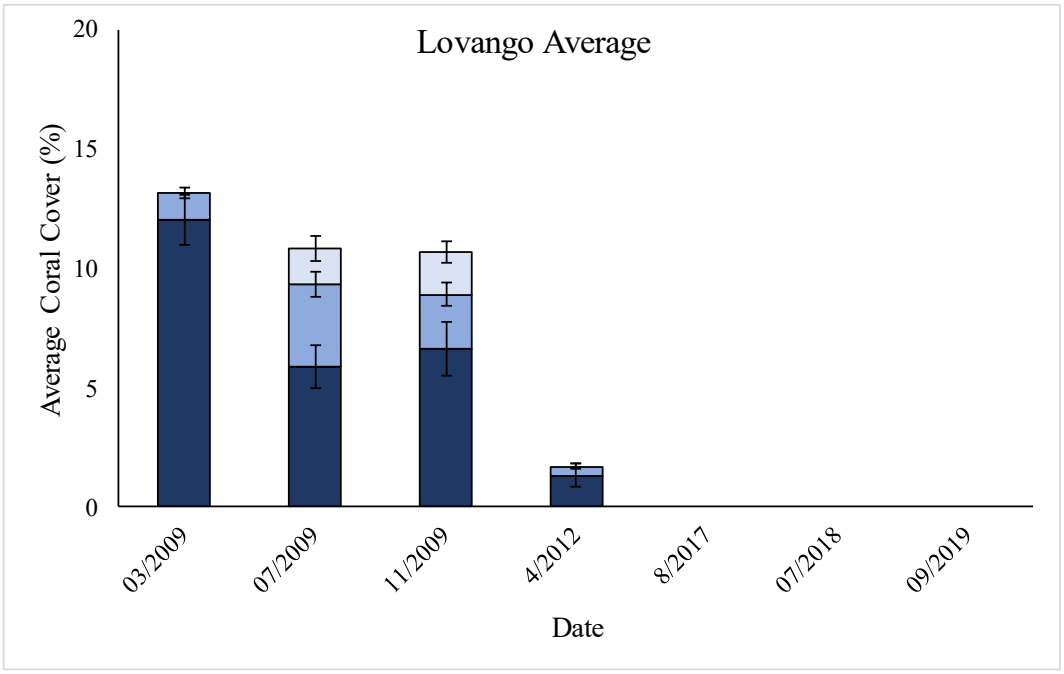


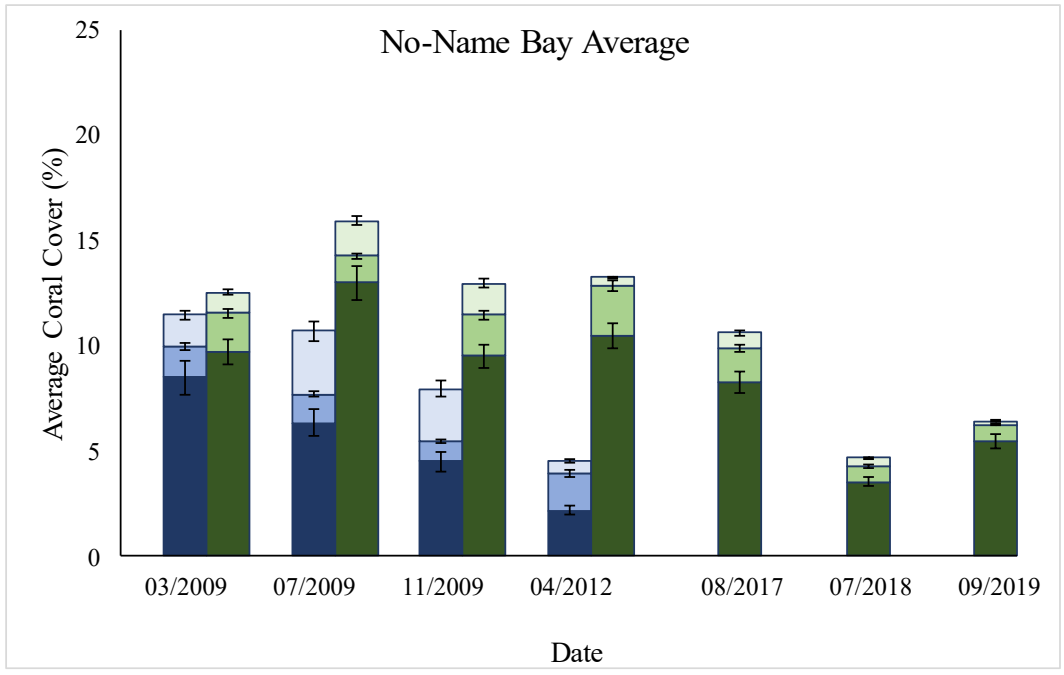




All sites


Thatch Average
Average Coral Cover (%)
40
30
20
10
0
03/2009
07/2009
11/2009
04/2012
08/2017
07/2018
09/2019
Date
White
Pale
Healthy


Lovango Average
Average Coral Cover (%)
20
15
10
5
0
03/2009
07/2009
11/2009
4/2012
8/2017
07/2018
09/2019
Date
White
Pale
Healthy


No-Name Bay Average
Average Coral Cover (%)
25
20
15
10
5
0
03/2009
07/2009
11/2009
04/2012
08/2017
07/2018
09/2019
Date
A. prolifera White
A. prolifera Pale
A. prolifera Healthy
A. cervicornis White
A. cervicornis Pale
A. cervicornis Healthy