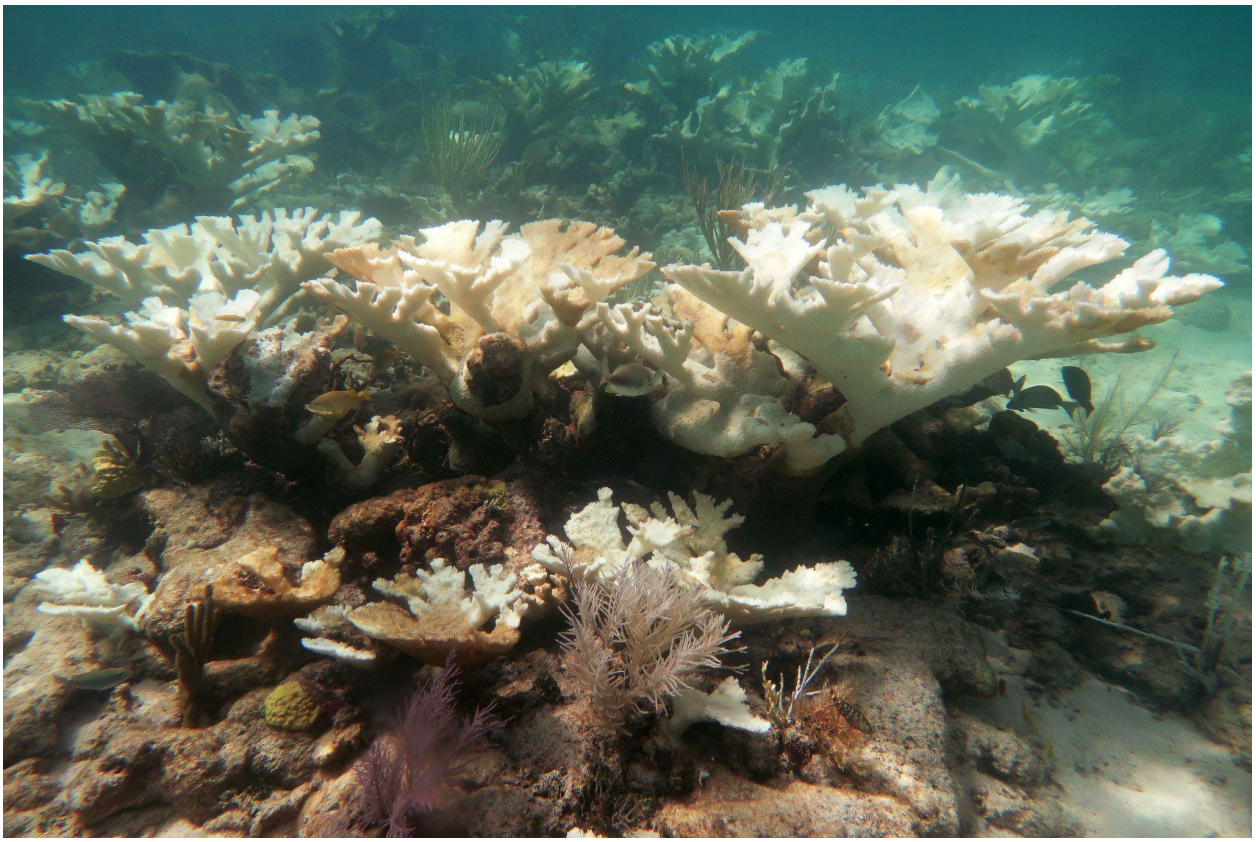
Genotypic inventory and impact of the 2023 marine heatwave on *Acropora palmata* (elkhorn coral) populations in the Upper Florida Keys, USA: 2020-2023

Dana Williams[1,2], Ken Nedimyer[3], Allan Bright[1,2], Mark Ladd[1]

[1] Population and Ecosystems Monitoring Division, NOAA Southeast Fisheries Science Center, Miami, Florida, USA
[2] University of Miami, Cooperative Institute for Marine and Atmospheric Studies, Miami, Florida, USA
[3] Reef Renewal USA, Inc, Tavernier, Florida, USA



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Cover Photo: Stand of *Acropora palmata* colonies at Turtles Rocks in the upper Florida Keys on July 24, 2023. Bright white areas are dead exposed skeleton not bleached tissue, indicating these areas died within the past few days. Light tan areas are dead skeleton with very light algal colonization indicating those portions of the colonies likely died a week before this photo was taken.

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EXECUTIVE SUMMARY

This report details the findings from extensive survey efforts conducted on reefs in the upper Florida Keys to characterize the abundance and distribution of genotypes for the ESA-listed coral species *Acropora palmata*. Between 2019 and 2023, we collected a total of 233 samples from *A. palmata* colonies present at reef sites spanning 50km of the Florida Reef Tract in the upper Florida Keys. These samples along with 57 archived samples collected prior to 2019 were evaluated using the Standardized Tools for *Acropora* Genotyping database (STAGdb) to complement genotyping results of additional upper Florida Keys samples (n=266) including those from nursery stock or contributed by other organizations. We used these genotype results to determine the total number of founder (unrelated native) genotypes sampled on reefs in the upper Florida Keys and to verify the number of founder genotypes currently held in offshore nurseries and gene banks. We identified 154 upper Florida Keys founder genotypes cataloged in STAGdb, 105 of which are believed to be in nursery holdings. Some nursery samples did not match the expected result indicating several potential resolutions including stock mixing or that there may be additional, unrecognized founder genotypes in the nursery stock. By 2022, only 12 of the upper Florida Keys founder genets that were missing from nursery holdings remained on the reef. Four of these founder genotypes were collected in 2022. In the summer of 2023, Florida's reefs experienced the most intense thermal stress event on record, causing widespread bleaching and mortality in the *A. palmata* population. The bleaching event prevented the collection of the remaining founders. Prior to the 2023 marine heatwave, 84 *A. palmata* upper Florida Keys founder genotypes remained alive on their native reefs. In late fall of 2023, we surveyed these known genets to record the fate of all known wild *A. palmata* colonies remaining on reefs in the upper Florida Keys. These surveys revealed that only 23 of the 84 founder genets remained on their native reefs. Moreover, the 23 surviving genets were restricted to five reefs in the upper Florida Keys, with all but five genets concentrated at a single site, Elbow Reef. Of the 18 genets remaining at Elbow Reef, only 14 genets had ramets that were large enough to be considered reproductive. Our findings highlight the urgent need to ensure that nursery stocks are correctly identified and that any remaining founders on the reef are identified and collected to preserve as much native *A. palmata* diversity as possible. Producing novel genotypes via the assisted sexual reproduction of surviving founder genotypes should be implemented to produce offspring that may be able to withstand future bleaching events.

Recommendations

- *Acropora palmata* nursery stock should be more thoroughly genotyped to ensure that known genotypes are represented in stocks and that genotypes are not mixed.
- Conduct more thorough searches of *Acropora palmata* habitat to locate any remaining surviving founder colonies and collect material for live gene-banking and nursery propagation. Surviving outplanted colonies should be genotyped to ensure they are represented in live gene banks and nursery stocks.
- Establish spawning hubs at offshore nurseries and on cooler reefs to facilitate both natural and human assisted reproduction.
- Create sexually produced offspring of known parentage for restoration aimed at minimizing back crossing.

GLOSSARY

founder genet stand - A collection of colonies sharing common contiguous reef structure that are believed to share a common genotype that originated from naturally occurring colonies rather than from human assisted crosses.

founder genotype - Genotype that originated from naturally occurring colonies rather than from human assisted crosses. Founder genets/genotypes have no known relationship to other founder genotypes in the population. They may exist as outplanted colonies on the reef but they originated from naturally occurring colonies.

genet - The collection of colonies or ramets belonging to the same genotype.

genotype - Identifier assigned to genetically distinct individuals.

live - Classification for colonies found to have any amount of live tissue during the post bleaching surveys conducted in late fall 2023, the alternate classification is 'summer dead' (see below).

native reef - Reef where a particular founder genotype was originally found.

putative genotype - Nursery stock originating from the same location and assumed to belong to a single genotype prior to formal genotype analysis.

ramet - An individual colony that shares the same genotype with other surrounding colonies (aka a clone or fragment).

remnant - Colonies estimated to be less than 20 cm in diameter.

reproductive - Colonies with more than 40 cm of contiguous live tissue.

SNP ID - The 6-character alphanumeric identifier assigned to a distinct genotype by the STAGdb. The IDs begin with HG followed by 4 numbers assigned sequentially as sample results are submitted.

stand - A collection of colonies sharing a common contiguous reef structure.

stock location - Colony-specific latitude and longitude where a particular putative genotype was originally collected for nursery stock.

stock samples - tissue samples collected from fragments propagated for nursery stock. While the samples were collected from fragments located in a nursery the stock location is the location on the reef where the stock was originally collected.

summer dead - Classification for colonies believed to be alive in early summer 2023 and found dead in post-bleaching surveys conducted in late fall 2023. These colonies likely died from heat stress but may also have died from disease in that timeframe. The alternate classification is 'live' (see above).

upper Florida Keys - Reef sites located from Turtle rocks (latitude 25.9° N) to Little Conch Reef (24.9°N).

wild colonies - Naturally occurring colonies that were not outplanted.

INTRODUCTION

The earliest documentation of the genotypic diversity of the upper Florida Keys *Acropora palmata* population occurred after Baums et al. (2005a) developed the necessary microsatellite markers to distinguish genotypes of *A. palmata*. In 2006 and 2010, colonies of *A. palmata* at several sites in the upper Florida Keys were sampled as part of NOAA SEFSC's Coral Research and Assessment Lab demographic monitoring program (Williams et al. 2014). At these sampled sites, genotypic diversity was found to be lower than reported at other Caribbean sites (Baums et al. 2005b) and declined from 2006 to 2010 (Williams et al. 2014). In addition to a decline in genotypic diversity, our annual demographic monitoring data showed a significant decrease in the abundance of *A. palmata* across the upper Florida Keys since 2004 (Williams et al. 2012, 2017) primarily attributed to several hurricanes and bleaching events. For example, following Hurricane Irma in 2017, we found that up to 70% of the total number of genotypes remaining in our monitoring plots had been lost (Williams et al. 2020). The substantial loss of *A. palmata* genotypes as a result of significant decline in colony abundance escalated the urgency to better document the remaining genotypes in the Florida Keys.

Prior to 2020, genotyping could only be done using microsatellite markers, which made it possible to distinguish unique genotypes within a single set of samples, but matching genotypes analyzed by different laboratories was not reliable. In 2020, Kitchen et al. (2020) developed the Standardized Tools for *Acropora* Genotyping database (STAGdb), which is based on a standardized array of single nucleotide polymorphism (SNP) markers (*Acropora* SNPchip), and provides a unified workflow that ensures a given genotype will be recognized as the same regardless of when or where it was analyzed, and also provides a uniform naming scheme. The STAGdb allows for comparison of corals held in nursery stocks with colonies on their native reefs which makes it possible to identify founder genotypes that should be prioritized for collections to ensure they are represented in nursery stocks and gene banks.

Our assessment of the status of the upper Florida Keys *A. palmata* founder genets coincided with an unprecedented thermal stress event in the summer of 2023 that caused mass bleaching and affected most coral species on reefs in the Florida Keys, including *A. palmata*. This thermal stress event was unprecedented in several ways: water temperatures exceeded thermal stress thresholds earlier than ever recorded (mid-July vs. previous timing of mid-August), water temperatures exceeded recognized coral bleaching thresholds (Manzello et al. 2007) for longer than ever recorded, and as a result, thermal stress accumulation, measured as Degree Heating Weeks (DHWs), was higher than ever recorded in Florida (Hoegh-Guldberg et al. 2023).

Past disturbance events affecting Florida Keys *Acropora palmata*

The decline of the *A. palmata* population in the upper Florida Keys during the past three decades is largely attributed to thermal stress events and physical disturbance from hurricanes. Prior to 2023, there were six recorded mass bleaching events in the Florida Keys: 1987, 1997, 1998, 2011, 2014, and 2015 (Manzello et al. 2007). Prior to the 2011 bleaching event, the impact of bleaching on Florida's *A. palmata* population was poorly documented. Dramatic losses between 1998 and 1999 were noted on reefs in the upper Florida Keys (Miller et al. 2002), and although the cause(s) for the losses could not be identified, they were likely driven by a combination of the 1998 bleaching event and Hurricane Georges that impacted Florida in early fall 1998. In contrast, the impacts of the 2011, 2014, and 2015 bleaching events were well documented (Williams et al. 2017) and revealed that the 2011 event had no

discernible impact on the abundance of *A. palmata*, while the 2014 and 2015 events each resulted in a loss of one third of the live tissue area. Our team's demographic monitoring efforts also documented severe declines in the upper Florida Keys' *A. palmata* populations as a result of an extremely active 2005 hurricane season, which contributed to more than half of live *A. palmata* tissue loss between 2004 and 2010 (Williams and Miller 2012). Hurricane Irma in 2017 caused substantial loss of *A. palmata* genotypic diversity in the Florida Keys (Williams et al. 2020). While *A. palmata* stands have the potential to recover from moderate physical disturbances, Hurricane Irma decimated entire stands making localized recovery impossible. Given the numerous disturbances experienced by the *A. palmata* population in the upper Florida Keys over the past 25 years, the genotypes that remained on their native reefs in 2023 were largely considered to be relatively 'hardy' genotypes that were able to persist in the current climate.

METHODS

Identifying *Acropora palmata* founder genets on their native reefs

Florida Keys *A. palmata* founder genotypes have been identified by directly sampling the colonies at their native reef or by sampling nursery stock that originated from known locations (henceforth referred to as 'stock locations'). As part of NOAA SEFSC's Coral Research and Assessment Lab demographic monitoring program, we exhaustively sampled many upper Florida Keys stands of *A. palmata* from Carysfort to Molasses reef between 2005 and 2010 to document the genotypic diversity of the monitored stands (Williams et al. 2014). After the *Acropora* SNPchip (Kitchen et al. 2020) was developed, we re-sampled as many of the previously identified distinct *A. palmata* founder genotypes as possible within our monitoring plots (n = 70). For instances where the original colonies had died, we re-analyzed archived samples (n = 57). In addition to colonies within our existing demographic monitoring plots, we also sampled wild *A. palmata* colonies (n = 163) located within 33 different stands spread across the upper Florida Keys that had not been genotyped before. This sampling approach was designed to identify as many distinct genets as possible rather than quantify diversity. Sampling all colonies within each stand was prohibitive from a time and cost standpoint. Consequently, for stands that included more than three colonies, we sampled a subset of colonies. In these instances, we targeted colonies that were farther apart within the stand or had differing morphologies (often indicative of different genotypes) when possible; otherwise, a haphazard selection of colonies was sampled. Other groups have collected samples from upper Florida Keys founder colonies on their native reefs (n = 10 samples), outplanted colonies (n = 67), and coral nursery organizations have genotyped their nursery stock/gene banks (n = 189) and contributed their results to the STAGdb (accessed on 7/10/2023) for a total of 556 samples from upper Florida Keys founders. There are an additional 289 samples from founders in the middle and lower Florida Keys in the STAGdb. However, all but 14 of the lower Florida Keys samples were collected by other groups that may have employed different sampling approaches making it difficult to integrate these results with those from the Upper Keys, and thus they are not included in this report.

Nursery stock evaluation

Nursery stock collected by restoration agencies for fragmentation-based nursery propagation and outplanting was originally differentiated as putative genotypes based on the stock collection locations.

Typically, fragments collected from spatially separated stands were assumed by the restoration agency to be distinct genotypes and assigned a nursery name. Nursery stock samples in the STAGdb are those collected from a fragment within the nursery stock or gene bank by one of the four nurseries that have collected *A. palmata* stock from reefs in the upper Florida Keys between 2014 and 2022 (n = 184) (henceforth referred to as 'stock samples'): The Coral Restoration Foundation (n = 74), Reef Renewal (n = 63), Mote Marine Laboratory (n = 21), and The Florida Aquarium (n = 19). Native reef samples are those collected from naturally occurring colonies at their native reef. There are 300 upper Florida Keys native reef samples in the STAGdb. Using the STAGdb, we compared the stock samples with the list of native reef samples collected from upper Florida Keys founder genotypes in the STAGdb.

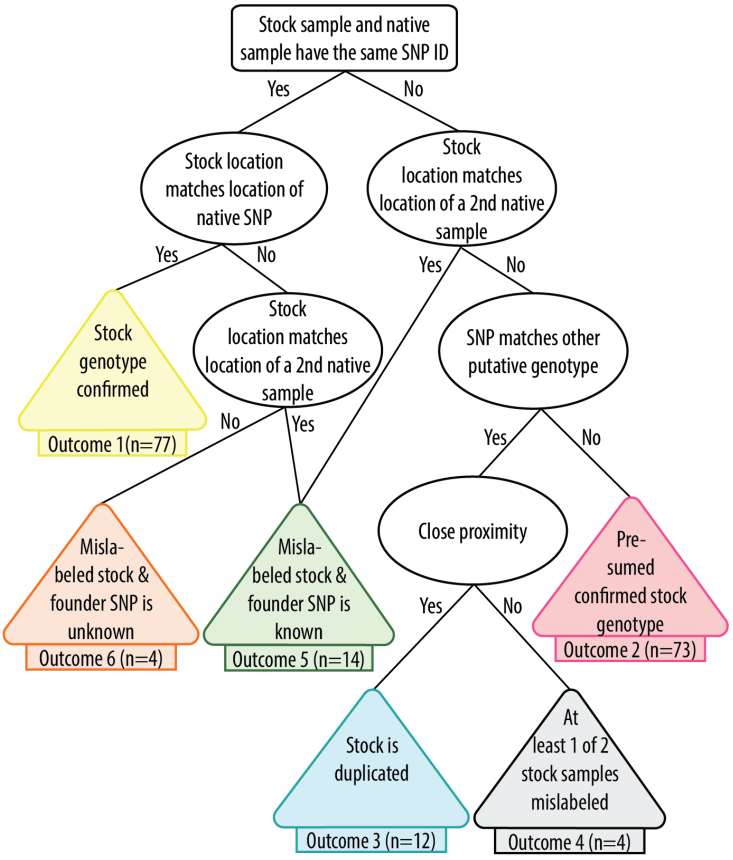

Figure 1. Decision tree used when comparing *Acropora palmata* genotyping results from nursery stock (n = 184) samples to upper Florida Keys native reef samples as reported in the Standardized Tools for Acropora Genotyping database (STAGdb).

Each sample in the STAGdb contributed by a nursery (n = 184) was mapped in Google Earth Pro (version 7.3.6.9345) using the corresponding colony-specific stock collection locations (i.e., 'stock location') along with each native reef sample (n = 300) to either confirm that locations matched, or to assist with reconciling any discrepancies. Because numerous outcomes were possible during this process, a decision tree (**Fig. 1**) was developed to arrive at one of six outcomes based on whether the SNP ID and collection locations of stock samples and native reef samples matched. The six possible outcomes were:

- ***Outcome 1:*** The stock sample matched both the location and SNP ID of a native reef sample and considered correct.
- ***Outcome 2:*** The stock sample did not share a SNP ID with any other samples and originated from a location where no other samples were collected and presumed to be correct.
- ***Outcome 3:*** Two stock samples from different putative genotypes were found to share a single SNP ID and the original stock collection locations for both were found to be on the same reef structure and designated as a duplicate collection.
- ***Outcome 4:*** Two stock samples from different putative genotypes were found to share a single SNP ID but the original stock collection locations for both were far apart. In this case the SNP ID of one stock sample was assumed to be correct and the other was mislabeled, but it is impossible to know which.

- ***Outcome 5:*** The stock sample SNP ID matched a native sample SNP ID from a different location than where the stock was originally collected but a second native sample was collected at the stock location, then the stock is mislabeled but the SNP ID of the stock is expected to be the same as the second native sample.
- ***Outcome 6:*** The stock sample SNP ID from stock originally collected from a location where no native samples were collected matched a native sample SNP ID then the true SNP ID of the stock sample is unknown.

2023 bleaching event

Beginning with the onset of the heat stress in early July of 2023, as many *A. palmata* founder genets as possible were monitored intermittently during the thermal stress event to document the extent of bleaching, mortality, and recovery. This monitoring was not conducted on a regular, pre-determined schedule due to a number of factors: the high number of upper Florida Keys founder genets coupled with their broad geographic spread, various logistical constraints related to our team's multiple on-going projects, and inclement weather.

As part of our ongoing *A. palmata* demographic monitoring program, we maintained temperature loggers (Onset HOBO MX2203) on the reef at our upper Florida Keys monitoring sites to record *in situ* water temperatures. Temperature loggers were deployed in locations out of direct sunlight and recorded water temperature every 30 minutes. We calculated the maximum daily average water temperature at selected sites and identified the maximum of the 30 minute temperature readings recorded between June and October. Based on bleaching temperature thresholds determined during the recent bleaching events in the summers of 2011, 2014, and 2015 (Williams et al. 2017), we tallied the number of days where the daily average water temperature reached 31.0 ^{o}C or greater. Additionally, we determined the date where each site had accumulated more than 21 days of daily average temperatures of 31.0 ^{o}C or over, a metric which serves as a predictor of bleaching in *A. palmata* (Williams et al. 2017).

Founder genet post-bleaching surveys

After water temperatures declined in late October of 2023, we conducted a survey of 99 stands where founder genets were previously identified either through direct sampling or nursery stock collections (henceforth referred to as 'founder genet stand') to record their post-bleaching status and identify as many upper Florida Keys surviving genets as possible. For these post-bleaching surveys, each founder genet stand was searched by SCUBA divers for any live or dead

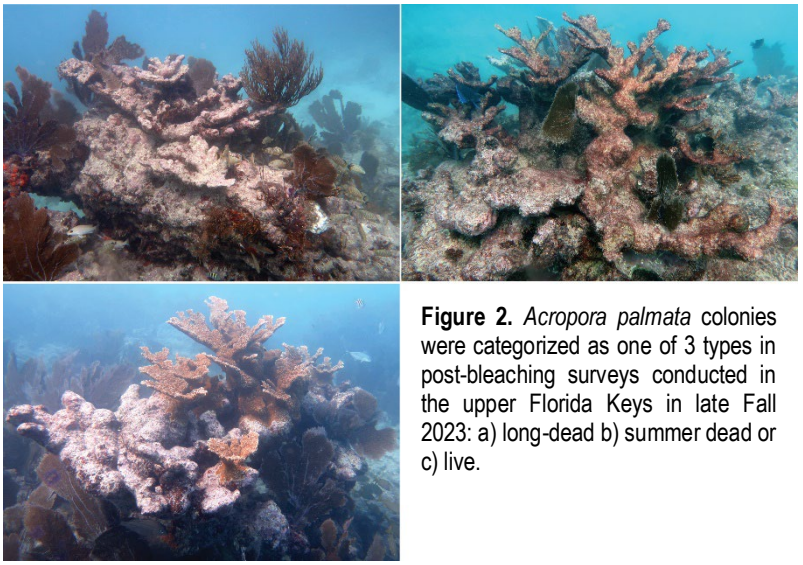

Figure 2. *Acropora palmata* colonies were categorized as one of 3 types in post-bleaching surveys conducted in the upper Florida Keys in late Fall 2023: a) long-dead b) summer dead or c) live.

A. palmata colonies. All colonies within a stand were assumed to be ramets of a single genet for the purposes of this survey. At founder genet sites where either no colonies or only colonies with highly eroded skeletal structures that were indistinguishable from the surrounding benthos were found, the founder genet was considered 'long dead' (i.e., died prior to 2023). For the remaining stands, we categorized colonies as either 'live' if they had any live tissue remaining, or 'summer dead' when the colonies had no live tissue but the skeletal structures still had well defined corallites and light to moderate algal turf colonization (**Fig. 2**; Leggat et al. 2019). Due to these characteristics, the 'summer dead' colonies were assessed to have been alive in early summer 2023 prior to the bleaching event. While we could not definitively determine the timing or cause of mortality, the condition of these skeletal structures was consistent with the condition of coral skeletons at sites where the timing of colony mortality was well documented (**Fig. 3**), allowing us to confidently determine that they died during the summer or early fall of 2023.

In founder genet stands where both 'live' and 'summer dead' ramets were present (n = 82), the approximate numbers of both types of colonies were further categorized into one of five size categories based on a coarse visual estimate of colony size: remnant, small, medium, large, and extra-large (**Suppl. S1**). These size categories were assigned a representative diameter for the purposes of approximating the amount of live tissue remaining for each genet. The assigned sizes (remnant [20 cm], small [40 cm], medium [80 cm] large [120 cm] and extra-large [170 cm]) were squared for each colony as an approximation of colony area. To approximate the live area of each *A. palmata* founder genet present during post bleaching surveys, the areas of all 'live' ramets in the stand were summed. To approximate the genet's live area in early summer, the area 'live' + area 'summer dead' was calculated. Founder genets with at least one ramet categorized as 40 cm or larger were counted as 'reproductive' genets (Williams et al. 2020) and genets with only remnant (20 cm) ramets were counted as remnant genets.

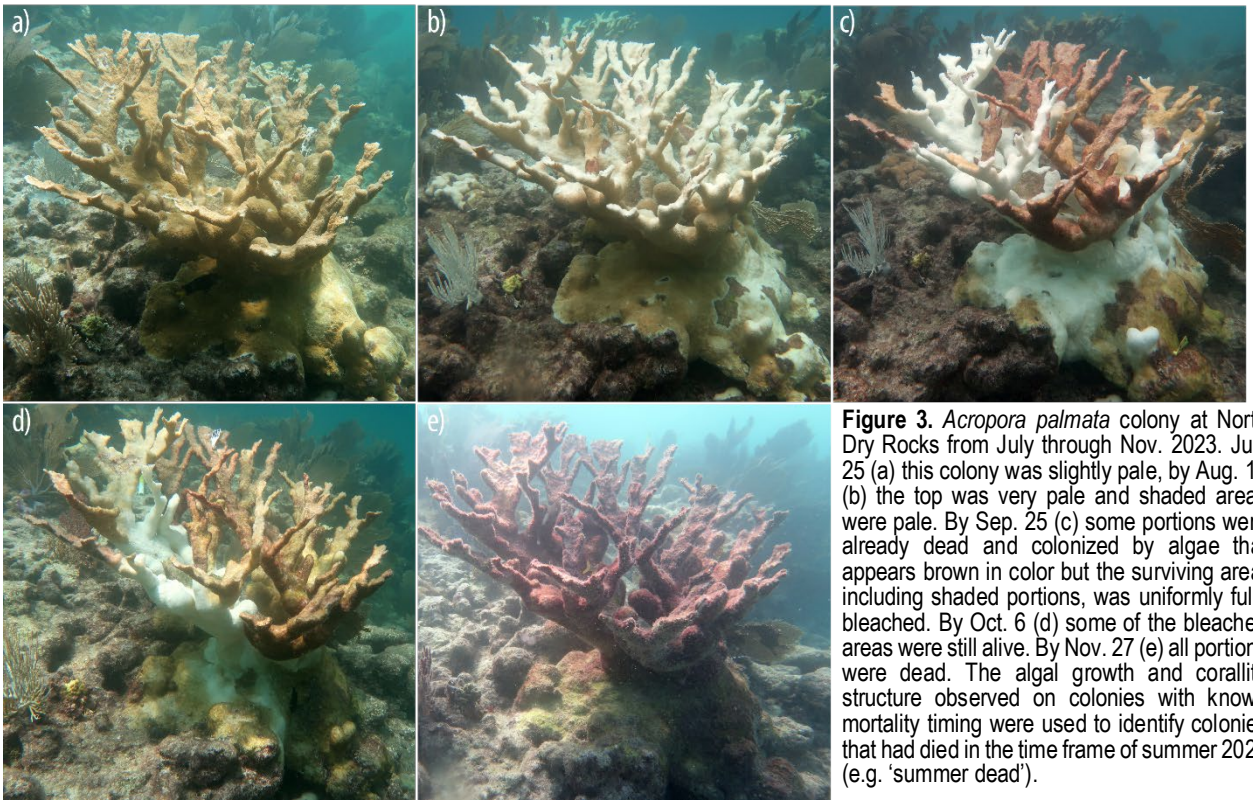

Figure 3. *Acropora palmata* colony at North Dry Rocks from July through Nov. 2023. July 25 (a) this colony was slightly pale, by Aug. 17 (b) the top was very pale and shaded areas were pale. By Sep. 25 (c) some portions were already dead and colonized by algae that appears brown in color but the surviving area, including shaded portions, was uniformly fully bleached. By Oct. 6 (d) some of the bleached areas were still alive. By Nov. 27 (e) all portions were dead. The algal growth and corallite structure observed on colonies with known mortality timing were used to identify colonies that had died in the time frame of summer 2023 (e.g. 'summer dead').

The location of all known founder genets and their estimated live areas were mapped in Google Earth Pro (version 7.3.6.9345) to visualize changes in the distribution of *A. palmata* founder genets on reefs in the upper Florida Keys following the marine heatwave of 2023.

RESULTS & DISCUSSION

Nursery stock evaluation

When samples collected from nursery stock fragments (n = 184) were matched with native reef sampled genotypes, each stock sample was assigned one of the six possible outcomes (**Figs. 1 and 4**). An example of genotype database entries illustrating how samples from nursery stock and native reef were reconciled (i.e. the above six scenarios) to determine the total number of founders expected and to verify nursery stock is provided as **Figure 3**. There were 77 stock samples that matched origin location and SNP ID with a known founder genet sampled on its native reef, providing high confidence that they are correctly identified (Outcome 1). There were 73 stock samples for which there was no native reef sample from the same location, and as expected, those samples do not match any other genotypes and are presumed to be correctly identified (Outcome 2). There are 12 stock samples that matched a putative genotype collected from a neighboring stand indicating that neighboring stands shared the same genotype and the nursery stock was likely duplicated (Outcome 3). There are four stock samples that match other stock samples from different stock locations that are expected not to match. Because they do not match any native reef samples, it is impossible to know which sample is correct and which sample is mislabeled (Outcome 4). There are 14 stock samples that match a native sampled founder on a reef other than one where the stock originated indicating the nursery stock is mislabeled. Because the stock location matches a native reef sample, the expected SNP ID is known (Outcome 5). There are four

	Sample Type	Origin Location	Sample Name	SNP ID	Conclusion	Assumed SNP Match	
1	Native Reef	KL Dry Rocks	KL-36	HG2628	Correct	HG2628	Native reef sample from location where no nursery stock collection was made, thus no match is expected.
2	Nursery Stock	Pickles	Apal-A	HG3159	Correct	HG3159	Stock sample from stock location where no native reef samples were collected thus no match is expected.
3	Nursery Stock	Grecian	Apal-B	HG4710	Duplicate stock	HG4710	Stock samples from neighboring stock locations on the same reef. It is most likely the neighboring stands shared the same genotype and the stock is duplicated.
	Nursery Stock	Grecian	Apal-C	HG4710	Duplicate stock	HG4710	
4	Nursery Stock	Sand Island	Apal-D	HG5822	Unknown	Unknown	Stock samples from different stock locations share the same SNP ID but no native reef samples exist from either location. Presumably one is correct and one is mislabeled but it is impossible to know which.
	Nursery Stock	French	Apal-E	HG5822	Unknown	Unknown	
5	Native Reef	Elbow	EL-57	HG6194	Correct	HG6194	Stock and native reef samples expected to match but don't, indicating that the stock sample is mislabeled but likely matches a known genotype.
	Native Reef	Carysfort	CF-88	HG7345	Correct	HG7345	
	Nursery Stock	Elbow	Apal-F	HG7345	mislabeled	HG6194	
	Nursery Stock	Carysfort	Apal-G	HG7345	Correct	HG7345	Stock and native reef samples match.
6	Native Reef	Molasses	ML-23	HG8550	Correct	HG8550	Stock and native reef samples match.
	Nursery Stock	Patch Reef X	Apal-H	HG8550	mislabeled	Unknown	Stock sample matches a native reef sample from a different location indicating it is mislabeled but no matching native reef sample exists so the SNP ID is unknown.
	Nursery Stock	Molasses	Apal-I	HG8550	Correct	HG8550	

Figure 4. Example Standardized Tools for *Acropora* Genotyping database (STAGdb) entries illustrating how samples from nursery stock and native reef were reconciled to determine the total number of *Acropora palmata* founders expected and to verify nursery stock. This table shows six examples of each of the six possible outcomes, see text for further explanation. Note that 'Origin Locations' are reported in the STAGdb or in stock collection records as colony-specific latitude and longitude that are more precise than general reef names shown in this example.

stock samples that originated from locations where no native reef samples were ever collected and therefore were not expected to match SNP ID with a native sample. Because these stock samples did match a native reef sample we can conclude that the stock sample is mislabeled but the stock is still believed to have originated from a unique founder genet but SNP ID is unknown (Outcome 6). In cases where we conclude that the stock sample is mislabeled, it is possible that the mislabeling occurred at the time that the sample was collected or analyzed, but it is also possible that it is an indication the nursery stock includes fragments of more than one genotype.

Stock samples that fell under Outcome 4 or Outcome 6 support the existence of four upper Florida Keys founder genets that have never been genotyped (**Fig. 2**), and thus, have no unique SNP ID but may still be present in the nursery stock and could potentially be identified with additional sampling of nursery stock. In Outcomes 5 and 3 (**Fig. 4**), the SNP results appear superficially the same: two putative nursery genotypes have the same SNP ID (putative genets Apal-B and Apal-C share the same SNP ID: HG4710, and putative genets Apal-F and Apal-G share the same SNP ID: HG7345). This result could be interpreted as two sets of nursery stock that are redundant and could be merged. However, by comparing the stock collection locations we deduce it is likely that Apal-B and Apal-C were duplicate collections from neighboring stands. In contrast, Apal-F and Apal-G originated from two different reefs; thus, they are expected to be different, and should not be merged.

There are 105 upper Florida Keys founder genotypes present in samples from at least one of the four Florida Keys nursery organizations sampled between 2014 and 2022. There may be an additional four founder genets that were not identified due to stock labeling issues, and additional sampling of nursery stock may uncover additional unrecognized genets.

Acropora palmata founder genotypes

As of December of 2023, there are 154 founder genotypes of *A. palmata* from upper Florida Keys reefs currently identified in the STAGdb. After reconciling stock samples with the native reef samples, we concluded that there are an additional four upper Florida Keys founders that are likely to be unique based on their original stock collection location bringing the total number of identified upper Florida Keys *A. palmata* founders to 158 genotypes distributed among 24 reefs ranging from Turtle Rocks in the north to Little Conch Reef in the south (**Suppl. S2**). The set of samples used to tally the number of founders does not represent the genotypic diversity at any one time point since the collections were made over a twenty-year time span; rather, it is a subset of genotypes that can be tracked over time.

Of the 53 founders that were not found among the 105 genotypes identified in nursery genotype samples (see *Acropora palmata* founder genotypes section below), 11 were no longer present on upper Florida Keys reefs in 2013 when nursery collections of *A. palmata* began in earnest. By 2022, when most nursery stock genotyping was completed, only ten of the 'missing' genotypes (i.e. those known to exist on the reef but not represented in a nursery stock) were believed to be remaining on upper Florida Keys reefs indicating that they should be prioritized for collection and incorporation into nursery stocks. Four of the identified missing founder genotypes from nursery stock were collected in 2022, prior to the 2023 bleaching event, and an additional four were collected during the 2023 bleaching event. However, these later four collections did not survive the event.

Two likely new founder genotypes were identified in 2023 and two more in early 2024. Colonies of these four founder genotypes have been sampled but not yet analyzed in STAGdb at the time of this

report. Based on their locations, it is reasonable to believe that they are unique founders that are not represented in any nursery stock. These previously unidentified founder colonies were located in areas that are not frequented by divers, or were adjacent to other known founder genotypes and assumed to already be collected and in nursery stock. Thus, it is possible that additional unidentified founder genets remain to be discovered on reefs in the upper Florida Keys.

Based on our sampling of 27 stands between 2019 and 2022, 20 stands had more than one live colony. Of those 20 multi-colony stands, only two stands (10%) included more than one genotype. In contrast, from the 24 stands sampled between 2006 and 2010, 16 stands were found to include more than one genotype (67%; Williams et al. 2014). However, by 2020, only 16 of those 24 remained alive and only two of the 16 remaining stands with live colonies consisted of more than 1 genet (12%; Williams et al. 2020). Thus, while our more recent sampling did not sample every colony within all stands, our finding of mostly monoclonal stands is consistent with what was observed within fully sampled plots in 2020.

2023 Bleaching event

Water temperatures in the summer of 2023 featured three heatwaves punctuated by wind events that likely helped lower temperatures down to near-typical summer temperatures for upper Florida Keys reefs (**Fig. 5**). The first heatwave began on July 4 and lasted through July 26. During this July heatwave, daily average water temperatures surpassed 31.3 ºC, the threshold for bleaching in *A. palmata*

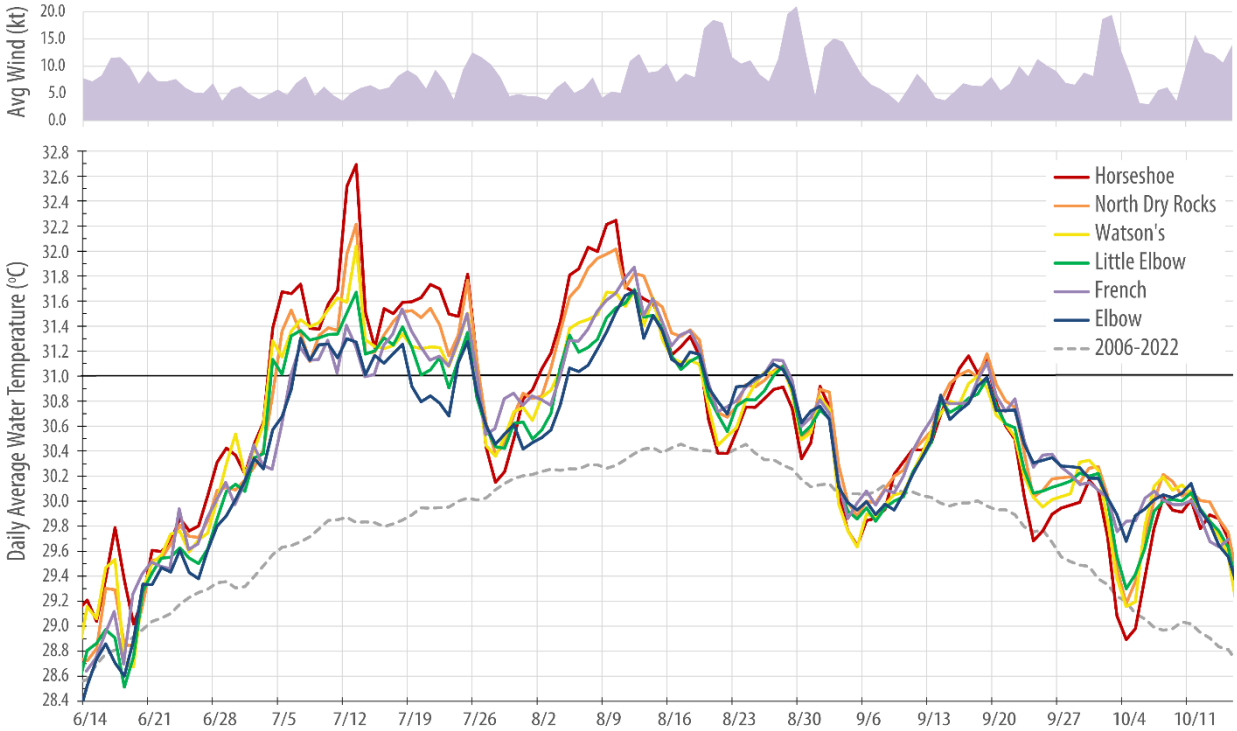

Figure 5. Daily average water temperatures from five selected reef sites with live *Acropora palmata* colonies at the start of summer 2023. This subset of sites shows the range of thermal regimes corals experienced during 2023 from Horseshoe Reef, the most inshore site to Elbow reef, the most offshore of the selected sites. The solid line depicts the 31.3 ºC threshold for bleaching in *A. palmata*. Dashed line shows the daily average water temperatures from typical upper Florida Keys fore-reef sites averaged from 2006 to 2022 (Williams et al. 2015). The top frame shows the daily average wind speed as measured at Carysfort Reef (wind data provided courtesy of WeatherFlow Networks).

(Williams et al. 2017), at all monitored sites except Elbow Reef. The intensity and duration of this heatwave were highest at the sites closest to shore that are surrounded by shallow water and decreased in intensity and, to some extent, duration as the distance from shore increased. During the July heatwave, water temperatures were not only higher than typical peak summer temperatures, but this heatwave also occurred four to six weeks earlier than the typical peak in water temperatures during the past 15+ years (**Fig. 5, Suppl. S3**). The second heatwave began around August 1 and persisted through August 19. During this heatwave, daily average temperatures surpassed 31.3 ^{o}C at all monitored sites including Elbow Reef. The third heatwave began on September 9 and persisted through September 23. During this heatwave, daily average temperatures did not reach 31.3 ^{o}C at any of the sites; however, they did exceed 31.0 ^{o}C for several days at some sites. Temperatures at all monitored sites dropped and remained below 30.5 ^{o}C after September 23. We assessed 47 genets at 17 upper Florida Keys reefs at various time points during the 2023 bleaching event (July through September). During this timeframe, all genets surveyed experienced some degree of bleaching.

Early mortality

The only remaining genet at Turtle Rocks in summer of 2023 was a sprawling stand of well over 100 *A. palmata* colonies in shallow back reef habitat that has persisted through several recent bleaching events and hurricanes. When we visited the site on July 24, 2023, all colonies were dead except for a single patch of bleached white tissue found remaining on one colony. Based on the very light to absent algal colonization on the skeletons, nearly all of the mortality had likely occurred within the past week or two; though, most mortality occurred within the past few days (**Suppl. S4**). One of two remaining genets at Horseshoe Reef in the Summer of 2023 was a large relatively dense thicket of *A. palmata* that has also persisted through numerous disturbances. When we visited this site on July 22, 2023, colonies within this thicket contained some areas that were still alive with some color, some top sides that were fully bleached, and areas of exposed dead skeleton in various stages of recent algal colonization indicating more incremental mortality than observed among the colonies at Turtle Rocks.

Such early mortality at these sites was particularly surprising as both of these sites are routinely warmer than outer fore reef sites and the genotypes that were thriving at these sites were observed to be relatively heat tolerant during recent bleaching events in 2014 and 2015 (unpubl. obs. Williams). Accordingly, the early mortality of wild *A. palmata* colonies at Turtle Rocks and Horseshoe Reef suggests a more acute stress response (Jones 2008) than observed at most other sites during the 2023 bleaching event. Unfortunately, the temperature logger deployed at Turtle Rocks failed prior to our July 24 survey, and thus, we do not have temperature data for early summer. However, based on the timing of our survey and temperature data from nearby reefs in similar habitats, it is unlikely that temperatures exceeded 31 ^{o}C for more than 21 days before colonies at Turtle Rocks experienced acute mortality. This contrasts our observations during the 2014 bleaching event, where the colonies of the Turtle Rocks genet survived 31 days over 31 ^{o}C, indicating high thermal tolerance relative to genets at other sites (**Suppl. S3**). Consequently, since temperatures in July of 2023 were likely only above 31.0 ^{o}C for ≤21 days, our observation of complete and acute mortality suggests that something other than accumulated thermal stress drove mortality at this site. One plausible explanation is that temperatures in July 2023 exceeded the maximum temperatures that this genet could tolerate even for short term exposure. Alternatively, high light intensity in early July near the summer solstice may have compounded what would normally be a non-lethal thermal stress exposure, causing the acute mortality of this thermally

tolerant genet. Interestingly, on July 22, a second genet at Horseshoe Reef that is found slightly deeper (~12 ft) on the offshore side of the reef, was pale but had no mortality. However, by August 7, it was observed fully bleached but alive and in late September it was observed colonized with algae consistent with it having died in mid to late August.

Post-bleaching surveys

Of the 162 (including the four discovered in 2023 and 2024) known upper Florida Keys founder genets, only 101 were believed to remain alive on their native reef prior to 2023. Many of the 61 founder genets no longer present on the reef in 2023 were likely lost during Hurricane Irma in September of 2017 (Williams et al. 2020). Of the 101 upper Florida Keys founder genet stands surveyed after the 2023 bleaching event, 17 had no identifiable colony structures that could have been alive in the past year, indicating that the founder genets originally located in these stands had died before 2023. Twenty-three of the founder genet stands had live *A. palmata* tissue remaining, and at the remaining 61 sites surveyed, we found no live *A. palmata*, only 'summer dead' *A. palmata* skeletal structures (**Fig. 1**). The 23 founder genet stands containing live *A. palmata* were confined to only five reefs in the upper Florida Keys: Carysfort, Elbow, French, Little Elbow, and Watson's Reefs (**Figs. 6** and **7**; **Table 1**). Only six of those 23 founder genets appeared roughly unchanged in their amount of live tissue area, the remaining 17 lost on average an estimated 75% of their live area.

Table 1. Distribution of the 84 *Acropora palmata* founder genets that were alive in early summer 2023 at upper Florida Keys reef sites and their status in surveys conducted after the bleaching event (10/31-12/5/2023). Genets classified as 'reproductive' had at least one ramet 40 cm or larger, 'remnant' genets had no ramets large enough to reproduce and 'summer dead' genets had only colony structures that were estimated to have died during the bleaching event remaining. Tallies include two colonies (one at Carysfort and one at French) discovered in early 2024.

Reef	Early Summer 2023		Post- Bleaching Fall 2023		
	Repro-ductive	Remnant	Repro-ductive	Remnant	Summer Dead
Allan's	1	0	0	0	1
Carysfort	3	2	0	1	4
Conch	2	1	0	0	3
Elbow	24	4	12	6	10
French	2	2	1	0	3
Grecian	2	2	0	0	4
Horseshoe	2	0	0	0	2
Little Conch	2	0	0	0	2
Little Elbow	2	0	1	1	0
Little Grecian	2	0	0	0	2
Molasses	4	0	0	0	4
North Dry Rocks	4	0	0	0	4
North North Dry Rocks	4	0	0	0	4
Phils	1	0	0	0	1
Pickles	1	1	0	0	2
Sand Island	1	1	0	0	2
South Carysfort	3	1	0	0	4
South Carysfort Patch	1	0	0	0	1
Triple A	1	0	0	0	1
Turtle Rocks	1	0	0	0	1
U91	1	0	0	0	1
Watson's	6	0	0	1	5
Total	70	14	14	9	61

While we did not systematically survey outplanted colonies, we encountered many outplanted stands of *A. palmata* during our post-bleaching surveys with no surviving colonies. Our past monitoring work included outplants of various known genotypes planted at Carysfort, Elbow, North Dry Rocks, Grecian Rocks, French, Sand Island, Molasses, and Pickles Reefs. In our post bleaching surveys, we found a small number of surviving outplanted *A. palmata* colonies at Carysfort, Elbow, French, Sand Island, and Molasses Reefs. While the same genotypes were not planted at all locations, at least eight outplanted founder genotypes survived at Carysfort Reef and four at Sand Island Reef (a total of 10 unique

genotypes between these two sites). At Elbow Reef, there were seven outplanted founder genotypes prior to the bleaching event and six of them survived the 2023 bleaching event at this site.

Concurrent disease-like mortality

Acute thermal stress-associated mortality can occur before corals have exhibited bleaching (Jones 2008). However, mortality that occurs in corals that have been bleached for weeks is expected to follow a relatively reliable pattern of progression. In *A. palmata*, the upper surfaces of colonies (e.g. those that are shallower and/or exposed to direct sunlight) typically pale and bleach earlier than the lower or shaded parts of the colony. When bleaching is severe and persists long enough to cause tissue mortality, it is common to see the portions of the colony that bleached first succumb to mortality first (**Suppl. S5**). Thus, when tissue mortality does not follow this expected bleaching-mortality sequence (**Fig. 3**) it is possible that a different process, such as disease, is causing tissue mortality. It is also plausible that elevated temperatures exacerbate typical disease processes (Rogers and Muller 2012, Muller and van Woesik 2014) in already compromised coral tissue (Muller et al. 2018) causing tissue mortality uncharacteristic of bleaching associated mortality. In surveys conducted during the bleaching event we could not reliably distinguish acute bleaching associated mortality from disease associated mortality on bleached coral, however we did observe tissue mortality that did not progress as expected for bleaching associated mortality, as well as tissue that was not fully bleached dying rapidly suggesting some cause other than accumulated thermal stress alone had contributed to tissue mortality.

Surviving genotypes

Of the 23 surviving wild *A. palmata* genets, 18 are at Elbow Reef where water temperatures were lower in Summer 2023 than those recorded at any other monitored upper Florida Keys sites, particularly during the periods of most intense thermal stress (**Fig. 5**). Elbow Reef typically maintains lower temperatures in the summer months compared to other sites in the upper Florida Keys due to it's unique location along the reef tract that provides increased exposure to deeper oceanic water. Thus, the higher survival of genotypes at Elbow Reef does not necessarily indicate that the surviving genets are more thermally tolerant than the genets at warmer reefs that did not survive. However, two of the more closely monitored genotypes at Elbow Reef, HG1548 (**Suppl. S6**) and HG0083 (not pictured) stand out as genets that tolerated the elevated temperatures better than most at this site based on relatively healthy coloration throughout the summer of 2023 and minimal loss of live tissue.

The lone surviving wild *A. palmata* genet at Watson's Reef could also be considered more thermally tolerant as Watson's Reef is one of the warmer sites included in our monitoring **(Fig. 5)**. This genet was not surveyed during the height of bleaching, but on October 31, and although it still had bleached upper surfaces it also had normal colored shaded areas characteristic of recovery after bleaching. This one surviving genet at Watson's Reef at least partly bleached and lost some live area during the summer, but the small surviving colony had fully recovered by post-bleaching surveys in November unlike the other five genets at that site that suffered complete mortality. The surviving genets at Carysfort and French Reefs that were discovered in early 2024 could be considered at least moderately thermally tolerant. Water temperatures at French (**Fig. 5; Suppl. S3**) and Carysfort (**Suppl. S3**) reefs were higher than at Elbow but lower than at Watson's Reef and peaked in August rather than July. Additionally, these colonies were found at water depths of 15-20 ft and at sites where water clarity (and thus light penetration) is typically lower, which may have reduced additional stressors that can interact with

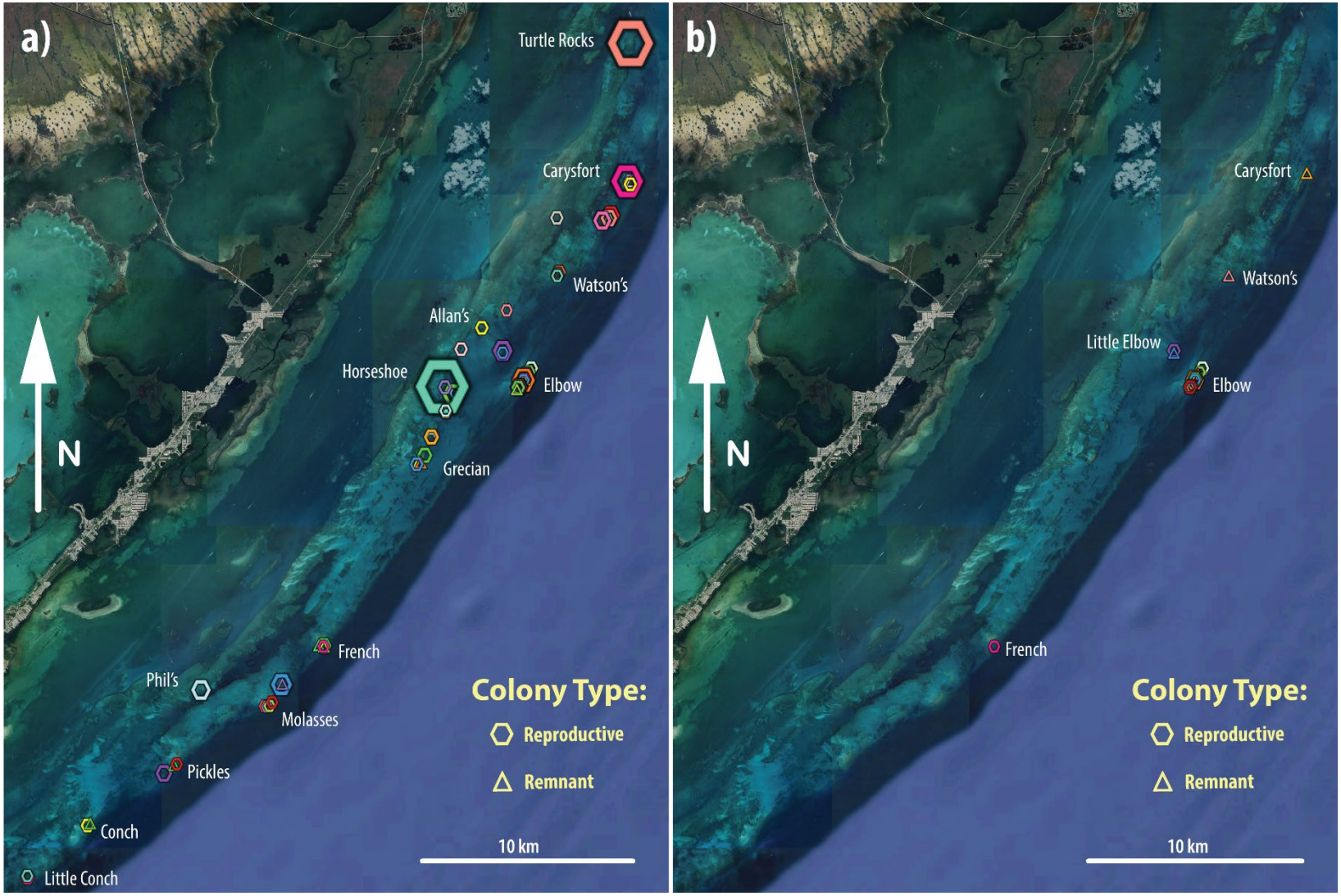

Figure 6. Map showing upper Florida Keys sites where *Acropora palmata* founder genets were located in (a) early summer 2023 and (b) late fall 2023 after the bleaching event. Points at Carysfort and French Reefs depict colonies found in early 2024. Symbol colors represent distinct genotypes within a reef (colors repeated at adjacent reefs are still distinct genotypes). Symbol size is scaled to indicate relative amount of live *A. palmata* at a site. Symbol shape indicates whether the genet includes colonies of reproductive size (>40 cm; hexagons) or only remnant colonies (~20 cm; triangles).

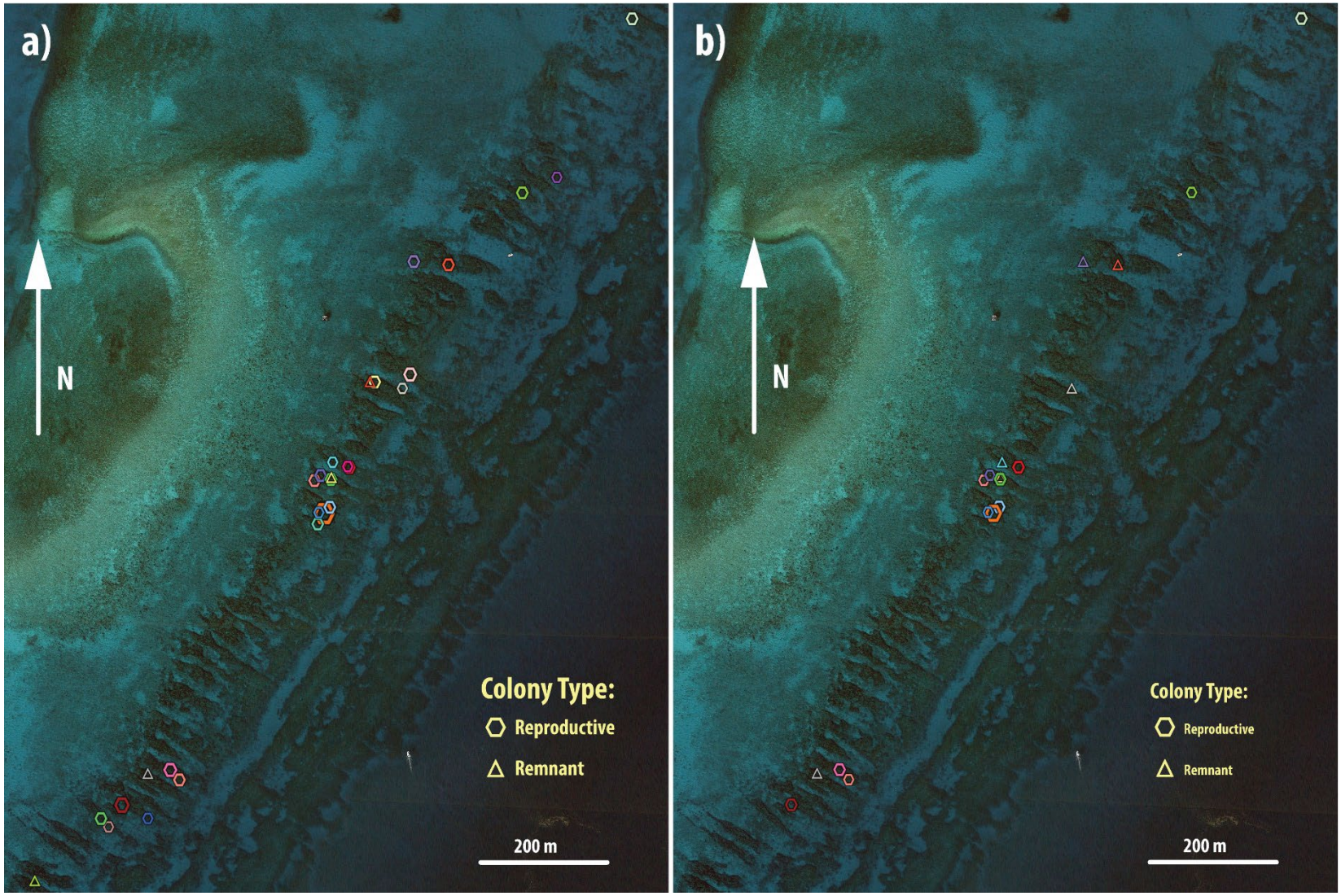

Figure 7. Map centered on Elbow Reef in the upper Florida Keys showing where stands of *Acropora palmata* founder genets were located in (a) early summer 2023 and (b) late fall 2023. Symbol color represents distinct genotypes, the symbol shape reflects whether the genet includes colonies of reproductive size and the size of the symbol is scaled to reflect the relative amount of live *A. palmata* tissue for each genet.

temperature. Habitats with similar environmental characteristics, such as deeper (15-30 ft) fore reef areas on the outer reef tract, are the most likely areas where additional founder genets may still be discovered, particularly in the upper Florida Keys or offshore of Miami-Dade or Broward counties.

CONCLUSIONS

Catastrophic loss of wild *A. palmata* in the Florida Keys

Of the 158 known wild founder *A. palmata* genotypes cataloged since 2002 from the upper Florida Keys, there are only 21 remaining on their native reefs. While we did not systematically survey the middle and lower Florida Keys founders, several agencies have reported that no wild *A. palmata* has been found alive following the bleaching event. Eight of these upper Florida Keys genets consist of only small remnant colonies that are especially vulnerable to complete mortality from disease, predation or storm damage, and are unlikely to persist or recover to an ecologically functional and reproductive size without intervention. Consequently, there are only 14 founder genets with wild colonies large enough to reproduce remaining at three reefs (Table 1), but only Elbow Reef has multiple founder genets (**Fig. 7**) that are large enough to contribute to sexual reproduction events. However, of the 12 reproductive founder genets at Elbow Reef, only 10 are spaced close enough to potentially cross fertilize with neighboring genets in the event of a synchronous spawning event. Given the currently low abundance of potentially reproductive wild *A. palmata* colonies at Elbow Reef, successful sexual reproduction at this site is unlikely without human intervention (i.e., gamete collection and mixing, larval rearing and settlement).

Consequences for reproduction among wild colonies

Elbow Reef has been one of three focal sites for wild *A. palmata* spawning collections in the upper Florida Keys over the past 20 years (Miller et al. 2018, Williams et al. 2023). Seven of the 18 founder genotypes that remain at Elbow Reef have been used in larval propagation efforts in the past (Miller et al. 2018) to create multiple cohorts, and their progeny have been propagated and outplanted to reefs in the Florida Keys (e.g. Merck et al. 2022; & unpubl. obs. Williams). Thus, genes from these genets are relatively well represented compared to genotypes that were used less, or not at all, to sexually propagate *A. palmata* in the past. Because nearly all of the reproductively sized colonies of *A. palmata* (wild or in nurseries) are located at Elbow Reef, it is still important to conduct spawning observations and collections at this site.

Spawning hubs

Prior to 2023, multiple sites with diverse stands of outplanted *A. palmata* originating from reefs throughout the Florida Keys were successfully acting as spawning hubs (Williams et al. 2023) making it possible for gametes from founders that originated from reefs hundreds of kilometers apart to cross fertilize on the same night. While efforts to accomplish this through captive breeding strategies are underway, recreating spawning hubs at reef sites that are more buffered from thermal stress should be a top priority. There are outplanted *A. palmata* at Elbow Reef that have been observed spawning in recent years (Williams et al. 2023) that survived the 2023 bleaching event. The surviving colonies belong to at least six founder genets originating from other upper Florida Keys reefs and are in relatively close proximity to reproductive native founders. Augmenting the remaining diversity of genets at this site is

the best chance to build a spawning hub that can withstand future bleaching events. Concurrently, establishing reproductively sized colonies of numerous genotypes in offshore nurseries that can be leveraged for spawning collections would facilitate the generation of new, genetically diverse cohorts of *A. palmata* that can be integrated into propagation and restoration pipelines. However, instead of creating multi-parent batch crosses as was commonplace for previous larval propagation campaigns, efforts should be made to conduct directed two-parent crosses that maximize the number of new cohorts with known parentage. Offspring of known parentage should be part of a restoration strategy that spatially distributes outplanted offspring to minimize the potential for backcrossing with parents or siblings.

Optimizing nursery stocks

Of the 23 surviving wild founder genotypes, only three potential founder genotypes remained missing from the known nursery stock prior to the 2023 bleaching event because they were discovered during the post-bleaching survey. Although the other 20 founder colonies were believed to be in nursery stocks prior to the bleaching event, the losses to nursery stock are still being assessed at the time of this report. If there is any doubt as to whether a founder genet is confidently identified or securely held within at least one nursery, these survivors should be collected again. Colonies that only exist on the reef as remnants with no live branches are especially unlikely to rebound as they are particularly susceptible to mortality, and they are not functionally contributing to the population. Accordingly, collection of the remaining tissue of these colonies is likely the best use of remnants allowing them to be propagated in a nursery setting.

Resolve nursery stock mixing to ensure founders are not lost

Based on the nursery samples in the STAGdb, there is indication that some nursery stock may be mixed with other genotypes. In most cases, only one sample was analyzed per putative genotype. There is no way to truly know how prevalent such cases are since a sample from a single fragment in a mixed genet may also end up matching the expected genet even if there are fragments of other genets included in that putative genet. When a putative genotype did not match the expected genotype, the match was predominantly one of two genotypes: HG0004 (from Horseshoe Reef) and HG0205 (from Conch Reef). The Horseshoe Reef genotype was one of the earliest genotypes to be propagated in Florida Keys nurseries (2009) and the Conch Reef genet was incorporated in 2012 so it is not unexpected that these two genets, having been in the nursery longer than most, would be the ones that have infiltrated other stock. However, both are also considered to be heat tolerant and fast-growing genotypes (unpubl. obs Williams & Nedimyer); so, it is particularly concerning that they have infiltrated other stock genotypes as they may out compete them in time if they have not already. Genotyping every stock fragment is cost prohibitive and unnecessary. Using grafting techniques in concert with formal genotyping may be a lower cost approach that will identify distinct genotypes within stock (Blanco-Pimentel et al. in press). Distilling all nursery held founder genotypes to pure known stock is a monumental task, but prioritizing it over propagating in the very near term should be considered. Special focus is needed on stock that was found to be duplicated or did not match the native genet found at the original collection point. Additionally, stock that originated from sites that were known to be genotypically diverse at the time of collection (e.g. Elbow, Sand Island, Molasses and Looe Key) should also be evaluated for any signs of mixing. A larger scale and more synchronous effort to genotype stock would greatly facilitate this process.

Future Bleaching Events

NOAA NCEI (2024) predicts that there is a one in three chance that 2024 global annual temperatures will be warmer than 2023 and a 99% chance that 2024 will rank in the top five hottest years on record. While the global average does not always predict local patterns, it is reasonable to expect that water temperatures in summer of 2024 will exceed coral bleaching thresholds. Manzello (2015) predicted annual bleaching events in the Florida Keys would begin between 2020 and 2034, so it seems prudent to expect that these unprecedented conditions will repeat frequently enough that some sites are not likely to support *A. palmata* until global temperatures decline. Restoration planning should acknowledge that the 2023 event will likely repeat in the near future.

Efforts to identify thermally tolerant founder genets has been a priority for restoration organizations in recent years (e.g. Cunning et al. 2021, Klepac et al. 2024, unpubl. obs. Williams & Nedimyer). Founder genets originating from sites that are warmer than typical could be expected to be somewhat resistant to bleaching. However, heat tolerant genets may also be lurking at sites that are relatively cooler. Although Elbow Reef did not experience the temperature extremes that occurred at inshore sites, temperatures there were similar to those experienced at other reefs in the 2014 and 2015 bleaching events, and all of these surviving genets could be seen as at least marginally heat tolerant. Some genets that did not survive were observed with normal tissue color and disease-like mortality in mid-September; while these colonies did not bleach they could still be considered as susceptible to heat stress since they exhibited rapid mortality. However, two genets, HG1548 (**Fig. S6**) and HG0083 (not pictured), were never observed bleached and survived with little to no tissue loss. These two could be considered to be on the higher end of the heat resistance spectrum, and could be capable of surviving at warmer sites. Both genotypes were only recently collected as nursery stock in 2022 and have not been outplanted to other reef sites to date.

Dwindling population for restoration applications

There are 105 upper Florida Keys founder genets that were present in nurseries or gene banks prior to the 2023 bleaching event. Insufficient information precluded an exhaustive review of middle and lower Florida Keys founder genets, however we estimate there were approximately 40 founder genets this region in nurseries prior to the 2023 bleaching event, and a smaller number of additional known founder genets north of the Florida Keys in Miami-Dade and Broward counties. Thus, there are likely approximately 150 Florida founder genets in existence in coral nurseries and gene banks that could be available for restoration. Given the mortality of the majority of founder genotypes at their native reefs during recent bleaching events, the number of these founder genets that could reasonably be expected to persist as outplanted colonies in the face of increasingly frequent and intense bleaching events is even smaller. However, thermally tolerant genotypes do exist and may also be found in the sexually propagated offspring of founder genets. It remains unknown if the thermal tolerance of the parent genets reliably increases the likelihood of thermally tolerant offspring, as has been demonstrated for some species of coral (e.g. Quigley & vanOppen, 2022). Furthermore, resilience to heatwaves likely involves a suite of characteristics beyond thermal tolerance (e.g., UV and disease resistance); thus, sexually propagating founder genotypes that are not especially thermally tolerant should not be ruled out. The relatively small pool of parents that are remaining in the Florida Keys that are available to contribute to sexual reproduction increases the chances of inbreeding if not carefully tracked. Encouragingly, tracking parent identities and designing outplanting to minimize the probability of closely

related individuals from fertilizing is straightforward and completely feasible. Importantly, these new genotypes would serve to preserve the native genetic diversity while potentially providing phenotypes that are more resilient in the changing Florida Keys environment.

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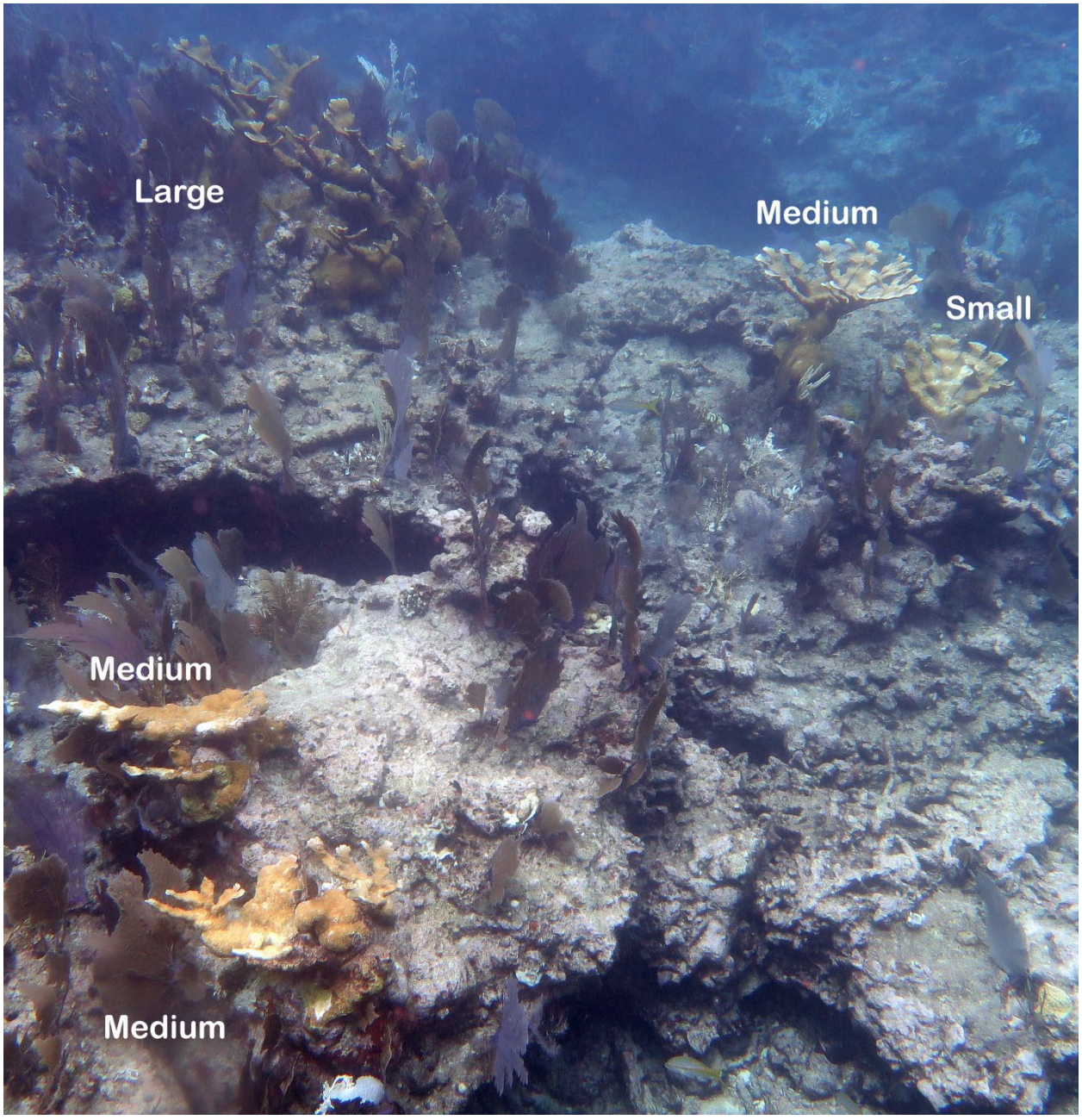
SUPPLEMENTARY MATERIALS



Supplement S1. *Acropora palmata* founder genet (HG0800) stand at Elbow Reef on August 17, 2023. This stand included 1 large (120 cm), 3 medium (80 cm) and 1 small (40 cm) colony. These estimated sizes were squared to calculate an approximate colony area which was summed for all colonies in this stand resulting in an estimated live area of 3.5 m² for this genet.

Supplement S2. Distribution of the identified upper Florida Keys *Acropora palmata* founder genotypes from samples collected between 2002-2022 and contributed to the Standardized Tools for *Acropora* Genotyping database (STAGdb). These numbers reflect where samples were collected only and they are not indicative of the total number of genotypes present at a reef at any given time. The totals do not include four founder colonies discovered in 2023 and early 2024 that have been sampled but not yet analyzed and added to the database. These colonies were found at Carysfort, Elbow, Little Grecian and French Reefs.

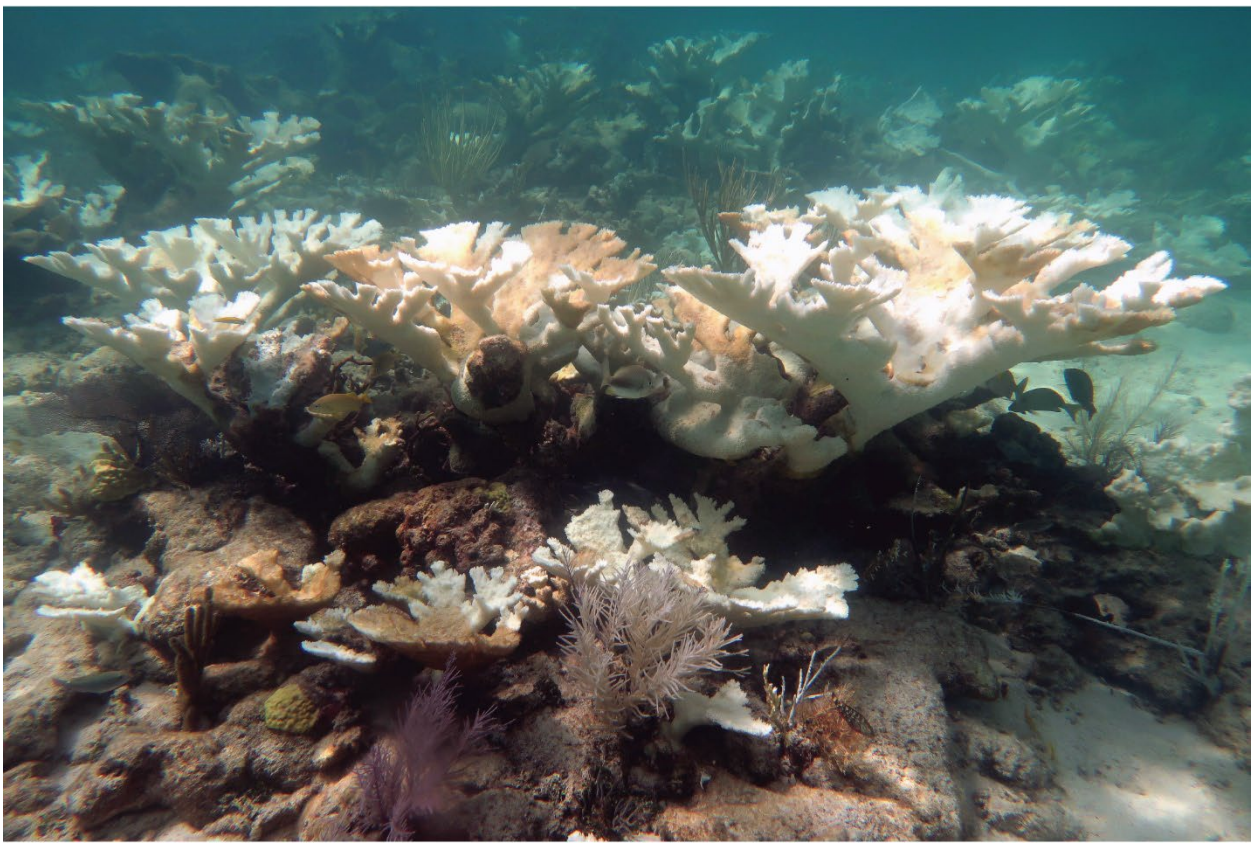
Reef	Founder Genets
Allans	1
Carysfort	10
Conch	4
Elbow	35
French	13
Grecian	4
Horseshoe	3
Key Largo Dry Rocks	8
Little Conch	2
Little Elbow	2
Little Grecian	3
Molasses	21
North Dry Rocks	4
North North Dry Rocks	6
Phils	1
Pickles	9
Sand Island	7
Snapper Ledge	1
South Carysfort	7
South Carysfort Patch	1
Triple A	3
Turtle Rocks	6
U91	1
Watsons	6
Grand Total	158

Supplement S3. Water temperature metrics from selected reefs sites in the upper Florida Keys as measured in 2023 and three recent bleaching years (Williams et al. 2015) for comparison. During recent bleaching events (2011, 2014 and 2015), three weeks exposure to daily averages of 31.0 °C or higher was associated with moderate prevalence of bleaching (Williams et al. 2017). Temperature readings are logged every thirty minutes with HOBO MX2203 loggers deployed in shaded areas on the bottom in monitored *A. palmata* stands.

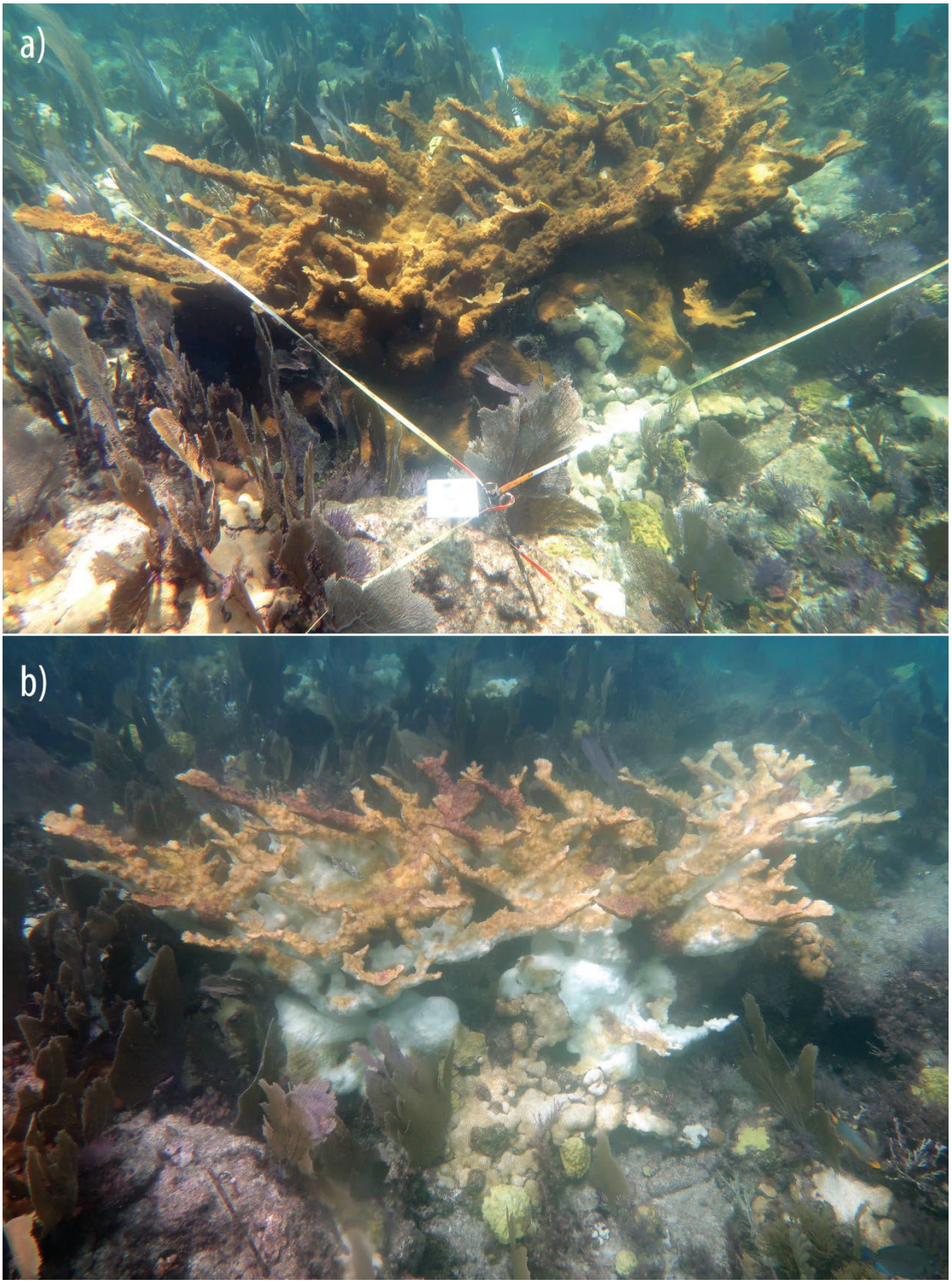
	Max Temp (30 min interval)				Max Daily Avg				Days ≥ 31.0 °C				Date where >21 days ≥ 31.0 °C			
Reef	2011	2014	2015	2023	2011	2014	2015	2023	2011	2014	2015	2023	2011	2014	2015	2023
Allans Reef	N/A	N/A	N/A	33.3	N/A	N/A	N/A	32.5	N/A	N/A	N/A	45	N/A	N/A	N/A	7/25
Carysfort Reef	32.1	31.9	32.3	32.4	31.4	31.4	31.5	31.8	14	8	34	41	~	N/A	8/27	8/5
Conch Reef	N/A	N/A	N/A	≥32.5	N/A	N/A	N/A	≥32.1	N/A	N/A	N/A	≥42*	N/A	N/A	N/A	8/2*
Elbow Reef	31.6	31.9	32.0	32.0	31.2	31.4	31.5	31.7	10	19	25	32	~	~	9/11	8/12
French Reef	31.8	N/A	32.1	32.1	31.3	N/A	31.5	31.9	11	N/A	39	38	~	N/A	8/24	8/7
Grecian Rocks	32.4	32.2	32.2	33.1	31.8	31.7	31.6	32.4	22	31	37	50	9/12	8/18	8/25	7/25
Horseshoe Reef	N/A	N/A	32.2	33.3	N/A	N/A	31.9	32.7	N/A	N/A	38	45	N/A	N/A	8/9	7/25
Little Elbow	N/A	N/A	N/A	32.4	N/A	N/A	N/A	31.7	N/A	N/A	N/A	39	N/A	N/A	N/A	8/4
Little Grecian	N/A	N/A	N/A	32.7	N/A	N/A	N/A	32.2	N/A	N/A	N/A	40	N/A	N/A	N/A	7/25
North Dry Rocks	N/A	N/A	32.0	32.9	N/A	N/A	31.7	32.2	N/A	N/A	37	44	N/A	N/A	8/11	7/26
North North Dry Rocks	N/A	N/A	N/A	32.7	N/A	N/A	N/A	32.1	N/A	N/A	N/A	41	N/A	N/A	N/A	7/25
Phils Reef	N/A	N/A	N/A	≥32.3	N/A	N/A	N/A	≥31.9	N/A	N/A	N/A	≥38*	N/A	N/A	N/A	8/2*
Pickles Reef	N/A	N/A	N/A	32.3	N/A	N/A	N/A	31.9	N/A	N/A	N/A	38	N/A	N/A	N/A	8/8
Sand Island	31.7	32.2	32.0	32.3	31.2	31.6	31.4	31.9	N/A	N/A	N/A	35	N/A	8/29	9/11	8/9
South Carysfort Reef	N/A	N/A	N/A	32.4	N/A	N/A	N/A	31.7	10	22	25	35	~	N/A	N/A	8/6
Turtle Rocks	≥32.2	32.5	≥32.5	≥33.1	≥31.7	31.9	≥31.8	≥32.3	N/A	31	N/A	≥44*	~	8/19	N/A	7/25*
Watsons Reef	N/A	N/A	N/A	32.8	N/A	N/A	N/A	32.0	N/A	N/A	N/A	42	N/A	N/A	N/A	7/25

* Loggers at Phils Reef and Turtle Rocks were deployed 7/24 and Conch Reef 7/25 at the end of the first heatwave so it's likely that the maximum readings were higher than shown here. While early July temperature data is not available at these sites, trends from similar sites were used to estimate the number of days exceeding 31.0 °C prior to the loggers being deployed.

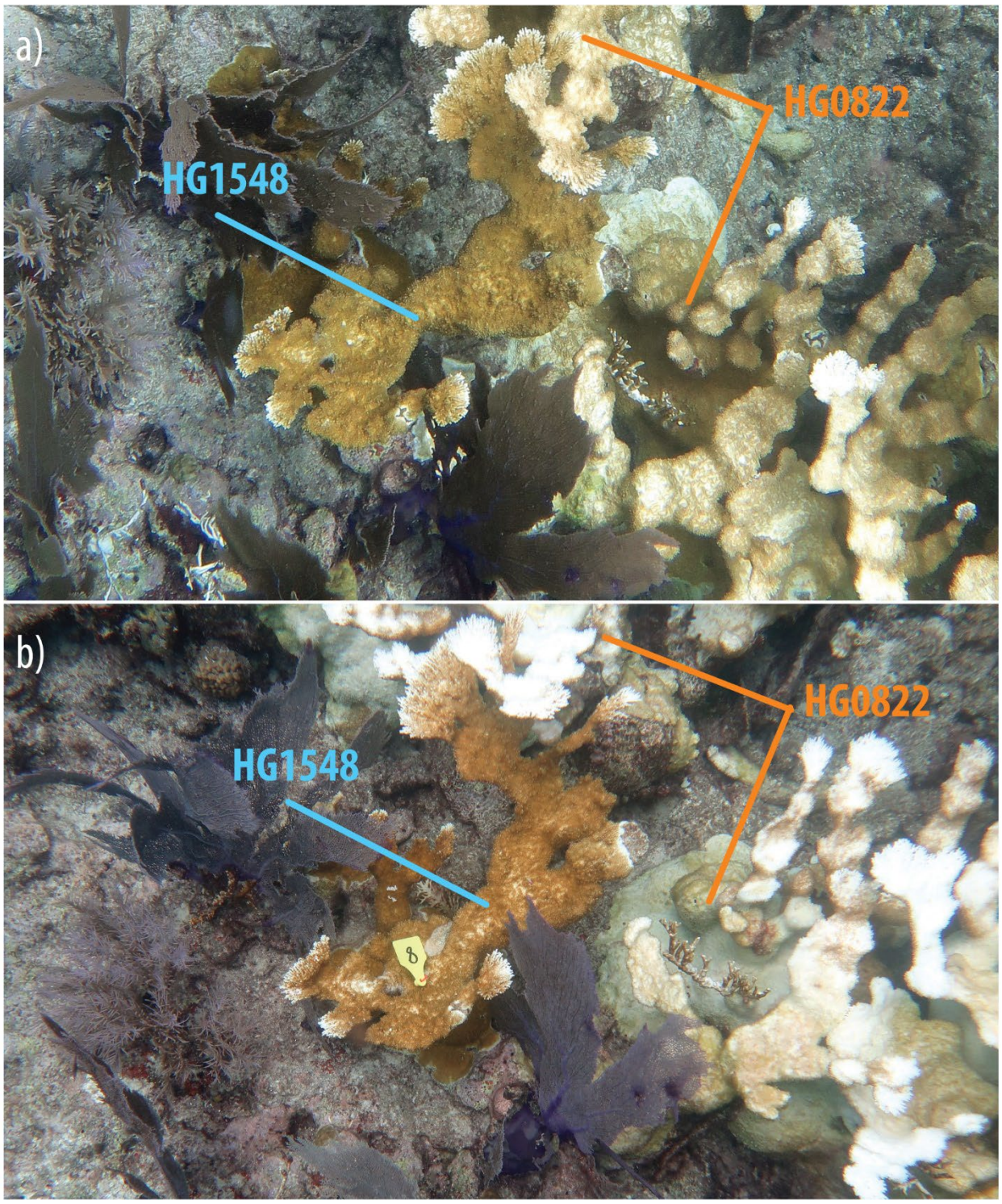
'N/A' indicates that temperature data are not available; '~' indicates that gaps in the temperature data prevent calculation of that metric.



Supplement S4. Stand of *Acropora palmata* colonies at Turtles Rocks in the upper Florida Keys on July 24, 2023. Bright white areas are dead exposed skeleton not bleached tissue indicating these areas died within the past few days. Light tan areas are dead skeleton with very light algal colonization indicating those portions of the colonies died earlier, likely within a week.



Supplement S5. *Acropora palmata* colony at Little Grecian in the upper Florida Keys on a) 7/7/2023 and b) 8/19/2023. The colony retained normal tissue color in early July but by mid-August, the upper surfaces are dead and colonized by algae indicating those areas died a few weeks earlier while the lower parts of the colony are fully bleached but still alive. This pattern suggests that sunlight likely hastened or compounded the heat stress. When resurveyed 10/11/2023 the colony was completely dead and the relatively heavier algal colonization at that time suggests that it died at least a month earlier (not shown).



Supplement S6. *Acropora palmata* colonies belonging to two distinct genets at Elbow Reef in the upper Florida Keys on a) 8/17/2023 and b) 9/19/2023. Genet HG1548 retained nearly normal coloration throughout the bleaching event suggesting that it was less stressed than the neighboring genet HG0822 which exhibited moderate to severe bleaching and rapid disease-like mortality in mid-September.