Supporting Information. Grottoli, A.G., R.J. Toonen, R. van Woesik, R. Vega Thurber, M.E. Warner, R.H. McLachlan, J.T. Price, K.D. Bahr, I.B. Baums, K.D. Castillo, M.A. Coffroth, R. Cunning, K.L. Dobson, M.J. Donahue, J.L. Hench, R. Iglesias-Prieto, D.W. Kemp, C.D. Kenkel, D.I. Kline, I.B. Kuffner, J.L. Matthews, A.B. Mayfield, J.L. Padilla-Gamiño, S. Palumbi, C.R. Voolstra, V.M. Weis, and H.C. Wu. 2020. Increasing comparability among coral bleaching experiments. Ecological Applications.

Appendix S1

A review of common analytical methods used for quantifying experimental conditions and coral response variables listed in Box 1 and Table 1.

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Section S1: EXPERIMENTAL CONDITIONS

S1.1: Tracking genets and ramets

Keeping track of all genets and ramets (**Fig. S1, Box 1**) throughout a study is necessary for identifying variation at the level of the individual and for ensuring sufficient biological replication in a study. Collection site information for corals ideally includes the latitude and longitude for each colony (in decimal degrees), the coordinate reference system, and collection depth (m). For all experiments, a clear labeling scheme using materials that are durable and readable for the entire duration is suggested. Such labels can be as simple as a pencil used to label the base that the coral are mounted on for short-term experiments. Many researchers use super glue, marine epoxy, or cable ties to affix semi-permanent labels such as Dymo tape, insect tags, or cattle tags directly to the coral or the base it is mounted on. However, it is still possible for labels to be lost during routine maintenance, so we recommend double labeling of ramets for longer-term experiments. Passive Integrated Transponder (PIT) tags epoxied to the base of a ramet or its mount offer a more permanent solution for tracking individuals through long-term experiments. Specific laboratory methods for identifying coral genets are described in Baums et al (2019) and detailed protocols are in (Baums et al. 2020). Specific strategies for collecting corals to optimize the probability of obtaining genetically unique individuals are addressed in section III.A. of the main text and detailed protocols are in (Baums 2020).

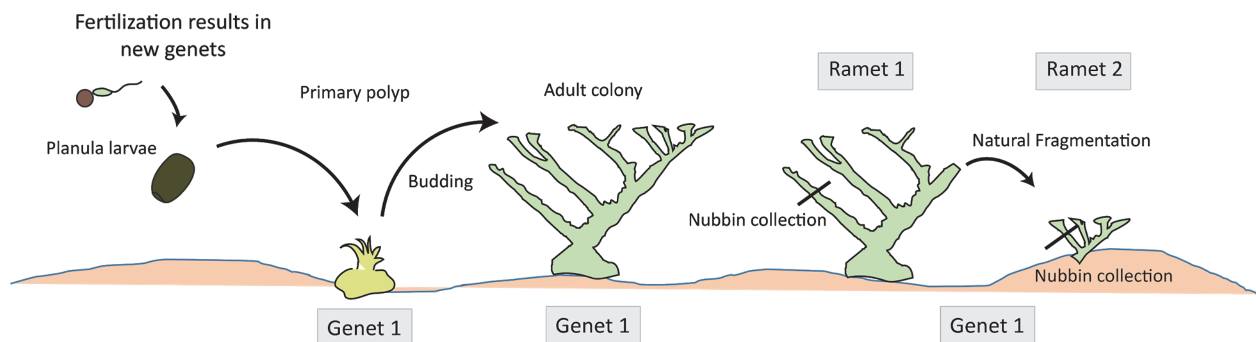

FIG. S1 Sexual reproduction leads to the formation of new genets via the development and metamorphosis of planula larvae into adult colonies. Colonies may fragment naturally resulting in a genet with many ramets. Genetic methods are the only way to definitively determine genet diversity and membership of nubbins (i.e., ramets) collected for experiments. Reproduced from Baums et al (2019).

S1.2: Temperature

In coral thermal bleaching experiments on coral, it is suggested that temperature is controlled and monitored as accurately and precisely as possible. There are two approaches to establishing the appropriate heat stress temperatures: 1) mimic heat stress profiles in the natural environment as much as possible, or 2) use a predetermined, static temperature regime. Both approaches require an understanding of the baseline temperature and variance at the coral collection site.

Baseline temperature and variance: ***In situ data from the coral collection site and depth*** (or as near as possible to that site), and ***during the same time of year as the proposed experiment*** (to ensure a representative range in seawater temperatures) ***should be used to determine the temperature baseline*** (i.e., maximum monthly mean, also known as MMM), average and maximum temperature, and absolute range of seawater temperatures for the corals in question. ***Ideally, continuous temperature loggers are used to collect the appropriate in situ data on an hourly basis*** where the loggers have been shaded in shallow water environments (Bahr et al. 2016) and calibrated regularly according to manufacturer specification to correct for drift. Alternatively, twice daily measurements (peak temperature at solar noon and minimum temperature at night) on the reef using a digital temperature probe or standard glass thermometer can be used. If gathering *in situ* data is logistically challenging or unavailable, sea surface temperature (SST) data can be obtained from the National Oceanic and Atmospheric Administration (NOAA) Coral Reef Watch (Liu et al. 2014, Heron et al. 2016), OI-SSTv2, or other available sources. However, the temporal and spatial resolution of satellite-derived data can be limiting because the spatial resolution of the satellite data is often much larger than the reef site in question and/or does not accurately capture near-shore temperatures.

Controlling temperature in experimental tanks: Selection of temperature stress profiles (i.e., mimic or static) are dependent on the research question and experimental setup. Temperature manipulation can occur either in the experimental tank or in header tanks/sumps using submersible heaters or chillers. Many makes and models are available (see suggestions in **Table S1**). When selecting a method of temperature control, it is important to consider the variance that will be experienced with any given system. Given that coral bleaching threshold can be as little as +1°C above MMM (e.g., Coles and Jokiel 1977, Jokiel and Coles 1977, Glynn and D'Croz 1990, Jokiel and Coles 1990), ***we suggest using a system with tight temperature control, such that the variance experienced at any set temperature is less than 0.5°C.*** In addition, high irradiance (both natural and artificial) and tank flow rates or turnover can affect the temperature in tanks and needs to be taken into account when testing the temperature control of the experimental system prior to initiating the experiment. It is suggested to use a heat-chiller combination in closed recirculating systems and to consider manipulating the turnover rate in open (flow-through) systems.

Monitoring temperature in experimental tanks: ***Continuous temperature loggers are suggested,*** with at least one logger per experimental treatment (i.e., one in the control tank, and one per tank of each different treatment). Temperature should be recorded every 15 minutes throughout the experimental period (including acclimation, ramp-up period, stress exposure, and recovery period if applicable). If possible, one logger is placed in every tank. When using multiple loggers, it is important to ensure they are all calibrated prior to beginning the experiment. Alternatively, calibrated temperature probes or thermometers can be used to measure the water temperature in all of your tanks at noon daily. In addition, it is suggested that hourly measurements be made for one full diel cycle in at least one tank of each treatment each month. We suggest taking multiple measurements at different locations within your experimental

Table S1. Examples of equipment that can be used to monitor and control seawater temperature in coral bleaching experiments. This list is by no means exhaustive, and CBRCN does not endorse any given company, brand, or system over another.

	Item / Device	Approx. price range	Things to consider
Monitoring temperature	Underwater Temperature Logger	~ $45 - $300	Keep shaded to prevent errors associated with high irradiance levels (see Bahr et al., 2016).
	Liquid-in-glass thermometer	~ $20	Avoid using mercury thermometers for environmental and personal safety reasons.
	Digital temperature probe		
Heaters	Advanced Aquarium Controller	Starting at ~$240	Expensive but with very low variance. Allows for tight control of experimental seawater temperatures.
	Electronic Aquarium Heater	Starting at ~$15	Inexpensive but temperature variance may be high.
Chillers	Thermoelectrical Chillers		Best for smaller aquariums (< 55 gallons). Quiet and energy efficient.
	In-line chillers		Appropriate for larger systems with in-line filtrations. Available in several sizes (⅕ to 1 HP). Requires extensive plumbing.
	Drop-in Chillers		Probe placed directly into tank. Available in a variety of sizes (⅕ to ⅓ HP). Ideal for systems with minimal space. No plumbing needed.

tank (i.e. closest to and farthest from heating source, closest to and farthest from water pump(s)/source of flow, and shallow and deep points within each tank) because the size and volume of the tank along with the placement of your heating element may create localized heating within the tank. If differences are found, we suggest either relocating your heating element and/or increasing the flow or direction of flow within your tank to promote uniform heating within your tank.

Reporting temperature in publications: ***Ideally, the temperature profile experienced by each of the experimental treatments throughout the experiment is reported.*** This includes temperatures experienced after collection from the reef, during the temperature ramp stage, the stress exposure period, and recovery period (if applicable). ***At a minimum, the mean temperature and variance experienced during the ramp stage and stress exposure period should be reported along with the number of measurements*** (i.e., sample size). In all cases, the method used for making the temperature measurements and targeted temperature profile (mimic or static) should be reported (i.e., frequency, duration, time of day, etc…) along with the temperature measurements in degrees Celsius.

S1.3: Light

Along with temperature, light is considered a critical component of the natural bleaching process and as such, investigators should strive to accurately record and report the light used in their bleaching experiments.

Light sources: Details of the light source used in the study (i.e., natural outdoor or artificial light) and manufacturer details on artificial light sources should always be provided. Photosynthetically Available (or active) Radiation (PAR) values represent an integrated light value across ~400–700 nm. However, the spectral quality of artificial light will vary with the spectral output of the light source as well as the total intensity. PAR levels should not exceed those measured at the collection site. ***Minimum PAR light levels of 250–500 µmol photons*** m^{-2} s^{-1} ***are typical, but this will depend on the light level required to saturate photosynthesis (*** E_K ***) which is also dependent on the long-term light history at the collection site*** and PAR light levels should not exceed those at the collection site. If investigators are examining very deep or shallow corals these values will require some empirical adjustment based on the particular collection depth and local conditions (e.g., a high turbidity site).

Measuring and reporting light in the experiment: At a minimum, the ***maximum PAR for the specific depth of coral collection*** on a clear cloudless day as well as the ***maximum PAR used in the experimental system should be reported in µmol photons*** m^{-2} s^{-1} [*]. If *in situ* PAR data is not available from the collection site, then photosynthesis to irradiance curves or active Chl *a* fluorescence to irradiance response curves on freshly collected corals can provide the light acclimation state of the coral needed to determine an appropriate PAR range for bleaching experiments. Inexpensive sensors that report light in Lux (luminous flux unit area^{-1}) are not recommended for bleaching studies as Lux represents light in a narrow waveband (peaking near 555 nm) and conversions between Lux and PAR sensors are only approximate at best and highly dependent on the light source used. Ideally, PAR is logged continuously over a diel cycle allowing one to calculate a daily light integral or photon exposure expressed in units of moles photons m^{-2} d^{-1}. This is especially important when working under naturally or artificially fluctuating light sources. If the absorption characteristics of the corals are known (Vasquez-

[*] A photon is a quantum of electromagnetic radiation, hence the use of either terms ‘quanta’ or ‘photons’ is applicable here. (Kirk 1994).

Elizondo et al. 2017), it is possible to calculate the light dose, allowing for more detailed comparisons between continuous physiological variables and the cumulative light history over the bleaching experiment. Reporting the spectral output of the light source used (measured in $\mu W\ cm^{-2}\ s^{-1}\ nm^{-1}$) is also very useful but requires further instrumentation and additional cost. If such instruments are not available, we encourage investigators to report spectral data for their particular light source as reported from the manufacturer.

PAR sensors: The two common light sensor designs are the flat and spherical (scalar) sensors. Scalar sensors have the advantage of sensing light in all directions, while flat sensors typically measure light within a 180° hemisphere above the sensor. Flat sensors lacking true cosine correction are more prone to positioning errors, as the amount of light intercepted by the sensor is affected by the angle of the light hitting the collector. Nevertheless, greater manufacturer choices and lower costs are two advantages for using flat sensors. In addition, PAR sensors are typically calibrated specifically for use in air or in water, and the investigator should pay close attention that appropriate calibration constants (typically provided by the manufacturer) are applied for the specific measurement location.

S1.4: Flow

Here we distinguish between water movement in the experimental system (i.e., water flow), exchange of water (i.e., turnover) held in the tanks, and water velocity (i.e., water motion relative to corals) in flow-through or recirculating systems. Flow has been shown to impact coral bleaching (Nakamura and van Woesik 2001, Nakamura et al. 2003, McLanahan et al. 2005), so it is important to have appropriate flow and water velocity in experiments and to report those conditions. The method for water movement generation can vary dramatically depending on the experiment goals (**Table S2**). If the goal is to examine coral responses under natural water velocity (i.e., mean and variability), then experimental water velocity should approximate that from which corals for the experiment were collected. For many back-reef environments, natural water velocity rates are on the order of 10 cm s^{-1} (Baird and Atkinson 1997, Nakamura and van Woesik 2001, Hench et al. 2008, Rogers et al. 2016). Low water velocity is generally on the order of ~5 cm s^{-1} and very low water velocity is on the order of 2 cm s^{-1} (Comeau et al. 2014). Flow conditions on forereefs are typically be much higher than 10 cm s^{-1}(Monismith 2007). Unless called for by the experimental question, keeping corals in standing water without circulation is largely unacceptable.

Recirculating experiments: ***Water velocity rates of 2-10 cm s^{-1} are suggested. At a minimum, use one of the many propeller pumps available for generating bulk flow in coral reef aquaria, and reporting the tank volume, circulating pump model and output rate (L h^{-1}) for each experimental tank***. Although basic aquarium powerheads are the most easily available and commonly used source of water flow, they are a poor option because the flow field is concentrated at the outlet of the jet and diffuses rapidly with distance (Riddle 2011). The pump should be sized to mix the tank volume at least 10 times per hour (i.e., a 100 L tank should have

a pump of at least 1000 L h^{-1}), which is usually sufficient to replicate very low water velocity backreef conditions. Turnover rates are dictated by biomass and should be high enough that water chemistry parameters (which should be measured periodically and reported as per **Table 2**) and nitrogenous wastes (see section **A.8**) are maintained as close to natural as possible throughout the experiment.

Flow-through experiments: ***Minimum water movement (flow) within the experimental tanks are the same whether experiments are flow-through or recirculating.*** Water input of 10 tank volumes per hour or more are likely needed to generate sufficient flow to avoid the use of circulating pumps in flow-through experiments. If pumps are used, the recommendations are the same as above for flow rates and reporting. In terms of turnover, rates are again dependent on biomass, but ***input of at least 1-4 tank volumes per day is recommended to minimize changes to experimental water parameters***, which should be tested periodically and reported to ensure they remain as close to natural as possible. Output flow rate (L h^{-1}) should be measured periodically and reported for flow-through experiments to ensure turnover rates are consistent through time and among experimental treatments.

Table S2: Methods for generating flow in experimental tanks

Flow type	Rationale	Equipment and Method
Laminar	Approximates constant currents	Flume
Oscillatory flow	Approximates reef conditions	Piston, wave box, flap wave generator, or paired aquarium propeller-type wavemaker pumps on opposite sides of the tank that cycle on and off to generate flow in alternating directions
Bulk water flow	More turbulent bulk water flow	Dump buckets
Random flow	To test flow effects	Wall of jets on each side of the tank triggered simultaneously but in random order

S1.5: Feeding

Feeding on zooplankton, dissolved organic carbon, and fine particulate organic carbon provides corals with fixed carbon (Ferrier-Pages et al. 1998, Anthony 2000, Grottoli et al. 2006, Palardy et al. 2008, Houlbreque and Ferrier-Pages 2009, Ferrier-Pages et al. 2010, Levas et al. 2013, Grottoli et al. 2014, Levas et al. 2016) as well as essential building blocks for tissue repair and synthesis (Piniak et al. 2003, Hughes et al. 2010, Hughes and Grottoli 2013, Baumann et al. 2014). When corals are bleached, heterotrophically derived carbon is even more important as bleached corals are not able to meet 100% of their daily metabolic demand through photosynthesis alone (Grottoli et al. 2006, Palardy et al. 2008, Ferrier-Pages et al. 2010, Grottoli et al. 2014, Levas et al. 2016). Some species of corals are able to increase heterotrophy in response to bleaching and supplement their metabolic demand (Grottoli et al. 2006, Palardy et al.

2008, Grottoli et al. 2014, Levas et al. 2016), or have high baseline heterotrophic capacity that buffers them from bleaching stress (Palardy et al. 2008, Levas et al. 2013, Grottoli et al. 2017). Thus, feeding corals during coral bleaching experiments provides nutritional opportunities that attempt to simulate zooplankton availability on reefs.

It is suggested that ***corals in experimental conditions for more than seven days be fed wild caught zooplankton or 2-day old Artemia nauplii to satiation starting one hour after dusk at least weekly***, but up to thrice weekly would be ideal. Coral do not eat every night; they eat every 2-3 nights, even if zooplankton is presented every night (Grottoli pers. obs.). Since corals naturally feed during dusk and dawn when zooplankton are migrating, this is the best time to provide supplemental food as well. Even if using unfiltered natural seawater in a flow-through system, corals will likely not be getting any zooplankton (Grottoli pers. obs.), necessitating supplemental feeding. During feeding, seawater inflow should be turned off to minimize zooplankton or *Artemia sp.* loss during feeding. However, some gentle mixing is optimal within the tanks to keep the zooplankton/*Artemia sp.* in suspension and facilitate capture by the coral polyps.

S1.6: Seawater

Natural reef-derived seawater contains all of the elements and nutrients needed by corals in natural proportions and is what corals are acclimated to at each site. However, artificial seawater is sometimes needed for recirculating experiments or when conducting studies where natural seawater is not available. Artificial seawater tends to have higher alkalinity and pH than natural seawater, as it is formulated to optimize the growth and appearance of corals in display aquaria and may not contain all of the micronutrients and elements that natural seawater provides. It is possible to get custom formulated artificial seawater that more closely mimics natural seawater (Schoepf et al. 2013) and some brands of artificial seawater more closely resembles natural seawater than others (Berges et al. 2001).

Natural, unfiltered, flow-through seawater is suggested whenever possible. In all cases, the type of seawater (natural vs artificial), the delivery system (flow-through vs recirculating), and the application of filters (filter type and pore size vs unfiltered) should be reported.

S1.7: Salinity

Corals typically live at tropical ocean salinities of 34–36 Practical Salinity Units (PSU), but exceptions occur (e.g., corals in the Arabian Seas with salinities ≥ 40 PSU). While corals can tolerate short-term variability in salinity from 27 and 40 PSU (Coles and Jokiel 1992, Ferrier-Pages et al. 1999), more prolonged exposure to decreases in salinity can cause bleaching or reduce thermal tolerance (van Woesik et al. 1995, D'Angelo et al. 2015), while increases in salinity can enhance thermotolerance (Gegner et al. 2017, Ochsenkuhn et al. 2017, Gegner et al. 2019). Thus it is important to monitor and control for salinity in bleaching experiments.

In a coral bleaching experiment, ***we suggest that if flow-through seawater cannot be used it would be best to match the mean salinity at the collection site***. Salinity should remain consistent throughout the experiment when it is not a variable of interest. Salinity of the tanks in the experimental setup should be monitored daily for fluctuations. This is especially important when increasing temperatures, as evaporative losses result in increased salinity. Measurements can be taken with multiple methods, including electrical conductivity probes, refractometer and specific gravity meter. Measurements of salinity are most often reported as PSU.

S1.8: Nutrients

Coral reefs are typically, but not always, found in oligotrophic habitats where primary production is limited by the availability of key nutrients. Nitrogen (N) and phosphorus (P) based compounds are the nutritional building blocks for compounds like amino acids and nucleic acids. Thus, N and P are key nutrients that influence coral respiration, photosynthesis, and growth (Kinzie III and Davies 1979, Stambler et al. 1991, Marubini and Davies 1996, Ferrier-Pages et al. 2000, Nordemar et al. 2003), the density, diversity, and function of Symbiodiniaceae (Cunning and Baker 2013, Shantz and Burkepile 2014) and other members of the coral microbiome (Zaneveld et al. 2016, Shaver et al. 2017, Ziegler et al. 2019). However, too much nutrient input from anthropogenic activities can reduce coral health via increased susceptibility to diseases, bleaching, and light stress (Bruno et al. 2003, Wooldridge and Done 2009, Kaczmarsky and Richardson 2011, Weidenmann et al. 2013, Vega Thurber et al. 2014, Wear and Vega Thurber 2015), although the forms and ratios of those nutrients are important to consider as well (Morris et al. 2019).

When preparing for an experiment, researchers should be aware of the local nutrient conditions as well as the range of nutrient concentrations in any experiment that uses sources of water that are different than the corals' native conditions. Most researchers try to maintain ammonia, nitrite, and phosphate concentrations close to zero and low concentrations of nitrate (typically < 5ppm) in recirculating aquaria (Delbeek and Sprung 1994, Adey and Loveland 2007). Using flow-through seawater from nearby sources is a common method for incorporating seawater that already contains ambient levels of nutrients. To maintain desired nutrient levels throughout an experiment, the seawater should be monitored at least weekly. Colorimetric test kits can monitor broad changes in the nutrient levels of an experimental setup. More precise colorimetric methods can also be used with a spectrophotometer or continuous flow autoanalyzer if more precise measurements of nutrient levels are desired.

S1.9: pH

Seawater pH can act synergistically with temperature to both exacerbate and diminish the effects of coral bleaching (Anthony et al. 2008, Schoepf et al. 2013, Van Hooidonk et al. 2014, Wall et al. 2014). In addition, coral biology can influence the surrounding seawater pH (i.e., corals will

raise the pH during the day due to photosynthesis and lower it at night due to respiration) (Kline et al. 2015). Thus, knowing the seawater pH during coral bleaching experiments is critical to interpreting any results.

We suggest that ***pH be measured*** with a simple, low-cost hand-held NBS (National Bureau of Standards) or NIST (National Institute of Standards and Technology) calibrated pH meter (i.e., glass electrode method, which measures the electrical potential difference between the pH electrode and a reference electrode) ***in all aquaria at least once per week***. These relatively quick measurements will reveal if flow is unequal in the replicate aquaria and if the tank turnover is sufficient to prevent the corals from changing the pH in the surrounding seawater. If the pH in coral-bearing aquaria is different from a replicate aquarium without corals, the turnover rate needs to be increased or the number of replicate corals in each aquarium should be reduced in order to stabilize the pH. Similarly, if the pH is not similar across all replicate aquaria, the seawater turnover rates should be increased and the turnover rate should be checked to determine if they are equal across all replicate tanks. Ideally, ***pH should be measured daily*** and adjustments made accordingly to ensure that pH is constant across all aquaria. If pH is one of the treatment factors, then pH within all aquaria of the same pH treatment should be the same following the recommendations above. It is important to realize that pH measurements alone are not sufficient for calculating seawater carbonate chemistry parameters and we refer to Riebesell et al. (2010) for best practices in ocean acidification research on measurement of seawater pH and carbonate chemistry.

S1.10: Dissolved oxygen

Dissolved oxygen (DO) is an important consideration in coral bleaching experiments, as small fluctuations in oxygen levels can induce stress responses in corals (Haas et al. 2014). DO levels are typically between 6-8 mg L^{-1} on tropical coral reefs (Manasrah et al. 2006, Nelson and Altieri 2019, Hughes et al. 2020). However, these levels can be depleted at night due to diel fluctuations in photosynthetic activity of reef organisms (Jones 1963, Nelson and Altieri 2019, Hughes et al. 2020). Furthermore, DO levels are forecasted to globally decrease due to climate change, making the assessment of the effect of DO levels on the bleaching response ever more important (Hughes et al. 2020).

DO should be monitored at the same time each day to ensure consistent levels throughout the experiment. These measurements can be taken with optical DO probes or colorimetrically. DO measurements are generally recorded in mg L^{-1}, but can also be recorded as percent of air saturation.

Section S2: CORAL RESPONSE VARIABLES

S2.1: Bleaching phenotype

S2.1a: Image Analysis of Coral Color

Subjective visual assessment of coral color has been a prevalent measurement of coral health for nearly a century (Yonge and Nicholls 1931). Direct observer comparison of coral color to the coral health color chart is widely used in field monitoring of bleaching because it is a noninvasive, inexpensive, and rapid assessment tool (Siebeck et al. 2006). However, the perception of color by humans is variable (Neitz and Jacobs 1986), and the color of submerged objects can be influenced by a variety of factors including light attenuation and turbidity (Winters et al. 2009). Recently, efforts have been made to standardize color score as a quantitative indicator of chlorophyll content and/or symbiont cell density (reviewed in (Winters et al. 2009, Chow et al. 2016).

Outlined below are the principle methods for quantifying coral color in order of increasing accuracy, which is also accompanied by increases in cost and effort. For all methods, we suggest researchers consider a minimum of two measures per experimental ramet: one at the beginning of the experiment (before heat treatment is applied) and one at the end to yield an assessment of the change in color over the course of the study. Additionally, if a scale bar is included in the photographs and the orientation of photographs is standardized (e.g. top-down for massive corals, or a lateral view for branching species), these photographs can also be used to obtain a metric of coral growth.

Direct observer-based qualitative assessment of color: Coral color can be recorded using a qualitative color scale based on a visual assessment (e.g., Rodrigues and Grottoli 2007, Cornwall et al. 2020), or based on a visual comparison with a color reference chart (e.g., Coral Watch Color Health Chart from (Siebeck et al. 2006)). These methods are rapid, inexpensive, allow for non-invasive assessment, and provide a linkage between field and laboratory measurements. These methods are more appropriate for measuring relative changes in bleaching state within the same colony or species over time rather than an absolute measure of coral color across species and locations (Siebeck et al. 2006). The ***Qualitative Color Scale*** is a simple five point comparative scale that can be used to quickly characterize each fragment: 1=None (no visible bleaching), 2=Visible (bleaching is just barely visible but not extensive), 3=Moderate (bleaching is obvious but not severe), 4=Severe (bleaching is widespread on the fragment it is not completely white), and 5=Total (fragment is completely white) and correlates with algal cell density (Cornwall et al. 2020). Scoring fragments by two independent observers may reduce variance. When using ***Coral Color Reference Charts,*** the observer makes a subjective assessment of coral color by comparison to color reference charts. This method is more rigorous than the visual assessment score method (Hoegh-Guldberg and Salvat 1995), but still suffers from observer bias and discontinuation of color differences in the chart.

Photographic Methods: Corals can be easily photographed and quantifications of color can be measured using the Red Green Blue (RGB) Color Model or the Grayscale Model on non-color-corrected photographs. Both methods can provide a potential link between field and laboratory observations but cannot pinpoint the mechanism of bleaching. The ***RGB Color Model*** is used to quantify relative increases in pixel intensities over all or any of the three color channels, which corresponds to an increasing in whitening or paling (Voolstra et al. 2020). Furthermore, chlorophyll density is highly correlated with the intensity of the red channel under a large range of lighting conditions, provided external light normalization corrections are applied (Winters et al. 2009). Coral color has also been linked to intensities of red, green, and blue (RGB) channels in photographs (Edmunds et al. 2003, Maguire et al. 2003, Siebeck et al. 2006) and through optical density measurements (Thieberger et al. 1995). The ***Grayscale Model*** is used to quantify the percent whiteness (bleaching intensity) of a colony using grayscale images. Corals are photographed with black and white reference cards and the resulting color images are subsequently converted to 8-bit gray scale. Percent whiteness is highly correlated with chlorophyll *a* and Symbiodiniaceae density (Chow et al. 2016, Amid et al. 2018) and is robust to variation in light conditions and water turbidity (Chow et al. 2016). One advantage of this method is the ease of application as it does not require the use of multivariate statistics to convert spectral values of RGB into a single measure.

S2.1b: Chlorophyll

Both chlorophyll *a* and *c2* are present in the chloroplasts of the Symbiodiniaceae endosymbiotic dinoflagellates (Symbiodiniaceae) that reside in all reef-building corals. Given the critical role of photosynthesis in the health and function of the coral-dinoflagellate mutualism, measurements of coral chlorophyll levels have been made for over 30 years (McLachlan et al. 2020b).

Although no field-standardized method has been advocated to date, that of Jeffrey and Humphrey (1975) is the most widely used. Chlorophyll pigments are extracted from a coral sample (slurry or ground) in acetone for 24 hours in the dark, before being centrifuged and the absorbance measured on a spectrophotometer. Chlorophyll measurements should be normalized to surface area (i.e., $\mu g\ cm^{-2)}$ or ash-free dry weight of the extracted sample (i.e., $\mu g\ g^{-1}$). Normalization per algal cell is also desirable, but requires Symbiodiniaceae density count data. More recently, chlorophyll extraction methods in methanol or ethanol solvents have been used (Ritchie 2006), though these approaches are currently less common.

S2.1c: Symbiodiniaceae cell density

The quantification of endosymbiotic Symbiodiniaceae microalgal cells residing in the tissues of reef-building corals is one of the central methods for establishing if a coral is bleached. Coral tissue is removed from the skeleton by water pik or airbrush (Johannes and Wiebe 1970, Szmant and Gassman 1990) or by means of NaOH treatment (Zamoum and Furla 2012). Care should be

taken to avoid only using the tips of branching corals (within 2 cm from growing tip) as this is the point of rapid tissue and skeletal growth, and symbiont densities are not reflective of the entire colony (Jones and Yellowless 1997). The microalgal cells are separated from the coral "blastate" via homogenization and centrifugation, then immediately counted or preserved ("fixed") for later enumeration using any of several common preservatives, such as formalin, Lugol's solutions, and glutaraldehyde.

Quantification of isolated cells is typically achieved by either of two methods: light microscopy-assisted counting using a hemocytometer (Guillard and Sieracki 2005) or flow cytometry (e.g., Krediet et al. 2015, Pogoreutz et al. 2017). Enumeration of algal cells using the former method is often completed via 6 −10 replicate hemocytometer counts. The latter method may increase precision and allow the differentiation of living and dead cells by means of their shape. In combination with fluorescence, flow cytometry can even be used to assess chlorophyll density or symbiont cell integrity, which may provide additional information with regard to the health state of Symbiodiniaceae. Recent methods of quantifying Symbiodiniaceae density using automated fluorescent cell counters (e.g. Countess™) have also been described (McLachlan et al. 2020a). The most common way to report Symbiodiniaceae densities is to normalize such counts to skeletal surface area. There are several methods that are commonly used to estimate surface area, including: foil wrapping (Marsh 1970), paraffin wax dipping (Stimson and Kinzie III 1991, Veal et al. 2010), geometric approximation (Naumann et al. 2009), or more recently 3D scanning (Enochs et al. 2014, Reichert et al. 2016). The latter may be accomplished with the assistance of smartphones and does not necessarily require any sophisticated equipment. Additionally, Symbiodiniaceae density can also be normalized to coral tissue (biomass) (Edmunds and Gates 2002) or host protein content (Cunning and Baker 2014).

Recently, genetic techniques using qPCR have been developed to calculate Symbiodiniaceae to coral host cell ratios (Mieog et al. 2009, Cunning and Baker 2013) as an additional metric of symbiont abundance (Cunning and Baker 2014). This can also be achieved based on flow cytometry counts of endosymbiotic Symbiodiniaceae and host cells (Rosental et al. 2017), effectively circumventing the need for intricate surface area determination.

Despite the broad diversity of methods now available, ***cell counting using a hemocytometer is*** a simple, cost-effective, and standardized method for determining symbiont densities that can be easily compared across coral species and studies. However, with the appropriate equipment and expertise, flow cytometry and molecular approaches may provide additional resolution and is more easily scaled when the number of samples is high. Irrespective of which method is use, it should be reported.

S2.2: Holobiont phenotype

S2.2a: Mortality

Differences in survivorship are a critical outcome for coral bleaching experiments that should be recorded. There are two approaches for documenting mortality that are relevant for coral: 1) documentation of colony mortality on a categorical scale (Bythell et al. 1993, Baird and Marshall 2002) and 2) documentation of mortality on a continuous scale (i.e., some portion of the living tissue has died) (Hughes and Jackson 1980, Meesters et al. 1997).

Categorical documentation of mortality: ***Mortality status of each coral ramet in the experiment should ideally be recorded on a daily basis for short-term and acute experiments and on a weekly basis for longer duration experiments.*** Coral ramet mortality is coded as alive (0), less than 50% of the tissue is dead (1), more than 50% of the tissue dead (2) or all of the coral tissue is dead (3). Regardless of how the data is summarized for presentation, the complete raw data file for this information should be used for statistical analyses. There are several statistical methods for analyzing mortality data including contingency tables (different distribution of deaths across groups), logistic regressions (differential probability of death between groups), and survival analysis (differential rate of mortality between groups).

Continuous scale documentation of mortality: ***Partial mortality should be recorded as the proportion of live tissue present at the beginning of the experiment that is dead at the end of the experiment***. For longer experiments, there are a number of challenges with this observation: repeated measures of partial mortality through time may document repeated losses, losses and then regrowth on one part of a fragment, or losses on one part of the fragment and gains on another. Another consideration is the potential for the death of one ramet in an aquarium to cause other ramets in the same aquarium to die. If this is a concern, we suggest removing the dying ramet from the aquarium and recording the partial mortality at that time.

S2.2b: Skeletal growth

Calcification rate is a useful response variable used to quantify coral growth during temperature-stress experiments regardless of duration. However, the method employed to quantify calcification will depend on the duration of the experiment and resources available for the study. **Calcification in short-term temperature stress experiment (<14 days):** The ***Total Alkalinity (TA)*** anomaly technique (Chisholm and Gattuso 1991) is a suitable method for determining net coral calcification rates in short-term experiments. This method is based on the principle that precipitation of 1 mol of $CaCO_3$ reduces the total alkalinity by two molar equivalents and has sufficient resolution to detect small changes in calcification (Chisholm and Gattuso 1991, Langdon et al. 2010), making it well-suited for shorter-time frame experiments (<14 days). Care should be taken to ensure values are blank corrected (Cohen et al. 2017), chambers are sealed and well stirred (Schoepf et al. 2017), incubations times are optimized (Schoepf et al. 2017), and

that sample analysis is completed immediately or within one year if mercuric chloride preserved (Pimenta and Grear 2018). Calcification rates can be normalized to skeletal dry weight, surface area (i.e., wax dip method, foil method, image method), or to coral planar-surface area (here termed ***Coral Shadow Area (CSA)***; **Fig. S2**) for universal comparison among experiments and species. The CSA can be easily calculated by measuring the maximum length and width of a live coral's elliptical planar-area footprint using calipers (preferable), or from a photographic or 3-D photogrammetry image, and applying the formula for an ellipse (approach modified for coral using Uzoh & Ritchie (1996); **Fig. S2**).

FIG. S2: Coral planar- area footprint or coral shadow area (CSA), can be used to normalize coral calcification rates for universal comparison across species and experiments. L = length, W = width, max = maximum. (Illustration by Kody R. Hargrave)

Calcification in longer-term temperature stress experiment (>14 days): The ***Buoyant Weight*** technique (Jokiel et al. 1978) is the most common method for determining net coral calcification rates in longer experiments. Dry mass gained per unit time is calculated by measuring buoyant mass at the start and finish of a defined experimental interval. Changes over time periods longer than 24 hours in the laboratory (Spencer Davies 1989) and weeks to months in the field (Kuffner et al. 2013, Morrison et al. 2013) are reliably detectable. Calcification rate measurements using this method could be normalized to CSA for universal comparison among experiments and species, and can be additionally normalized to contoured surface area (i.e., as determined by foil method (Marsh 1970), wax dipping (Meyer and Schultz 1985), or 3-D modeling (Bythell et al. 2001)). The CSA and 3-D methods are preferred approaches for quantifying surface area as they introduce less uncertainty into data expressed as ratios. Calcification measurements normalized to planar CSA have the advantage that they can be used in carbonate budgets and are comparable with rates measured at reefscape scales (Langdon et al. 2010). Additionally, changes in CSA can be used as a low-cost approach to quantify coral growth in longer experiments and in field settings where buoyant weight is not an option. ***Linear Extension*** is another way to quantify coral growth and is typically measured beyond an Alizarin Red-S line (Dustan 1975, Wellington et al. 1996, Grottoli and Wellington 1999, Holcomb et al. 2013, Morrison et al. 2013) or Calcein dye line (Wilson et al. 1987, Bove et al. 2019). Alizarin is visible in the skeleton and Calcein lines can be visualized with fluorescence microscopy. However, this approach is destructive because the skeleton needs to be sectioned at the end of the study, and it is less applicable to some coral morphologies. All growth rates, regardless of method used, are most universally comparable when reported in SI units as opposed to percent change.

S2.3: Other

S2.3a: Active chlorophyll fluorometry

Chl *a* fluorometry is a common and non-destructive technique that provides a wealth of information on the photochemical state of *in hospite* Symbiodiniaceae. A comprehensive review on these methods can be found in Sugget et al (2011) and for measurements specific to corals, we encourage review of Warner et al (2010) and Ralph et al (2016). Although non-intrusive and straightforward to measure, active fluorescence data is easily misinterpreted if one does not have background knowledge of photosynthetic processes, including photoacclimation, photoadaptation, and photoinhibition.

The most frequently recorded metric by active Chl *a* fluorescence in coral bleaching studies is the ***dark-acclimated maximum quantum yield of photosystem II (PSII) commonly referred to as F_v/F_m***. F_v/F_m is the probability of a photon absorbed to elicit primary photochemistry at PSII and provides a rapid measure of PSII physiological function. For multi-turnover instruments such as the pulse-amplitude modulation (PAM) fluorometers, typical F_v/F_m values for 'healthy' algae may range from 0.5–0.7 but are highly dependent on the coral light acclimation state at the collection site (see references above for details). Dark acclimation time (often 15–60 min) is critical and should be empirically determined to ensure adequate relaxation of non-photochemical processes, but not so long as to induce dark reduction of PSII (and hence artificially low F_v/F_m). Another useful variable that is often measured (though not as frequently) is the effective photochemical efficiency of PSII in the light activated state (recommended abbreviation as F_q'/F_m', but also referred to by $\Delta F/F_m'$, or Φ_{PSII}). F_q'/F_m' measurement timing depends on the type of lighting used, experimental questions, and total duration of the experiment, but is typically recorded in the middle of a light phase (e.g., at peak diurnal or static light also referred to as the 'growth light intensity'). For experiments lasting several days, recording these two parameters in tandem (e.g., F_v/F_m after sunset and F_q'/F_m' during peak sunlight exposure) enables the investigator to assess declines of each parameter as well as the rate of recovery from F_q'/F_m' back to F_v/F_m at the end of each day or longer.

PAM fluorometry is the most common technique in coral research. An advantage of this instrument is the ability to measure fluorescence in the presence of artificial or natural light. While the submersible Diving-PAM is also frequently used, there are other devices that provide a similar range of functionality and some at considerably lower cost. For example, other commercially available fluorometers operate on the saturation pulse method, thereby providing a relatively cheap way to measure basic fluorescence parameters such as F_v/F_m. However, some of these models are designed for terrestrial plants and may not use fiberoptic-based sensors, requiring one to remove their samples from water for measurement and/or making it more difficult to measure corals with complex morphology.

For reported F_v/F_m values to be most meaningful, the following parameters should be measured and reported: instrument measuring light and saturation light pulse intensity (in PAR if available

or, at a minimum, the specific instrument setting), type of lights used (e.g., LED, halogen), saturation pulse duration and frequency, any settings related to the instrument gain or signal damping functions, dark acclimation times, and specific light treatments corals were kept under (including duration and intensity). Note that active Chl *a* fluorescence is not typically a direct substitute for other photosynthesis methods such as measuring oxygen production or carbon assimilation, and we encourage investigators to consider these and other techniques if a deeper understanding of coral photosynthesis is warranted.

S2.3b: Symbiodiniaceae identity

Determination of symbiont identity is a valuable part of any bleaching study and may facilitate the interpretation of bleaching susceptibility and coral responses. There are many approaches to identify Symbiodiniaceae with varying levels of taxonomic resolution (LaJeunesse et al. 2018). Identification of Symbiodiniaceae at the genus level can be achieved using restriction fragment analysis of the 18S rDNA (Rowan and Powers 1991), PCR assays targeting rDNA (Correa et al. 2009, Rouze et al. 2017), genotyping arrays (Kitchen et al. 2020), or other markers (Mieog et al. 2009) conserved within genera. Finer scale approaches include fragment or sequence analysis of the chloroplast 23S rDNA (Santos et al. 2003) that provides within-genus resolution, and sequence analysis of microsatellite flanking regions (Santos et al. 2004) that can provide species level resolution. Multi-locus genotyping of species-specific microsatellite loci provides within species, strain-level resolution and so may help elucidate genotype-genotype interactions between hosts and symbionts (Baums et al. 2014). In general, a number of gene marker loci with different levels of taxonomic resolution exist (LaJeunesse et al. 2018), the most commonly used marker is the non-coding ribosomal ITS2 region. Given its multicopy nature, it amplifies well in PCR reactions even with degraded or low concentration DNA (Hume et al. 2018). Recently, next-generation sequencing (NGS)-based strategies are becoming more commonplace. NGS approaches are motivated by the greater sequencing depth (i.e., the capacity to sequence 1000s of ITS2 copies per sample) to improve characterization of symbiont communities. From the current available methods to analyze ITS2 NGS data, only the analytical framework SymPortal (Hume et al. 2019) makes explicit use of the intragenomic sequence diversity in comparison to other NGS methodologies that collapse intragenomic diversity. SymPortal employs novel logic to identify within-sample informative intragenomic sequences, termed defining intragenomic variants (DIVs), and uses combinations of these DIVs to achieve superior resolution, resolving putative taxa. Notably, ITS2 NGS data can be submitted to symportal.org, an accompanying online platform for free-of-charge analysis, increasing standardization and comparability. This approach provides within genus- and sometimes species-level resolution.

When possible, coral bleaching studies should characterize Symbiodiniaceae with the finest level of taxonomic resolution. The ***ITS2 marker is the most commonly used method*** with near-species-level resolution that can be achieved by DGGE or ideally NGS with SymPortal analysis. At a minimum, genus-level resolution can be obtained by PCR and/or RFLP assays.

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