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Increasing comparability among coral bleaching experiments

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Abstract. Coral bleaching is the single largest global threat to coral reefs worldwide. Integrating the diverse body of work on coral bleaching is critical to understanding and combating this global problem. Yet investigating the drivers, patterns, and processes of coral bleaching poses a major challenge. A recent review of published experiments revealed a wide range of experimental variables used across studies. Such a wide range of approaches enhances discovery, but without full transparency in the experimental and analytical methods used, can also make comparisons among studies challenging. To increase comparability but not stifle innovation, we propose a common framework for coral bleaching experiments that includes consideration of coral provenance, experimental conditions, and husbandry. For example, reporting the number of genets used, collection site conditions, the experimental temperature offset(s) from the maximum monthly mean (MMM) of the collection site, experimental light conditions, flow, and the feeding regime will greatly facilitate comparability across studies. Similarly, quantifying common response variables of endosymbiont (Symbiodiniaceae) and holobiont phenotypes (i.e., color, chlorophyll, endosymbiont cell density, mortality, and skeletal growth) could further facilitate cross-study comparisons. While no single bleaching experiment can provide the data necessary to determine global coral responses of all corals to current and future ocean warming, linking studies through a common framework as outlined here, would help increase comparability among experiments, facilitate synthetic insights into the causes and underlying mechanisms of coral bleaching, and reveal unique bleaching responses among genets, species, and regions. Such a collaborative framework that fosters transparency in methods used would strengthen comparisons among studies that can help inform coral reef management and facilitate conservation strategies to mitigate coral bleaching worldwide.

Key words: common framework; coral bleaching; coral heat stress; cross-study comparisons; experimental design methods; feeding; flow; light; phenotype; standardization; temperature.

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Introduction

Temperature stress from ocean warming due to climate change is now the single largest threat to coral reefs globally (Veron et al. 2009, Cantin et al. 2010, Frieler et al. 2012, Hughes et al. 2018). Reef ecosystems are experiencing unprecedented declines in coral colony abundance, coral diversity, and reef growth as a result of temperature-induced coral bleaching, a phenomenon that is becoming more frequent and severe (e.g., Hoegh-Guldberg et al. 2007, Eakin et al. 2009, Veron et al. 2009, Hoegh-Guldberg 2011). By the end of this century, tropical seawater temperatures are expected to rise by 1°C–3°C (IPCC 2013), and severe bleaching is expected to occur annually in some regions by 2030 and globally by 2055 (van Hooidonk et al. 2014). Coral bleaching is the visual manifestation of the breakdown in the symbiosis between the coral host and its endosymbiotic dinoflagellates (family Symbiodiniaceae; LaJeunesse et al. 2018) whereby the coral loses its endosymbiotic algae or pigments resulting in a pale or "bleached" appearance. Bleaching results in decreased coral health, growth, and reproductive output, as well as increased coral susceptibility to disease and mortality (e.g., Brown 1997, Hoegh-Guldberg 1999, Omori et al. 1999, Buddemeier et al. 2004, Jokiel 2004, Maynard et al. 2015).

Despite the wide impact of bleaching events, the magnitude and extent of bleaching can vary substantially across scales, ranging from the individual colony to the ocean basin (e.g., Rowan et al. 1997, Fitt et al. 2000, Loya et al. 2001, Grottoli et al. 2006, 2014, Palumbi et al. 2014, Muller et al. 2018, Morikawa and Palumbi 2019). Although it is well documented that temperature and irradiance are key drivers of coral bleaching, the processes causing broad variation in bleaching susceptibility and recovery across reefs, corals, and colonies are not fully resolved. Manipulative experiments remain a critical tool for elucidating the underlying mechanisms and responses of corals to thermal stress (McLachlan et al. 2020). However, few studies conduct detailed comparisons of results across data sets because it is not always straightforward to ascertain whether the variation in bleaching and recovery responses are due to (1) differences in experimental design (e.g., differences in light, baseline temperature, rate of temperature increase, experimental duration, etc.), (2) differences in bleaching and recovery measurements, (3) differences in coral biology, or (4) some combination of these differences.

A detailed review of coral bleaching experiments by McLachlan et al. (2020) revealed that many important details about how experiments are designed and executed are sometimes missing from published papers, making comparisons between studies challenging. For example, knowing experimental heating temperature, heating duration, and lighting conditions are essential for cross-study comparisons because all three variables can influence coral bleaching responses. In addition, some bleaching studies use a heat-hold or heat-pulse strategy of heating that mimics daily heat stress over a mid-day low tide (Oliver and Palumbi 2011), whereas others mimic the onset and duration of a natural reef-wide bleaching event with gradual increases in temperature and prolonged temperature exposure (Rodrigues and Grottoli 2007). Whether corals are exposed to pulse or gradual exposure may influence responses (Mayfield et al. 2013*b*). Therefore, clear reporting of experimental details and results is necessary for meaningful comparisons among studies (Gerstner et al. 2017) and for reliably identifying patterns in coral bleaching and recovery across species, habitats, reefs, and regions.

One way to increase comparability and transparency among ongoing and future coral bleaching studies is to develop a common framework for reporting the conditions and results of coral bleaching experiments, while neither being overly prescriptive nor diminishing scientific innovation. A common framework for coral bleaching should include consideration of coral provenance, experimental conditions, and husbandry. Similar approaches have been successful in advancing other fields (e.g., ocean acidification research; Riebesell et al. 2010, Cornwall and Hurd 2015), while also allowing for the rapid development of creative approaches to understanding underlying mechanisms. Doing so for experimental coral bleaching research will markedly improve our ability to detect important trends, identify species vulnerabilities and tolerances, and help coral researchers and managers devise solutions for coral persistence over the coming decades (Warner et al. 2016).

The state of coral bleaching experimental design and methods

Prior to the 1970s, the phenomenon of coral bleaching was relatively unknown. In 1971, coral bleaching was reported on a Hawaiian nearshore reef adjacent to a power plant that discharged warm water (Jokiel and Coles 1974). The first experimental research connecting coral bleaching with high-temperature stress followed (Jokiel and Coles 1977). One of the first records of large-scale heat-induced coral bleaching was in Panama, which was attributed to a thermal anomaly associated with the 1982–1983 El Niño event at that time (Glynn 1983). Since then, experimental research on coral bleaching has accelerated, with at least 243 peer-reviewed journal articles published since 1990, two-thirds of which were published in the last 10 years alone (McLachlan et al. 2020). Manipulative experiments have been, and remain, critical for elucidating the triggers and responses of the coral holobiont to thermal stress and assessing their subsequent recovery. Research to date reveals that bleaching susceptibility and recovery vary among coral species, populations, seasons, reef habitats, and genetically distinct individuals (i.e., genets, Box 1) as well as among corals harboring similar or different algal endosymbionts or bacteria (e.g., Rowan et al. 1997, Fitt et al. 2000, Loya et al. 2001, Grottoli et al. 2006, 2014,

Palumbi et al. 2014, Ziegler et al. 2017, Muller et al. 2018, Morikawa and Palumbi 2019, Voolstra et al. 2020). Yet, it is unclear how much of the variation in bleaching responses is a consequence of biological differences in bleaching among coral holobionts, differences in experimental conditions (e.g., differences in light, baseline temperature, rate of temperature increase, experimental duration, flow, etc.), or methodologically inherent biases in how coral bleaching is measured (McLachlan et al. 2020). We know that the scientific understanding of coral bleaching relies heavily on experimental outcomes from three coral species (*Pocillopora damicornis*, *Stylophora pistillata*, and *Acropora millepora*), that experimental conditions are sometimes not reported (e.g., missing information on water flow, experimental location, heating rate), and that measurements of bleaching phenotypes are weighted heavily by responses of the endosymbiotic algae (McLachlan et al. 2020). Thus, direct comparisons among studies can be challenging. While experimental methods ultimately depend on the research question, this paper outlines a strategy for providing a common framework for coral bleaching experiments to enhance cross-comparisons and strengthen coral bleaching meta-analyses. The details were developed by 27 coral research scientists from 21 institutions, spanning research expertise in biological, geological, physical, and computational disciplines, who participated in the first Coral Bleaching Research Coordination Network (CBRCN) workshop at The Ohio State University in May of 2019.

Experiments were separated into three temporally defined categories: (1) short-term and acute (0–7 d of thermal stress), (2) moderate duration (8–30 d of thermal stress), and (3) long-term and chronic (>31 d of thermal stress) experiments. The methods used and the experiments conducted within each category are clearly different from each other (McLachlan et al. 2020) and thus were considered separately. A summary of the common framework for coral bleaching experiments in each category is given in Table 1 (see details in the *Proposed Common Framework* section). Our summary is not intended to be prescriptive, but instead should be considered as a heuristic guide to help facilitate and strengthen comparisons among studies. One common finding that emerged from discussions of all three experimental categories was to provide guidance on the number of replicates in experiments. This topic will be discussed first as it applies to all experimental categories. In addition, we find that including measurements for common coral response variables in coral bleaching experiments would further enhance cross-study comparisons by providing common physiological reference points across studies. A list of potential response variables is provided at the end of Table 1. A brief review of common methods for measuring each listed variable is provided in Appendix S1. A full discussion of the proposed common framework is detailed below.

Proposed Common Framework

Number of genets and ramets

For all types of coral bleaching experiments, it is essential to control for potential sources of variation in the response of experimental corals across scales of biological organization. For example, there may be measurable

Box 1. Glossary of Terms

Ambient temperature: temperature at time of collection.
Baseline temperature: temperature from which heat-stress offset is calculated (typically MMM).
MMM: maximum monthly mean (i.e., average daily temperature of the hottest month of the year for the previous several years).
Genets[†]: formed by sexual reproduction. All colonies and tissue that can trace their ancestry back to the same fertilization event belong to the same genet (Appendix S1: Fig. S1).
Genotype[†]: the genetic makeup of a sample for a given (set of) genetic marker(s). When enough markers are assayed, a sample can be assigned to a genet based on its genotype.
Ramets[†]: physically independent modules arising from colony fragmentation or other asexual means of dispersion. A genet can have one or many ramets. Ramets can be experimentally generated nubbins, naturally occurring fragments, or attached colonies (Appendix S1: Fig. S1).
Phenotype: the set of observable characteristics of an individual resulting from the interaction of its genotype with the environment.
Water flow rate: volumetric water flow rate per unit time (L/s^{-1}). In a tank, this would be the fluid output from the exhaust of the pump or tank outflow in flow-through systems.
Water turnover time: time required to replace the entire volume of water in a tank(s), assuming the tank is continuously well mixed. Calculated by dividing the tank volume by the flow rate.
Water flow velocity: motion of water relative to sessile coral (cm/s^{-1}).
[†]Baums et al. (2019).

Table 1. Framework for coral bleaching experimental methods and coral response variables.

Variable	Appendix section	Suggested target or range		
		Acute and short-term experiments (<7 d at BST)	Moderate duration experiments (7–30 d at BST)	Long-term experiments (>30 d at BST)
No. genets	S1.1	5 minimum; >5 if possible	5 minimum; >5 if possible	≥5
No. replicate tanks per treatment		Minimum two tanks per treatment	ANOVA design, minimum of 3 tanks per treatment factor; regression design, gradient study with >3 treatment levels; avoid pseudo-replication	Avoid pseudo-replication
Acclimatization to experimental tanks		Typically none	7–12 d following fragmentation and mounting	7–12 d following fragmentation and mounting
Control temperature	S1.2	Ambient temperature at collection site at time of collection	Ambient temperature at collection site at time of collection	Ambient temperature at collection site during the experimental period
Baseline temperature	S1.2	MMM and/or rapid temperature profiles corresponding to in situ temperature patterns if appropriate	MMM	MMM
Bleaching stress temperature above local MMM		Typically +3 to +9°C; increase temperature from MMM until death is observed, then set target temperature lower; if the goal is to observe phenotypic variability, expose corals to several temperatures to find the temperature at which half of the corals bleach; stress exposure should happen at the same time of day; temperature stress duration should be standardized within experiments	+1 to +4°C depending on local ecological relevance and species, may need to be higher in extreme environments	+1°C or more depending on local ecological relevance and species
Temperature ramp-up rate		None recommended as it will depend on temperature stress duration; heating rates should be adjusted to take the same time across treatment temperatures	0.1–1°C/d	Mimics increase in temperature rate observed during previous bleaching events at that site
Temperature modulation		Temperature ramp-up to static elevated temperature, followed by recovery at baseline temperatures; profiles can be run once or multiple times	May be static or diurnally modulated; choice of modulation should be the same in treatments and controls	Static or diurnal for indoor experiments; diurnal and seasonal for outdoor experiments
Control conditions		At ambient temperature; exact same conditions as treatment, except for temperature	At ambient temperature; exact same conditions as treatment, except for temperature	At ambient temperature; exact same conditions as treatment, except for temperature; mimics natural conditions
Light	S1.3	Ideally, static light conditions for short-term thermal exposures (with no light at night) or possibly diurnal variability if over several days; light levels match natural light conditions; minimum 250–500 μmol photons·m^{-2}·s^{-1}	Ideally, diurnal variability with 80% of maximum PAR light at collection site; minimum 250–500 μmol photons·m^{-2}·s^{-1}	Indoor tanks, diurnal variability (with moonlight cycles); outdoor tanks, apply shade to mimic PAR at collection depth; minimum 250 μmol photons·m^{-2}·s^{-1}

Table 1. (Continued)

Variable	Appendix section	Suggested target or range		
		Acute and short-term experiments (<7 d at BST)	Moderate duration experiments (7–30 d at BST)	Long-term experiments (>30 d at BST)
Flow				
Flow system	S1.4			
Flow-through		Report pump rate in liters pumped per hour	2–20 cm/s	2–20 cm/s, mimic natural conditions
Closed		Report pump rate in liters pumped per hour, tank volume	Record flow rates, pump size, tank volume, and try to base the flow rate on in situ data	Record flow rates, pump size, tank volume
Tank volume turnover	S1.4			
Flow-through		100% within 3–6 h	1–4 times per day	1–4 times per day
Closed		100% within 3–6 h	Case-dependent and depends on system biomass	Case-dependent and depends on system biomass
Feeding	S1.5	None typically	Minimum once per week to satiation; report feeding amount, rate, and food type	Minimum once per week to satiation; ideally feed up to three times per week; report feeding amount, rate, and food type; mimic food availability in nature
Seawater	S1.6	Filtered or unfiltered; natural or artificial	Filtered or unfiltered; natural or artificial	Filtered or unfiltered; natural or artificial
Post heat-stress monitoring		Hours to a few days (longer than the stress duration); this doubles the number of fragments needed	If possible, immediate (0.2–1 month) and long-term monitoring (>1 month) depending on the question	0.2–3 months depending on the question
Other environmental conditions				
Salinity	S1.7			
Nutrients	S1.8			
pH	S1.9			
Dissolved oxygen	S1.10			
Coral bleaching responses				
Bleaching phenotype				
Image analysis of color	S2.1a			
Chlorophyll concentration	S2.1b			
Symbiodiniaceae cell density	S2.1c			
Holobiont phenotype				
Mortality	S2.2a			
Skeletal growth	S2.2b			
Other				
Active chlorophyll fluorescence†	S2.3a			
Symbiodiniaceae identity	S2.3b			

Notes: BST, bleaching stress temperature; MMM, maximum monthly mean (i.e., men temperature of the warmest month; ANOVA, analysis of variance; PAR, photosynthetically active radiation. A review of commonly used methods is summarized in Appendix S1. Glossary of terms is given in Box 1.

†E.g., PAM fluorometry.

differences in performance among genets when comparing the performance of their ramets (i.e., fragments, asexually produced, originating from the same genet) in different experimental conditions (Appendix S1: Fig. S1; Box 1) (Parkinson et al. 2018, Muller et al. 2018, Jury and Toonen 2019, Morikawa and Palumbi 2019, Wright et al. 2019, Voolstra et al. 2020). Investigating multiple ramets of the same genet across treatments allows for a more direct inference of treatment effects. Such "identical twin"-type designs have proven useful in short-, moderate-, and long-term bleaching studies (Grottoli et al. 2014, Ziegler et al. 2017). Furthermore, there is increasing evidence that heritable genetic effects that are attributable to distinct coral genets can significantly affect holobiont physiology and thermal tolerance (Meyer et al. 2009, Dixon et al. 2015, Kenkel et al. 2015, Kuffner et al. 2017, Jury et al. 2019). To control for this source of variation, genets and their ramets should be identified and tracked, and sufficient numbers of genets should be included in a given study.

Recent work by Baums et al. (2019) indicated that for Caribbean corals, four genets capture the most common genetic diversity within a population (though this minimum could vary for corals in other ocean basins). Thus, a minimum of five genets from each species, population, region, or habitat would add sufficient representation across each experimental treatment and allow for a minimum of four genets if one genet is lost due to unforeseen circumstances. A larger sample size would more effectively characterize a population, especially if the experimental goals include measuring the variance as well as the mean responses. We recognize that this minimum recommendation may not be sufficient in some cases and power analyses prior to the start of the experiment would facilitate determining the appropriate number of genets needed.

Tracking the identity of each genet and ramet throughout the duration of an experiment is useful for survival analysis, which can factor into variance among genets (see methods for tracking genets and ramets in Appendix S1: Section S1.1). Ideally, unique genets are confirmed with genetic markers, but we recognize that this may not be a reasonable expectation in many studies. Alternatively, distinct colonies sampled at least 5 m apart on the reef decreases the chances that collections will include clonal ramets (Baums et al. 2019). For species known to engage more heavily in asexual proliferation, particularly Acroporids (Baums et al. 2006, Gorospe et al. 2015, Manzello et al. 2019), even greater spacing of field-sampled corals may be needed, or secondary genetic analysis performed, to verify the uniqueness of the sourced corals (Gorospe et al. 2015, Riginos 2015, Manzello et al. 2019).

Acute and short-term (0–7 d) thermal-stress experiments

Acute and short-term thermal-stress experiments are here defined as those designed to be completed in 0–7 d. The advantages of such experiments are three-fold. First, many corals can be rapidly tested for their responses to a variety of temperatures and their responses can be compared among species, populations, genets, and experimental treatments. Quick testing of hypotheses further allows for the rapid validation of interesting and unexpected results. Second, the phenotype of the coral of interest is captured soon after collection, thereby avoiding potential behavioral and physiological changes arising from acclimation in captivity. Third, these experiments can be used to mimic strong, rapid swings in temperature that some corals are exposed to in shallow-water settings, especially in localities with large tidal cycles (Green et al. 2019). Corals exposed to the latter are among some of the most heat resistant (Oliver and Palumbi 2011) and serve as important subjects to understand thermal tolerance and stress resilience. Overall, short-term experiments provide a mechanism to test a large number of colonies and reef sites for their immediate and extreme physiological responses to acute-heat exposure that are not possible in longer experiments.

However, the short-duration and fast-temperature-ramping rates inherent to these types of experiments do not mimic most natural bleaching events, and care must be taken when using results from acute and short-term bleaching experiments to infer outcomes or make predictions about natural bleaching. These experiments are also limited by the types of responses that can be quantified over short periods of time. For instance, pigmentation and –omics level responses are easily quantified, but processes such as calcification that typically require more time to measure are not as amenable to short heat-stress studies. Thus, acute and short-term thermal-stress experiments may be most ecologically relevant for understanding corals from reef flats and shallow lagoons that experience natural short-term heating associated with low tide (Brown et al. 2002, Palumbi et al. 2014, Herdman et al. 2015, van Oppen et al. 2018). The extent to which acute-stress experimental outcomes relate to results obtained from long-term heat-stress experiments, and how both inform our knowledge of thermal resilience in situ is under active investigation. Results from one study suggest that the thermal tolerance of corals in acute heat-stress studies are indicative of thermal resilience of corals to natural heat-stress events (Voolstra et al. 2020).

Mechanistically, acute and short-term thermal-stress experiments use small-scale, highly portable instrumentation that house small tanks where physical variables such as temperature, light, and flow can be highly controlled, facilitating downstream comparisons among studies (Fig. 1). While these experiments can be done with as few as two tanks per treatment, four to six tanks provide additional statistical power (Table 1) and serve as a fail-safe in case a tank malfunctions. The relatively simple design is flexible and more repeatable than moderate and long-term experiments, amenable to

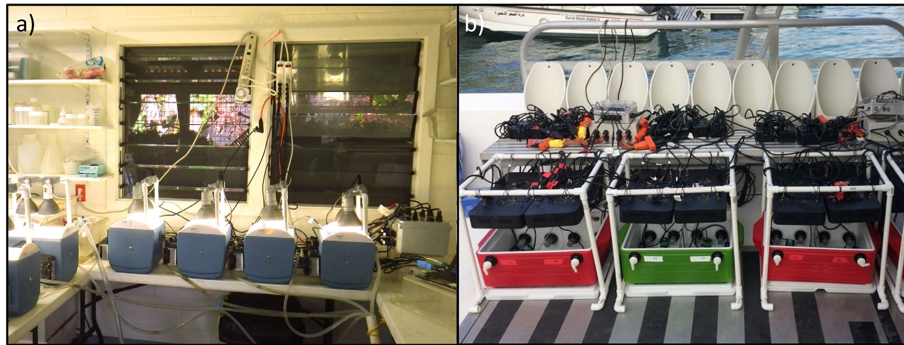

Fig. 1. Two examples of acute and short-term coral heat-stress experimental setups. Photo in panel (a) by S. Palumbi and panel (b) by C. R. Voolstra.

deployment in remote locations, and accessible to those working with limited resources. These features may make acute and short-term thermal-stress experiments readily adoptable by researchers, teachers, and students. In addition, acute and short-term studies typically use small coral ramets allowing for conservative use of coral material and the opportunity to obtain repeatable phenotype diagnostics with a large number of samples at a relatively low effort per sample. Reporting the average and range of as many physicochemical conditions as possible in an experiment enhances comparisons among studies since differences in any one of the non-temperature variables can influence how corals respond to temperature stress. A common framework for acute and short-duration coral bleaching experiments is summarized in Table 1.

Acute and short-term thermal-stress experimental conditions.—1. Temperature.—In all heat-stress experiments, treatment temperatures are typically based on in situ temperature measurements or previous bleaching records from the coral collection site. Given the dramatic heat-stress conditions in short-term and acute studies, pilot studies to empirically assess coral responses to a range of temperature levels are helpful in determining the exposure temperature at which the corals in question bleach and die. These pilot experiments are relevant for setting a target temperature and should be set below the temperature that caused mortality (Table 1). Treatment temperature may fall within a range of +3°C to +9°C above the monthly mean maximum (MMM; Voolstra et al. 2020). This initial testing is particularly important when in situ temperature data are lacking. Reef temperature at the time of collection should provide the most realistic control temperature. Precision and accuracy of temperature in control and treatment tanks is achieved by using continuous temperature logging devices (Appendix S1: Section S1.2), which enhance the ability to compare results across studies.

Temperature profiles of acute and short-term heat-stress experiments are either of a heat-pulse or a heat-hold design (Fig. 2; Mayfield et al. 2011, Parkinson et al. 2018, Morikawa and Palumbi 2019, Voolstra et al. 2020). Heat-pulse experiments are often designed to mimic natural temperature fluctuations over diel cycles, across tidal cycles, and during internal wave or upwelling events, but may also be used to rapidly test the response of corals to a range of elevated temperatures that are not typically recorded in a natural setting (Fig. 2a). The profile encompasses cycles of ramp-up heating, exposure to a target high temperature, and ramp-down cooling, often followed by a recovery phase (i.e., with the latter often lasting longer than the heat cycle(s) themselves). In the simplest case, heat-pulse experiments run through one such cycle, but any number of cycles may be explored (e.g., to assess the effect of repeated heat exposures on recovery and resilience). They can also explore the maximum thermal tolerance of corals with multiple tanks at temperatures ranging from MMM to +9°C (Fig. 2b). Heat-pulse designs explicitly allow the exploration of the holobiont response to thermal extremes, as well as examination of the potential for acclimation, given that the heat-stress exposures are brief. Starting and stopping times typically mimic natural diel variability, are only run during the day, and ideally finish at the same time of the day each day. Consistency in ramp duration (minutes–hours) and heating duration at the target temperatures helps to facilitate comparisons among heat-pulse coral-bleaching studies. We recognize that this protocol may result in variable temperature ramp rates (°C/h) to reach the desired heat-stress target temperatures (Table 1; Fig. 2b).

In heat-hold experiments, the temperature ramp-up rate is high compared with long-term experiments, and the duration of heating at the target temperature is extended compared to heat-pulse experiments (Fig. 2c). For this type of experiment, thermal stress is continuously accumulated, and could be considered a short-term model for bleaching events in which the entire water column is rapidly heated.

2. Light.—Coral bleaching is a response to both temperature and light (Jokiel and Coles 1990, Brown et al. 1994, Warner et al. 1999, Brown et al. 2002). Natural

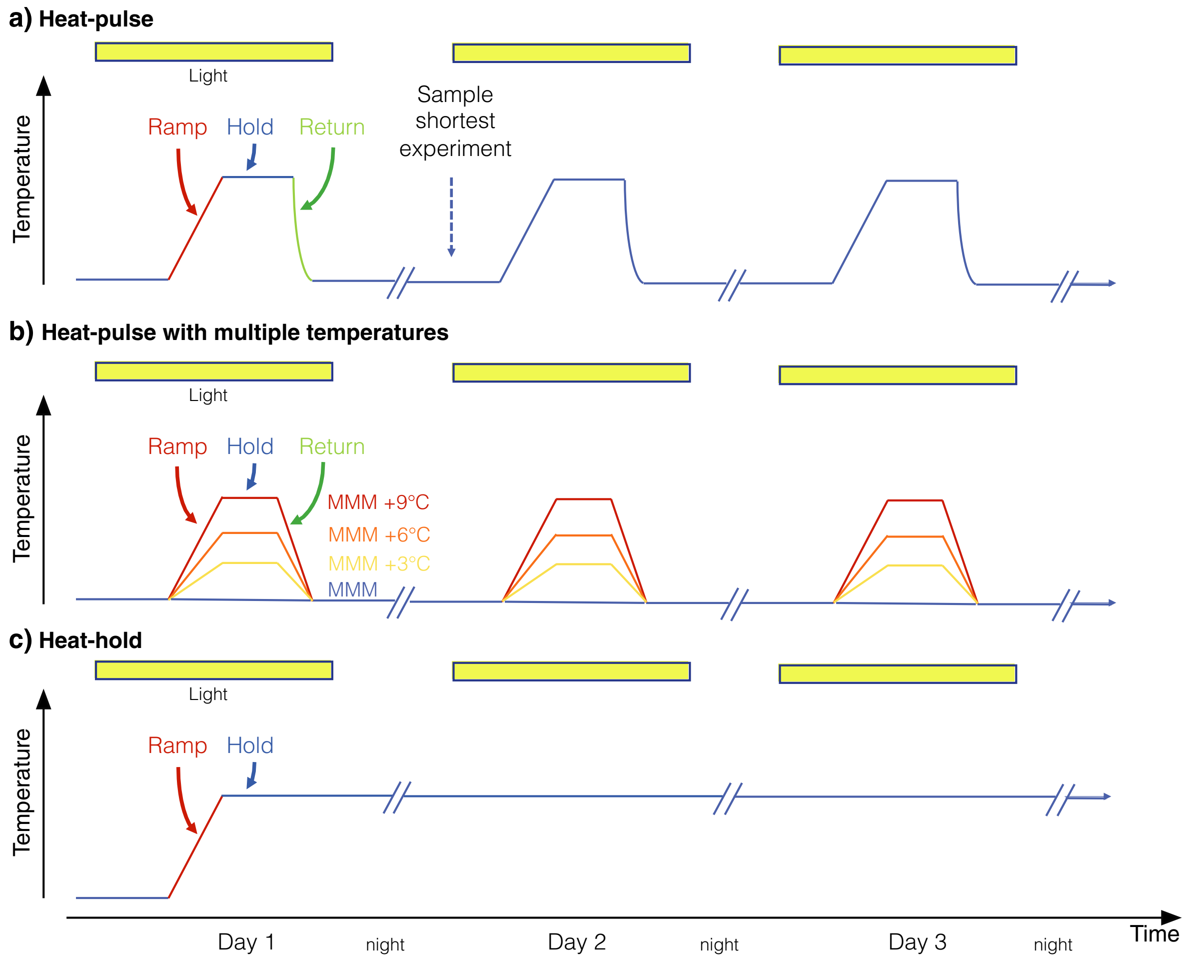

FIG. 2. Temperature profiles of coral (a) heat-pulse, (b) heat-pulse with multiple temperatures, and (c) heat-hold acute and short-term thermal stress experiments. Number of days will depend on the specific study. Yellow bars indicate light cycles. Line breaks indicate night. MMM, maximum monthly mean temperature.

bleaching often correlates strongly with maximal light conditions (Mumby et al. 2001), and there is often a relationship between temperature-related photodamage to Symbiodiniaceae and light intensity (Warner and Suggett 2016). Artificial light that is modulated over day/night cycles (see yellow bars in Fig. 2) mimics the diel cycle providing realistic light cues for these photosynthetically active animals with strong circadian rhythms (Hoadley et al. 2016). If light is not a dependent variable, in situ light data from the coral collection site can be used to determine the maximum irradiance on a clear cloudless day and thus the maximum experimental light levels. If replicating natural light conditions is not possible, minimum light levels from 250 to 500 μmol photons·m^{-2}·s^{-1} are typically sufficient to stimulate maximal photosynthesis (P_{max}; Warner et al. 1999, Falkowski and Raven 2007, Suggett et al. 2013; Table 1; Appendix S1: Section S1.3). Given the short nature of acute heat-stress experiments, use of static light intensities during the day is more practical over fluctuating light levels that incorporate dawn and dusk. Light levels that are standardized within experiments enhance comparability of results among runs.

3. Seawater flow and turnover.—Adequate flow within the tanks is important as static water creates temperature, pH, and oxygen gradients, chemical changes, and pockets of high microbial growth (Mass et al. 2010, Osinga et al. 2017), whereas higher current flow reduces bleaching (Nakamura and van Woesik 2001, Nakamura et al. 2003, Lenihan et al. 2008, Schmidt et al. 2016, Fujimura and Riegl 2017). Thus, adequate flow as well as consistent flow rates among tanks are needed for valid comparisons within and among studies. Thus, flow and tank volume turnover need to be sufficient in acute and short-term studies (Table 1) where flow effects may manifest quickly. Water flow velocity can be measured with a velocimeter (i.e., distance travelled per unit time) and seawater turnover rate within tanks can be estimated by measuring the volume exchanged over a defined time

period. Submersible water pumps can provide additional circulation in cases where tank turnover and/or flow is limited for logistical reasons. In flow-through systems, we suggest a 100% water turnover rate every 3–6 h (Table 1).

4. Feeding.—Unlike long-term experiments, direct feeding is not critical in acute and short-duration studies (assuming sufficient light is provided to the corals; Table 1). However, the type of seawater used (i.e., filtered, unfiltered, natural, artificial) is important as the chemical composition and particulate organic matter content can vary substantially among different seawater types.

5. Applications for early life stages.—Acute and short-term thermal-stress experiments allow for the assessment of temperature stress on early-life stages of coral larvae and juveniles. In the estimated 85% of coral species where eggs are not provisioned with algal symbionts by the parent colony, larvae provide access to naturally aposymbiotic tissue, which can be used to understand the coral host response to temperature stress (Voolstra et al. 2009, Baums et al. 2013, Dixon et al. 2015), albeit against the background of ontogenetic change. Endosymbiont-host associations are often manipulated more easily during larval and juvenile stages when the coral may be able to associate with a wider array of symbionts than during the adult stage (Abrego et al. 2009, van Oppen 2015, Quigley et al. 2017, Poland and Coffroth 2019). Furthermore, the small size of coral larvae allows for comparison across many individuals in the same experiment.

Moderate-duration (8–30 d) thermal-stress experiments

Moderate-duration thermal-stress experiments are defined as those in which thermal stress lasts between 8 and 30 d above the baseline temperature (Glynn and D'Croz 1990; Table 1). These experiments typically seek to simulate natural conditions by assessing the coral phenotypic responses while maximizing biological realism and ecological relevance. For experiments conducted at remote field sites, moderate duration experiments are often more practical and cost-effective than long-term experiments. Key advantages of moderate-duration experiments is that they can be used to measure compensatory mechanisms, holobiont responses, mortality, and recovery that are typically included in long-term experiments, but with a more ecologically relevant heat-stress duration than acute and short-term experiments. In addition, moderate-duration experiments do not limit the range and type of coral responses that can be quantified and are sufficiently long to detect genet-level responses.

Mechanistically, moderate-duration thermal-stress experiments are typically conducted using standard indoor or outdoor aquaria where physical variables such as temperature and flow can be reasonably constrained, facilitating subsequent comparisons between studies (Fig. 3). Light conditions may be natural or artificial (see *Light* section below) and tank replication of at least three tanks per treatment reduces the problem of tank effects. Coral ramets in these studies are typically medium to large in size (e.g., 3–8 cm tall), making them easy to manipulate experimentally and providing sufficient material for a large number of downstream analyses. Coral ramets are typically allowed to recover for 7–12 d after fragmentation providing time for initial wound healing (Traylor-Knowles 2016, Edmunds and Yarid 2017, Counsell et al. 2019). It is generally assumed that 7–12 d is sufficient time for acclimation to the experimental conditions prior to the start of the experiment. Mimicking natural conditions in terms of baseline temperature, light, flow, salinity, pH, nutrient levels, and dissolved oxygen, as closely as is reasonably possible, helps to provide ecologically relevant findings. Reporting the average and range of as many physicochemical conditions as possible in an experiment enhances comparisons among studies since differences in any one of the non-temperature variables can influence how corals respond to temperature stress (Finelli et al. 2006, Anthony et al.

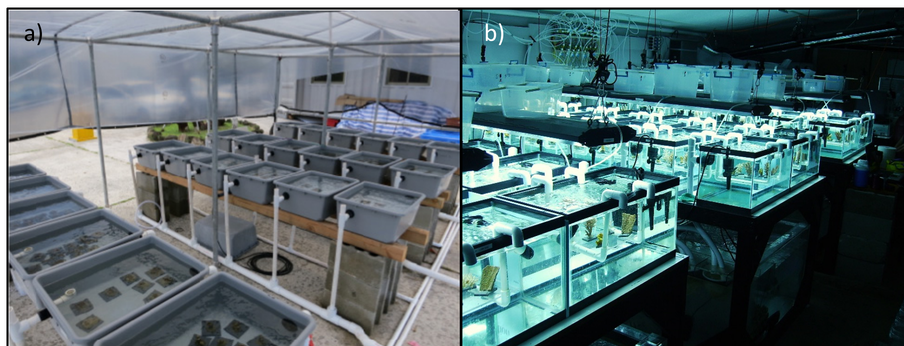

Fig. 3. Example of an (a) outdoor and (b) indoor moderate-duration coral heat-stress experiment setup. Long-term experimental setups are similar. Photo in panel (a) by D. Kemp and panel (b) by A. Grottoli.

2008, Wiedenmann et al. 2013, Vega Thurber et al. 2014). A common framework for moderate-duration coral bleaching experiments is outlined in Table 1.

Moderate-duration thermal-stress experimental conditions.—1. Temperature.—The duration and severity of thermal stress is determined by the experimental question. Thermal stress of +1–4°C above the local thermal baseline (i.e., MMM) typically produces a bleaching response within 30 d (Jokiel and Coles 1990, Fitt et al. 2001, Grottoli et al. 2006, Mayfield et al. 2013*b*; Table 1; Appendix S1: Section S1.2). The upper temperature threshold depends on what is realistic for the species studied, and what is ecologically relevant for that location. Gradual temperature ramp-up rates over several days minimizes the chances of heat-shock and mimics the rate of warming in natural bleaching events (Table 1). In general, a temperature ramp-up rate of no more than 1°C/d can prevent an acute stress response, although this is still rapid in relation to many natural bleaching events (Jokiel 2004, Ainsworth et al. 2016, Bahr et al. 2017). Ideally, the warming rate should simulate natural profiles when possible so as not to induce an acute stress response (Table 1; Appendix S1: Section S1.2). How long corals are experimentally maintained at bleaching stress temperatures will depend on the desired phenotypic response (i.e., such as disruption of photosynthesis, loss of pigmentation/endosymbionts, or onset of mortality), but without unintended mortality over the course of the experiment.

2. Light.—Similar to the recommendations above for acute experiments (see *Acute and short-term thermal-stress experimental conditions: Light*), light requires special consideration in moderate-duration experiments as well (Table 1; Appendix S1: Section S1.3). When light is not an experimental treatment, light conditions that mimic natural irradiance conditions as closely as possible at the depth from which the colonies were collected will be most ecologically relevant. For outdoor experiments, neutral-density shade cloth is useful for attenuating full sunlight and to ensure that light intensity mimics photosynthetic available radiation (PAR) experienced at the depth from which the corals were collected (Grottoli et al. 2014, Jury and Toonen 2019). Recommended peak PAR levels should follow the same guidelines provided in *Acute and short-term thermal-stress experimental conditions: Light*. For indoor systems, diurnal light cycling is most realistic though it is often difficult to generate daytime light levels that are as high as those experienced in shallow reef environments. When replicating natural light conditions is not possible, minimum light levels close to saturating photosynthesis are typically sufficient (*Acute and short-term thermal-stress experimental conditions: Light*), but this is dependent on the collection location and ideally empirically tested prior to starting experiments. For corals from deeper locations, maximum light levels are more easily matched to those at the collection site. Since high light can modulate bleaching responses in corals (Anthony et al. 2007, Ferrier-Pagès et al. 2007, Hawkins et al. 2015), an adequate acclimation period is especially important in experimental systems where light conditions differ from those at the collection sites.

3. Seawater flow and turnover.—Adequate water flow minimizes unwarranted temperature gradients and localized pH or chemical changes in experimental tanks. For comparative purposes clear reporting of the various flow parameters is useful (i.e., circulating pump size, brand, and model, the tank volume, water flow, and tank volume turnover time; Table 1; Appendix S1: Section S1.4). For many reef environments, near-bottom water velocities are on the order of 2–20 cm/s (Nakamura and van Woesik 2001, Hench et al. 2008, Lowe et al. 2009, Hench and Rosman 2013) depending on the location (e.g., lagoon vs. barrier reef crest). Velocity variability due to wave exposure can be quantified using the root mean squared (rms) velocity (Reidenbach et al. 2006, Falter et al. 2007, Lowe et al. 2008). Flow rates within experimental tanks should attempt to replicate flow conditions at the corals collection site to minimize any unintended flow effects. Complete water exchange (i.e., tank volume turnover) is also important for ensuring adequate mixing and temporally stable physicochemical conditions in tanks during an experiment. Tank volume turnover times of once per day may be all that is feasible for some types of experiments, although higher daily turnover is better for providing physicochemical conditions in the system that are more consistent with natural reef environments (Table 1, Appendix S1: Section S1.4).

4. Feeding and post heat-stress recovery.—Corals are mixotrophic, relying on both autotrophy and heterotrophy for proper nourishment. Heterotrophic feeding on zooplankton, particulate, and dissolved organic particles is a natural part of their diet and an essential source of nutrition, especially when stressed (Anthony 2000, Grottoli et al. 2006, Houlbreque and Ferrier-Pages 2009, Edmunds 2011, Hughes and Grottoli 2013, Baumann et al. 2014). In moderate-duration heat-stress experiments, supplemental feeding at least once a week to satiation provides corals with some of that essential nutrition (though coral have access to zooplankton nightly on the reef, so up to three times a week is more realistic; Tables 1; Appendix S1: Section S1.5). Even if using natural seawater flow-through systems, corals will likely not be getting zooplankton or adequate nutritional resources, necessitating supplemental feeding. Little to no zooplankton are available in many natural seawater flow-through systems (A. G. Grottoli, *personal observation*), although there can be fine particulate and dissolved organic carbon available. Finally, moderate-duration experiments present an opportunity to monitor responses to post heat-stress treatment (i.e., recovery; Table 1). How corals physiologically recover from heat-stress is an understudied area of research (McLachlan

et al. 2020), yet vital to understanding how corals might recover or continue to decline following bleaching events (Hughes and Grottoli 2013, Grottoli et al. 2014).

Long-term and chronic (>31 d) thermal-stress experiments

Long-term bleaching experiments are here defined as those in which thermal stress above the baseline temperature (i.e., MMM temperature) lasts for 31 d or more. These experiments may include a single prolonged heat-stress, multiple heat-stress events with similar or different heating profiles (i.e., repeat or annual bleaching), and/or preconditioning and recovery periods (Mayfield et al. 2013*a*, Grottoli et al. 2014; Fig. 3). These experiments are best-suited for reproducing naturally occurring heat-stress conditions and bleaching events followed by observations on recovery. As such, long-term and chronic experiments have maximum ecological relevance and provide real-world responses of coral phenotypes to thermal stress. Experiments on these time-scales can capture seasonal variability and evaluate acclimatization responses that integrate over long timespans, which include photo-acclimation, changes in gene expression, symbiont shuffling, calcification, changes in energy reserves, and feeding behaviors. In addition, the long-term nature of these studies also enables time-series analysis and can facilitate more collaborative and comprehensive measurements.

Despite the advantages of long-term heat-stress experiments, they require much more investment in resources and effort than short-term and moderate-duration experiments. Long-term studies also have a greater risk of tank effects that compound over time (although these problems can be minimized by rotating treatments among experimental tanks, or rotating corals among tanks of the same treatment), or other unforeseen issues that may cause the experimental conditions to deviate from those that are realistic in nature (e.g., an outbreak of algae, micro-predator, or disease). Therefore, backup equipment, maintenance of power, adequate plumbing, robust scientific equipment, and careful monitoring are critical for these types of experiments.

Mechanistically, long-term experiments are typically conducted in outdoor tank systems where ambient light and flow-through seawater best replicate conditions on the reef. Alternatively, they are conducted in an indoor laboratory setting where conditions are carefully controlled to mimic natural environments. However, since this can be expensive and difficult, outdoor settings are typically more practical. In most studies, pseudoreplication is avoided by including two or more tanks per treatment (Table 1). As with moderate-duration experiments, sufficient time for wound healing post-collection under control conditions ensures corals can acclimate to the system prior to experimentation (Table 1). Coral ramets in these studies typically start off as small to medium in size but can grow to be very large in studies lasting more than a year. This allows for many downstream analyses, but the projected growth of the corals should be taken into account in the planning stages of long-term experiments. Since these types of experiments are designed to mimic naturally occurring heat-stress events, the physical conditions other than those being experimentally manipulated are ecologically relevant when they mimic local conditions as closely as possible. When local environmental data are not available for the area where the experimental corals were sourced, data from nearby or comparable sites are often used to establish the physical conditions in the experiment. Measuring and reporting as many physicochemical conditions (i.e., temperature, light, flow, salinity, pH, etc.) at the highest resolution possible is especially important in longer studies as their changes can have cumulative effects over the course of the study and influence the measured coral response variables. A common framework for long-term duration coral bleaching experiments is outlined in Table 1.

Long-term and chronic thermal-stress experimental conditions.—1. Temperature.—Control temperatures are most realistic when they mimic the ambient diel temperature and the seasonal variability where the corals were collected (Table 1; Appendix S1: Section S1.2). While this is reasonable for outdoor flow-through systems, it can be challenging in an indoor environment. The heat-stress temperature will depend on the local ecological relevance and species of interest. An MMM +1°C or more (i.e., enough to elicit a bleaching response without being so severe as to cause unintended mortality over the experimental duration) often realistically mimics natural bleaching events (Table 1). Likewise, the rate of thermal ramping will depend on the observed natural warming rate observed in one or more previous local bleaching events (Table 1).

2. Light.—Optimal experimental light conditions mimic natural irradiance at the coral collection depth and site, including the daily light integral for the region on both diel and seasonal timescales. The lighting requirements in long-term experiments are the same as those for moderate heat-stress experiments and discussed in *Moderate-duration thermal-stress experimental conditions: Light* above. Due to the longer duration of these studies, indoor systems that also simulate moonlight provide an important regulator of coral physiology, particularly reproduction, over longer timescales (Table 1).

3. Seawater flow and turnover.—The common framework structure for flow and turnover in long-term heat-stress experiments is the same as those for moderate heat-stress experiments and discussed in *Moderate-duration thermal-stress experimental conditions: Seawater flow and turnover* above.

4. Feeding and post heat-stress recovery.—The common framework structure for feeding and monitoring of

recovery are the same in long-term heat-stress studies as for moderate-duration heat-stress studies and are discussed in *Moderate-duration thermal-stress experimental conditions: Feeding and post heat-stress recovery.*

Common Currencies for Quantifying Coral Bleaching Responses

Bleaching is often based on characteristics of the algal endosymbionts (i.e., color, appearance) or the coral holobiont (i.e., growth, mortality). Yet, in some experiments, no quantified measure of bleaching is reported (McLachlan et al. 2020). This creates difficulty in comparing coral bleaching studies because there is no common experimental "currency" among them. For example, one study might measure the microbiome and endosymbiont algal density, whereas another study might measure calcification and gene expression. Even if the two studies are on the same coral species from the same location, without a common response variable between them it is more difficult to compare and draw inferences. This is especially true when there are different bleaching thresholds among different genets of the same species, or different species that are morphologically indistinguishable (Boulay et al. 2014, Johnston et al. 2018). Reporting one or more common currency measures of coral bleaching could provide a quantitative reference to enhance physiological comparisons among studies and provide greater potential for meta-analyses. Examples of measurements that could serve as common currencies include color image analysis, chlorophyll concentration, Symbiodiniaceae cell density, mortality rate, and skeletal growth rate. While there are many other methods for quantifying coral bleaching, the response variables listed in Table 1 were prioritized for their effectiveness in quantifying bleaching and holobiont phenotypes as well as for their ease of measurement, minimal training necessary to execute the measurements, and low per sample cost, making them accessible to as many researchers as possible. Measuring and reporting at least one endosymbiont response variable (i.e., color, chlorophyll, cell density) and one holobiont response variable (i.e., mortality, growth) would be a valuable means of establishing common physiological reference points between studies (Table 1; Appendix S1: Sections S2.1, S2.2). Reporting these response variables in International System of Units (SI units), as opposed to percentage change, would further facilitate cross-study comparisons, future data reuse, and statistical analyses. If resources permit, measurements of active chlorophyll fluorescence (e.g., pulse-amplitude modulating [PAM] fluorometry) can be an effective and non-destructive way of quantifying endosymbiont photosystem performance. Further, Symbiodiniaceae diversity (i.e., genus, species, or strain) can provide incredibly insightful information as it is an important correlate of bleaching severity and recovery (Table 1; Appendix S1: Section S2.3). We recognize that the latter two analyses require substantial instrumentation, cost, and training, and therefore may not be feasible in many instances.

Implications of Accurate Reporting for Meta-Analysis

McLachlan et al. (2020) noted that many basic environmental and experimental conditions are underreported in coral bleaching experiments. For example, at least 95% of the studies examined do not report any measure of flow (i.e., flow within tanks or tank turnover rates), 25% do not report light intensity, and 21% do not provide any quantitative measurement of the bleaching phenotype or the precise geographic location of the study. Yet, flow and light can have dramatic interactive effects on thermal-stress responses (Nakamura and van Woesik 2001, Nakamura et al. 2003, McLanahan et al. 2005, Nakamura et al. 2005). A quantitative measure of bleaching severity can have a profound effect on how the results might be interpreted, and the geographic location is critical for placing results into a broader ecological context (e.g., bleaching threshold temperature above MMM of corals in the Red Sea are a lot higher than elsewhere, Bellworthy and Fine 2017, Osman et al. 2018). Being able to effectively compare findings among studies requires accurate reporting of experimental conditions. Thus, we have compiled a summary of some metadata that are valuable to accurately report in Table 2 to increase transparency in experimental methods, enhance comparability among studies, and facilitate a more global understanding of coral bleaching patterns across space and time. We recognize that not all metadata types will apply to all experiments.

Beyond Coral Bleaching Experiments

While the development of a common framework for coral bleaching experiments is a step in the right direction, there is more to consider. Every year, researchers conduct coral bleaching experiments, measure some response variable(s) of interest, and publish their results. Too often, remaining coral material is disposed of, or not archived in a way that could be utilized or made available to other researchers for additional studies. The next step for the coral research community is to evaluate how coral samples are collected, preserved, and archived to determine how researchers might effectively share existing coral material to conduct additional complementary research without duplicative experimentation. This approach has the advantage of limiting the amount of wild coral material harvested for research, increasing the return on investment for a given experiment, fostering new collaborations and exchanges of ideas, and reducing the time to discovery. Sample preservation and archiving are strategies that have been effectively used in other communities (e.g., International Ocean Drilling Program) and are models for coral researchers to consider developing.

TABLE 2. Summary of metadata that can be reported in coral bleaching experiment research to increase cross-study comparisons.

Metadata type	Conditions or methods	Units or other identifier(s)
Coral collection	Latitude and longitude at collection site	Decimal degrees
	Collection depth	Meters
	Collection date(s)	Year-month-day
	Coral species	
	Coral morphology (i.e., plating, encrusting, mounding, branching, foliose)	
	Symbiodiniaceae for all coral colonies†	
	Acclimation post collection prior to experiment	Days
Experimental design	Name of location	Institution, city, state/province, country
	Bleaching stress temperature period	Start and end dates in year-month-day
	System type (flow-through or recirculating, outdoor or indoor)	
	No. tanks per treatment	
	No. coral genets (colonies) per treatment	
	No. coral genets (colonies) per tank within treatments	
	No. recovery days post heat-stress	
Experimental temperature conditions‡	Heat stress temperature above MMM per treatment	°C
	Control temperature	°C
	Baseline temperature (MMM)	°C
	Temperature ramp-up rate	°C/h or °C/d
	Duration at heat stress temperature	Hours or days
	Temperature modulation	Static, diurnal, seasonal
Other experimental conditions	Light conditions§	μmol photons·m^{-2}·s
	Light cycle	Static, diurnal, seasonal
	Water flow velocity¶ or tank volume with pump circulating capacity	Flow rate, cm/s
	Tank turnover¶	No./d or L/min
	Seawater filtration	Filtered or unfiltered
	Seawater source#	Natural or artificial
	Salinity‖	
	Nutrient concentrations†† (i.e., ammonia, nitrite, nitrate, phosphate)	
	Feeding‡‡ (i.e., fed/not fed, frequency, concentration, and food type)	
	pH§§	
	Dissolved oxygen¶¶	

Notes: MMM, maximum monthly mean (i.e., mean temperature of the warmest month). A review of commonly used methods for many of the measurements and analyses is included in Appendix S1. Not all conditions or methods will apply to all studies.

†Appendix S1: Section S2.3b.
‡Appendix S1: Section S1.2.
§Appendix S1: Section S1.3.
¶Appendix S1: Section S1.4.
#Appendix S1: Section S1.6.
‖Appendix S1: Section S1.7.
††Appendix S1: Section S1.8.
‡‡Appendix S1: Section S1.5.
§§Appendix S1: Section S1.9.
¶¶Appendix S1: Section S1.10.

CONCLUSIONS

The common framework for coral bleaching experiments outlined in this paper provides some insights and suggestions that could help increase comparability among coral bleaching experiments. We recognize that studies are driven by specific research questions that may differ in scope or have requirements that are outside the framework parameters outlined here. Nevertheless, it is our hope that the common framework discussed here will encourage researchers to consider measuring and reporting more of the physicochemical conditions and variables (Table 1), better appreciate the value of reporting all of the relevant metadata (Table 2), and perhaps incorporate new analytical techniques or approaches in their research (see Appendix S1). The broad adoption of a common framework for coral bleaching experiments would increase the comparability of studies and enhance collaboration, which would have the net effect of increasing the efficacy and creativity of coral bleaching

research. As coral reefs continue to change globally, every effort we can make to accelerate the pace of discovery will bring us that much closer to innovative solutions for protecting and restoring coral reefs.

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Supporting Information

Additional supporting information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/eap.2262/full

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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).

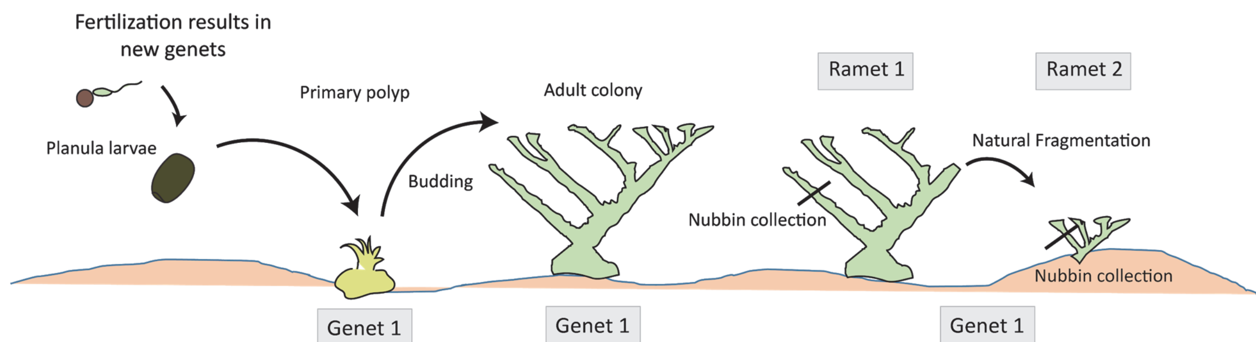

FIG. S1 Sexual reproduction leads to the formation of new genets via the development and metamorphosis of planula larvae into adult colonies. Colonies may fragment naturally resulting in a genet with many ramets. Genetic methods are the only way to definitively determine genet diversity and membership 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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