Unified methods in collecting, preserving, and archiving coral bleaching and restoration specimens to increase sample utility and interdisciplinary collaboration (#67954)

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Unified methods in collecting, preserving, and archiving coral bleaching and restoration specimens to increase sample utility and interdisciplinary collaboration

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Coral reefs are declining worldwide primarily because of bleaching and subsequent mortality resulting from thermal stress. Currently, extensive efforts to engage in more holistic research and restoration endeavors have considerably expanded the techniques applied to examine coral samples. Despite such advances, coral bleaching and restoration studies are often conducted within a specific disciplinary focus, where specimens are

collected, preserved, and archived in ways that are not always conducive to further downstream analyses by specialists in other disciplines. This approach may prevent the full utilization of unexpended specimens, leading to si l research, duplicated efforts, and increased co A recent National Science Foundation-sponsored workshop set out to consolidate our collective knowledge across the disciplines of Omics, Physiology, and Microscopy & Imaging garding the methods used for coral sample collection, preservation, and archiving. Here, we hig ht knowledge gaps, and propose some simple steps for collecting, preserving, and archiving coral-bleaching specimens that can increase the impact of individual coral bleaching and restoration studies and foster additional analyses and future discoveries through collaborati Rapid freezing of samples in liquid nitrogen lacing at −80℃ to −20℃ is optimal for most Omics and Physiology studies with a few exceptions, however, freezing samples removes the potential for many Microscopy & Imaging-based analyses due to the alteration of tissue integrity during freezing. For Microscopy & Imaging, samples are best stored in aldehydes. The use of sterile gloves and receptacles during collection supports the downstream analysis of host-associated bacterial and viral communities which are particularly germane to disease and restoration efforts. Across all disciplines, the use of aseptic techniques during collection, preservation, and archiving maximizes the research potential of coral specimens and allows for the greatest number of possible downstream analyses.

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Abstract: Coral reefs are declining worldwide primarily because of bleaching and subsequent mortality resulting from thermal stress. Currently, extensive efforts to engage in more holistic research and restoration endeavors have considerably expanded the techniques applied to examine coral samples. Despite such advances, coral bleaching and restoration studies are often conducted within a specific disciplinary focus, where specimens are collected, preserved, and archived in ways that are not always conducive to further downstream analyses by specialists in other disciplines. This approach may prevent the full utilization of unexpended specimens, leading to siloed research, duplicative efforts, and increased costs. A recent National Science Foundation-sponsored workshop set out to consolidate our collective knowledge across the disciplines of Omics, Physiology, and Microscopy & Imaging regarding the methods used for coral sample collection, preservation, and archiving. Here, we highlight knowledge gaps and propose some simple steps for collecting, preserving, and archiving coral-bleaching specimens that can increase the impact of individual coral bleaching and restoration studies, as well as foster additional analyses and future discoveries through collaboration. Rapid freezing of samples in liquid nitrogen or placing at −80℃ to −20℃ is optimal for most Omics and Physiology studies with a few exceptions; however, freezing samples removes the potential for many Microscopy & Imaging-based analyses due to the alteration of tissue integrity during freezing. For Microscopy & Imaging, samples are best stored in aldehydes. The use of sterile gloves and receptacles during collection supports the downstream analysis of host-associated bacterial and viral communities which are particularly germane to disease and restoration efforts. Across all disciplines, the use of aseptic techniques during collection, preservation, and archiving maximizes the research potential of coral specimens and allows for the greatest number of possible downstream analyses.

Keywords: coral, reef, provenance, storage, methodology, protocols, pipelines, analytics, physiology, Omics, microscopy, imaging, restoration, conservation

Introduction

Coral reefs provide sustenance, goods, and services for coastal communities worldwide and likely harbor more than one third of all marine species (Fisher et al., 2015). However, corals and reef frameworks are increasingly being degraded due to anthropogenic disturbances. Climate change has severely affected coral reef health on a global scale, primarily through increased sea surface temperatures, leading to devastating coral bleaching ever The increased frequency and intensity of these events reduces the capacity for reef recovery and restoration efforts (Heron et al., 2016; van Hooidonk et al., 2016; Sully et al., 2019), and successive bleaching events have decreased live coral cover by up to 60% in some localities (Miller et al., 2009; Raymundo et al., 2019; Dalton et al., 2020). As a result, up to one third of all reef-building c species may be at risk of extinction from the combined effects of bleaching and local stressors such as nutrient pollution, overfishing, and habitat destruction (Pandolfi et al., 2003; Carpenter et al., 2008; Plaisance et al., 2011; Hughes et al., 2017, 2018, 2019). Given the increased frequency and severity of bleaching events, scientists and restoration practitioners need to study coral bleaching and disease more efficiently as a matter of urgency. One way to achieve greater efficiency is through the implementation of a common framework recently developed for coral bleaching experiments (Grottoli et al., 2021). Another is by reducing the number of duplicative efforts more broadly and maximizing the number of analyses that can be performed on sampled specimens through greater collaboration.

Identifying Common Methodological Pipelines in Collecting, Preserving, and Archiving

The technology and methods commonly used in coral biology research have quickly progressed in recent decades (Cziesielski, Schmidt-Roach & Aranda, 2019; Grottoli et al., 2021). The combination of traditional and modern genomic insights, physiological metrics, and microscopy & imaging analytics have together given scientists an ever-expanding toolkit to interrogate the mechanisms and results of coral bleaching and restoration efforts at the subcellular, cellular, tissue, and organismal levels. Integration of these approaches thus allows individual specimens to be used for multiple downstream applications and expands the potential utility of every coral sample collected. Despite this, scientists and practitioners tend to sample, preserve, and archive specimens in a manner specific to their own specialized applications or aims, and on average only conduct one or two downstream analyses per study. For example, in a review of 197 studies on coral bleaching, it was found that nearly 75% of studies performed only one or two analyses: 42.1% used a single downstream analysis (n=83), while 30.5%, 14.7%, 7.1%, and 5.6% conducted two, three, four, or five downstream analyses (McLachlan et al., 2021). These data suggest that more could be done with each set of samples collected during coral bleaching and restoration studies and efforts. Yet, limits exist on how many tools individual researchers can manage, conduct, and financially support. Trained in increasingly complicated fields of study, it is impractical for any one scientist, or even a team of scientists, to have the breadth of knowledge, skills, and resources to conduct the full range of possible Omics, Physiology, and Microscopy & Imaging analyses on any given set of specimens. However, with effective documentation during sampl coupled with strategic preserving and archiving decisions, specimens could be available to additional research teams, who could increase the number of analyses ultimately conducted on a given set of samples, contributing to a better understanding of bleaching mechanisms with less sampling and experimental damage to reefs.

As the numbers and expertise of scientific investigators expand, so do the tools, methods, and perspectives at their disposal. Our recent survey of the literature quantified the

techniques coral scientists recently used for collecting, preserving, processing, and archiving coral bleaching specimens (McLachlan et al., 2021). A subsequent formal workshop brought together investigators from around the world to further synthesize research methods in order to identify low-cost and practical ways to share specimens, reduce duplicative efforts, and increase the end-use potential of samples generated in coral bleaching research and restoration programs (Figure 1). We identified and consolidated working pipelines that could 1) expand the number of potential analyses on existing specimens, and 2) assist in future project planning to maximize the number of potential downstream analyses while minimizing any extra work, time, or funds required. While no single methodological pipeline can be all-inclusive, several critical steps in these methodological pipelines were found to optimize the potential utility of each coral specimen within the constraints of a given study design.

Consolidating Methods for Broadening Participation

To build on a common platform recently developed for coral bleaching experiments (Grottoli et al., 2021), we sought to identify relatively easy-to-perform and low-cost collection, preservation, and archiving methods that would maximize the analytical potential of each specimen derived from both coral bleaching and restoration experiments and field-based surveys. Cheap and unifying methods can serve to increase participation and inclusion in coral bleaching and restoration research, particularly for those with minimal funding. Clear, simple guidelines for specimen and sample collection, manipulation, and preservation can also make it easier for experts working on parallel questions in non-coral systems to bring their hypotheses and approaches to bear on the coral bleaching and restoration fields. Adapting and expanding sampling, preserving, and archiving of specimens in ways that allow for additional downstream analyses can generate research opportunities for early career scientists and students, providing a mechanism for additional collaboration and more entry points into the field of coral research, as well as creating new opportunities for collaborations and networking between researchers with distinct yet complementary areas of inquiry, thereby fostering advances and new ideas within the field. These efforts support the inclusion of researchers in the field who may not currently conduct marine fieldwork due to lack of access to resources (e.g., funding, SCUBA gear, boat access, laboratory equipment), training (e.g., scientific dive certifications), and/or physical or logistical capability. A separate challenge in promoting diversity and inclusion in the broader field of coral research is to connect researchers that have samples with other scientists and managers (including undergraduate trainees and volunteers) from diverse disciplines and backgrounds that can run additional analyses. A database of samples and researchers (and their research interests/skill sets) could be useful in identifying and jump-starting fruitful collaborations and sample sharing. Between 2014 and 2021 over 20,000 coral specimens and samples were collected for bleaching studies (McLachlan et al., 2021), many of which are suitable for additional analyses that could address new questions concerning various aspects of bleaching. Going forward, implementation of specific collection, preservation, and archiving pipelines developed herein could further maximize and foster more collaboration among diverse community members and stakeholders.

Consolidating Methods for Restoration Specimens

Coral restoration and rehabilitation programs aim to assist in the recovery of reef ecosystems through passive and active means, and for the ultimate goal of creating a reef that can independently continue to develop without further intervention (Boström-Einarsson et al.,

2020). Recent efforts to explore the success and failure of some restoration programs have revealed a lack of coordinated efforts among restoration practitioners, scientists, and manag Similar to concerns within the coral bleaching community these often small-scale and disparate efforts can result in non-standardized reporting methods (McLachlan et al., 2020). Further, some restoration programs remain unlinked to scientific endeavors that could track natural biological, chemical, and oceanographic phenomena that provide mechanistic context for why some coral propagation and outplanting efforts result in success while others do not. Collaborative work to engage in scientific inquiries both before, during, and after restoration efforts, along with standardized practices, could accelerate and advance restoration programs. For example, genetic, physiological, and microbiome sampling of specimens from restoration corals that are successfully outplanted have revealed key aspects of why some genotypes and species are more resistant or resilient to local and global stressors (Baums, 2008; Lohr & Patterson, 2017; Morikawa & Palumbi, 2019; Klinges et al., 2020; van Woesik et al., 2021; Voolstra et al., 2021). Thus, the consolidated methods presented herein can be used to bridge the gaps between the restoration and research communities more readily and completely.

General Considerations for Collecting, Preserving, & Archiving Coral Bleaching Specimens

The central aim of our workshop was to identify simple and low-cost methods that could increase the impact of every coral bleachi udy in an effort to understand basic scientifi principles and increase restoration and co ation success. In the process, we uncovered several key issues that all researchers and managers can consider regardless of individual subfields, including specimen: 1) provenance and metadata, 2) collection considerations, and 3) storage temperature, handling, and sterility. It is also important to consider how collection, preservation, and storage methods may shift the accuracy or precision of downstream analyses. For a more elaborate discussion of specific methods see the Supplemental Materials.

Specimen and Sample Provenance:

Museums and private collections have dard protocols for documenting the history, or origin, of individual specimen (Smithsonian In tion, 2006; National Science and Technology Council, Interagency Working Group on Scientific Collections, 2009; National Academies of Sciences, Engineering, and Medicine, 2020). Researchers and practitioners can optimize the use of their data and samples by rigorously cataloguing, and formally documenting as many experimental (e.g., temperature ramp rate, light level, flow), biological (e.g., coral color, morphotype, taxonomy), and environmental (e.g., depth, nutrient concentrations, reef type) variables as possible (Grottoli et al., 2021) because these meas ments provide needed context for each collection. We refer to these descriptive, contextual data as metadata. Sample provenance also includes the documentation of how and where samples and their resulting data and metadata are physically and digitally stored. Growing recognition of the value of historical data and appreciation for FAIR (findability, accessibility, interoperability, and reusability) data standards (Wilkinson et al., 2016) is leading the efforts to archive sample data and metadata in ways that facilitate reuse and ensure archived data is available to future researchers (Zerbino et al., 2018; Davis et al., 2019; Percie du Sert et al., 2020). Numerous community-based resources can also provide data storage options to both facilitate data archiving and reuse, including those

specific to coral research, restoration and biodiversity (e.g., GEOME (Deck et al., 2017; Riginos et al., 2020)). Further, many funding agencies have specific data management and dissemination requirements (e.g., BCO-DMO at the National Science Foundation, GenBank at the National Center for Biotechnology Information, Environmental Data Service at the Natural Environment Research Council). However, relevant details concerning these samples are often overlooked. For example, a recent sampling of the Sequence Read Archive (SRA) of GenBank found that only ~14% of archived specimens included both collection year and site as basic metadata that would be required for the reuse of archived genomic data in future studies (Toczydlowski et al., 2021). As the culture of global research and reef conservation and restoration have moved toward more open and collaborative models, there is growing pressure from funding bodies, journals, management agencies, and researchers alike to provide these data in open-access formats (Sibbett, Rieseberg & Narum, 2020), and emerging cyberinfrastructure to support the discovery and reuse of material samples (e.g., iSamples (Davies et al., 2021)). Such consolidated efforts stand to benefit the advancement and accessibility of the field of coral bleaching research and restoration science and effort as a whole.

Sample Collection Considerations

There is a myriad of possible techniques for collecting, processing, and archiving most coral specimens (for more details see Supplemental Materials). However, unique differences among coral taxa, their morphotypes, tissue thicknesses, skeletal density, and variation in life stages demand special consideration as these variables may affect the biology and chemistry of collected coral samples and could dictate the applicability of many downstream procedures. Additionally, colony and specimen/sample size as well as species-specific variation can affect how corals respond to and recover from stress (Brandt, 2009; Thomas & Palumbi, 2017; Álvarez-Noriega et al., 2018; Levas et al., 2018). The quantity of available sample material can also affect what downstream techniques are possible. Precise measurements of colony and specimen size is an active area of research (Table 1) with the advent of new technological developments such as 3D laser scanning and photogrammetry (House et al., 2018; Vivian et al., 2019; Zawada, Dornelas & Madin, 2019). Information about the original size of the parent colony or outplant specimen can provide helpful information for interpreting resulting data because size has been shown to be an important bleaching predictor (Álvarez-Noriega et al., 2018). Finally, collection permits may restrict the number of samples that can be collected, which can affect the types of analytical methods that are possible downstream and how much excess material may or may not be available for archiving and future research. Lastly, developmental stage can have significant impacts on which methods are suitable and practical for any methodological pipeline. For example, the amount of material required for some analyses may be prohibitive when working with coral larvae or gametes, but easily performed on adult tissues. Thus, the types of research questions that can be addressed will vary depending on the life stage of the specimen and dictate the types of downstream analyses and collaborations that are most productive.

Temperature and Sample Storage Considerations

When collecting and preserving coral bleaching and restoration specimens for short and long-term use, documenting a sample's temperature history is critical (see Box 1 on Freezing and Cryopreservation). In general, altered temperatures can cause rapid state changes in live specimen physiology, microbiology, and geochemistry. Many subcellular and cellular processes

can change within minutes to hours when corals undergo shifts in ambient temperature (Hillyer et al., 2017a), and swift sample processing is important to capture those responses. Once samples are preserved, temperature can further influence the integrity of each sample for some types of analyses. For example, cells could lyse if samples are too cold, thus making them unsuitable for imaging of intact cells. Each scientific discipline has guidelines for optimal preservation temperatures suitable to ensure the integrity for their analytic process (see Table 2). The duration of storage for these specimens can also dictate ideal archiving temperature conditions. If samples are intended to be stored for tens of years (e.g., in coral gamete biobanks), cryopreservation and downstream restoration, rapid-freezing in liquid nitrogen, and storing at −80°C are the safest holding temperatures. If the tissues or cells can tolerate freeze-drying, and the final packaging is vacuum-sealed, then such specimens can be maintained for many years at room temperature.

However, coral bleaching and restoration research is often conducted in locations where adequate freezing agents and materials (e.g., liquid nitrogen, dry shippers, or even ice) may not always be available. Although not all methods require temperature stabilization, many do (Table 2). Therefore, if possible, all researchers should record 1) transport holding temperature, 2) any altered temperatures during transport, and 3) the duration of transport. For example, if live or dead specimens were removed from an offshore reef, transported to shore, and placed in new containment, the method and duration of transport as well as the temperature of any onshore activities (e.g., freezer storage, water temperature manipulation) should be documented.

BOX 1: Freeze it and Forget it?

Freezing material is at the heart of maintaining robust tissue archives. But what are the limits of some of these freezing processes in terms of tissue quality over time? Before deciding how to store samples, both the sensitivity of the measurement and how long that process needs to be viable should be considered. The cryopreservation field is rapidly evolving, especially for human samples. For example, standard practice for understanding tumor physiology was to fix in formalin, embed in paraffin, and store at room temperature. However, delicate RNA can degrade over time under these conditions but remains robust if stored at −80°C (Baena-Del Valle et al., 2017). Thus, coral RNA and enzyme specimens may best be stored at −80°C, potentially remaining stable for up to 10 years at these low temperatures and making them suitable for additional downstream analyses. For corals, storing at −80°C allows for the highest number of downstream analyses (Table 2). However, longer-term stable storage (> tens of years) at liquid nitrogen temperatures (−196°C) is preferable (Ortega-Pinazo et al., 2019; Kelly et al., 2019), though highly impractical for many researchers due to the cost and equipment needs associated with ultra-cold storage. In contrast, many laboratory analyses can be reliably performed on specimens stored at −20°C (Table 2) for two to five years.

Frozen But Alive: Cryopreservation Holds Material Safely for Many Years

Cryobiology is the study of cells and tissues at cold temperatures. The central principle in cryopreservation is to avoid the formation of lethal intracellular ice. Generally, cryopreservation uses permeating cryoprotectants or solutes, such as dimethyl sulfoxide (DMSO), methanol or propylene glycol, and non-permeating solutes, such as sugars (e.g., glycerol), to allow the permeating cryoprotectants to enter cells and block ice crystal formation, and to permit the non-permeating solutes to dehydrate and remove intracellular water to reduce and avoid ice formation. Once cells and tissues are safely cryopreserved and held at liquid nitrogen

temperatures, most biological processes are reduced. Theoretically, if cells are maintained at liquid nitrogen temperatures, they can survive for thousands of years with minimal damage. Thus, cryopreservation of living coral tissue and maintenance in liquid nitrogen (e.g., cryobanks) provides access to a multitude of scientific and restoration uses because the tissues are frozen, but also alive. Once the cryoprotectants are warmed and the cells are rehydrated, they are alive, and any number of analyses can be done post-thawing. However, cryopreserved cultured cells are equally robust at either −196ºC or −80ºC using a number of metrics over 8 years (Miyamoto et al., 2018). Even in properly cryopreserved samples, tissue degradation can occur if samples are removed from a freezer to subsample and then refrozen or exposed to heat transients by opening and closing of a freezer door. Thus, avoiding any changes in freezer temperatures is ideal.

To date, cryopreservation processes have been used to preserve coral sperm from over 48 species worldwide (Hagedorn et al., 2012). This international collaboration has used frozen sperm to subsequently fertilize coral eggs and create new coral larvae (Hagedorn et al., 2017). Moreover, frozen sperm has also been used to demonstrate the feasibility of assisted gene flow in the critically threatened coral *Acropora palmata* (Hagedorn et al., 2021). Frozen coral material is now archived in biorepositories around the world and some of the material for the assisted gene flow experiments was stored for up to 10 years before successful use in fertilization experiments.

Specimen Handling and Sterility Considerations:

There is increased interest in how the coral holobiont microbiome (i.e., Symbiodiniaceae, bacteria, viruses, and other microscopic eukaryotes) responds to, and may be involved in, preventing or exacerbating coral bleaching and/or increasing or reducing restoration success. Many ecological and physiological bleaching studies can be easily paired with Symbiodiniaceae analyses (e.g., cell densities, gene sequencing) through shared samples, but the potential for coral bacterial and/or viral analyses is severely compromised when sterile collection tools (e.g., gloves, bone-cutters) and sterile receptacles (bags or tubes) are not used. The use of aseptic handling techniques during coral collection and processing is a relatively small and inexpensive change in the methodological pipeline that can enable additional downstream microbiome analyses (Figure 2). For example, a suitable aseptic technique in the field may be as simple as wearing nitrile gloves when handling corals and using sterile receptacles, such as Whirl-Pak® sample bags. Importantly, while aseptic techniques are ideal for many downstream applications, it is impractical to not impossible to maintain underwater and in some handling situations.

Caveats and Considerations for Methodologies, Accuracy, and Usability

In each discipline there may be recommended and, in some cases, well benchmarked standard operating protocols for each individual method discussed below and in the Supplemental. However, many of the methodological pipelines discussed below may also be suitable for some aspects of coral-bleaching and restoration research but have not yet been fully evaluated in terms of their accuracy and precision. Therefore, deviations from standard procedures for a given discipline could potentially result in data that is inaccurate, uninterpretable, or unusable. It is important to consider the potential caveats when using any non-standard procedure in one's work. Yet, as research techniques improve and additional methods and protocols are confirmed as having high precision and accuracy, more of the potential pipelines discussed below may be employed with confidence in any given discipline. For example, using chemically fixed (e.g., in formaldehyde) samples for genomic-based analysis was non-standard

in the past, but new work shows that these preserved specimens can be used to gain insight into various aspects of corals biology retrospectively (Green et al., 2020).

Identification of Consolidated Methodological Pipelines for General Use in Coral Bleaching and Restoration Studies

A previous literature review identified 34 methodologies in coral-bleaching studies (McLachlan et al., 2021), broadly categorized into three disciplinary areas: Omics (e.g., genomics, epigenomics, transcriptomics, metagenomics, amplicon analysis, proteomics, and metabolomics), Physiology (e.g., chlorophyll, lipids/protein/carbohydrate concentrations, biomass, tissue and skeletal stable isotopes), and Microscopy & Imaging-based analyses (e.g., Symbiodiniaceae density, skeleton ultrastructure, electron microscopy, histology, Raman spectroscopy). To quantitatively determine which methodological pipelines can maximize the number of downstream procedures across these three disciplinary areas, we assigned several broad categorical terms to determine whether a step in the pipeline was 'optimal,' 'acceptable,' 'undesirable,' or 'not acceptable'. Pipelines marked undesirable indicate that there may be research to show the method is not ideal, or that it is illogical to pursue a particular pipeline based on basic biology. Thus, caution should be taken when evaluating these incomplete pipelines. Further, in many cases it was unclear if limitations existed for a particular downstream method or pipeline due to a lack of existing references, and thus we also designated many cells in the matrix as 'unknown' (Table 2). These 'unknowns' are likely to have resulted from insufficient testing or knowledge in a particular area as opposed to the method being truly unacceptable; testing these approaches may present fruitful areas for future research.

Once the table was complete, we then summed the number of 'optimal' and 'acceptable' cells to determine which pipelines best served a given set of downstream methodologies. In evaluating the various methodological approaches used in specimen collection, preservation, and archiving, we were able to identify several pipelines that maximize the number of downstream analyses that are possible (Table 2 green cells; Figure 2 hot pink cells). However, it is assumed that aseptic techniques, such as wearing nitrile gloves and using sterile receptacles for storage and transport, were used in the initial step in any given pipeline (Step 1; Table 2).

Freezing or Fixation Methods Dictate Most Methodological Pipelines

Instantaneous freezing or 'rapid freezing' in liquid nitrogen upon initial collection followed by ultra-cold storage (e.g., −80℃) is optimal for maximizing the number of possible downstream analyses (supports ~43% or 21 of 49 methods) (Table 2, green cells column D; Figure 2). Analyses that could concurrently or sequentially be conducted after specimen rapid-freezing and cold storage fell primarily within the Omics and Physiology disciplines, while rapid freezing is inappropriate for most tissue Microscopy & Imaging because it alters tissue integrity (see Box 1). Freezing post-collection using −80℃ and more conventional −40℃ or −20℃ freezers were also deemed acceptable for several procedures within the Omics- and Physiology-based methods (supports ~44% or 19 of 49 methods), except for some RNA-based analyses, which always require immediate rapid freezing or preservation (e.g., in RNAlater®).

Within the Microscopy & Imaging discipline, preserving in paraformaldehyde and glutaraldehyde allowed for the greatest number of downstream methods (27% or 13 of 49 methods), including some Omics methods. However, few if any of the Physiological methods could be conducted on samples initially or secondarily stored in these aldehydes.

406
407 *Methodological Analyses are Needed to Determine the Suitability of Some Collecting,*
408 *Preserving and Archiving Sample Pipelines*
409 A few analyses, including metabolomics, mycosporine-like amino acids (MAAs), soluble
410 lipid, histology, and electron microscopy-based techniques stood out as highly restrictive in their
411 requirements for initial and secondary storage methods. Each of these analyses had less than 3
412 recommended pipelines for collecting and storage (Table 2, Column AZ). Such methodological
413 limitations could be due to stringency in storage requirements or, as suggested by the large
414 number of unknowns in Table 2, due to insufficient testing of potential alternative methods.
415 Thus, we summed the number of 'unknown cells' to determine which methods had the most
416 uncertainty in terms of how samples could be collected, preserved, and archived.
417 Numerous methods had many 'unknowns' (Table 2 Column BA) limiting our ability to find
418 suitable additional pipelines to recommend outside of their standard procedures. For example,
419 biomass quantification and tissue isotope analysis each respectively had 47% and 26% unknowns
420 for the 49 different possible methodological pipelines we tracked.
421 Below we discuss considerations specific to each major discipline: Physiology, Omics,
422 and Microscopy & Imaging, given these methodological differences. Furthermore, we add more
423 details about standard operating procedures for each of the major downstream analyses within
424 Table 2 and throughout the Supplementary Material. While not an exhaustive list, we aimed to
425 give researchers enough information to consider how to collect, preserve, and archive their
426 specimens for many potential applications. We also recognize that methods are continuously
427 evolving with the advent of new technologies. It is likely that newer, better methods will
428 eventually become available and, thus, future researchers should take steps to confirm that
429 additional procedures have not become available following the publication of this work.
430
431
432 Considerations for Individual Fields of Study
433
434 **Omics Methods**
435 'Omics' are a collection of methods that focus on the identification, characterization, and
436 quantification of macromolecules (e.g., nucleic acids, proteins, lipids, and carbohydrates) and
437 biochemical compounds (e.g., metabolites and vitamins). Typically, high throughput procedures
438 are applied, such as DNA and RNA sequencing, liquid chromatography, mass and nuclear
439 magnetic resonance spectroscopy, or X-ray crystallography. Such methods span approaches in
440 proteomics, metabolomics, transcriptomics, meta-barcoding, phylogenetics, epigenetics,
441 microbiology, and virology and often require computationally intensive bioinformatic analysis of
442 large datasets.
443 *Sample Collection*: In general, the use of aseptic techniques is strongly encouraged across
444 all coral bleaching and restoration research collections, allowing for the most complete set of
445 downstream analyses, including the characterization of the microbiome (which includes the coral
446 virome) for which aseptic technique is necessary to ensure further analysis accuracy and integrity
447 (Table 2). However, Omics methods targeting the coral host and/or Symbiodiniaceae do not
448 require sterile tools and receptacles unless total Symbiodiniaceae community diversity is being
449 examined in high resolution. Additionally, reagents and materials can contaminate
450 samples with off-target cellular materials, foreign nucleic acids, viral particles, and exogenous
451 chemicals. Moreover, compounds or enzymes that degrade, damage, or alter macromolecules

and biological compounds (e.g., proteases, R/DNAses) can disrupt or inhibit many molecular processes (e.g., cations and polymerase chain reaction) needed to create Omic datasets or intermediate sample products (e.g., sequencing libraries). Thus, care should be taken to avoid the use of non-sterile materials and/or reagents that are not certified as molecular biology grade.

Preservation for Short Term Storage: Rapid freezing and storage at −80°C is the optimal collection and storage technique for most Omics work such as genomics, metagenomics, proteomics, and RNA-Seq methods (see below). While RNA-based methods (e.g., transcriptomics) are notoriously sensitive to initial collection and storage conditions and require rapid freezing or immediate storage in salt buffers, DNA is more stable and thus can be collected and preserved in a variety of conditions. Several methods (e.g., DNA- and RNA-based host, symbiont, and microbiome approaches) can also be used when corals are initially preserved in salt buffers, some aldehydes, or DMSO (G er et al., 2011; Michael A., Zoe A. & Christina A., 2013). These buffered specimens can be stored short-term at a variety of temperatures because the compounds stabilize the nucleic acids in specimens (Hopwood, 1975; Seutin, White & Boag, 1991; Dawson, Raskoff & Jacobs, 1998; Douglas & Rogers, 1998; McKenzie, 2019). For metabolo , rapid freezing in liquid nitrogen (which inhibits metabolic processes) and storage at –80˚C is optimal and generally considered best practice, especially if subsequent separation of host and symbiont is desired (Lohr et al., 2019) as storing samples in methanol immediately restricts analyses to holobiont metabolome due to the potential for salt contamination (Hillyer et al., 2017b). However, for epigenetic pecific sub-applications have different sample preservation requirements (see Table 2). All in all, the approaches available for preserving specimens for Omics work are limited by the requirements of the most stringent aspect of the molecules under study (see Supplementary Material for details).

Processing: The amount of material necessary for each downstream Omics procedure varies significantly. Procedures that use amplification steps (e.g., metabarcoding of the bacterial and archaeal 16S rRNA, Symbiodiniaceae ITS2, or eukaryotic 18S) will require little material (~1 cm^2). Genomics, metagenomics, transcriptomics, epigenetics, and metabolomics will require more starting material (e.g., > 1 cm^2) with typically higher quality standards. Single coral fragments are frequently analyzed with multiple different, complementary procedures (e.g., genomics, transcriptomics, and metabarcoding).

Archiving for Long Term Storage: Almost all macromolecules in specimens are more stable long-term when ultra-frozen and many of them can be stored at freezing temperatures indefinitely if in the appropriate fixative or buffer. However, some methods such as Assay for Transposase-Accessible Chromatin using sequencing (ATAC-Seq)-based epigenetics require unusual storage conditions, such as cryopreservation in liquid nitrogen.

Caveats: Delays or alterations in sampling, processing, and preserving any specimen for these Omics techniques may alter the accuracy and precision of the resulting analysis. However, many alternative preservation and storage methods (e.g., formaldehyde) for Omics work remain untested or are incompletely benchmarked, thus it is unknown how these methods may affect downstream analyses. Future Omics research could scrutinize how these under-studied pipelines may alter a specimen's true biological and chemical composition in the short-term and when stored for long periods of time.

Physiology Methods

Coral physiological measurements are a staple of coral bleaching research, with 51% of coral bleaching studies published since 1992 measuring at least one physiological trait

(McLachlan et al., 2020). Table 2 lists the 10 physiological measurements most commonly performed. Rapid freezing in liquid nitrogen followed by storage in an ultra-cold freezer was the optimal practice across all physiological methods that we evaluated, although direct freezing at −40°C to −80°C is also acceptable. In all cases, common practices in collection and archiving exist for many of these measurements.

Sample Collection: In contrast to several of the microbial analyses described above (see Omics section), sample sterility and aseptic conditions are not an absolute requirement for downstream physiological assays. As a general guide, a coral ramet height >1 cm or tissue area >1 cm^2 are minimal requirements. However, larger fragment sizes (i.e., several cm^2) are generally desirable to minimize edge effects associated with tissue heterogeneity and avoiding tissue damaged in the sampling process. Larger fragments also allow for multiple laboratory analyses and to facilitate better cross-comparisons among corals and studies because stress conditions may decrease the amount of a given variable (e.g., lipids), making it harder to measure in very small specimens. In addition, a small sample size (1cm^2) may provide enough material for one analysis (e.g., endosymbiont chlorophyll *a*), whereas considerably more material is required for other analyses (e.g., coral tissue-based isotopes that can require four times this amount). When combining these requirements with the added benefit of long-term archiving for later potential use, investigators will often want to double their ramet size when possible. Large fragments or ramets also have the benefit of providing a more representative sample and minimizing fragment edge effects associated with sampling, as well as any positional effects within the coral fragment itself (e.g., top vs side of a branch or coral-mound). If the sample is not immediately processed at the time of collection, immediate rapid freezing in liquid nitrogen or immediate freezing at −80°C followed by storage in liquid nitrogen or at −80°C are ideal. Unless the goal is cryopreservation, in many cases freezing at −20°C or colder is suitable for several physiological analyses.

Preservation for Short Term Storage (days-months): Storage at −40°C or colder for days to months is typically suitable for all physiological analyses, though some methods have additional requirements (Table 2). Storage at −20°C for up to several months is also acceptable for many, but not all, analyses. In general, storage in liquid preservatives or fixatives (e.g., methanol, formalin, etc.) is considered either not acceptable or the efficacy of such preservatives is unknown when considered for many types of physiological analyses. Freeze-drying was noted as a suitable method for storing samples for physiological assays and is beginning to be used in coral bleaching studies (Wall et al., 2021; Pupier et al., 2021; Baumann et al., 2021). Freeze-drying is especially conducive to isotopic analysis (Wall et al., 2020) and provides for easy storage and transport as freeze-dried samples can be stored at room temperature. Notably, these samples should be stored in the dark.

Processing: For most physiological analyses, the processed subsample or specimen will be completely consumed and thus not available for long-term archiving. For example, a coral subsample which is ground and burned in a muffle furnace for ash-free dry weight biomass quantification cannot subsequently be used for chlorophyll concentration analysis. Notable exceptions include lipid extracts, skeletal material prepared for elemental analyses, and cryopreserved samples, all of which can be archived long-term for additional downstream analyses. Nevertheless, given the desire for technical replication and repeatability, many investigators typically collect coral fragments large enough to have remaining samples that were not processed and may be placed into a long-term archive and potentially used for other downstream analyses (summarized in Figure 2).

Archiving for Long Term Storage: Future physiological analyses are possible with frozen or freeze-dried fragments, ground whole coral, and frozen tissue homogenates and isolated fractions (e.g., host and microbial fractions), dried skeletal material, and cryopreserved tissues. Except for analyses that rely on live samples (e.g., cryobiology of reproductive cells and nanofragments; see section above on cryopreservation), all non-skeletal physiological analyses are possible with material that has been stored long-term at −80℃ or freeze-dried and then stored between −80℃ and −40℃. However, the efficacy and accuracy of using material that has been in long-term storage between −20℃ to −40℃ is currently unknown. Due to protein degradation, denaturation, or other decomposition, physiological analyses are not possible on coral material that has been stored long-term at 4℃ or warmer. In most cases, long-term storage of more than a year is reasonable at temperatures of −80℃ or less, but it is unknown if some analyses could be reliably performed on material that was archived for more than 10 years (see above for cryopreservation where the efficacy of long-term storage is established, e.g., sperm storage >10 years). In this regard, more study is required to determine the maximum duration that samples can be archived for each storage method (i.e., liquid nitrogen, −80℃, and freeze-drying) and still be suitable for physiological analyses. An exception to this rule is with the coral skeleton (usually in the form of cores, cross-sections, ramets, or ground powder), which is best stored dry at room temperature or refrigerated at 4℃ indefinitely if no future analysis of the skeletal organic matrix is intended.

Caveats: For all but the skeletal isotopic and elemental analyses, the subsamples for each individual physiological analysis are drawn from a representative, homogeneous mixture of either ground coral or tissue blastate that is collected from a larger fragment in both height and surface area than what is needed to make the specific measurement. In addition, obtaining a larger sized fragment or subsample than what is strictly needed to conduct the analysis is recommended to obtain a representative sample, to minimizing edge effects potentially associated with the way the fragment was cut, and any positional effects within the coral fragment itself (e.g., top vs. side of branch or mini mound).

Microscopy & Imaging Methods

Microscopy & Imaging of coral specimens is essential to many aspects of coral-bleaching and restoration surveillance and experimentation, and 58% of bleaching studies published over the last 30 years utilized at least one Microscopy & Imaging technique (McLachlan et al., 2020). Imaging can be at the gross-colony or micro-corallite morphological level (Table 2), at the tissue or cellular level (often referred to as histology), or at the subcellular level using techniques like electron microscopy and Raman spectroscopy.

Sample Collection: Typically, coral samples collected from the field for Microscopy & Imaging should not be rapidly frozen, but instead placed in temporary storage, preferably in a cooler (with some fresh sea water), and out of direct sunlight. Microscopy & Imaging techniques differ from other methods in the requirement that samples must be as intact as possible (e.g., cells not lysed, corallites undamaged, or any fragment alteration). A variety of imaging techniques such as 3-D photogrammetry and CT-scanning for surface area must be conducted on intact colonies or fragments prior to any additional analyses on ground skeleton or blastates. Similarly, photographs for coral color (e.g., to assess bleaching by loss of pigment) ideally are collected while the animal is alive before any other alteration to the colony has taken place and is thus best conducted underwater alongside a white standard or color chart. The need for intact skeletons and tissue extends to a variety of other Microscopy & Imaging techniques at smaller

scales. Histology, XRF-scanning, Raman 2-D mapping, Nanoscale secondary ion mass spectrometry (NanoSIMS), and scanning electron microscopy (SEM) all require tissue or skeleton in their original shape or arrangement, on the scale of the analysis. For instance, using SEM to describe corallite morphology is possible on broken skeletal fragments, as long as those fragments are intact at the appropriate scale (e.g., an entire corallite, which may be <1 mm to 10s of mm depending on the coral species). There are, however, a few methods that can use ground skeleton or tissue (e.g., Raman spot measurements, geochemistry), which can be conducted on altered or broken samples as long as they are suitably preserved. Investigations concerning the localization of nucleic acids, proteins, or microbes require immediate fixation upon collection.

Preservation for Short Term Storage: The short-term preservation of samples for Microscopy & Imaging varies with the objectives of the research. For analyses requiring tissue fixation, the type of fixative used varies greatly between researchers and applications, and there is no single best method. There are several commercial preservative kits available (e.g., Bouin's, Z-fixed (buffered aqueous zinc formalin), Glutaraldehyde/Paraformaldehyde solution mix), and many preservative types that can be prepared (e.g., formalin, glutaraldehyde, formaldehyde, methanol, ethanol, salt buffer). The proportion of each fixative (e.g., paraformaldehyde and glutaraldehyde) and time of fixation can significantly affect the accessibility of epitopes for immunolocalization (for both light and electron microscopy) and may require extensive optimization for each target and tissue type. Over-fixation is of particular concern for small samples like gametes and larvae. Techniques targeting nucleic acids (e.g., Fluorescence *in situ* hybridization (FISH)) also require special handling and electron microscopy (EM) grade or molecular grade (RNAse/DNAse-free) reagents (Wada et al., 2016). The temperature and time of preservation varies with the preservative used and the type of sample collected (e.g., fragment, larvae, gamete).

By contrast, some preservation methods are simply unacceptable as they directly induce alterations that significantly affect the capability of researchers to adequately investigate the sample. Examples of this are histological and electron microscopy artefacts in coral tissue integrity and structure induced by freezing, changes in skeletal structure induced by chemical exposure, or the deterioration or alteration of nucleic acids when improperly cooled or stored in particular compounds or at incompatible temperatures.

While there are numerous benchmarked methods for different types of light- and electron-based microscopy, most samples can handle different initial preservation types, such as storage in glutaraldehyde ~2% or formalin ~2-5% at 4℃ without being altered by the technique. Similarly, it is highly likely that any chemicals that are added to coral skeletons will change their geochemistry, therefore coral skeletons are best stored dry at room temperature or refrigerated at 4℃ when conducting geochemical focused imaging.

For imaging or light-based methods of Symbiodiniaceae quantification (e.g., hemocytometry, coulter counter, flow cytometry), short term sample preservation (e.g., of a tissue blastate) is recommended at 4℃ without fixation for two reasons: 1) freeze/thaw cycles can lyse symbiont cells, and 2) fixation can alter cell counts. However, coral fragments are commonly stored frozen (−20℃ to −80℃) prior to tissue homogenization, and tissue homogenates can be stored frozen prior to analysis over the short term with the caveat that cell counts may be affected, and multiple freeze/thaw cycles are best avoided. Additionally, although cleaning skeletons (e.g., with hydrogen peroxide or sodium hypochlorite bleach) have potential to alter the ratios of certain isotopes (Grottoli et al., 2005; Holcomb et al., 2015), there is sparse information available on the effects of preservatives on coral skeletons. However, we suggest

that adding any form of chemical preservative prior to geochemical analyses should be done with caution because these chemicals could alter the composition of coral skeletons by adding contaminants or causing partial dissolution.

Archiving for Long Term Storage: Derivatives of Microscopy & Imaging methods of coral skeletons are usually solid and exist in the form of cores, thin sections, or powder, and are best stored dry at room temperature or refrigerated at 4℃. These can be kept indefinitely, although the true shelf life of each of these has not been thoroughly benchmarked for all downstream analyses. Coral tissues for histology-based methods are typically stored as fixed tissues embedded in paraffin blocks or sections mounted on microscopy slides, and can be stored at room temperature or 4℃. For immunolocalization and fluorescence in situ hybridization (FISH), these blocks can retain their quality indefinitely, whereas any generated thin sections from these blocks can deteriorate much more quickly (Wakai et al., 2014; Alamri, Nam & Blancato, 2017). Sections treated with dyes should be stored in the dark, whereas skeletons and tissue blocks are typically not light sensitive.

Derivatives of coral skeletons, in particular skeleton cores and thin sections, can be used for various downstream analyses, including skeletal imagining (e.g., using CT scanning, X-ray, and XRF) and skeleton geochemistry analysis (e.g., Raman spectroscopy, isotopic and elemental analysis). Tissue-containing derivatives can be used for similar downstream analysis, and other techniques. There are no derivatives from the symbiont count techniques (i.e., hemocytometry, flow cytometry, countess cell counter), as the small sub-sample volume is typically consumed by these methods, though any remaining original sample may be available for additional analyses depending on its storage method (i.e., tissue blastate, Symbiodiniaceae pellet). Digital imagery produced from many of these techniques (e.g., electron microscopy, CT scanning, X-ray, coral color analysis), may be used for different image analysis techniques downstream.

While most fixed tissue derivatives can be stored at 4℃ or room temperature for long periods of time, stained (i.e., dyed) samples should be stored in the dark, and there is little known about the long-term preservation of samples for imaging. Long-term storage of coral tissues or homogenates for symbiont quantification that have not been chemically fixed is possible at −20℃ to −80℃ for >1 year if subsequential cell counts are performed with a hemocytometer, but not recommended at −20℃ due to Symbiodiniaceae cell degradation. Coral skeletons should be stored in a dry location at room temperature, and properly curated with metadata on collection dates, locations, water depths, etc. (Reich et al., 2012), and ideally with unique accession numbers. The preservation of photographic imagery, particularly those that document reef conditions during the previous century (e.g., (Shinn, EA & Kuffner, IB, 2017), is also an important community goal.

Future Considerations for the Coral Bleaching and Restoration Community Members Regarding Collecting, Preserving, and Archiving of Coral Specimens

This work is intended to provide a consolidated resource regarding specimen collection, preservation, and storage for current and future coral bleaching and restoration researchers and managers. We identified methodological pipeline overlaps that can be leveraged to expand the utility of experiments and specimens, as well as provide opportunities for collaborations. We also found that many potential method amendments are either untested or have yet to be fully benchmarked. Thus, we recommend that researchers and funding agencies work together to explore additional methods. The ‘unknowns’ in our summary (Table 2) will hopefully encourage the community at large to publish methodology reports that demonstrate both positive and

negative results in their method development. Often only positive results are published, limiting our view of what has been attempted previously. At the same time, we recognize that there is no single method that can be used for all downstream analyses; specimens from single corals can be collected in various ways and still expand future research possibilities. We recommend that, when possible, researchers and managers collect original samples in such a way that will optimize as many downstream analyses as possible, such as other target methods mentioned here, regardless of the focus of each experiment. Clearly, this requires more planning, more materials, and the means to store and distribute samples. Archiving samples long-term requires more storage capacity, and thus we recommend that funding agencies and research groups invest research and infrastructure dollars into the development, design, generation, and maintenance of long-term storage banks and freezers that can house specimens for collaborations and future investigations using new methods that might provide greater insight into the causes, mechanisms, and consequences of coral bleaching as well as enhancing and potentially increasing the success and impact of restoration science. Such archives/storehouses would also require an accompanying publicly available database of each specimen and their metadata so that researchers would be able to identify the most suitable samples for additional s

Glossary

Airbrushing: The use of pressurized and focused air, sometimes accompanied with a liquid to remove the surface tissue of corals from the skeleton.

Archiving: Temperature and/or chemical fixative or preservation techniques for samples post-processing for potential future use.

Aseptic techniques: Laboratory practices, procedures, and methods used to keep equipment and samples free from contamination from living microorganisms and nucleic acids such as DNA or RNA.

Blastate: semi-liquid mixture of fine coral skeleton particles, tissue, and mucus, usually in combination with seawater or a chemical stabilizer/preservative.

Blue ice: Regular ice (ice cubes, ice packs, etc.). Storage temperature at or near 0℃.

Computed tomography (CT) scanning: a technique where skeleton is exposed to X-rays from multiple angles, and the resulting 2-dimensional X-ray images are processed to produce a 3-dimensional image of skeletal density.

Cryopreservation: a process where organelles, cells, tissues, extracellular matrix, organs, or any other biological constructs susceptible to damage caused by unregulated chemical kinetics are preserved by cooling to very low temperatures.

Destructive: causing irreparable damage, rendering any sample unusable for further analyses.

Downstream analysis: The eventual laboratory analysis of the variable(s) of interest (e.g., chlorophyll concentration, lipid concentration, gene expression).

Epigenetics: the assessment of the modifications to the genome outside of the nucleic acid sequence. Often used to understand non-genetic mechanisms of acclimatization and/or plasticity.

Fixative: a chemical substance used to preserve and/or stabilize some aspect (e.g., protein and/or cellular structure) of a specimen, such as formaldehyde or paraformaldehyde.

Genet: a genetically unique coral colony or a collection of colonies (ramets) that can trace their ancestry back to the same sexual reproductive event (i.e., they stem from the same settler and, hence, share the same genome) (definition from Baums et al. 2019).

Genomics: Analysis of partial or whole genomes including assessments of nucleotide sequence, genetic organization, and putative gene and gene family function.

Long-term storage: Temperature and/or chemical fixative or preservation techniques for samples >24 hours after initial collection.

Lyophilization (freeze-drying): A shelf stable method of preservation. Lyophilization or freeze-drying removes water from a sample while the sample is under vacuum. As such, ice can be changed directly from solid to vapor without passing through a liquid phase. After dehydration, samples are shelf stable and can be stored in the lab away from sunlight.

Metabolomics: the assessment of metabolites found in a specimen.

Metagenomics (or metatranscriptomics): methods aimed at analyzing partial or whole genomes from all organisms within a mixed community, including assessment of the composition and potential function of DNA (or RNA) found in a specimen. Often used to look at genetic potential, microbial community composition and function, and/or genetic background of a specimen.

Micro-XRF scanning: Micro-X-ray fluorescence scanning is a non-destructive analysis for major and minor elements at down to 5 µm resolution by scanning the surface of skeletal slabs. XRF is based on the excitation of material with X-ray radiation and detection of the emitted fluorescence radiation spectrum whereby each element reacts at characteristic energy lines.

Mycosporin like amino-acids (MAA): Metabolites induced by high-light and high wavelength light in diverse marine organisms in order to either absorb damaging UV rays (e.g., act as sunscreens) or offset their effects (e.g., act as antioxidants).

Nanoscale secondary ion mass spectrometry (NanoSIMS): A method that uses primary ions beam to interact with sample surfaces resulting in the generation of secondary ions which can be analyzed for their specific mass.

Parent colony: Coral colony growing on the reef from which specimens were removed.

Preservative: A chemical solution in which specimens are placed to avoid decay, such as ethanol >70% or a nucleic acid stabilizing salt buffer.

Proteomics: The high throughput analysis of peptides, proteins, and protein modifications from a given sample typically using either nuclear magnetic resonance or mass spectrometer techniques.

Provenance: a record of sample origin, collection, sampling, processing, and storage methods over lifetime usage of samples and their associated specimen.

Raman Spectroscopy: liquid, gas, or solid samples are exposed to a laser beam, and the changes in wavelength of the scattered light produces a Raman spectrum, which provides information about sample mineralogy and chemical composition.

Rapid Freezing ("flash" freezing): Immediate sample preservation via freezing. In the field this may include placing samples on dry ice or liquid nitrogen. In the lab, this may include placing samples on dry ice, liquid nitrogen, or in an ultra-cold freezer.

Ramet: Replicate fragments or colonies originating from the same genet.

Receptacle: A piece of laboratory equipment that receives and contains something (e.g., test tube, vial, bottle). Synonyms: container, holder, vessel.

Sample [noun]: 1) A representative part or single item from a larger whole or group (e.g., fragment from a coral colony or a whole colony from a reef); 2) a finite part of a statistical population whose properties are studied to gain information about the whole.

Sample [verb]: to take a sample of or from.

Scanning electron microscopy (SEM): High resolution microscopy using focused electron beans rasterized across a surface and visualization using the secondarily emitted electrons that result from the beam interacting with atoms on the surface.

Short-term storage: Temperature and/or chemical fixative or preservation techniques for samples <24 hours after initial collection.

Skeletal elemental analysis (non-isotopic): Measurement of element ratios in coral skeletons, typically relative to Ca. Examples include Mg/Ca, Sr/Ca, and U/Ca.

Skeletal stable isotopes: Measurement of stable carbon, oxygen, and boron isotope ratios in coral skeleton.

Specimen processing: Laboratory manipulation to prepare specimens for desired downstream analysis (e.g., airbrushing, freeze-drying, tissue homogenizing).

Specimen: 1) an individual, item, or part considered typical of a group, class, or whole; 2) a portion or quantity of material for use in testing, examination, or study.

Specimen collection: The removal of coral specimens from the reef or from experimental tanks.

Specimen preservation: The method by which coral specimens are sacrificed, preserved, and stored immediately following collection (e.g., rapid-freeze with liquid nitrogen and stored at −80°C).

Sterile equipment: Tools (e.g., gloves, forceps, cotton swabs), receptacles (e.g., test tubes, vials, bottles), and other equipment (e.g., fume hood, laboratory work bench) which have been decontaminated, and thus, are free from living microorganisms and nucleic acids such as DNA or RNA.

Tissue stable isotopes: Measurement of stable carbon and nitrogen isotope ratios in coral tissues.

Transcriptomics: the assessment of the composition and often function of mRNAs and sometimes small regulatory RNAs found in a specimen. Often used to look at physiological changes/responses to particular focal conditions and /or the phylogenetic placement of a specimen.

Transmission electron microscopy (TEM): High resolution microscopy using beams of electrons transmitted through a specimen and then captured for visualization on some device or material. For electron transmission samples are typically required to be ultra-thin sectioned at thicknesses of less than 100nm.

Ultra-cold freezing: Storage of samples at ultra-cold temperatures (−40ºC to −86°C)

Water-piking: The use of a Water-Pik (oral irrigator device) to remove the tissue from a coral skeleton using a jet of high-pressure water.

XRF scanning: X-ray fluorescence scanning is a non-destructive analysis for major and minor elements at cm to mm d to 200 micron-scale resolution by scanning the surface of split skeletal cores or of skel labs. XRF is based on the excitation of material with X-ray radiation and detection of the emitted fluorescence radiation spectrum whereby each element reacts at characteristic energy lines.

Figure 1. Flow chart of conceptual design for workshop on methods of collecting preserving and archiving coral bleaching specimen.

Figure 2. Pictogram outlining some of the most commonly used different methodological pipelines starting with coral specimen handling and collection, incorporating preservation techniques, and ending with downstream analysis and the number of possible techniques. The use of aseptic techniques in sample collection, preservation, storage, and archiving increases the number of possible downstream analyses, rela o specimens handled using non-aseptic tools and receptacles, particularly in the Omics category. Similarly, freezing of samples at any point in

the pipeline may limit the number of Microscopy & Imaging analyses that can be applied, though higher temperature storage points (>4ºC) and sample state changes limit the utility of specimens for many Omics and Physiological analyses as detailed in Table 2.

Table 1. Measures of coral-specimen size/growth are essential metadata when normalized to other variables (e.g., symbiont density, calcification, etc.). Measures of size/growth over time can also be experimental response variables if measured through time (e.g., before and after a treatment is applied, before and after a natural bleaching event etc.). This table summarizes possible measures of size/growth and their utility for normalization. Methods which are *italicized* are categorized as invasive, impacting the coral colony through substantial contact or destruction.

Table 2. Table of field-specific (omics, physiology, and microscopy) collecting, preserving, and archiving pipelines.

Literature Cited

Alamri A, Nam JY, Blancato JK. 2017. Fluorescence In Situ Hybridization of Cells, Chromosomes, and Formalin-Fixed Paraffin-Embedded Tissues. In: Espina V ed. *Molecular Profiling: Methods and Protocols*. Methods in Molecular Biology. New York, NY: Springer, 265–279. DOI: 10.1007/978-1-4939-6990-6_17.

Álvarez-Noriega M, Baird AH, Bridge TCL, Dornelas M, Fontoura L, Pizarro O, Precoda K, Torres-Pulliza D, Woods RM, Zawada K, Madin JS. 2018. Contrasting patterns of changes in abundance following a bleaching event between juvenile and adult scleractinian corals. *Coral Reefs* 37:527–532. DOI: 10.1007/s00338-018-1677-y.

Atkinson MJ, Carlson B, Crow GL. 1995. Coral growth in high-nutrient, low-pH seawater: a case study of corals cultured at the Waikiki Aquarium, Honolulu, Hawaii. *Coral Reefs* 14:215–223. DOI: 10.1007/BF00334344.

Baena-Del Valle JA, Zheng Q, Hicks JL, Fedor H, Trock BJ, Morrissey C, Corey E, Cornish TC, Sfanos KS, De Marzo AM. 2017. Rapid Loss of RNA Detection by In Situ Hybridization in Stored Tissue Blocks and Preservation by Cold Storage of Unstained Slides. *American Journal of Clinical Pathology* 148:398–415. DOI: 10.1093/ajcp/aqx094.

Bak RPM. 1973. Coral weight increment in situ. A new method to determine coral growth. *Marine Biology* 20:45–49. DOI: 10.1007/BF00387673.

Barnes D, Beck RE, Schultz JS. 1970. Coral Skeletons : An Explanation of Their Growth and Structure Author (s): David J . Barnes Published by : American Association for the Advancement of Science Stable URL : http://www.jstor.org/stable/1730509 REFERENCES Linked references are available on. 170:1305–1308.

Barnes DJ, Crossland CJ. 1980. Diurnal and seasonal variations in the growth of a staghorn coral measured by time-lapse photography. *Limnology and Oceanography* 25:1113–1117. DOI: 10.4319/lo.1980.25.6.1113.

Baumann JH, Bove CB, Carne L, Gutierrez I, Castillo KD. 2021. Two offshore coral species show greater acclimatization capacity to environmental variation than nearshore counterparts in southern Belize. *Coral Reefs* 40:1181–1194. DOI: 10.1007/s00338-021-02124-8.

Baums IB. 2008. A restoration genetics guide for coral reef conservation. *Molecular Ecology* 17:2796–2811. DOI: 10.1111/j.1365-294X.2008.03787.x.

Boström-Einarsson L, Babcock RC, Bayraktarov E, Ceccarelli D, Cook N, Ferse SCA, Hancock B, Harrison P, Hein M, Shaver E, Smith A, Suggett D, Stewart-Sinclair PJ, Vardi T, McLeod IM. 2020. Coral restoration – A systematic review of current methods, successes, failures and future directions. *PLOS ONE* 15:e0226631. DOI: 10.1371/journal.pone.0226631.

Brandt ME. 2009. The effect of species and colony size on the bleaching response of reef-building corals in the Florida Keys during the 2005 mass bleaching event. *Coral Reefs* 28:911–924. DOI: 10.1007/s00338-009-0548-y.

Buddemeier RW. 1974. Environmental controls over annual and lunar monthly cycles in hermatypic coral calcification. *Proc 2nd Int Coral Reef Symp.* 2:259–267.

Bythell J, Pan P, Lee J. 2001. Three-dimensional morphometric measurements of reef corals using underwater photogrammetry techniques. *Coral Reefs* 20:193–199. DOI: 10.1007/s003380100157.

921 Carpenter KE, Abrar M, Aeby G, Aronson RB, Banks S, Bruckner A, Chiriboga A, Cortés J,
922 Delbeek JC, DeVantier L, Edgar GJ, Edwards AJ, Fenner D, Guzmán HM, Hoeksema
923 BW, Hodgson G, Johan O, Licuanan WY, Livingstone SR, Lovell ER, Moore JA, Obura
924 DO, Ochavillo D, Polidoro BA, Precht WF, Quibilan MC, Reboton C, Richards ZT,
925 Rogers AD, Sanciangco J, Sheppard A, Sheppard C, Smith J, Stuart S, Turak E, Veron
926 JEN, Wallace C, Weil E, Wood E. 2008. One-Third of Reef-Building Corals Face
927 Elevated Extinction Risk from Climate Change and Local Impacts. *Science* 321:560–563.
928 DOI: 10.1126/science.1159196.
929 Chalker BE, Barnes DJ. 1990. Gamma densitometry for the measurement of skeletal density.
930 *Coral Reefs* 9:11–23. DOI: 10.1007/BF00686717.
931 Cocito S, Sgorbini S, Peirano A, Valle M. 2003. 3-D reconstruction of biological objects using
932 underwater video technique and image processing. *Journal of Experimental Marine*
933 *Biology and Ecology* 297:57–70. DOI: 10.1016/S0022-0981(03)00369-1.
934 Courtney LA, Fisher WS, Raimondo S, Oliver LM, Davis WP. 2007. Estimating 3-dimensional
935 colony surface area of field corals. *Journal of Experimental Marine Biology and Ecology*
936 351:234–242. DOI: 10.1016/j.jembe.2007.06.021.
937 Cziesielski MJ, Schmidt-Roach S, Aranda M. 2019. The past, present, and future of coral heat
938 stress studies. *Ecology and Evolution* 9:10055–10066. DOI: 10.1002/ece3.5576.
939 Dalton SJ, Carroll AG, Sampayo E, Roff G, Harrison PL, Entwistle K, Huang Z, Salih A,
940 Diamond SL. 2020. Successive marine heatwaves cause disproportionate coral bleaching
941 during a fast phase transition from El Niño to La Niña. *Science of The Total Environment*
942 715:136951. DOI: 10.1016/j.scitotenv.2020.136951.
943 Davies N, Deck J, Kansa EC, Kansa SW, Kunze J, Meyer C, Orrell T, Ramdeen S, Snyder R,
944 Vieglais D, Walls RL, Lehnert K. 2021. Internet of Samples (iSamples): Toward an
945 interdisciplinary cyberinfrastructure for material samples. *GigaScience* 10. DOI:
946 10.1093/gigascience/giab028.
947 Davis AP, Grondin CJ, Johnson RJ, Sciaky D, McMorran R, Wiegers J, Wiegers TC, Mattingly
948 CJ. 2019. The Comparative Toxicogenomics Database: update 2019. *Nucleic Acids*
949 *Research* 47:D948–D954. DOI: 10.1093/nar/gky868.
950 Dawson MN, Raskoff KA, Jacobs DK. 1998. Field preservation of marine invertebrate tissue for
951 DNA analyses. *Molecular marine biology and biotechnology* 7:145–152.
952 Deck J, Gaither MR, Ewing R, Bird CE, Davies N, Meyer C, Riginos C, Toonen RJ, Crandall
953 ED. 2017. The Genomic Observatories Metadatabase (GeOMe): A new repository for
954 field and sampling event metadata associated with genetic samples. *PLOS Biology*
955 15:e2002925. DOI: 10.1371/journal.pbio.2002925.
956 D'Olivo JP, McCulloch MT, Judd K. 2013. Long-term records of coral calcification across the
957 central Great Barrier Reef: Assessing the impacts of river runoff and climate change.
958 *Coral Reefs* 32:999–1012. DOI: 10.1007/s00338-013-1071-8.
959 Douglas MP, Rogers SO. 1998. DNA damage caused by common cytological fixatives. *Mutation*
960 *Research/Fundamental and Molecular Mechanisms of Mutagenesis* 401:77–88. DOI:
961 10.1016/S0027-5107(97)00314-X.
962 Edmunds PJ, Elahi R. 2007. The demographics of a 15-year decline in cover of the Caribbean
963 reef coral Montastraea annularis. *Ecological Monographs* 77:3–18. DOI: 10.1890/05-
964 1081.

965 Enochs IC, Manzello DP, Carlton R, Schopmeyer S, van Hooidonk R, Lirman D. 2014. Effects
966 of light and elevated pCO2 on the growth and photochemical efficiency of Acropora
967 cervicornis. *Coral Reefs* 33:477–485. DOI: 10.1007/s00338-014-1132-7.
968 Felis T, Pätzold J, Loya Y. 2003. Mean oxygen-isotope signatures in Porites spp. corals: Inter-
969 colony variability and correction for extension-rate effects. *Coral Reefs* 22:328–336.
970 DOI: 10.1007/s00338-003-0324-3.
971 Fisher R, O'Leary RA, Low-Choy S, Mengersen K, Knowlton N, Brainard RE, Caley MJ. 2015.
972 Species Richness on Coral Reefs and the Pursuit of Convergent Global Estimates.
973 *Current Biology* 25:500–505. DOI: 10.1016/j.cub.2014.12.022.
974 Gaither MR, Szabó Z, Crepeau MW, Bird CE, Toonen RJ. 2011. Preservation of corals in salt-
975 saturated DMSO buffer is superior to ethanol for PCR experiments. *Coral Reefs* 30:329–
976 333. DOI: 10.1007/s00338-010-0687-1.
977 Goreau TJ. 1959. The physiology of skeleton formation in corals. I. A method for measuring the
978 rate of calcium deposition by corals under different conditions. *Biological Bulletin*
979 116:59–75.
980 Grottoli AG, Rodrigues LJ, Matthews KA, Palardy JE, Gibb OT. 2005. Pre-treatment effects on
981 coral skeletal δ13C and δ18O. *Chemical Geology* 221:225–242. DOI:
982 10.1016/j.chemgeo.2005.05.004.
983 Grottoli AG, Toonen RJ, Woesik R van, Thurber RV, Warner ME, McLachlan RH, Price JT,
984 Bahr KD, Baums IB, Castillo KD, Coffroth MA, Cunning R, Dobson KL, Donahue MJ,
985 Hench JL, Iglesias-Prieto R, Kemp DW, Kenkel CD, Kline DI, Kuffner IB, Matthews JL,
986 Mayfield AB, Padilla-Gamiño JL, Palumbi S, Voolstra CR, Weis VM, Wu HC. 2021.
987 Increasing comparability among coral bleaching experiments. *Ecological Applications*
988 31:e02262. DOI: 10.1002/eap.2262.
989 Hagedorn M, Carter VL, Henley EM, van Oppen MJH, Hobbs R, Spindler RE. 2017. Producing
990 Coral Offspring with Cryopreserved Sperm: A Tool for Coral Reef Restoration. *Scientific*
991 *Reports* 7:14432. DOI: 10.1038/s41598-017-14644-x.
992 Hagedorn M, Carter V, Martorana K, Paresa MK, Acker J, Baums IB, Borneman E, Brittsan M,
993 Byers M, Henley M, Laterveer M, Leong J-A, McCarthy M, Meyers S, Nelson BD,
994 Petersen D, Tiersch T, Uribe RC, Woods E, Wildt D. 2012. Preserving and Using
995 Germplasm and Dissociated Embryonic Cells for Conserving Caribbean and Pacific
996 Coral. *PLOS ONE* 7:e33354. DOI: 10.1371/journal.pone.0033354.
997 Hagedorn M, Page CA, O'Neil KL, Flores DM, Tichy L, Conn T, Chamberland VF, Lager C,
998 Zuchowicz N, Lohr K, Blackburn H, Vardi T, Moore J, Moore T, Baums IB, Vermeij
999 MJA, Marhaver KL. 2021. Assisted gene flow using cryopreserved sperm in critically
1000 endangered coral. *Proceedings of the National Academy of Sciences* 118. DOI:
1001 10.1073/pnas.2110559118.
1002 Herler J, Dirnwöber M. 2011. A simple technique for measuring buoyant weight increment of
1003 entire, transplanted coral colonies in the field. *Journal of Experimental Marine Biology*
1004 *and Ecology* 407:250–255. DOI: 10.1016/j.jembe.2011.06.022.
1005 Heron SF, Maynard JA, van Hooidonk R, Eakin CM. 2016. Warming Trends and Bleaching
1006 Stress of the World's Coral Reefs 1985–2012. *Scientific Reports* 6:38402. DOI:
1007 10.1038/srep38402.
1008 Hillyer KE, Dias DA, Lutz A, Roessner U, Davy SK. 2017a. Mapping carbon fate during
1009 bleaching in a model cnidarian symbiosis: the application of 13C metabolomics. *New*
1010 *Phytologist* 214:1551–1562. DOI: 10.1111/nph.14515.

1011 Hillyer KE, Dias DA, Lutz A, Wilkinson SP, Roessner U, Davy SK. 2017b. Metabolite profiling
1012 of symbiont and host during thermal stress and bleaching in the coral Acropora aspera.
1013 *Coral Reefs* 36:105–118. DOI: 10.1007/s00338-016-1508-y.
1014 Hoegh-Guldberg O. 1988. A method for determining the surface area of corals. *Coral Reefs*
1015 7:113–116. DOI: 10.1007/BF00300970.
1016 Holcomb M, Cohen AL, McCorkle DC. 2013. An evaluation of staining techniques for marking
1017 daily growth in scleractinian corals. *Journal of Experimental Marine Biology and*
1018 *Ecology* 440:126–131. DOI: 10.1016/j.jembe.2012.12.003.
1019 Holcomb M, DeCarlo TM, Schoepf V, Dissard D, Tanaka K, McCulloch M. 2015. Cleaning and
1020 pre-treatment procedures for biogenic and synthetic calcium carbonate powders for
1021 determination of elemental and boron isotopic compositions. *Chemical Geology* 398:11–
1022 21. DOI: 10.1016/j.chemgeo.2015.01.019.
1023 Holmes G. 2008. Estimating three-dimensional surface areas on coral reefs. *Journal of*
1024 *Experimental Marine Biology and Ecology* 365:67–73. DOI:
1025 10.1016/j.jembe.2008.07.045.
1026 van Hooidonk R, Maynard J, Tamelander J, Gove J, Ahmadia G, Raymundo L, Williams G,
1027 Heron SF, Planes S. 2016. Local-scale projections of coral reef futures and implications
1028 of the Paris Agreement. *Scientific Reports* 6:39666. DOI: 10.1038/srep39666.
1029 Hopwood D. 1975. The reactions of glutaraldehyde with nucleic acids. *The Histochemical*
1030 *Journal* 7:267–276. DOI: 10.1007/BF01003595.
1031 House JE, Brambilla V, Bidaut LM, Christie AP, Pizarro O, Madin JS, Dornelas M. 2018.
1032 Moving to 3D: relationships between coral planar area, surface area and volume. *PeerJ*
1033 6:e4280. DOI: 10.7717/peerj.4280.
1034 Hughes TP, Anderson KD, Connolly SR, Heron SF, Kerry JT, Lough JM, Baird AH, Baum JK,
1035 Berumen ML, Bridge TC, Claar DC, Eakin CM, Gilmour JP, Graham NAJ, Harrison H,
1036 Hobbs J-PA, Hoey AS, Hoogenboom M, Lowe RJ, McCulloch MT, Pandolfi JM,
1037 Pratchett M, Schoepf V, Torda G, Wilson SK. 2018. Spatial and temporal patterns of
1038 mass bleaching of corals in the Anthropocene. *Science* 359:80–83. DOI:
1039 10.1126/science.aan8048.
1040 Hughes TP, Kerry JT, Álvarez-Noriega M, Álvarez-Romero JG, Anderson KD, Baird AH,
1041 Babcock RC, Beger M, Bellwood DR, Berkelmans R, Bridge TC, Butler IR, Byrne M,
1042 Cantin NE, Comeau S, Connolly SR, Cumming GS, Dalton SJ, Diaz-Pulido G, Eakin
1043 CM, Figueira WF, Gilmour JP, Harrison HB, Heron SF, Hoey AS, Hobbs J-PA,
1044 Hoogenboom MO, Kennedy EV, Kuo C, Lough JM, Lowe RJ, Liu G, McCulloch MT,
1045 Malcolm HA, McWilliam MJ, Pandolfi JM, Pears RJ, Pratchett MS, Schoepf V, Simpson
1046 T, Skirving WJ, Sommer B, Torda G, Wachenfeld DR, Willis BL, Wilson SK. 2017.
1047 Global warming and recurrent mass bleaching of corals. *Nature* 543:373–377. DOI:
1048 10.1038/nature21707.
1049 Hughes TP, Kerry JT, Baird AH, Connolly SR, Chase TJ, Dietzel A, Hill T, Hoey AS,
1050 Hoogenboom MO, Jacobson M, Kerswell A, Madin JS, Mieog A, Paley AS, Pratchett
1051 MS, Torda G, Woods RM. 2019. Global warming impairs stock–recruitment dynamics of
1052 corals. *Nature* 568:387–390. DOI: 10.1038/s41586-019-1081-y.
1053 Jokiel PL, Maragos JE, Franzisket L. 1978. Coral growth: buoyant weight technique. In: *Coral*
1054 *Reefs: Research Methods*.

Jones AM, Cantin NE, Berkelmans R, Sinclair B, Negri AP. 2008. A 3D modeling method to calculate the surface areas of coral branches. *Coral Reefs* 27:521–526. DOI: 10.1007/s00338-008-0354-y.

Kelly R, Albert M, de Ladurantaye M, Moore M, Dokun O, Bartlett JMS. 2019. RNA and DNA Integrity Remain Stable in Frozen Tissue After Long-Term Storage at Cryogenic Temperatures: A Report from the Ontario Tumour Bank. *Biopreservation and Biobanking* 17:282–287. DOI: 10.1089/bio.2018.0095.

Klinges G, Maher RL, Vega Thurber RL, Muller EM. 2020. Parasitic 'Candidatus Aquarickettsia rohweri' is a marker of disease susceptibility in Acropora cervicornis but is lost during thermal stress. *Environmental Microbiology* 22:5341–5355. DOI: 10.1111/1462-2920.15245.

Kuffner IB, Hickey TD, Morrison JM. 2013. Calcification rates of the massive coral Siderastrea siderea and crustose coralline algae along the Florida Keys (USA) outer-reef tract. *Coral Reefs* 32:987–997. DOI: 10.1007/s00338-013-1047-8.

Kuffner IB, Toth LT, Hudson JH, Goodwin WB, Stathakopoulos A, Bartlett LA, Whitcher EM. 2019. Improving estimates of coral reef construction and erosion with in situ measurements. *Limnology and Oceanography* 64:2283–2294. DOI: 10.1002/lno.11184.

Laforsch C, Christoph E, Glaser C, Naumann MS, Wild C, Niggl W. 2008. A precise and non-destructive method to calculate the surface area in living scleractinian corals using X-ray computed tomography and 3D modeling. *Coral Reefs* 27:811–820. DOI: 10.1007/s00338-008-0405-4.

Lavy A, Eyal G, Neal B, Keren R, Loya Y, Ilan M. 2015. A quick, easy and non-intrusive method for underwater volume and surface area evaluation of benthic organisms by 3D computer modelling. *Methods in Ecology and Evolution* 6:521–531. DOI: 10.1111/2041-210X.12331.

Levas S, Schoepf V, Warner ME, Aschaffenburg M, Baumann J, Grottoli AG. 2018. Long-term recovery of Caribbean corals from bleaching. *Journal of Experimental Marine Biology and Ecology* 506:124–134. DOI: 10.1016/j.jembe.2018.06.003.

Logan A, Anderson H. 1991. Skeletal extension growth rate assessment in corals, using CT scan imagery. *Bulletin of Marine Science* 49:847–850.

Lohr KE, Khattri RB, Guingab-Cagmat J, Camp EF, Merritt ME, Garrett TJ, Patterson JT. 2019. Metabolomic profiles differ among unique genotypes of a threatened Caribbean coral. *Scientific Reports* 9:6067. DOI: 10.1038/s41598-019-42434-0.

Lohr KE, Patterson JT. 2017. Intraspecific variation in phenotype among nursery-reared staghorn coral Acropora cervicornis (Lamarck, 1816). *Journal of Experimental Marine Biology and Ecology* 486:87–92. DOI: 10.1016/j.jembe.2016.10.005.

Lough JM, Barnes DJ. 2000. Environmental controls on growth of the massive coral Porites. *Journal of Experimental Marine Biology and Ecology* 245:225–243. DOI: 10.1016/S0022-0981(99)00168-9.

Madin JS, Baird AH, Dornelas M, Connolly SR. 2014. Mechanical vulnerability explains size-dependent mortality of reef corals. *Ecology Letters* 17:1008–1015. DOI: 10.1111/ele.12306.

Marsh JA. 1970. Primary productivity of reef-building calcareous red algae. *Ecology* 51:255–263. DOI: 10.1038/news050808-1.

McKenzie A. 2019. Glutaraldehyde: A review of its fixative effects on nucleic acids, proteins, lipids, and carbohydrates. DOI: 10.31219/osf.io/8zd4e.

McLachlan RH, Dobson KL, Schmeltzer ER, Thurber RV, Grottoli AG. 2021. A review of coral bleaching specimen collection, preservation, and laboratory processing methods. *PeerJ* 9:e11763. DOI: 10.7717/peerj.11763.

McLachlan RH, Price JT, Solomon SL, Grottoli AG. 2020. Thirty years of coral heat-stress experiments: a review of methods. *Coral Reefs* 39:885–902. DOI: 10.1007/s00338-020-01931-9.

Meyer JL, Schultz ET. 1985. Tissue condition and growth rate of corals associated with schooling fish. *Limnology and Oceanography* 30:157–166. DOI: 10.4319/lo.1985.30.1.0157.

Michael A. G, Zoe A. P, Christina A. K. 2013. Comparison of DNA preservation methods for environmental bacterial community samples. *FEMS Microbiology Ecology* 83:468–477. DOI: 10.1111/1574-6941.12008.

Miller J, Muller E, Rogers C, Waara R, Atkinson A, Whelan KRT, Patterson M, Witcher B. 2009. Coral disease following massive bleaching in 2005 causes 60% decline in coral cover on reefs in the US Virgin Islands. *Coral Reefs* 28:925–937. DOI: 10.1007/s00338-009-0531-7.

Miller MW, Weil E, Szmant AM. 2000. Coral recruitment and juvenile mortality as structuring factors for reef benthic communities in Biscayne National Park, USA. *Coral Reefs* 19:115–123. DOI: 10.1007/s003380000079.

Miyamoto Y, Ikeuchi M, Noguchi H, Hayashi S. 2018. Long-term Cryopreservation of Human and other Mammalian Cells at −80 °C for 8 Years. *Cell Medicine* 10:2155179017733148. DOI: 10.1177/2155179017733148.

Morikawa MK, Palumbi SR. 2019. Using naturally occurring climate resilient corals to construct bleaching-resistant nurseries. *Proceedings of the National Academy of Sciences* 116:10586–10591. DOI: 10.1073/pnas.1721415116.

National Academies of Sciences, Engineering, and Medicine. 2020. *Biological Collections: Ensuring Critical Research and Education for the 21st Century*. Washington, DC: The National Academies Press. DOI: 10.17226/25592.

National Science and Technology Council, Interagency Working Group on Scientific Collections. 2009. *Scientific collections: mission-critical infrastructure for federal science agencies: a report of the Interagency Working Group on Scientific Collections (IWGSC)*. Washington DC: Office of Science and Technology Policy.

Naumann MS, Niggl W, Laforsch C, Glaser C, Wild C. 2009. Coral surface area quantification-evaluation of established techniques by comparison with computer tomography. *Coral Reefs* 28:109–117. DOI: 10.1007/s00338-008-0459-3.

Neal BP, Lin TH, Winter RN, Treibitz T, Beijbom O, Kriegman D, Kline DI, Greg Mitchell B. 2015. Methods and measurement variance for field estimations of coral colony planar area using underwater photographs and semi-automated image segmentation. *Environmental Monitoring and Assessment* 187. DOI: 10.1007/s10661-015-4690-4.

Odum HTO& EP. 1995. Trophic Structure and Productivity of a Windward Coral Reef Community on Eniwetok Atoll Author (s): Howard T . Odum and Eugene P . Odum Published by : Ecological Society of America Stable URL : http://www.jstor.org/stable/1943285 . Your use of the JSTOR. *Ecological Monograph* 25:291–320.

Ortega-Pinazo J, Díaz T, Martínez B, Jiménez A, Pinto-Medel MJ, Ferro P. 2019. Quality assessment on the long-term cryopreservation and nucleic acids extraction processes

implemented in the andalusian public biobank. *Cell and Tissue Banking* 20:255–265. DOI: 10.1007/s10561-019-09764-9.

Pandolfi JM, Bradbury RH, Sala E, Hughes TP, Bjorndal KA, Cooke RG, McArdle D, McClenachan L, Newman MJH, Paredes G, Warner RR, Jackson JBC. 2003. Global Trajectories of the Long-Term Decline of Coral Reef Ecosystems. *Science* 301:955–958. DOI: 10.1126/science.1085706.

Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen OH, Rawle F, Reynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T, Würbel H. 2020. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research*. *Journal of Cerebral Blood Flow & Metabolism* 40:1769–1777. DOI: 10.1177/0271678X20943823.

Plaisance L, Caley MJ, Brainard RE, Knowlton N. 2011. The Diversity of Coral Reefs: What Are We Missing? *PLOS ONE* 6:e25026. DOI: 10.1371/journal.pone.0025026.

Pupier CA, Grover R, Fine M, Rottier C, van de Water JAJM, Ferrier-Pagès C. 2021. Dissolved Nitrogen Acquisition in the Symbioses of Soft and Hard Corals With Symbiodiniaceae: A Key to Understanding Their Different Nutritional Strategies? *Frontiers in Microbiology* 12:657759. DOI: 10.3389/fmicb.2021.657759.

Rahav O, Ben-Zion M, Achituv Y, Dubinsky Z. 1991. A photographic, computerized method for in situ growth measurements in reef-building cnidarians. *Coral Reefs* 9:204. DOI: 10.1007/BF00290422.

Raymundo LJ, Burdick D, Hoot WC, Miller RM, Brown V, Reynolds T, Gault J, Idechong J, Fifer J, Williams A. 2019. Successive bleaching events cause mass coral mortality in Guam, Micronesia. *Coral Reefs* 38:677–700. DOI: 10.1007/s00338-019-01836-2.

Reich, Streubert, Matt, Dwyer, Brendan, Godbout, Meg, Muslic, Adis, Umberger, Dan. 2012. St. Petersburg Coastal and Marine Science Center's Core Archive Portal, U.S. Geological Survey Data Series 626.

Riginos C, Crandall ED, Liggins L, Gaither MR, Ewing RB, Meyer C, Andrews KR, Euclide PT, Titus BM, Therkildsen NO, Salces-Castellano A, Stewart LC, Toonen RJ, Deck J. 2020. Building a global genomics observatory: Using GEOME (the Genomic Observatories Metadatabase) to expedite and improve deposition and retrieval of genetic data and metadata for biodiversity research. *Molecular Ecology Resources* 20:1458–1469. DOI: 10.1111/1755-0998.13269.

Seutin G, White BN, Boag PT. 1991. Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology* 69:82–90. DOI: 10.1139/z91-013.

Shinn EA. 1966. Coral growth-rate, an environmental indicator. *Journal of Paleontology* 40. DOI: 10.2110/palo.

Shinn, EA, Kuffner, IB. 2017.Florida Keys Corals: A Photographic Record of Changes from 1959 to 2015. *Available at https://cmgds.marine.usgs.gov/catalog/spcmsc/FL_corals-photo-record_metadata.faq.html* (accessed July 21, 2021).

Sibbett B, Rieseberg LH, Narum S. 2020. The Genomic Observatories Metadatabase. *Molecular ecology resources* 20:1453–1454. DOI: 10.1111/1755-0998.13283.

Smith S V. 1973. Carbon Dioxide Dynamics: a Record of Organic Carbon Production, Respiration, and Calcification in the Eniwetok Reef Flat Community. *Limnology and Oceanography* 18:106–120. DOI: 10.4319/lo.1973.18.1.0106.

Smithsonian Institution. 2006. *Smithsonian Directive (SD) 600: Collection Management ; Implementation Manual*. Smithsonian Institution.

Spencer Davies P. 1989. Short-term growth measurements of corals using an accurate buoyant weighing technique. *Marine Biology* 101:389–395. DOI: 10.1007/BF00428135.

Stimson JS. 1985. The Effect of Shading by the Table Coral Acropora Hyacinthus on Understory Corals. *Ecology* 66:40–53.

Stimson JS, Kinzie RA. 1991. The temporal pattern and rate of release of zooxanthellae from the reef coral Pocillopora damicornis (Linnaeus) under nitrogen-enrichment and control conditions. *Journal of Experimental Marine Biology and Ecology* 153:63–74. DOI: 10.1016/S0022-0981(05)80006-1.

Storz D, Gischler E. 2011. Coral extension rates in the NW Indian Ocean I: Reconstruction of 20th century SST variability and monsoon current strength. *Geo-Marine Letters* 31:141–154. DOI: 10.1007/s00367-010-0221-z.

Sully S, Burkepile DE, Donovan MK, Hodgson G, van Woesik R. 2019. A global analysis of coral bleaching over the past two decades. *Nature Communications* 10:1264. DOI: 10.1038/s41467-019-09238-2.

Tambutté E, Allemand D, Bourge I, Gattuso JP, Jaubert J. 1995. An improved 45Ca protocol for investigating physiological mechanisms in coral calcification. *Marine Biology* 122:453–459. DOI: 10.1007/BF00350879.

Tanzil JTI, Brown BE, Dunne RP, Lee JN, Kaandorp JA, Todd PA. 2013. Regional decline in growth rates of massive Porites corals in Southeast Asia. *Global Change Biology* 19:3011–3023. DOI: 10.1111/gcb.12279.

Thomas L, Palumbi SR. 2017. The genomics of recovery from coral bleaching. *Proceedings of the Royal Society B: Biological Sciences* 284:20171790. DOI: 10.1098/rspb.2017.1790.

Toczydlowski RH, Liggins L, Gaither MR, Anderson TJ, Barton RL, Berg JT, Beskid SG, Davis B, Delgado A, Farrell E, Ghoojaei M, Himmelsbach N, Holmes AE, Queeno SR, Trinh T, Weyand CA, Bradburd GS, Riginos C, Toonen RJ, Crandall ED. 2021. Poor data stewardship will hinder global genetic diversity surveillance. *Proceedings of the National Academy of Sciences* 118. DOI: 10.1073/pnas.2107934118.

Vago R, Gill E, Collingwood JC. 1997. Laser measurements of coral growth. *Nature* 386:30–31.

Vivian DN, Yee SH, Courtney LA, Fisher WS. 2019. Estimating 3-dimensional surface areas of small scleractinian corals. *Caribbean journal of science* 49:192–200. DOI: 10.18475/cjos.v49i2.a8.

Voolstra CR, Suggett DJ, Peixoto RS, Parkinson JE, Quigley KM, Silveira CB, Sweet M, Muller EM, Barshis DJ, Bourne DG, Aranda M. 2021. Extending the natural adaptive capacity of coral holobionts. *Nature Reviews Earth & Environment* 2:747–762. DOI: 10.1038/s43017-021-00214-3.

Wada N, Pollock FJ, Willis BL, Ainsworth T, Mano N, Bourne DG. 2016. In situ visualization of bacterial populations in coral tissues: pitfalls and solutions. *PeerJ* 4:e2424. DOI: 10.7717/peerj.2424.

Wakai S, Shibuki Y, Yokozawa K, Nakamura S, Adegawa Y, Yoshida A, Tsuta K, Furuta K. 2014. Recycling and Long-Term Storage of Fluorescence In Situ Hybridization Slides. *American Journal of Clinical Pathology* 141:374–380. DOI: 10.1309/AJCPYX1UTI7LDAUY.

1236 Wall CB, Kaluhiokalani M, Popp BN, Donahue MJ, Gates RD. 2020. Divergent symbiont
1237 communities determine the physiology and nutrition of a reef coral across a light-
1238 availability gradient. *The ISME Journal* 14:945–958. DOI: 10.1038/s41396-019-0570-1.
1239 Wall CB, Wallsgrove NJ, Gates RD, Popp BN. 2021. Amino acid δ13C and δ15N analyses
1240 reveal distinct species-specific patterns of trophic plasticity in a marine symbiosis.
1241 *Limnology and Oceanography* 66:2033–2050. DOI: 10.1002/lno.11742.
1242 Wilkinson MD, Dumontier M, Aalbersberg IjJ, Appleton G, Axton M, Baak A, Blomberg N,
1243 Boiten J-W, da Silva Santos LB, Bourne PE, Bouwman J, Brookes AJ, Clark T, Crosas
1244 M, Dillo I, Dumon O, Edmunds S, Evelo CT, Finkers R, Gonzalez-Beltran A, Gray AJG,
1245 Groth P, Goble C, Grethe JS, Heringa J, 't Hoen PAC, Hooft R, Kuhn T, Kok R, Kok J,
1246 Lusher SJ, Martone ME, Mons A, Packer AL, Persson B, Rocca-Serra P, Roos M, van
1247 Schaik R, Sansone S-A, Schultes E, Sengstag T, Slater T, Strawn G, Swertz MA,
1248 Thompson M, van der Lei J, van Mulligen E, Velterop J, Waagmeester A, Wittenburg P,
1249 Wolstencroft K, Zhao J, Mons B. 2016. The FAIR Guiding Principles for scientific data
1250 management and stewardship. *Scientific Data* 3:160018. DOI: 10.1038/sdata.2016.18.
1251 van Woesik R, Banister RB, Bartels E, Gilliam DS, Goergen EA, Lustic C, Maxwell K, Moura
1252 A, Muller EM, Schopmeyer S, Winters RS, Lirman D. 2021. Differential survival of
1253 nursery-reared Acropora cervicornis outplants along the Florida reef tract. *Restoration*
1254 *Ecology* 29:e13302. DOI: 10.1111/rec.13302.
1255 Zawada KJA, Dornelas M, Madin JS. 2019. Quantifying coral morphology. *Coral Reefs*
1256 38:1281–1292. DOI: 10.1007/s00338-019-01842-4.
1257 Zerbino DR, Achuthan P, Akanni W, Amode MR, Barrell D, Bhai J, Billis K, Cummins C, Gall
1258 A, Girón CG, Gil L, Gordon L, Haggerty L, Haskell E, Hourlier T, Izuogu OG, Janacek
1259 SH, Juettemann T, To JK, Laird MR, Lavidas I, Liu Z, Loveland JE, Maurel T, McLaren
1260 W, Moore B, Mudge J, Murphy DN, Newman V, Nuhn M, Ogeh D, Ong CK, Parker A,
1261 Patricio M, Riat HS, Schuilenburg H, Sheppard D, Sparrow H, Taylor K, Thormann A,
1262 Vullo A, Walts B, Zadissa A, Frankish A, Hunt SE, Kostadima M, Langridge N, Martin
1263 FJ, Muffato M, Perry E, Ruffier M, Staines DM, Trevanion SJ, Aken BL, Cunningham F,
1264 Yates A, Flicek P. 2018. Ensembl 2018. *Nucleic Acids Research* 46:D754–D761. DOI:
1265 10.1093/nar/gkx1098.
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Table 1(on next page)

Table 1. Measures of coral specimen size/growth are essential metadata

Table 1. Measures of coral specimen size/growth are essential metadata when normalized to other variables (e.g., symbiont density, calcification, etc.). Measures of size/growth over time can also be experimental response variables if measured through time (e.g., before and after a treatment is applied, before and after a natural bleaching event etc.). This table summarizes possible measures of size/growth and their utility for normalization.Methods which are *italicized* are categorized as invasive, impacting the coral colony through substantial contact or destruction.

Measure of size/growth	Example unit	For Normalization	Economical method	Resource-intensive method
Length/height/width/ diameter	cm	Not ideal	Direct measurement with tape or calipers	NA
Contoured surface area	cm^2	Highly desirable	*Wax dipped (Stimson & Kinzie, 1991)*, *foil wrapped (Marsh, 1970)*, *latex dipped (Meyer & Schultz, 1985)*, *geometric shape fitting* (Odum, 1995; Jones et al., 2008), *spectrophotometry using dye* (Hoegh-Guldberg, 1988),	Photogrammetry (Bythell, Pan & Lee, 2001; Courtney et al., 2007; Lavy et al., 2015), X-ray computed tomography (Laforsch et al., 2008; Naumann et al., 2009), handheld laser scanning (Holmes, 2008), 3D laser scanning (Enochs et al., 2014), stereo video (Cocito et al., 2003)
Planar area footprint	cm^2	Highly desirable	Direct measurement with tape or calipers (Kuffner, Hickey & Morrison, 2013)	From photographs (Rahav et al., 1991; Edmunds & Elahi, 2007; Madin et al., 2014; Neal et al., 2015; Kuffner et al., 2019)
Volume	cm^3	Not ideal	Calipers or tape	Photogrammetry (Lavy et al., 2015)
Linear extension rate	cm yr^{-1}	Highly desirable	*Tagging* (Shinn, 1966), *staining with alizarin/ alizarin complexone /calcein/oxytetracycline* (Barnes, Beck & Schultz, 1970; Holcomb, Cohen & McCorkle, 2013), time lapse photography (Barnes & Crossland, 1980), direct measurement with tape (Miller, Weil & Szmant, 2000) or calipers (Stimson, 1985)	*X-radiography* (Lough & Barnes, 2000), *luminescent lines* (D'Olivo, McCulloch & Judd, 2013; Tanzil et al., 2013), *stable isotope profiling* (Felis, Pätzold & Loya, 2003; Storz & Gischler, 2011), *in situ* laser measurements (Vago, Gill & Collingwood, 1997),
Bulk density	g cm^{-3}	Not commonly used	Wet weight of skeleton divided by height (Atkinson, Carlson & Crow, 1995)	*X-ray densitometry* (Buddemeier, 1974), *gamma densitometry* (Chalker & Barnes, 1990), *Computerized tomography densitometry* (Logan & Anderson, 1991)
Calcification rate *NB: can be calculated as the product of extension and density*	g $CaCO_3$ cm^{-2} y^{-1}	Highly desirable	*Buoyant weight* (Jokiel, Maragos & Franzisket, 1978; Spencer Davies, 1989), *in situ* buoyant weight (Bak, 1973; Herler & Dirnwöber, 2011)	*Radioisotope incorporation* (Goreau, 1959; Tambutté et al., 1995), alkalinity anomaly (Smith, 1973)

Table 1. Measures of coral specimen size/growth are essential metadata when normalized to other variables (e.g., symbiont density, calcification, etc.). Measures of size/growth over time can also be experimental response variables if measured through time (e.g., before and after a treatment is applied, before and after a natural bleaching event

etc.). This table summarizes possible measures of size/growth and their utility for normalization. Methods which are *italicized* are categorized as invasive, impacting the coral colony through substantial contact or destruction.

Figure 1

Flow chart of conceptual design

Flow chart of conceptual design for workshop on methods of collecting preserving and archiving coral bleaching specimen.

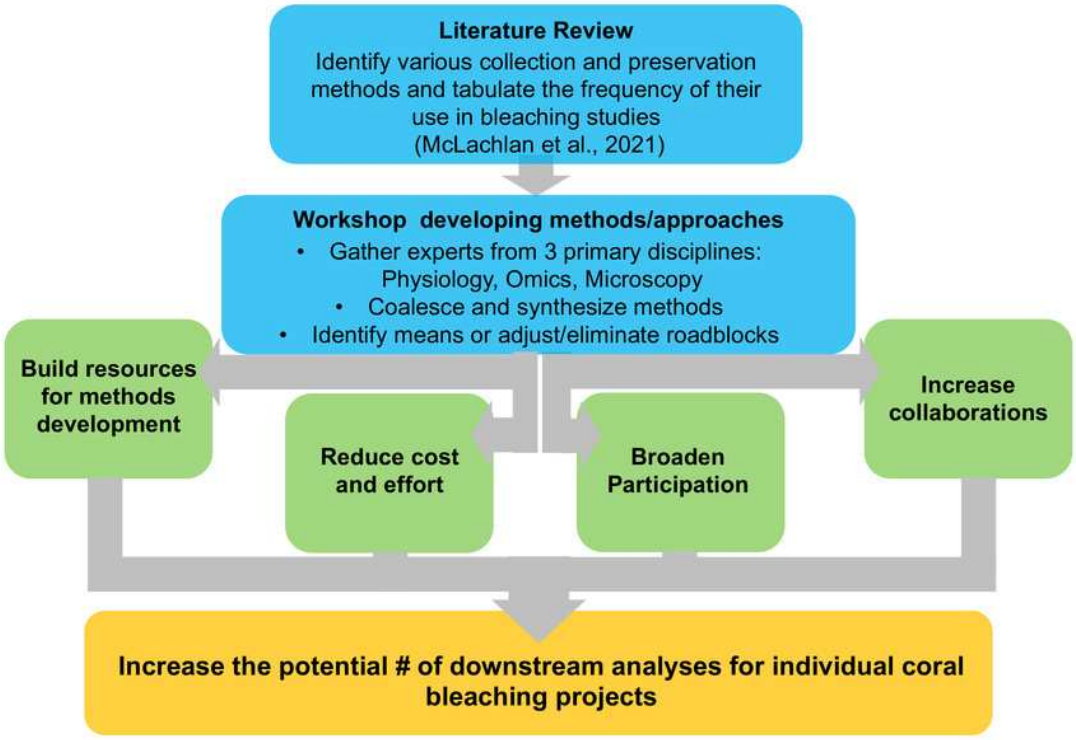


Figure 2

Figure 2. Pictogram outlining some of the most commonly used different methodological pipelines

Pictogram outlining some of the most commonly used different methodological pipelines starting with coral specimen handling and collection, incorporating preservation techniques, and ending with downstream analysis and the number of possible techniques. The use of aseptic techniques in sample collection, preservation, storage, and archiving increases the number of possible downstream analyses, relative to specimens handled using non-aseptic tools and receptacles, particularly in the Omics category. Similarly, freezing of samples at any point in the pipeline may limit the number of Microscopy & Imaging analyses that can be applied, though higher temperature storage points (>4ºC) and sample state changes limit the utility of specimens for many Omics and Physiological analyses as detailed in Table 1.

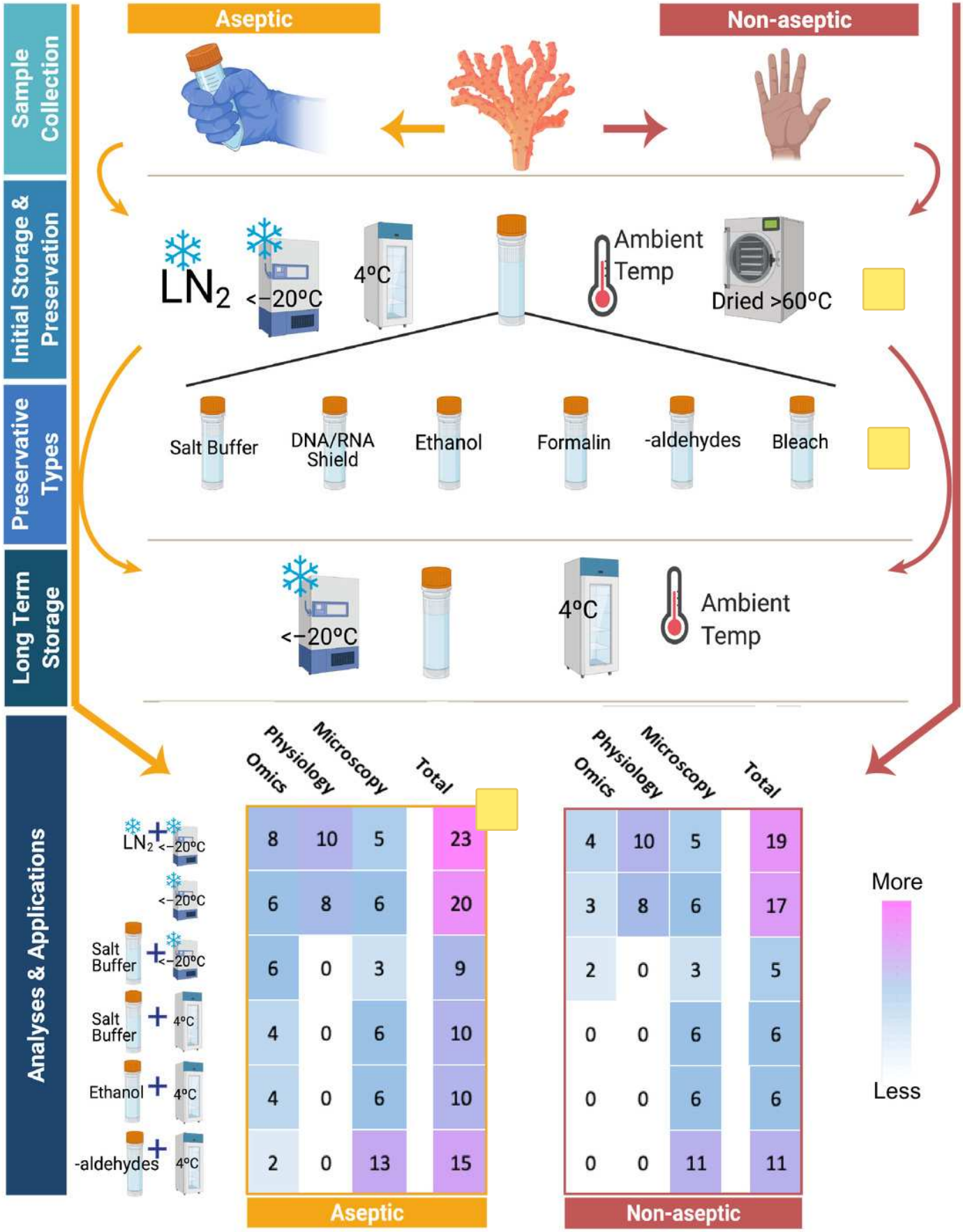
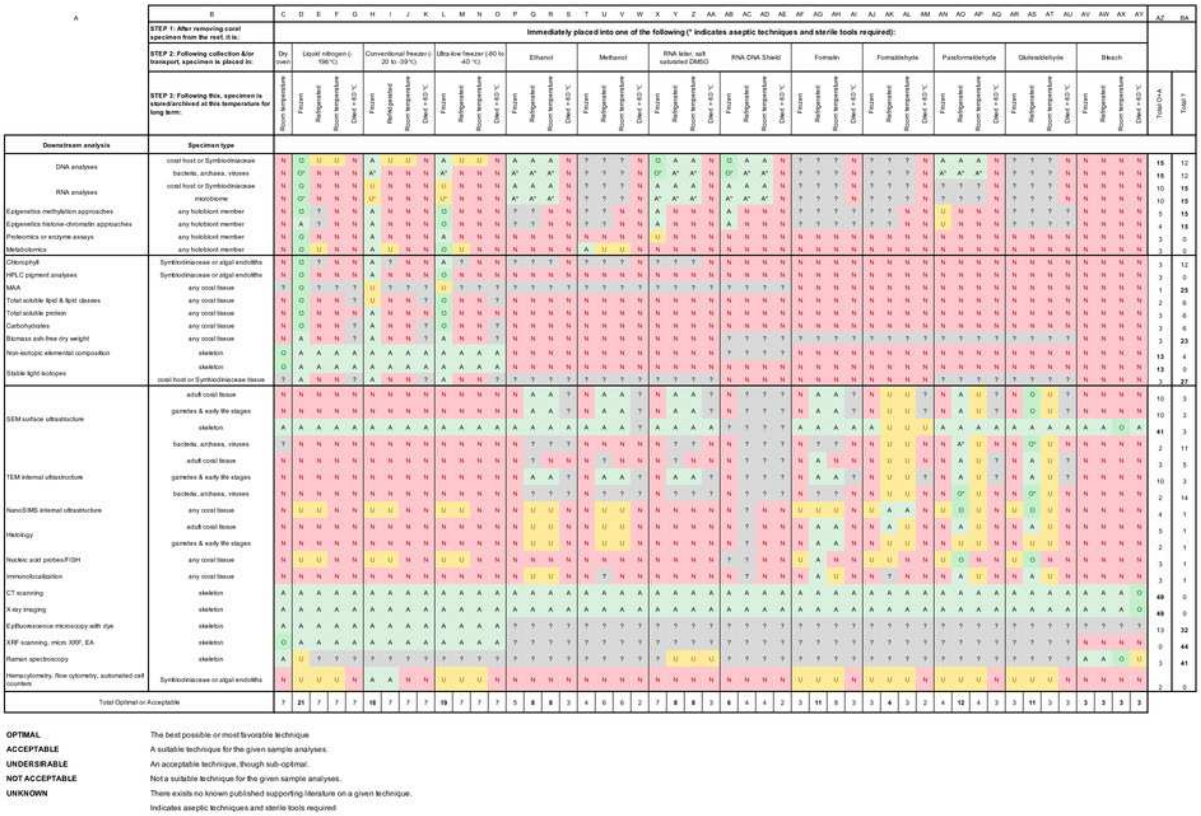

Sample Collection
Initial Storage & Preservation
Preservative Types
Long Term Storage
Analyses & Applications
Aseptic
Non-aseptic
LN2
<−20°C
4°C
Ambient Temp
Dried >60°C
Salt Buffer
DNA/RNA Shield
Ethanol
Formalin
-aldehydes
Bleach
<−20°C
4°C
Ambient Temp
Omics
Physiology
Microscopy
Total
LN2 + <−20°C
<−20°C
Salt Buffer + <−20°C
Salt Buffer + 4°C
Ethanol + 4°C
-aldehydes + 4°C
8 10 5 23
6 8 6 20
6 0 3 9
4 0 6 10
4 0 6 10
2 0 13 15
Aseptic
4 10 5 19
3 8 6 17
2 0 3 5
0 0 6 6
0 0 6 6
0 0 11 11
Non-aseptic
More
Less

Figure 3

Table 2. Field-specific (omics, physiology, and microscopy) collecting, preserving, and archiving pipelines

Table 2. Field-specific (omics, physiology, and microscopy) collecting, preserving, and archiving pipelines.



SUPPLEMENTAL MATERIALS
Discipline-Based Methods Appendices:

Omics-Based Methods

DNA-Based Work

Sample Collection: DNA samples should be collected using aseptic techniques to maximize the number of analyses that can be completed without additional sampling effort. Non-sterile tools and receptacles are acceptable for DNA analysis of the coral host and Symbiodiniaceae but are not desirable for certain DNA analyses. However, if total Symbiodiniaceae community diversity is being assessed, care should be taken to avoid cross-contaminating specimens by wiping and/or rinsing tools and receptacles between samples. Aseptic sampling techniques and aseptic equipment (e.g., wearing nitrile gloves and storing each sample in separate sterile transport receptacles) are optimal when characterizing microbial communities, and choosing to proceed without using those techniques significantly limits the reliability of DNA-based microbial community analysis. DNA analyses do not require large or high biomass samples, as the optimal sample should have a surface area of 1–2 cm^2 for most DNA analyses, or 1–3 polyps if sampling via syringe extraction (e.g., (Kemp et al. 2008; Correa et al. 2009)). Although DNA is stable for extended periods of time, such samples should be fixed in preservative and/or frozen at −80°C as quickly as possible. The amount of time between sampling and preservation of DNA can be upwards of one hour if stored in seawater or on ice but minimizing the length of this interim period will benefit most analyses, particularly those focused on microbial communities. Following the initial preservation of the sample, most storage options below −20°C are acceptable (e.g., −40°C) for maintaining the quality of DNA in samples. When freezing, samples should be rapidly frozen in liquid nitrogen as soon as possible following transport on ice. However, freezing at −20°C can be acceptable if other options are unavailable. In most instances, storage of the samples at 4°C or room temperature for any extended period is not desirable (Rubin et al. 2013). The addition of liquid preservatives or salt buffers may help maintain the quality of the DNA in the sample if it is being preserved for months to years prior to analysis. Several preservatives (e.g., formaldehyde) have yet to be thoroughly tested in terms of their ability to maintain quality of coral, Symbiodiniaceae, and coral-associated bacteria or virus DNA. The utility of these solutions for short- and long-term storage are therefore considered unknown. If a preservative is not immediately used following sample collection, the samples can be transported in a cooler with ice for up to an hour but longer amounts of time may lead to some changes in the microbial community composition (Rubin et al. 2013).

Short-Term Preservation of Samples: There are several preservatives and storage methods that are optimal for DNA analyses, including concentrated ethanol (as high as possible, up to 95%), salt buffers including DMSO (Gaither et al. 2011; Michael A. et al. 2013; Hernandez-Agreda et al. 2018), RNA/DNA Shield, Paraformaldehyde (PFA) (Ainsworth et al. 2015) to Phosphate Buffered Saline (PBS) (Hernandez-Agreda et al. 2018; Greene et al. 2020), and freezing at −80°C. Storage at −20°C, 4°C, or room temperature tends to be acceptable if combined with one of the previously listed preservatives (Dawson et al. 1998).

Long-Term Preservation of Processed Samples: Following DNA isolation, any remaining product not used for the present analyses should optimally be archived at −80°C with few to no freeze-thaw cycles to maintain the quality of the DNA. Samples most likely could be stored

long-term at −40°C, although that has not been explicitly tested on coral holobiont DNA. Variability in DNA extraction and amplification success is common among different species of cnidarians, however, so testing of preservation methods for the specific species being studied is advised (Dawson et al. 1998; Gaither et al. 2011). While stored DNA should remain usable for several years, confirming the quality of the DNA is important regardless of storage duration because significant DNA degradation can occur within the first 24hrs following preservation in a suboptimal preservative for some corals (Gaither et al. 2011). Several freeze-thaw cycles over one year may degrade the quality of a DNA sample, while a sample stored for 5–10 years with minimal disruption could be in good condition (Dawson et al. 1998; Shao et al. 2012).

Archiving Extra Coral Fragments: Additional material from the original sample to be archived for future work can be stored as an unprocessed fragment or in the form of ground coral or airbrushed blastate. Such samples may be stored for more than 10 years if maintained at −80°C with minimal freeze thaw cycles; sterile glass or plastic receptacles are acceptable for all downstream DNA-based analyses. Preservatives like RNA/DNA Shield or other salt/salt-saturated buffers are also acceptable for long-term storage if paired with −80°C to −40°C temperatures.

RNA-Based Work

Sample Collection: Coral specimens for RNA-based analyses should be collected using aseptic techniques to maximize the number of analyses that can be completed without additional sampling effort, and to minimize sample contamination and degradation by foreign RNA and RNAses, etc. Aseptic sampling techniques and sterile equipment are optimal when characterizing RNA viruses from corals or their symbionts. Small amounts of tissue are potentially sufficient for RNA-based analyses, particularly for phylogenomics. However, if sample degradation or changes of gene expression (due to tissue disruption) are a concern, it may be advantageous to collect a larger initial fragment in the field, then sub-sample and preserve a small undisturbed portion. For gene expression work, sample collection should be standardized to specific hours of the day as diel changes in expression patterns are well documented (e.g., (Levy et al. 2003, 2007; Hemond and Vollmer 2015; Wright et al. 2019)). Care should be taken to preserve samples as quickly as possible to avoid alteration of gene expression patterns and/or degradation (note that temporary storage temperatures and times are significantly more restrictive for RNA than DNA in Table 1).

Short-Term Preservation of Samples: Samples for RNA-based analyses should be stored using sterile tools and receptacles, when possible, as non-sterile equipment will lead to contamination and/or RNA degradation. RNA-based short-term preservation closely mirrors that for DNA-based analyses. For many preservatives in Table 1, there are data available for other model systems, but no literature available for coral holobionts on the efficacy or caveats of a given preservative (e.g., formalin, glutaraldehyde, methanol).

Long-Term Preservation of Processed Samples: The main difference in long-term preservation approaches for RNA- versus DNA-based downstream analyses is that for RNA, it is preferable to rapid-freeze whole tissue samples (i.e., intact coral fragments) to −80°C without freeze-thaw cycles, rather than to preserve partially processed tissues (e.g., blastate) long-term.

Archiving Extra Coral Fragments: Considerations and caveats for archiving extra coral fragments for RNA-based analyses closely follow the information above (for RNA-based short-term and long-term processed sample preservation and storage). Since RNA is more sensitive to

degradation than DNA (Ji et al. 2017), it may be difficult to achieve sufficient RNA yields from samples that have been stored for years or decades, or in suboptimal preservatives.

Protein-Based Work

Sample Collection: The optimal method for collection of specimens for protein analyses is to use aseptic techniques (clean collection tools and storage containers) and to get the samples frozen as quickly as possible. Non-sterile tools and containers are acceptable, though sterile practices are optimal if samples are to be analyzed for bacteria, viruses or other microbes; though we are not aware of any studies to date explicitly targeting non-Symbiodiniaceae microbes of corals for protein analysis. Small amounts of tissue (~1cm^3) are potentially sufficient for protein analysis, though it is common practice to collect a larger fragment (2-3 cm^3) and sub-sample during the protein extraction phase.

Short-term Preservation of Samples: The optimal approach would be to flash freeze the samples as soon as possible after collection (e.g., on the boat or once returned to shore), though keeping samples on ice packs that have been incubated in an ultra-cold freezer (−40°C or below) is a potential alternative if liquid nitrogen is not available. Short-term storage in a cooler on regular ice is acceptable up to 1 hour, as is short-term freezing at −20°C. It is unknown how much protein degradation occurs on ice or at −20°C for whole coral fragments, so longer times may be acceptable if high quality protein is able to be extracted from samples that were not frozen in < 1hr. The biggest difference with protein preservation compared to nucleic acid analyses is that we are not aware of any field preservation buffers that are reliable for stabilizing proteins and inactivating proteases in an intact coral sample, thus freezing is the optimal approach.

Long-term Preservation of Processed Samples: Rapid-freezing of remaining fragments of whole tissue (i.e., intact coral fragments) to −40 to −80°C without freeze-thaw cycles is optimal, though rapidly frozen tissue blastate is also acceptable and may be optimal if subsequent protein activity assays are the desired end point analysis. Alternatively, extracted protein may be preserved frozen (at −20°C or below) in a reliable protein extraction buffer or lyophilized and stored frozen as a solid. Slow degradation of samples is possible, though the authors have experience using frozen protein extracts up to 2 years post-extraction with high quality yields. A total protein gel or other suitable QA/QC is recommended in general, but particularly for samples that have been stored under less-than-optimal conditions or for extended (>2 years) periods of time.

Archiving Extra Coral Fragments: Considerations and caveats for archiving extra coral fragments for protein-based analyses closely follow the information above (for protein-based short-term and long-term processed sample preservation and storage), including the recommendations for QA/QC to verify protein integrity.

Epigenetics

Epigenetic research is relatively new, and thus does not have the same wealth of previous studies from which to draw general recommendations as exists for DNA, RNA, and protein work (Allis et al. 2015). Depending on the type of epigenetic research being pursued, current protocols fall largely in line with the DNA, RNA, or protein analyses sections. In general, methylation approaches (e.g., MeDIP-seq, EpiRAD, RRBS, etc.) tend to align with recommendations typical of the DNA-based analyses above, whereas RNA, nucleosome or genomic architecture (e.g.,

histone or chromatin configuration) approaches (e.g., miRNA, ATAC-seq, ChIP-seq, 3C, etc.) tend to fall closer to those for RNA or proteomics outlined below.

Sample Collection: As with the nucleic acid sections, the use of aseptic techniques is not absolutely critical for these techniques but if used will allow for expanded downstream applications. Small amounts of tissue are generally sufficient, again in keeping with the DNA and RNA recommendations. While it is possible to perform some methods using DNA preserved in the least stringent methods outlined above, epigenetic studies tend to be most informative when multiple classes of molecules (DNA, RNA, and protein) are collected and processed from the same sample. Although it may not be desirable or possible to process all these classes simultaneously, the ability to return to the same specimens and expand on previous studies could provide invaluable insights into epigenetic mechanisms. Samples therefore can ideally be collected in a manner consistent with DNA, RNA, and protein recommendations to allow for downstream epigenetics work.

Short-Term Preservation of Samples: Specific applications will have differing requirements for sample preservation, but the number of approaches available to explore for epigenetic studies are limited by the requirements of the most stringent aspect of the molecules under study. For DNA methylation, short-term storage at room temperature in a suitable preservative is possible, but for miRNA or ATAC-seq, such treatment would destroy the sample. Frequently, multi-molecule studies will require rapid-freezing coral fragments of moderate size (1–2 cm^2) to allow for multiple analyses, and storage at −80°C or below with subsampling of the frozen fragment for each of the analyses performed.

Long-Term Preservation of Processed Samples: As with the short-term preservation above, the storage method depends on the approach. If the sample is a DNA extract for methylation studies only, the sample could be stored as outlined in that section above. For multi-molecule studies, samples should ideally be rapid-frozen and continue to be stored at −80°C without freeze-thaw cycles.

Archiving Extra Coral Fragments: To maintain the quality and integrity of the range of molecules containing epigenetic information for coral samples, ideally the sample would be rapid-frozen and stored at −80°C without thawing. The maximum time for which cryopreserved samples remain useful is unknown, particularly for corals, in which only a handful of epigenetic studies have been performed to date (e.g., (Putnam et al. 2016; Torda et al. 2017; Dimond and Roberts 2020; Rodríguez-Casariego et al. 2020)). It is expected that samples are likely to be useful for a couple of years, limited to the most sensitive of the molecule classes.

Metabolomics

The application of metabolomics has proved especially powerful for elucidating the metabolic basis of the cnidarian-dinoflagellate symbiosis and how it responds to thermal stress (Hillyer et al. 2017a, 2018; Williams et al.). Moreover, given the especially close link between the metabolite composition and an organism's physiology and health, it is thought that metabolomics has greater potential for environmental monitoring and provides a more direct measure of organism functioning than other Omics platforms such as transcriptomics and proteomics (Bahamonde et al. 2016). However, as with epigenetics, its application to study corals is still relatively new, and sample collection and preservation techniques are still being optimized (Matthews 2022). Optimal sample preservation can depend on whether the profiling is untargeted or targeted, as well as the analytical platform destination, including proton-nuclear magnetic resonance spectroscopy (^{1}H-NMR), liquid chromatography-mass spectrometry (LC-

MS), and/or gas chromatography-mass spectrometry (GC-MS). While discussion of specific approaches is beyond the scope of this manuscript, basic in-field and in-lab techniques for sample collection, preservation and storage are more generalizable and a conservative (platform- and metabolite-wide) approach can be described.

Sample Collection: When examining variable responses, like in the case of coral bleaching, the time between sample collection and preservation (i.e., time taken to quench metabolism) is critical to capture the original response. The goal should be to preserve a sample as quickly as possible after collection, preferably via rapid freezing in liquid nitrogen, to prevent changes in metabolite composition due to sample degradation (Mushtaq et al. 2014). The time of collection period should also be taken into consideration as diurnal processes in both the host and algal members will lead to radically different metabolic profiles depending on the time a day the sample is taken. For example, circadian rhythms for both members and daylight vs. nighttime involve different metabolic processes affecting the analytical outcome. The target metabolites should also be considered prior to sampling as the downstream preservation and analysis might differ depending on the metabolite class of interest (e.g., carbohydrates or lipids). Final biomass is also a critical consideration, particularly for coral bleaching experiments, as algal symbiont density is typically reduced, and untargeted metabolomic analyses of Symbiodiniaceae fractions will require sufficient biomass for metabolomics platforms (~15mg dry weight).

If rapid preservation is not possible, all samples should be treated equally throughout the whole sampling protocol and have a similar sampling to freezing time window. This will enable sample preservation homogeneity to correct for any possible degradation artifacts. The Metabolomics Standards Initiative (Fiehn et al. 2007; Sumner et al. 2007) states that the preferred minimum sample size is five replicates (n=5).

Short-Term Preservation of Samples: Upon collection, the sample should be immediately rapid frozen in liquid nitrogen for optimal results although some other preservation methods may work with caveats (see Table 1). Ideally, samples will be frozen at −80℃, and freeze-thaw cycles avoided, in light-blocked containers until processing.

Long-Term Preservation of Processed Samples: Samples should always be maintained at −80℃ temperatures, preferably in light-blocked containers.

Physiology Methods:

Chlorophyll and other pigments

Collection, Sacrifice and Preservation: Samples for chlorophyll quantification can be collected from field or experimental samples using sterile or non-sterile equipment. Whole coral fragments, tissue samples or planulae are acceptable and generally require 1 cm^2 or more of coral tissue (however, more may be required if samples are significantly bleached). Rapid freezing and short-term storage with dry ice is acceptable, but CO_2 gas can create acidic conditions that may lead to some degradation (Roy et al. 2011). Both liquid nitrogen and ultra-cold freezing are suitable for rapid-freezing samples intended for pigment analysis, including accessory pigments that may rapidly convert under certain light levels (Southerland and Lewitus 2004; Warner and Berry-Lowe 2006). Sample storage in liquid nitrogen (−196℃) or ultra-cold freezing (−80℃) are commonly employed for long-term storage prior to extraction (Roy et al. 2011), and algal samples stored on filters at −80℃ have remained stable for up to one year. It is also

recommended to store samples in air-tight packaging with as much air evacuated as possible to prevent oxidation (Roy et al. 2011).

Processing: Extraction in 100% acetone or acetone:water (90:10) are historically the most common solvent mixtures for Symbiodiniaceae, followed by spectrophotometry with the calculations of Jeffrey and Humphrey (Jeffrey and Humphrey 1975). Fluorescence-based measurements are also possible and typically more sensitive to low concentrations (Holm-Hansen and Riemann 1978). Acetone extracts from other microalgae tend to show minimal degradation when stored for <20 days at −15℃, and total chlorophyll and carotenoid degradation rates are ~ –0.2% d^{-1} (Hooker, SB 2005). In addition to acetone, alternative methods, such as extraction in methanol and spectrophotometry are also available (Porra et al. 1989; Hoadley et al. 2019). For Symbiodiniaceae extracted at room temperature from the scleractinian coral *Pocillopora capitata*, both acetone and methanol-based extraction methods achieved peak fluorescence within 1 hour, indicating complete extraction (Holm-Hansen and Riemann 1978). Longer extraction times of up to 12 hours are also acceptable but may be best at lower temperatures (−20℃). While not as common, lyophilization (freeze-drying), immediately followed by extraction, is also an effective method for pigment extraction and in some cases improves the chlorophyll extraction efficiency (van Leeuwe et al. 2006), including in Symbiodiniaceae from soft corals (Pupier et al. 2018).

Mycosporin-Like Amino Acids (MAAs)

Collection, Sacrifice and Preservation: Samples for mycosporin-like amino acid (MAA) determination can be collected from field or tank-based experiments using sterile or non-sterile tools. Whole coral fragments (skeleton + tissue), tissue, or Symbiodiniaceae samples are acceptable. MAAs can also be determined from mucus, as it has already been done for fish (Reverter et al. 2018), or for corals (Teai et al. 1998). Planulae can also be analyzed for MAAs (Zhou et al. 2016). Coral surface area of ~2cm^2 or greater is needed for the quantification and identification of MAAs. The best practice for sample storage is to rapid freeze in liquid nitrogen and transfer to a −80℃ freezer. Short-term storage (immediately after collection) in cooler ice or in bags or tubes filled with seawater and kept at 4℃ on the boat are also acceptable for up to 4–6 hours as long as temperature is monitored and samples are preserved from light (Corredor et al. 2000). Additionally, coral tissue, Symbiodiniaceae, or larvae can be kept at −20℃ for a week or freeze-dried and kept at −80℃ for long-term storage. Preservatives such as formalin, ethanol, peroxide, and bleach should not be used for these samples, as such chemicals can interact with those used for the extraction of MAAs.

Storage of Processed Samples: Typically, there is little archiving of processed material because MAA assays generally consume the specimen.

Long-Term Storage: For long-term storage of samples before analysis it is optimal to preserve samples at −80℃.

Lipid, Protein, Carbohydrates, and Biomass Assessments

Collection, Sacrifice and Preservation: Samples collected for energy reserve quantification can be collected from field or tank-based experiments using sterile or non-sterile tools. Whole coral fragments (skeleton + tissue), tissue samples (airbrushed or water-pik), mucus, or larvae (planula) are acceptable. Coral surface area of ~1cm^2 or greater is needed for quantification of each energy reserve though new microplate methods are in development that may allow for smaller fragment sizes in future. The best practice for sample storage is to rapid

freeze in liquid nitrogen or dry ice or at −80 °C and store in a −80°C freezer. While storage at −20°C is also acceptable, storage at refrigerator or room temperature is not advised. In the absence of immediate freezing capacity, short-term storage (immediately after collection) on blue ice (cooler + ice) for up to 1 hour or storage in live-well buckets or bags on the boat or during transportation is also acceptable for up to 4-6 hours as long as water is changed often and temperature is monitored. Additionally, corals can be shipped to the laboratory live using wet paper towels and sealed plastic receptacles or wet bubble wrap. This live transport method affords roughly 48 hours of viability but can have significant impacts on energy reserves. Preservatives such as formalin, methanol, ethanol, peroxide, and bleach should not be used for these samples, as such chemicals can damage cells, leach energy reserves, and alter energy quantification. It is important to acknowledge that lipid, protein, carbohydrate, and biomass values may be underestimated when using airbrushed tissue, as a significant proportion of organic tissue resides within the skeletal organic matrix (Conlan et al. 2017). Though lipid, protein, and carbohydrates can be standardized to surface area, standardization to ash free dry weight is more robust for comparisons among coral species and specimens with different tissue thicknesses (Edmunds and Gates 2002). Biomass is standardized to surface area.

Total Soluble Lipids

One-gram wet weight of ground coral (whole tissue plus skeleton) is suitable for lipid extraction. Ideally samples are freeze-dried prior to analysis. A similar wet weight of air-brushed tissue blastate or collected mucus can be used as well, though the use of these methods may result in lipid concentrations (and classes) that differ from methods that utilize whole, ground coral fragments. Lipid extractions should be extracted using 2:1 Chloroform: Methanol (Hara and Radin 1978; Harland et al. 1991; McLachlan et al. 2020a). Notably, a lipid extraction method that utilizes 2:1 Dichloromethane (DCM): Methanol, which has been successful in extracting lipids from other organisms (Christie and Han 2010) has proven unsuccessful and unreliable for at least some species of corals (Baumann et al. 2014). Lipid concentrations can be quantified via weight (McLachlan et al. 2020a) or through colorimetric assays in microplates (Cheng et al. 2011). It should be noted that ideally extracted lipids should be stored in air-tight receptacles. Amber glass receptacles are useful to prevent oxidation and photodegradation, but frozen lipid samples can also be stored in plastic receptacles. Following lipid extraction into solvents, glass storage receptacles are required to avoid reactions of solvents with plastics. It is recommended that all glassware be washed with non-phosphate soap and pre-baked (suggested method: (McLachlan et al. 2020a)).

Storage of Processed Samples: If lipids are extracted via chloroform:methanol and dried (to determine lipid weight) they can be stored for additional analyses (e.g., lipid class determination via HPLC or Iatroscan) by resuspending the dried lipids in chloroform (Christie 2003). Lipids should be stored in sealed amber glass vials in the dark and are viable for additional analyses for multiple years (<10). Acceptable storage methods include −80°C freezer and liquid nitrogen / cryopreserved are likely acceptable though not tested.

Proteins

Half a gram of ground whole coral (wet weight) or 0.5–1.0ml of tissue blastate can be used for most protein extractions. Similar wet weights or volumes are likely suitable for collected mucus. In all cases slightly higher amounts of weight wet or volume are recommended to allow for replicate sampling, method testing, and as a buffer should the protein content of your

preferred sample type be low. Soluble proteins (host, *Symbiodiniaceae*, or holobiont) can be quantified using a colorimetric method (McLachlan et al. 2020b).

Storage of Processed Samples: There is no archiving of processed materials because protein assays using the colorimetric BSA protein method render sample unusable for additional downstream analyses

Carbohydrates

Whole coral samples of ~1cm^3 or 1g are recommended for carbohydrate quantification. More sample may be required for bleached corals. Carbohydrate concentrations can be measured following phenol and sulfuric acid extraction using a colorimetric procedure (DuBois et al. 1956). Smaller coral sample amounts may be utilized for more modern, microplate-based colorimetric carbohydrate protocols (*sensu* (Masuko et al. 2005)). However, 1cm^3 is still recommended to allow for triplicate analysis of each sample.

Storage of Processed Samples: There is no archiving of processed materials because carbohydrates assays using the colorimetric method render sample unusable for additional downstream analyses.

Biomass

One gram of ground whole coral (wet weight) can be used for most ash-free dry weight biomass determinations. Similar wet weights are likely suitable for airbrushed tissue blastate or collected mucus, but slightly higher amounts of weight wet are recommended to allow for replicate sampling, method testing, and as a buffer should the biomass content of your preferred sample type be low. It is important to acknowledge that biomass values may be underestimated when using airbrushed tissue, as a significant proportion of organic tissue resides within the skeletal organic matrix (Conlan et al. 2017), and this is also true for protein, lipid, and carbohydrate analyses. Tissue biomass (host, *Symbiodiniaceae*, or holobiont) can be quantified by drying coral material to a constant weight (60℃ for 24 hrs) and burning it (450℃ for 6 hrs) to yield the ash-free dry weight (McLachlan et al. 2020c).

Storage of Processed Samples: There is no archiving of processed materials because biomass analyses completely consumes the sample leaving no materials for additional downstream analyses (McLachlan et al. 2020c).

Stable Isotopes In Tissues (Whole, Host, and Symbiodiniaceae $\delta^{13}C$ and $\delta^{15}N$)

Natural abundance stable isotopes in coral tissue samples have been used to determine the proportion of heterotrophy vs photoautotrophy in corals (Muscatine et al. 1989; Rodrigues and Grottoli 2006; Grottoli et al. 2017; Ferrier-Pagès et al. 2021; Price et al. 2021), the trophic status of corals (Conti-Jerpe et al. 2020; Price et al. 2021) and to determine the proportionate contribution of various food sources to coral tissues (Price et al. 2021). Isotopic enrichment experiments and pulse-chase isotope labeling using both $\delta^{13}C$ and $\delta^{15}N$ have successfully identified pathways of C and N acquisition and allocation within corals (Piniak et al. 2003; Hughes et al. 2010; Hughes and Grottoli 2013; Baumann et al. 2014; Tanaka et al. 2015; Ezzat et al. 2017; Pupier et al. 2021).

Coral tissue is removed from the skeleton by air-brushing or water-picking. The resulting blastate is then processed for isotopic analysis of the whole coral, or the blastate is further processed to separate the coral host from the endosymbiont via a series of sonication and centrifugation steps. Preparation for stable isotopic analysis can be completed for dried down

whole tissue, host tissue, and Symbiodiniaceae tissue (Price et al. 2020). Other methods are similar, but tissue material may be loaded onto a pre-burned GFF filter (Rodrigues and Grottoli 2006). However, GFF filters necessitate a larger tin for packing the samples, reducing the number of samples that can be analyzed in a single run, and clog the combustion column resulting in more frequent column cleaning and higher analytical costs.

Isotopes and Element/Calcium in Coral Skeletal Material

Stable isotopes and element/calcium ratios in coral skeletal samples are widely used to reconstruct past climate and environmental changes at annual to monthly resolutions (Grottoli and Eakin 2007; Felis 2020), although a reliable geochemical identification of bleaching events in skeletal records is still in its infancy (D'Olivo and McCulloch 2017). Skeletal $\delta^{13}C$ has been used to reconstruct the autotrophy-heterotrophy contribution of carbon in the coral skeleton (Felis et al. 1998; Grottoli and Wellington 1999), seasonal changes in light levels (Grottoli 2002), and the ^{13}C Suess effect as a function of anthropogenic CO_2 emissions (Swart et al. 2010). Skeletal $\delta^{18}O$ (Wellington et al. 1996; Boiseau et al. 1998; Quinn et al. 1998), Sr/Ca and U/Ca (Felis et al. 2009), Li/Ca and Li/Mg (Hathorne et al. 2013), Sr-U (Alpert et al. 2017) and clumped isotopes (Saenger et al. 2012) have been used to reconstruct the temperature and hydrology of the surface ocean (Felis 2020).

Skeletal boron isotopes ($\delta^{11}B$) and boron/calcium (B/Ca) are used to determine the effects of ocean acidification on coral calcification (McCulloch et al. 2017) and to reconstruct the history of ocean pH (Hemming and Hanson 1992; Hönisch et al. 2004). Nitrogen isotopes ($\delta^{15}N$) in skeleton-bound organic matter have been used to provide information about the oceanic nitrogen cycle and the influence of anthropogenic nitrogen on the open ocean (Wang et al. 2018). Skeletal barium isotopes ($^{138/134}Ba$) and cadmium (Cd/Ca) have been suggested as a proxy for oceanic barium cycling (LaVigne et al. 2016; Liu et al. 2019) and upwelling (Shen et al. 1987), respectively. The combination of element/Ca and skeletal boron ($\delta^{11}B$) isotope records have been successful at detecting the response of coral calcification and calcifying fluid to thermally induced bleaching stress (D'Olivo and McCulloch 2017).

Sample Collection: Coral skeletal cores are collected using underwater pneumatic or hydraulic coring devices. Coral skeletons of ramets and whole colonies are also collected, depending on the study. Aseptic techniques are not necessary. Coral tissue is removed with an airbrush or water-pik. Coral cores, colonies, and fragments are cut into longitudinal 1cm thick slabs along the major axis of growth and dried thoroughly, preferably at 60°C for several days if possible. Coral skeleton sub-samples are collected by hand using a micro-milling or rotary tool under a dissecting microscope for monthly to annually resolved analyses, and with high-precision micromilling/microdrilling for annually to monthly resolved analyses. All samples are collected along the major axis of growth (Giry et al. 2010). The resulting skeletal powder is then processed for isotopic and elemental analysis using specific preparation steps depending on the various analytical methods. For $\delta^{13}C$ and $\delta^{18}O$ analyses, care must be taken not to chemically clean samples prior to stable isotopic analyses as this can cause uncorrectable isotopic fractionation (Grottoli et al. 2005). High-pressure water is sufficient to remove debris from the surface prior to drying and drilling a skeletal core. However, element/Ca analyses typically involve significant chemical cleaning steps (Matthews et al. 2006). An alternative to drilling is direct "non-destructive" analysis of coral skeletal slabs for element/Ca ratios by Laser Ablation Inductively Coupled Plasma Mass-Spectrometry (LA-ICP-MS) (Matthews et al. 2006; Hathorne et al. 2011) and X-Ray Fluorescence (XRF) scanning (Ellis et al. 2019).

Short-Term, Long-term, And Archiving of Skeletal Samples: Coral cores, whole fragment or colony skeleton, and ground coral skeletal powder can all be stored dry at room temperature or at 4℃ indefinitely. The most important is that the skeletal material be initially dried following collection in a drying oven at 60℃ until completely dry, then stored dry so that mold does not grow on the skeleton.

Microscopy & Imaging Methods:

Tissue and Skeleton Ultrastructure

While many features of corals undergoing bleaching can be viewed using visible light, others require methods that can resolve smaller external and internal features such as the structure and dynamics of cell membranes and organelles as well the intracellular and extracellular placement and morphology of microbial symbionts, viral particles, or proteins. In these cases, techniques such as scanning and transmission electron microscopy (EM) can be used. Although the varieties of staining procedures for EM are beyond the scope of this manuscript, basic in-field and in-lab techniques for preservation and storage methods of coral samples are more generalizable and have been published on extensively in the past.

Sample Collection: Coral tissues collected for scanning and transmission electron microscopy (SEM and TEM, respectively) should be fixed and never frozen (with the exception of cryo-EM, which requires special preparation prior to freezing, see Box 1). Samples collected for skeletal analyses do not require fixation and can be stored at room temperature. Depending on the application, tissues should be fixed immediately upon collection (e.g., Nano-SIMS, immunolocalization, microbiome work). Fixing tissues with EM-grade reagents is optimal to avoid background fluorescence. Sterile tools are only required for microbiome applications but samples exploring other aspects of the holobiont need not use aseptic techniques.

Short-Term Preservation of Samples: No preservation is required for skeletal samples. However, preservation methods for tissues vary, but typically consist of a combination of paraformaldehyde and glutaraldehyde and some salt buffer such as phosphate buffered saline or sodium cacodylate (Price and Peters 2018; Greene et al. 2020). Fixed tissues are best stored in glass receptacles at 4ºC.

Sample Processing: Tissue removal from skeletal samples is typically done by immersion in dilute bleach solution to remove all organic material. Skeletons are coated with gold-palladium prior to imaging (Tambutté et al. 2007). Fixed tissues can be fractured or mounted directly onto EM grids for SEM. For TEM of tissues, samples must first be decalcified typically in high molar EDTA and must then be sectioned using a microtome, mounted, dehydrated and stained (Correa et al. 2016). No further processing is required unless immunolocalization is desired.

Long-Term Preservation of Processed Samples: Once tissues have been mounted on EM grids either whole (SEM) or sectioned (TEM) and imaged, they can be stored at room temperature and reimaged at a later date. Some damage to tissue sections can occur during TEM imaging. Skeletal samples can be stored indefinitely at room temperature for future use.

Archiving of Unprocessed Samples: Tissues embedded in blocks or cryopreserved can be stored indefinitely; skeletons can be stored indefinitely at room temperature ideally with low humidity.

Histology: Gross Morphology

Histological techniques can provide wealth of information about coral bleaching at the cell and tissue levels. Histology provides evidence on several coral aspects such as cell structure and integrity, presence of endosymbionts (Symbiodiniaceae and other microorganisms) and presence of diseases and pathogens (Bythell et al. 2002; McClanahan et al. 2004; Work and Meteyer 2014; Gierz et al. 2020). Important efforts have been made for the last four decades to optimize coral sampling, preservation, and observation for histological investigations, leading to numerous valuable protocols (Hayes and Bush 1990; Brown et al. 1995; Greene et al. 2020). Also, the difference in term of morphology and proteins and lipids compositions induce the use of different protocols among coral species, life stage, endosymbiont type (Rinkevich and Loya 1979; Harii et al. 2009). While the diversity in laboratory techniques is beyond the scope of this manuscript, universal basic procedures are highlighted here.

Sample Collection: Coral tissues collected for histological investigations should be fixed immediately (or transported briefly on ice) upon collection and never frozen in order to preserve cell integrity for future observations. Samples can be collected without concern for sterility and require at least $1cm^2$ of adult coral tissue for histological analysis or entire larvae.

Short-Term Preservation of Samples: Short-term preservation type and time vary among studies, but mostly consist of a combination of formalin/formaldehyde with seawater, or paraformaldehyde/glutaraldehyde with phosphate-buffered saline solution (Hayes and Bush 1990; Brown et al. 1995; Bythell et al. 2002; McClanahan et al. 2004; Harii et al. 2009). The relative proportions of each preservative and the time of preservation differs slightly among studies but preserving in 10% formalin in seawater is common practice. EM-grade fixatives are preferred for applications requiring fluorescence imaging (see next section); however, non-EM grade reagents are acceptable for downstream applications that do not require fluorescence (e.g., hematoxylin and eosin staining).

Sample Processing: For tissue observations, coral samples are decalcified in a short-term preservative, but sometimes the use of formic acid, ascorbic acid, or EDTA can help the decalcification process (Hayes and Bush 1990). The samples are then dehydrated using a graded series of ethanol and then embedded in commercial resin. Sections are made using a microtome and slices mounted onto a glass slide (dye can be added) for observation of cells and/or tissues under photomicroscope (Hayes and Bush 1990; Brown et al. 1995; Bythell et al. 2002).

Long-Term Preservation of Processed Samples: Histology processing is not destructive, allowing the slices to be stored for years and be re-used for further observations. The slices need to be stored in a dark, cool, and dry location. Ideally, samples need to be stored in specialized boxes that protect slides from dust deposition.

Archiving of Unprocessed Samples: The unprocessed samples in the preservative solutions can be stored for a short (days to weeks) time. Once transferred to ethanol, fixed tissues can be stored at 4 °C for months to years. Alternatively, samples embedded in resin can be archived for years for future sections if stored in a dark, cool, and dry location.

Histology: Localization of Nucleic Acids and Proteins

Sample Collection: Coral tissues collected for localization of nucleic acids or proteins should be fixed immediately upon collection (or transported briefly on ice) using EM-grade reagents and kept at 4°C but never frozen. Samples collected for nucleic acid localization (FISH) require molecular grade reagents and equipment. Sterile tools are only required for microbiome

applications. At least 1 cm^2 of adult coral tissue for histological analysis or entire larvae is sufficient.

Short-Term Preservation of Samples: Tissue fixation protocols vary, but mostly consist of a combination of paraformaldehyde with or without glutaraldehyde in a phosphate-buffered saline solution. The relative proportions of each preservative and the time of preservation differs slightly among studies, but is generally conducted <24 hours at 4C. EM-grade fixatives are preferred for applications requiring fluorescence imaging; non-EM grade reagents are acceptable for downstream applications that do not require fluorescence (e.g., colorimetric immunoperoxidase development).

Sample Processing: Coral samples are decalcified in a short-term preservative, but sometimes the use of formic acid, ascorbic acid, or EDTA can help the decalcification process (Hayes and Bush 1990). The samples are then dehydrated using a graded series of ethanol, xylene (or xylene substitutes), and then embedded in commercial resin (e.g., paraffin wax). Sections are made using a microtome and slices mounted onto a glass slide, wax removed, and tissues rehydrated. It is recommended to process tissue sections the same day, either with nucleic acid probes, antibodies, and/or nucleic acid stains (e.g., DAPI; Hoescht). If slide will not be hybridized (antibodies or FISH probes) within 24–48 hours of sectioning, the slide may be stored in a sealed receptacle in the freezer at −20℃ for up to 2 weeks (Wakai et al. 2014). It is recommended to view and image slides within a few days of hybridization.

Long-Term Preservation of Processed Samples: Formalin-fixed samples probed with nucleic acids (e.g., FISH) can be re-used once they are observed, in which case the probe is removed with formamide and the slide can then be stored for future re-probing at −20℃ or −80℃ for at least 100 days. Fixed cell suspensions should be stored in cryovials in the freezer (−20℃) (Wakai et al. 2014). Labeled tissue sections can be stored for further observations and imaging. FISH slides can be stored for up to 1 year at −20℃ in the dark (Alamri et al. 2017). Immunochemistry slides can be stored at −20℃ to 4℃ in the dark for years. Ideally, slides need to be stored in specialized boxes that protect them from dust deposition and light.

Archiving of Unprocessed Samples: Fixed tissue samples can be stored in 100% ethanol at 4℃ for years (Schimak et al. 2012). Tissues embedded in resin can be archived for years for future sectioning if stored in a dark, cool, and dry location.

<u>Skeletal Imaging (CT Scanning, X-RAY, Dyes & XRF Scanning)</u>

Sample Collection: Skeletal imaging techniques are commonly applied to skeletal cores but are also applicable to coral fragments. Skeleton samples must be rinsed thoroughly immediately upon collection to remove seawater and avoid salt contamination.

Short-Term Preservation: Prior to analysis, all skeletal imaging techniques require that samples are cleaned of seawater and dried. Ideally, samples should not be stored in chemical preservatives to avoid dissolution of the skeleton, precipitation of new minerals on the sample, or any other alteration to the skeletal integrity. However, CT scanning and X-Ray analysis have been applied to skeleton samples previously frozen or stored in ethanol, and these techniques (at least when investigating features on the mm or cm scale) are not highly sensitive to minor dissolution/precipitation on the micron scale.

Sample Processing: The processing procedures for skeletal imaging vary among techniques. The benefit of CT scanning is that entire samples (e.g., cores) can be scanned intact, without any alterations. Conversely, X-ray and XRF require cores to be sliced, while analysis of dyes typically requires the preparation of polished sections embedded in epoxy.

Long-Term Preservation of Processed Samples: Processed skeletal samples should be stored in a cool, dry location. The samples must be entirely dry, and ideally should be wrapped in plastic to prevent mold growth or dust deposition. Fluorescent dyes should be stored in the dark to avoid slow photo-bleaching of the dye.

Archiving of Unprocessed Samples: Skeletal cores and coral fragments can all be stored dry at room temperature or at 4°C indefinitely. The most important is that the skeletal material be initially dried following collection in a drying oven at 60°C until completely dry, then stored dry so that mold does not grow on the skeleton. For sliced cores, the same long-term preservation noted above applies to the unprocessed halves. It may be beneficial to keep one half of cores undisturbed for long-term preservation.

Skeletal Chemistry (Raman)

Sample Collection: Raman spectroscopy can be applied to skeletal cores or coral fragments. Coral fragments are initially soaked in ~3% sodium hypochlorite (bleach) for at least 1 hour, or until white (bleach can be replaced if necessary) to remove tissue.

Short-Term Preservation of Samples: Samples for Raman analysis should ideally never be stored in any chemical preservative or in water. Since Raman analyses are conducted on skeletal surfaces at micron-scales, any minor dissolution or precipitation of new aragonite crystals has the potential to influence the Raman data.

Sample Processing: Heating of skeleton samples prior to Raman analysis can substantially reduce the data quality (DeCarlo et al. 2018). This is because heating causes "annealing" of samples, a process in which disorder in the crystal structure of the skeleton is alleviated. Since Raman analyses depend on characterizing skeletal disorder, heating can thus change the Raman data in such a way that comparisons among samples may not be meaningful. The sensitivity of Raman analyses to heated coral skeletons is poorly known, though. Heating to 60°C for minutes to several hours to dry skeletal powders does not seem to have a substantial effect, whereas heating to 140°C for 16 hours dramatically changes the resulting Raman spectra (DeCarlo et al. 2018), but heat procedures in between these two have not yet been tested.

Long-Term Preservation of Processes Samples: Skeletal material can be stored at room temperature indefinitely if stored in a sealed receptacle.

Archiving of Unprocessed Samples: Bleached coral skeletons which were not ground may be stored indefinitely at room temperature.

Symbiodiniaceae Density and Mitotic Index

Sample Collection: Samples for Symbiodiniaceae quantification are typically collected as whole coral fragments, but planulae and gametes are acceptable and generally require 1cm^2 of coral tissue, although more may be required if corals are significantly bleached. Samples are best kept cold on ice or at 4°C. While rapid freezing and short-term storage with dry ice is acceptable, one should ensure that cell breakage is not occurring by freezing, or that the percent loss in Symbiodiniaceae cells due to freezing is consistent and quantifiable.

Short-Term Preservation of Samples: Sample storage in an ultra-cold freezer is commonly employed for short and long-term storage. Samples can also be kept short-term at −20°C or 4°C, although not optimal. If samples are chemically preserved (e.g., by glutaraldehyde or formaldehyde fixation) storage at 4°C is acceptable.

Sample Processing: Tissues must be homogenized prior to counting symbionts and is typically done using filtered seawater (natural or artificial). Tissue homogenization can be done

on airbrushed/water-piked tissues using a glass or electronic homogenizer, or by grinding whole fragments with mortar and pestle. Hemocytometers, flow cytometry, and other automated cell counter methods benefit from separation of symbiont cells from host tissue/mucus/skeleton via differential centrifugation prior to counting to avoid clogging the instrument (Krediet et al. 2015; McLachlan et al. 2020d). Fixation can aid in long-term storage prior to counting but can affect cell counts. Freeze/thaw cycles can lyse symbiont cells and depress cell counts (Krediet et al. 2015) and repeat freeze-thaw cycles should be avoided. A study of the effects of freezing and fixation on the Symbiodiniaceae density of anemones found that the order in which samples are frozen/fixed and homogenized is important, and can alter the density of cells which are subsequently counted (Krediet et al 2015).

Long-Term Preservation of Processed Samples: Methods for symbiont counts are destructive and no processed sample remains.

Archiving of Unprocessed Samples: Remaining fragments, planulae, gametes, and/or tissue homogenates can be stored long-term at −80°C, but, as noted above, freeze/thaw cycles can lyse symbiont cells and repeat cycles should be avoided.

Coral Color Analysis from Digital Imagery

Collection of Digital Images: Coral color analysis is conducted using digital images taken of a live coral colony either *in situ* or *ex-situ* in a photo studio (Amid et al. 2018). Photographs must be taken prior to any preservation or processing of tissue, such as freezing, use of preservatives or fixatives, airbrushing etc., to ensure no alteration of the original coral color occurs. Depending on the method of image analyses intended (e.g., the Red Green Blue Color Model (Edmunds et al. 2003; Siebeck et al. 2006; Winters et al. 2009; Voolstra et al. 2020), or the Greyscale Model (Chow et al. 2016; Amid et al. 2018), it may be necessary to photograph the coral colony next to a black, white, or color standard reference card. It is essential that the coral colony and the reference card receive the same uniform illumination/light field. If possible, image coral colonies from multiple angles in order to get a good representation of bleaching degree and color (McLachlan and Grottoli 2021). Capturing images using the camera's RAW settings will avoid loss of information during the image compression compared to other photo formats (e.g., JPEG).

Processing of Digital Images: Depending on the method of image analysis used, digital image corrections may be necessary, for example external light normalization (Winters et al. 2009) or conversion of digital images to 8-bit grey scale (Chow et al. 2016; Amid et al. 2018) using an image analysis software such as ImageJ (Rasband, W.S. 1997) or Adobe Photoshop (2004).

Preservation of Digital Images: Digital images may be stored indefinitely via cloud or physical storage.

Measuring Coral Size

Data Collection: Coral specimen size is a basic but essential measurement that is often necessary as the denominator used to standardize the quantity of another measured variable to adjust for the amount of coral analyzed. Researchers can inflate the uncertainty of their measured variables of interest if there is considerable measurement error in what is used to normalize the sample measurement. To avoid error inflation, there are several ways to measure coral size (Supplementary Material Table 1), and many of these can be used as an integrated measure of

640 coral “health” if measurements are taken at two time points (e.g.., measuring growth, see
641 appendix in Grottoli et al. 2021).
642