

Establishment of a long-term reef-building coral biopsy archive as a tool for the marine biology research community

Anderson B. Mayfield¹⁻³

¹ Coral Reef Diagnostics, Miami, FL, USA

² International Coral Reef Society, Tavernier, FL, USA

³ Coral Research and Development Accelerator Platform, Thuwal, Saudi Arabia

*Corresponding author. Email: anderson@coralreefdiagnostics.com or anderson.mayfield@cordap.org

Abstract

Coral reefs are in demise across the planet, with many relics of their former selves. In most instances, samples collected in one experiment, or during a particular field expedition, are never again used, nor are the biopsies (or molecules extracted from them) shared with colleagues or archived in biorepositories. Herein I advocate that a greater degree of sample sharing be adopted by marine biologists for at least two reasons. First, the corals from which many such biopsies have been taken have likely perished, meaning that the only information (aside from images & raw data) that will exist for these invaluable research subjects must be derived from these very biopsies. Secondly, reuse of previously obtained biopsies lessens environmental damage; although sampling of small (~100 mg) tissue specimens from entire coral colonies is unlikely to impact their survival, extraction of whole cores with drills, or sacrificing of entire fragments from branching species, could pose a significant risk to source colonies (especially if they are not sustainably harvested). In this article, I have outlined pertinent information (e.g., whereabouts & storage conditions) of coral samples obtained from not only the world's largest coral reef survey, the Living Oceans Foundation's "Global Reef Expedition," but also select aquarium and field experiments undertaken in Taiwan and Florida. I anticipate that this global reef coral sample archive, which is associated with a dataset whose levels of biological organization span molecules to entire ocean basins, will serve as a long-term research tool for the research community, if only to serve as a "physiological snapshot" of these corals in the period just prior to the fundamental shift in their biology caused by global climate change.

Keywords: bio-archive, biopreservation, biorepository, coral reefs, marine ecology

Introduction

Justification for biopreservation & biobanking

In many research projects, samples are discarded at the completion of the analysis, or once the article is published. This could be due to spatial constraints (e.g., limited freezer space) or simply because it was thought at the time that the samples could yield no new information (beyond what was presented in the article). Given the great expense (money+human effort) required to collect and analyze reef-building coral samples (Demerlis *et al.*, 2022), both in terms of accessing

oftentimes difficult-to-reach sites and the costs for expensive laboratory kits and other research consumables, neither excuse is particularly acceptable. In laboratories with limited funding, in which long-term cold storage is infeasible due to unstable power supplies, shipping samples to dedicated biorepositories may be an attractive option that should be more widely considered (assuming 1) samples were collected legally & 2) existence of funds for shipping).

Only coral skeletons are ever typically archived in repositories or museums since they inherently fossilize

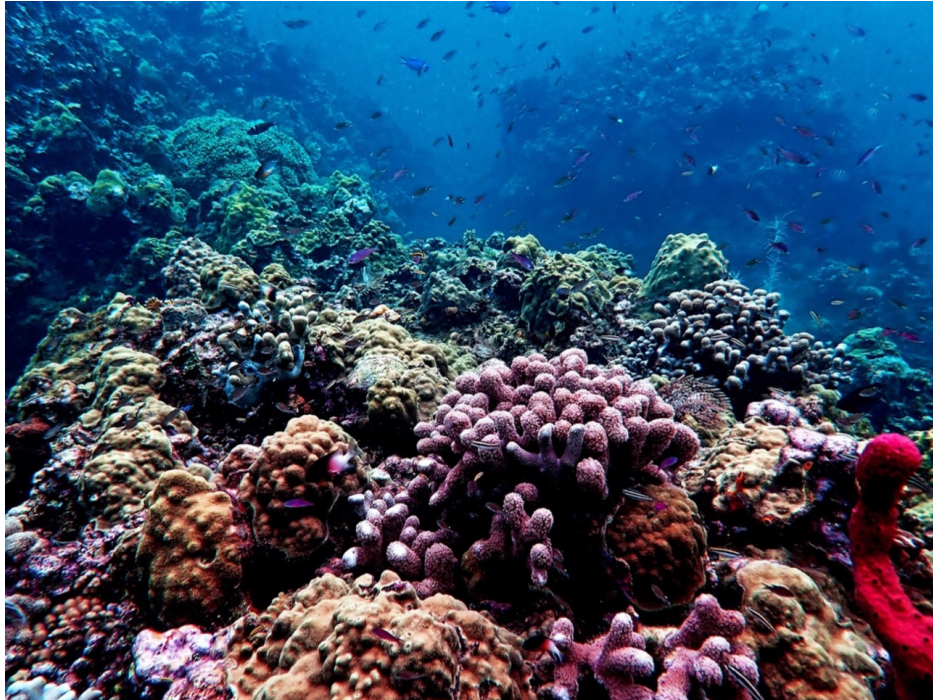


Figure 1. A colorful coral reef at Toucari Bay, Dominica (Southeastern Caribbean).

Few disease-free brain corals (Figure 2) can now be found due to the stony coral tissue loss disease (SCTLD) outbreak.

and can be stored for many years even at room temperature (RT; see Table 1 for a list of non-standard abbreviations.). Tissue samples, or molecules derived from living tissues-RNAs, DNAs, proteins, lipids, and polar metabolites-are not generally stored for more than a few years, when in fact the majority of these samples could be incredibly valuable to other researchers. For one, in many instances the coral colony from which the sample was taken may no longer exist; the biopsy (or derived macromolecules) might be the only remnant of its existence on the planet (beyond images), and it could be useful for environmental forensics (Mayfield *et al.*, 2019b), reconstruction of past climate or seawater quality (Doo *et al.*, 2012), evolutionary analyses, or a plethora of other topics (e.g., eco-physiology; Tortolero-Langarica *et al.*, 2016). Brain corals are now virtually extinct in many parts of the Caribbean (Figure 1) due to the recent emergence of stony coral tissue loss disease (SCTLD); diver photographs and the odd skeleton may now be all we have left to remember them. Only time will tell whether it would have been a research or conservation benefit to have bio-banked any brain corals prior to this unprecedented marine pandemic.

As the coral biology field grows in number of researchers and our collective geographic reach, with more scientists sampling corals now than ever before (Grottoli *et al.*, 2021), we have an

opportunity to not only probe the biology of sampled coral colonies in new and exciting ways (Figure 2), but also biopreserve these specimens for future study. I will give a personal anecdote to provide an example in which long-term biopreservation of coral specimens was fortuitous. When I began graduate school with the late Dr. Ruth Gates at the Hawaii Institute of Marine Biology (HIMB, HI, USA) in 2003, researchers in all biological disciplines were already tapping into the power of gene expression technologies with great enthusiasm, especially next-generation (RNA or DNA) sequencing (NGS). Twenty years later, in fact, gene expression research remains popular (e.g., Monteiro *et al.*, 2020; Rubin *et al.*, 2021; Morris *et al.*, 2023). I tasked myself with the development of protocols tailored to measuring gene expression in dual-compartmental organisms like reef-building scleractinians (e.g., Mayfield *et al.*, 2009), which house dinoflagellate endosymbionts within half of their cells (those of the gastrodermal layer; Figure 3). Hundreds of coral and model anemone (Mayfield *et al.*, 2014c; Chen *et al.*, 2016) articles focused on gene expression have since been published, with narratives reaching the likes of *Science* and *Nature* (in which gene expression data are regularly used to make inferences into cellular behavior). Such stories, however, have been predicated on the proteins encoded by these gene mRNAs showing

strong, positive correlations in concentration, a hypothesis I decided to first test at the ‘Omics-scale in 2014 (Mayfield *et al.*, 2016b-c) with a model reef coral *Seriatopora hystrix* (Figure 4). As it turned out, NGS-based transcriptomics and proteomics yielded very different suites of cellular molecules as being involved in key cellular processes like high-temperature adaptation; the R^2 values were effectively 0 for both host corals and their photosynthetic endosymbionts.

Upon reproducing these findings in other corals (Mayfield *et al.*, 2018b, 2021a), I had to face the reality that many of my past, exclusively gene expression-focused studies were associated with

“castle in the sky” story lines; having boldly assumed that the proteins would show commensurate shifts, I had made conjectures about coral health in the Anthropocene (Mayfield & Chen, 2020) that were, in the end, unsupported by the molecules that actually enact physiological changes in cells (i.e., proteins). The “silver lining,” and the moral of this story (which is not merely to cast doubt on my early research projects), is that I had co-extracted and then biopreserved nearly all proteins from my past projects. I could then “revisit” the studies and instead employ proteomics approaches, which is exactly what I did (Mayfield *et al.*, 2018a-b). This happens in science; technologies improve over time.

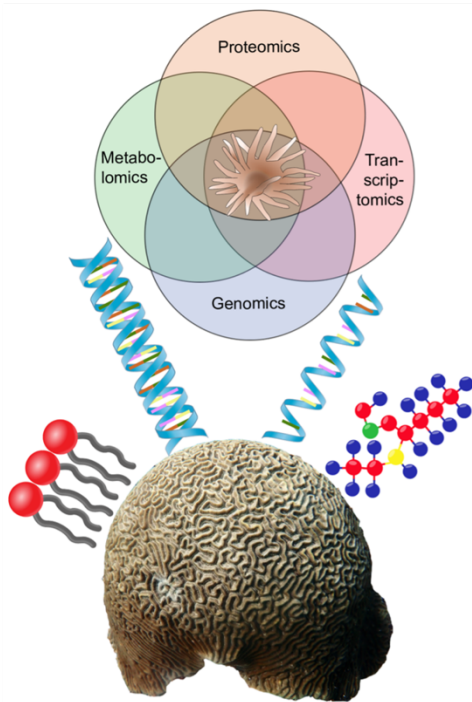
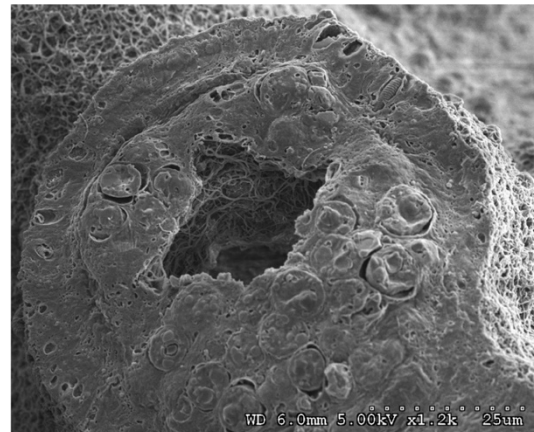


Figure 2. Molecular analysis of scleractinian corals. Molecular biotechnology has revolutionized our understanding of life on the planet, and corals are no exception (Chen *et al.*, 2012; Mayfield *et al.*, 2013d; Wang *et al.*, 2013). “Multi-‘Omics” (Mayfield *et al.*, 2014d; Mayfield, 2023) now enables us to make powerful inferences into the complex, long-obscured biology of cells (Peng *et al.*, 2011), including the crowded gastrodermal cells of reef corals, which are occupied in their near-entirety by photosynthetic dinoflagellates of the family Symbiodiniaceae (Mayfield & Gates, 2007; Figure 3).

However, it is rare for researchers to go back through their old samples to re-analyze them with new approaches (Mayfield, 2020a). For one, these samples may no longer exist for reasons addressed above. Secondly, in most instances, new personnel would be tasked with this, and many supervisors may be hesitant to deprive their students/employees of what

funded marine laboratories adjacent to easy-to-access reefs (e.g., HIMB, NMMBA), a good justification must be made for repeating a past study with living reef corals (vs. simply asking someone to provide the samples). For one, many corals are going extinct before our eyes. Is it ethical to repeat an experiment if many corals must be sacrificed in the process?

Fig. 3. A scanning electron micrograph (SEM) of a freeze-fractured *Pocillopora acuta* tentacle. The outer epidermal tissue layer and the inner gastrodermal layer featuring dinoflagellate endosymbionts of the family Symbiodiniaceae (as the pronounced, ~10 μm spherical objects) can be seen.



many consider to be the more “fun” parts of the marine biology scientific process: field work and/or aquarium studies. Rather than hand a student a set of proteins from a prior work (or a collaborator’s), it is more common to just repeat the experiment (or re-sample the corals *in situ*).

In a model systems lab with culturable animals (e.g., Peng *et al.*, 2020), repeating a study makes sense; surely something in every hypothetical project can be improved. Maybe a power analysis could be performed to justify a superior sampling regime. However, even in well-

This has happened recently in the Republic of Palau. The health and resilience of the reefs were extensively characterized in 2015 as part of the Living Ocean Foundation’s (LOF) “Global Reef Expedition” (GRE), yet researchers today are repeating this analysis. To be fair, much has changed since 2015 in terms of anthropogenic impacts, so a justification could be made to study what has happened on these reefs in the interim. However, the carbon costs required to reach Palau from the United States are incredibly high.

I would argue that this new cohort of

researchers should reach out to LOF for data and samples, but this would mean scientists from the USA would not have the chance to explore a veritable underwater paradise (Figure 5; albeit one that is far from pristine). It is not to say that there is never a need to repeat a study, nor should scientists refrain from re-surveying previously monitored locations; indeed, surveys should be repeated. However, I do think the level of sample-sharing with the coral research field is minimal, and this needs to change. A biopsy from a Palauan coral sampled in 2015 (Mayfield *et al.*, in

very similar projects are being repeated every few years, without acknowledgement of those that came before. Whether this is due to not having found the reference, or, more surreptitiously, the realization by the author(s) that the study is not novel yet happens to be convenient to conduct, cannot typically be known.

The goal of this article is to provide a key for finding a large number of coral samples that have been archived in biorepositories across the globe (Table 2). This is not an exhaustive list of all corals

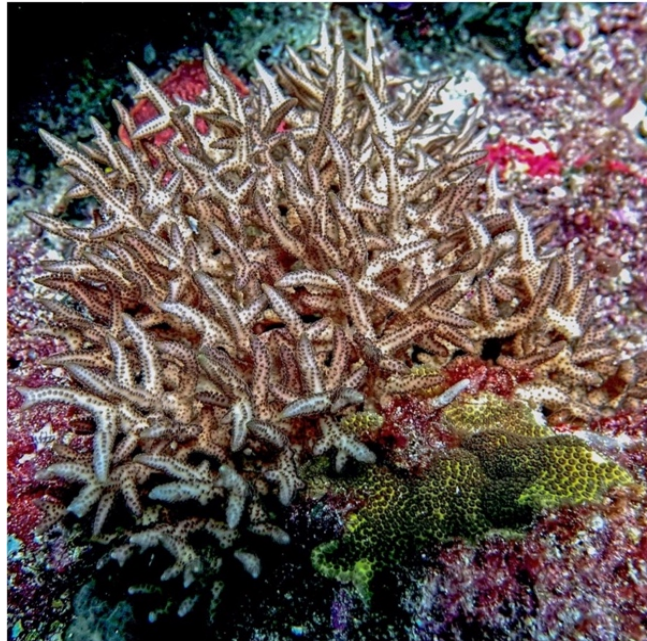


Figure 4. An *in situ* image of a model coral for research: *Seriatopora hystrix* (taken during the LOF-GRE mission to New Caledonia).

prep.) is valuable in and of itself and would also be of use to one interested in the thermal biology of Palau's reefs in 2023. I fear we have fallen into a cycle in which

ever sampled, but instead those from my prior laboratory experiments (Tables 3 & 9) and field excursions (Tables 2 & 4-9) that could be valuable to other researchers. In

Table 1. Non-standard abbreviations. When applicable, hyperlinks have been overlaid on the full names, and more obscure terms are defined in parentheses.

Abbreviation	Full name
AmmonBicarb	ammonium bicarbonate (commonly used to dissolve proteins for mass spectrometry)
AOML	Atlantic Oceanographic and Meteorological Laboratory (Miami, FL, USA)
BIOT	British Indian Ocean Territory (i.e., Chagos)
DEPC	diethyl-pyrocabonate (a potent inactivator of RNAses)
DESS	DMSO+EDTA+NaCl (a solution for DNA preservation)
GBR	Great Barrier Reef (Australia)
GRE	Global Reef Expedition
HSS	high salt solution (used for superior precipitation of RNA)
ISRS/ICRS	International Society for Reef Studies (ISRS; DBA International Coral Reef Society)
iTRAQ	isobaric tags for relative and absolute (protein) quantification
LOF	Living Oceans Foundation
MassIVE	Mass spectrometry interactive virtual environment (San Diego, CA, USA)
MS	mass spectrometry
NCEI	National Centers for Environmental Information (Boulder, CO, USA)
NF	not featured (in tables, figures, or article text)
NGS	next-generation (RNA or DNA) sequencing
NMMBA	National Museum of Marine Biology and Aquarium (Checheng, Pingtung, Taiwan)
NOAA	National Oceanic and Atmospheric Administration (Washington, D.C., USA)
Northwestern	Northwestern University (Evanston, IL, USA)
NUS	National University of Singapore
PWII	protein wash II: 95% ethanol with 2.5% glycerol
RSMAS	Rosenstiel School of Marine and Atmospheric Sciences (Miami, FL, USA)
RT	room temperature
SCS	South China Sea (typically referring to Taiwan's Dongsha Atoll)
SEM	(prepared for) scanning electron microscopy
SmithBioRep	National Museum of Natural History's Biorepository (Washington, D.C., USA)
SCTLD	stony coral tissue loss disease
TEAB	triethylammonium bicarbonate (a common dissolution buffer in proteomics)
TEM	(prepared for) transmission electron microscopy
TMT	tandem mass tags (a proteomic technology developed by Thermo-Fisher Scientific)
TORI	Taiwan Ocean Research Institute (Kaohsiung County, Taiwan)
UCSD	University of California, San Diego (San Diego, CA, USA)
UMBP	University of Miami-Bascom Palmer Eye Institute (Miami, FL, USA)

the instances of many of the samples collected from the field, the respective coral colonies have indeed perished on account of climate change-induced or local anthropogenic stressors (Chen *et al.*, 2022). These biopsies (or the molecules extracted from them), then, represent the only hard evidence of their existence (with photographs & other data providing “softer” evidence). As seawater conditions continue to change, eliciting both ephemeral and sustained physiological changes in all manner of marine organisms (Enochs *et al.*, 2020), future generations may wish to peer into the cells of these corals to see how they functioned in the years before their milieu was fundamentally altered on account of human activities (*sensu* Mayfield & Lin, 2022).

Data & sample access

All data found within the published articles associated with/describing the archived samples are freely available either through the journal article itself (normally accessed via the online/electronic supplemental material); public, open-access data archives (e.g., Dryad); or the coral health data hub coralreefdiagnostics.com. Proteomic data have been deposited on the University of California, San Diego’s (UCSD) “[MassIVE](#)” data repository, which is cross-listed with [Proteome Xchange](#). Images of sampled corals in the case of the

Orbicella faveolata field experiment (Table 9) are hosted on NOAA’s National Centers for Environmental Information ([NCEI](#)) repository; several of the proteomic datasets deposited at MassIVE are also archived on NCEI (see below). The samples themselves (as biopsies or macromolecular extracts) can be requested a number of ways, the easiest of which being sending an email to anderson@coralreefdiagnostics.com. Secondly, you may fill out the online form on <https://coralreefdiagnostics.com/sample-sharing>. Third, consult Table 10 for the contact information of the individuals overseeing the respective biorepositories.

Concluding remarks

The expense, human effort, and carbon footprint of repeating an experiment or field expedition are immense, making it increasingly more difficult to justify overall cost with the predicted long-term benefit with respect to scientific discovery and/or coral reef conservation. For certain, return visits to sites of interest *should* be undertaken if and when possible, with data preferably taken by non-invasive means (e.g., images or diver-scribed notes; Lin *et al.*, 2018). Ideally, locals in the nearby vicinity would tackle such objectives since the associated carbon costs would inherently be far lower than a scientist flying in from abroad. And if a shoddy experiment is conducted, one

lacking in controls (e.g., Seveso *et al.*, 2017)? In this case, the experiment could very well be repeated. Those carrying out population genetic analyses or otherwise analyses of DNA, however, would do well to request samples from those with them already in hand. This is surely less glamorous than collecting the samples for one's self in regions of utter beauty (Figure 6), but reproducing efforts to obtain them given the costs mentioned above is not providing a net benefit to the coral reef ecosystems we are aiming to ultimately protect through these monumental efforts. It is my hope, then, that the archived samples presented herein will not only

benefit future coral reef research but lessen environmental impacts in the process.

Acknowledgments

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Figure 5. A hard coral-dominated reef off the west coast of the Republic of Palau. Photograph taken by the author at 10-15 m.

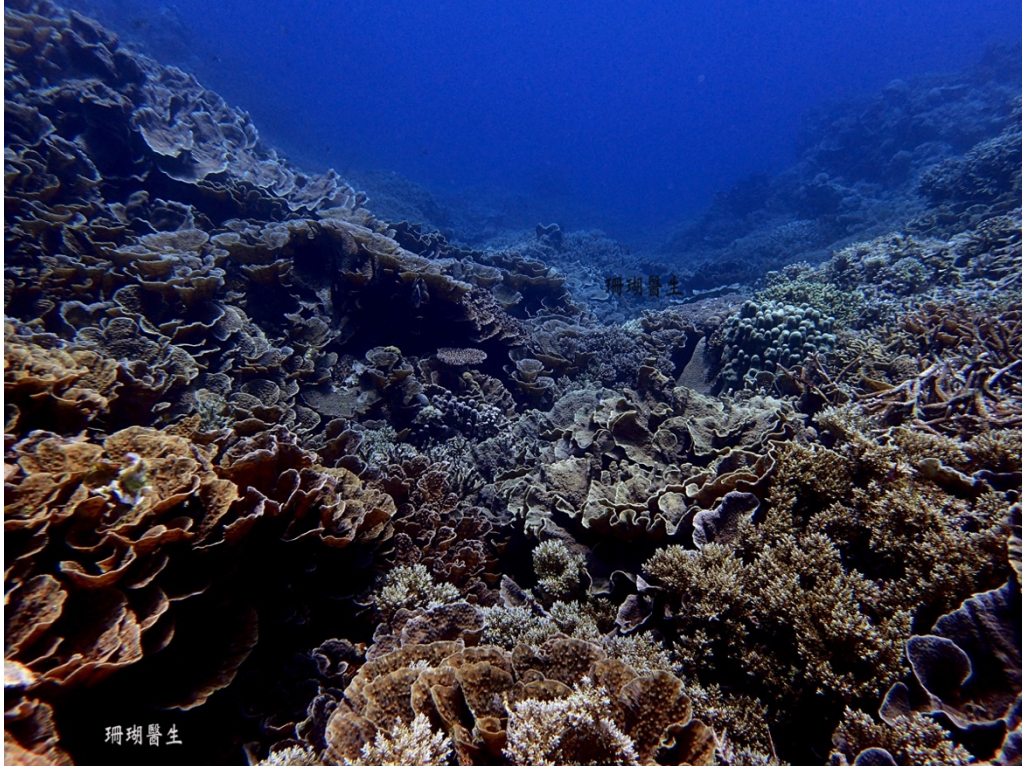


Figure 6. A coral community overgrowing a lava flow in [Maluku, Indonesia \(Banda Islands\)](#). This image constitutes the background of [coralreefdiagnostics.com](#), the website archiving all imagery and other data associated with the coral samples described herein. Watermark translation = “Dr. Coral.”

Table 2. Summary of samples and data collected to date. Unless otherwise noted, all samples were collected *in situ* between 2008 and 2022 (with rows ordered from earliest to most recent). See subsequent tables for details on the samples themselves. NA=not applicable (only imagery & survey data collected). NF=not featured in main text tables.

Location	Taxa	Images	Tissues	Tissue cassettes	RNA	DNA	Protein	Other	Table
Taiwan (mainland)^a	<i>Pocillopora</i> spp.	✓	✓	✓	✓	✓	✓	SEM & TEM	3
Taiwan (mainland)^a	<i>Seriatopora</i> spp.	✓	✓	✓	✓	✓	✓		3
French Polynesia	<i>Pocillopora</i> spp.	✓	✓	✓	✓	✓	✓	TEM	4
Cook Islands	<i>Pocillopora</i> spp.	✓	✓	✓	✓	✓	✓	TEM	4
Fiji	<i>Pocillopora</i> spp.	✓	✓	✓	✓	✓	✓		5
Tonga	<i>Pocillopora</i> spp.	✓	✓	✓	✓	✓	✓		5
New Caledonia	<i>Pocillopora</i> spp.	✓	✓	✓	✓	✓	✓		6
Australia (GBR)	NA	✓							NF
Solomon Islands	<i>Pocillopora</i> spp.	✓	✓	✓	✓	✓	✓		6
Solomon Islands	<i>Seriatopora</i> spp.	✓	✓	✓	✓	✓	✓		6
Palau	<i>Pocillopora</i> spp.	✓	✓	✓	✓	✓	✓		7
Palau	<i>Seriatopora</i> spp.	✓	✓	✓	✓	✓	✓		7
Chagos (BIOT)	<i>Pocillopora</i> spp.	✓	✓	✓	✓	✓	✓		8
Chagos (BIOT)	<i>Seriatopora</i> spp.	✓	✓	✓	✓	✓	✓		8
Maldives	<i>Pocillopora</i> spp.	✓	✓						8
Maldives	<i>Acropora</i> spp.	✓	✓						8
Taiwan (SCS)	<i>Pocillopora</i> spp.	✓	✓ ^d						NF
Florida^b	<i>Orbicella faveolata</i>	✓	✓		✓	✓	✓	lipids+metabolites	9
Florida^c	<i>Acropora cervicornis</i>	✓	✓		✓	✓	✓	lipids+metabolites	9

^aLab samples only (mesocosms). ^bMix of microcosm & field samples. ^cLab samples only (microcosms). ^dn=28 biopsies.

Table 3. Samples from experimental studies carried out at Taiwan’s National Museum of Marine Biology and Aquarium (NMMBA) with the model corals *Pocillopora acuta* and *Seriatopora hystrix* between 2008 and 2020. Studies denoted by asterisks (*) feature corals that were stressed (e.g., bleached) or died over the course of the study on account of the imposed stressor regime. “Tissue-molecular” and “Tissue-micro” correspond to tissues preserved for molecular (e.g., TRIzol™) and microscopic (e.g., paraffin) analyses, respectively. Note that NMMBA is a hub of coral reef research, and this list does not reflect myriad experimental studies performed there on other symbiotic anthozoans (e.g., Chen *et al.*, 2015, 2017; He *et al.*, 2023) nor data from local field monitoring (Ye *et al.*, 2023; the exception being Dongsha Atoll, South China Sea [SCS], whose samples are briefly described in Table 2). NA=not applicable.

Target species	Temp. treatment (°C)	High pCO ₂ (µatm)	Time-scale	Tissue-molecular	Tissue-micro	R N A	D N A	Protein	Reference(s)/data repository
<i>S. hystrix</i>	NA	NA	hours			✓	✓		Mayfield <i>et al.</i> (2010, 2012b)
<i>S. hystrix</i>	27 vs. 30	NA	hours			✓	✓		Mayfield <i>et al.</i> (2011, 2014b)
<i>S. hystrix</i>	26 vs. 23-29 over 6-hr	NA	days	✓		✓	✓	✓ ^d n=12	Mayfield <i>et al.</i> (2012a, 2013c, 2014b, 2016b-c, 2018a), Mayfield (2016, 2020b); MassIVE accession: MSV000085863 ; NCEI accession: 0216077
<i>P. acuta</i>	26 vs. 29 ^a	415 vs. 635	days			✓	✓		Putnam <i>et al.</i> (2013)
<i>P. acuta</i>	26.5 vs. 29.7	NA	months	✓	✓	✓	✓	✓ ^c	Mayfield <i>et al.</i> (2013b, 2014d, 2018b)
<i>P. acuta</i>	31.5-sustained*	NA	weeks			✓	✓	✓	Mayfield <i>et al.</i> (2013a, 2014a)
<i>P. acuta</i>	26 vs. 29	415 vs. 850	weeks	✓	✓	✓	✓	✓ ^d n=9	Mayfield <i>et al.</i> (under review) MassIVE accession: MSV000085868
<i>P. acuta</i>	31.5-return to ambient at night	NA	weeks		✓		✓		Mayfield <i>et al.</i> (2013a)
<i>P. acuta</i>	25	400 vs. 1,000	months	✓					Liu <i>et al.</i> (2020)
<i>S. hystrix</i>	25	400 vs. 1,000	months	✓					Liu <i>et al.</i> (in prep.)
<i>P. acuta</i>	25, 28, or 31	400 vs. 800 ^b	months	✓					Liu <i>et al.</i> (in prep.)
<i>P. acuta</i>	26 vs. 30	NA	months						McRae <i>et al.</i> (in prep.)
<i>P. acuta</i>	26 vs. 32	NA	hours						McRae <i>et al.</i> (in prep.)
<i>P. acuta</i>	26 vs. 30 ^c	NA	months			✓	✓	✓	McRae <i>et al.</i> (2021); MassIVE accession: MSV000087874

^aLarval study. ^bNutrient effects also tested. ^cTrans-generational study. ^dArchived at SmithBioRep. ^eArchived at UMBP (n=12).

Table 4. Pocilloporid coral samples from French Polynesia's Austral Islands and the Cook Islands. Please see Table 1 for abbreviations and Supplemental table 1 for the exact species sampled (e.g., *Pocillopora damicornis*, *P. acuta*, & others). Note that some sample sizes are estimates for RNAs and DNAs, as some aliquots were used in their entirety during laboratory analysis. All images of the corals *in situ* (n=123) can be found on coralreefdiagnostics.com. Apple Photo libraries of whole-colony and polyp-scale images can be provided as Adobe Lightroom and/or Apple Photo libraries (please send request to anderson@coralreefdiagnostics.com or use the sample request form posted [here](#)).

Sample type	Sample size	Sample condition	Sample storage media	Storage temp.	Location #1	Location #2 (if applicable)	Manuscript(s)
Austral Islands, French Polynesia (Apr. 2013): corals were sampled from Raivavae, Tubuai, Rurutu, & Maria Atoll.							
tissues+skeleton	61	homogenized	RNA Later®	-80°C	NMMBA	RSMAS	Mayfield <i>et al.</i> (2015)
tissues only	28	embedded	paraffin	RT	NMMBA		unanalyzed
RNA	30-60	purified	DEPC-H ₂ O	-80°C	NMMBA	SmithBioRep	Mayfield <i>et al.</i> (2016b, 2019a) 11/7/23 11:02:00 AM
DNA	30-60	purified	Tris (pH 8.5)	-20°C	NMMBA	NUS	Mayfield <i>et al.</i> (2015)
protein	50	precipitated	PWII	-150°C	SmithBioRep		unanalyzed
Cook Islands (Apr.-May 2013): corals were sampled from Rarotonga, Aitutaki, & Palmerston Atoll (see Mayfield <i>et al.</i> , 2015 for details.)							
tissues+skeleton	62	homogenized	RNA Later®	-80°C	NMMBA	RSMAS	Mayfield <i>et al.</i> (2015)
RNA	30-60	purified	DEPC-H ₂ O	-80°C	NMMBA	SmithBioRep	Mayfield <i>et al.</i> (2016b, 2019a)
DNA	30-60	purified	Tris (pH 8.5)	-20°C	NMMBA	NUS	Mayfield <i>et al.</i> (2015)

Table 5. Pocilloporid coral samples from Fiji and Tonga. Please see Table 1 for abbreviations, Supplemental table 1 for the exact species sampled (as well as other sampling data+meta-data), and Table 10 for the individuals in care of the samples at the respective repositories. Image access is as described in Table 4, with select habitat photos found [here](#).

Sample type	Sample size	Sample condition	Storage media	Storage temp.	Location #1	Location #2	Manuscript(s)
Fiji (June 2013): please see Mayfield <i>et al.</i> (2017b) for locations of the sampled corals within Lau Archipelago.							
tissues+skeleton	153	homogenized	RNA Later	-80°C	NMMBA	RSMAS	Mayfield <i>et al.</i> (2017b)
tissues only	33	embedded	paraffin	RT	NMMBA		unanalyzed
RNA	61	purified	DEPC-H ₂ O	-80°C	NMMBA	SmithBioRep	Mayfield <i>et al.</i> (2017b, 2018c)
DNA	90-96	purified	Tris (pH 8.5)	-20°C	NMMBA	NUS	Mayfield <i>et al.</i> (2015)
protein	69	precipitated	PWII	-80°C	SmithBioRep		unanalyzed
Tonga (Sept. 2013): corals were sampled from Ha'apai & Va'vau (see Mayfield <i>et al.</i> , 2017a for details & Dryad for images of colonies.)							
tissues+skeleton	115	homogenized	RNA Later	-80°C	NMMBA	RSMAS	Mayfield <i>et al.</i> (2017a)
tissues+skeleton	73	unhomogenized	RNA Later	-80°C	Northwestern		unanalyzed ^a
RNA	90-97	purified	DEPC-H ₂ O	-80°C	NMMBA	SmithBioRep	Mayfield <i>et al.</i> (2017a, 2021b)
DNA	90	purified	Tris (pH 8.5)	-20°C	NMMBA	NUS	Mayfield <i>et al.</i> (2017a)
protein	106	precipitated	PWII	-150°C	SmithBioRep		unanalyzed

^aWill be analyzed via a variety of microscopic approaches to assess light scattering properties (*sensu* Spicer *et al.*, 2019).

Table 6. Pocilloporid coral samples from Melanesia (New Caledonia & Solomon Islands). Please see Table 1 for abbreviations, Supplemental table 1 for the exact species sampled, and Table 10 for the individuals in care of the samples at the respective repositories. Image access is as described in Table 4. Note that in the Solomon Islands, two coral genera were sampled: *Pocillopora* and *Seriatopora* (mainly a mix of *S. hystrix* & *S. caliendrum*).

Sample type	Sample size	Sample condition	Storage media	Storage temp.	Location #1	Location #2	Manuscript(s)
<u>New Caledonia</u> (Oct.-Nov. 2013): please see Mayfield <i>et al.</i> (2017c) for locations of the sampled corals.							
tissues+skeleton	139	homogenized	RNALater	-80°C	NMMBA	RSMAS	Mayfield <i>et al.</i> (2017c)
tissues+skeleton	36	unhomogenized	RNALater	-80°C	Northwestern		unanalyzed ^a
tissues only	28	embedded	paraffin	RT	NMMBA		unanalyzed
RNA	120	purified	DEPC-H ₂ O	-80°C	NMMBA		Mayfield & Dempsey
DNA	120	purified	Tris (pH 8.5)	-20°C	NMMBA	NUS	(2022)
protein	120	precipitated	PWII	-80°C	NMMBA		unanalyzed
<u>Solomon Islands</u> (Sept. 2015): please see Mayfield <i>et al.</i> (2022a) for locations of the sampled corals.							
<i>Genus Pocillopora</i>							
tissues+skeleton	126	homogenized	TRIzol	-80°C	NMMBA		Mayfield <i>et al.</i> (2022b)
tissues+skeleton	76	unhomogenized	RNALater	-80°C	Northwestern		unanalyzed ^a
RNA	120	purified	DEPC-H ₂ O	-80°C	NMMBA		Mayfield <i>et al.</i> (2022b)
DNA	120	purified	Tris (pH 8.5)	-20°C	NMMBA	NUS	Mayfield <i>et al.</i> (2022b)
DNA	120	diluted	Tris+water	-20°C	RSMAS		partially analyzed
protein	120	precipitated	PWII	-80°C	NMMBA		unanalyzed
<i>Genus Seriatopora</i>							
tissues+skeleton	200	unhomogenized	RNALater or 80% ethanol	-80°C	TORI		unanalyzed

^aWill be analyzed via a variety of microscopic approaches to assess light scattering properties.

Table 7. Pocilloporid coral samples from Micronesia (Palau). Please see prior tables for abbreviation and Supplemental table 1 for the exact species sampled. Note that while 185 biopsies were archived, they were from only 150 pocilloporid coral colonies (i.e., 35 colonies were sampled twice.).

Sample type	Sample size	Sample condition	Storage media	Storage temp.	Location #1	Location #2	Manuscript(s)
<u>Palau</u> (Jan.-Feb. 2015): please see coralreefdiagnostics.com for sample collection locations and other details.							
Genus <i>Pocillopora</i>							
tissues+skeleton	185	unhomogenized	RNALater & TRIzol	-80°C	NMMBA	RSMAS	Mayfield & Dempsey (in prep.)
tissues only	35	embedded	paraffin	RT	NMMBA		unanalyzed
RNA	120	purified	DEPC-H ₂ O	-80°C	NMMBA		Mayfield & Dempsey (in prep.)11/7/23 11:02:00 AM
DNA	160	purified	Tris (pH 8.5)	-20°C	NMMBA	NUS	Wainwright <i>et al.</i> (in prep.)
DNA	160	diluted	Tris+water	-20°C	RSMAS		
protein	160	precipitated	PWII	-80°C	NMMBA		unanalyzed
Genus <i>Seriatopora</i>							
tissues+skeleton	217	unhomogenized	75% ethanol	-80°C	TORI		unanalyzed

Table 8. Pocilloporid coral samples from the Indian Ocean (Chagos & the Maldives). Please see prior tables for abbreviations and Supplemental table 1 for the exact species sampled. In the case of the Maldives samples, the vast majority of the source colonies perished on account of back-to-back bleaching events that occurred in 2016 and 2017.

Sample type	Sample size	Sample condition	Storage media	Storage temp.	Sample location	Manuscript(s)
Chagos (i.e., BIOT; March-May 2015): see coralreefdiagnostics.com for sample collection locations and other details.						
Genus <i>Pocillopora</i>						
tissues+skeleton	166	unhomogenized	RNA Later & TRIzol	-80°C	NMMBA	Mayfield <i>et al.</i> (in prep.)
tissues only	70	embedded	paraffin	RT	NMMBA	unanalyzed
RNA	150	precipitated	isopropanol	-80°C	NMMBA	Mayfield <i>et al.</i> (in prep.) 11/7/23 11:02:00 AM
DNA	150	purified	Tris (pH 8.5)	-20°C	NMMBA ^a	Wainwright <i>et al.</i> (in prep.)
DNA	150	diluted	Tris+water	-20°C	RSMAS	unanalyzed
protein	150	precipitated	PWII	-80°C	NMMBA ^b	unanalyzed
Genus <i>Seriatopora</i>						
tissues+skeleton	174	unhomogenized	DESS	-80°C	TORI	unanalyzed
tissues+skeleton	30	homogenized	TRIzol	-80°C	NMMBA	unanalyzed
RNA	27	purified	DEPC-H ₂ O	-80°C	NMMBA	unanalyzed
DNA	27	purified	Tris (pH 8.5)	-20°C	NMMBA	unanalyzed
protein	24	precipitated	PWII	-80°C	UMBP	unanalyzed
Maldives (Jan. 2016 & mid-2017): see coralreefdiagnostics.com for sample collection locations and other details.						
Genus <i>Pocillopora</i> -2016 (pre-bleaching event)						
tissues+skeleton	85	unhomogenized	TRIzol	-80°C	NMMBA	unanalyzed
tissues+skeleton	19	unhomogenized	RNA Later	-80°C	Northwestern	unanalyzed
Genus <i>Acropora</i> -2016 (pre-bleaching event)						
tissues+skeleton	82	unhomogenized	TRIzol	-80°C	NMMBA	unanalyzed
tissues+skeleton	18	unhomogenized	RNA Later	-80°C	Northwestern	unanalyzed
Genus <i>Pocillopora</i> -2017 (post-bleaching)						
tissues+skeleton	58	unhomogenized	RNA Later	-150°C	SmithBioRep	unanalyzed
Genus <i>Acropora</i> -2017 (post-bleaching)						
				-10°C	SmithBioRep	unanalyzed

^aA subset is also at NUS. ^bA subset of 39 is also at UMBP.

Table 9. Massive coral samples (*Orbicella faveolata*) from [Florida](#) (USA). See prior tables for abbreviations. Online locations of imagery and proteomic data are provided as hyperlinks overlaid upon the accession numbers. Proteins from six *Acropora cervicornis* genotypes exposed to a temperature challenge study (n=89 over the two-week experiment; Shaw *et al.*, in prep.) are also stored at UMBP (analyzed via Thermo-Fisher Scientific’s “tandem mass tag” [TMT] labeling followed by nano-liquid chromatography+mass spectrometry [MS]). Sample sizes in parentheses in right-most column are shown when number differed from that shown in the “Sample size” column. *Aliquots also stored at AOML.

Sample type	Sample size	Sample condition	Storage media	Storage temp.	Sample location	Manuscript(s)/biorepository accession
<i>Orbicella faveolata</i> -laboratory experiments (described in Mayfield <i>et al.</i> , 2021a)						
tissues+skeleton	92	homogenized	none	-80°C	AOML	Mayfield (2022)
RNA	92	purified	DEPC-H ₂ O	-80°C	AOML	Aguilar <i>et al.</i> (unpublished)
RNA	40	precipitated	isopropanol+HSS	-150°C	SmithBioRep*	
DNA	41	precipitated	isopropanol	-150°C	SmithBioRep*	Manzello <i>et al.</i> (2019)
protein	46	precipitated	PWII	-80°C	SmithBioRep*	MassIVE accession: MSV000086098 ; Proteome Xchange accession: PXD021349
protein-shotgun proteomics	16	various	PWII	-80°C	SmithBioRep*	MassIVE accession: MSV000086530 (n=16); Proteome Xchange accession: PXD022796 ;
protein-iTRAQ	21	precipitated	PWII	-80°C	SmithBioRep*	NCEI accession: 0242879 (n=21)
protein-iTRAQ	41	purified	TEAB	-80°C	UMB*P	Manzello <i>et al.</i> (in prep.) ^a
<i>Orbicella faveolata</i> -field experiment (described in Mayfield & Lin, 2023)						
tissues+skeleton	124	preserved	TRIZol	-80°C	AOML	NCEI accession: 0243645 (<i>in situ</i> images)
tissues+skeleton	124	preserved	RNALater	-80°C	AOML	
RNA	42	precipitated	isopropanol	-80°C	AOML	
DNA	42	precipitated	isopropanol	-80°C	AOML	
protein	42	precipitated	PWII	-80°C	cannot find*	MassIVE accession: MSV000089240 ;
protein-iTRAQ	36	purified	TEAB	-80°C	cannot find*	NCEI accession: 0254274 (*Omic data)

^aSamples from an unpublished reciprocal transplant study (n=35 out of 41 proteins analyzed via iTRAQ).

Table 10. Contact information of the biorepositories. As this information is subject to change, please first email me at anderson@coralreefdiagnostics.com, and I will identify the best point-of-contact. See Table 1 for full names of institutes, as well as hyperlinks to institute websites.

Biorepository	Location	Samples	Contact person/people	Contact email
<i>Asia</i>				
NMBA	Pingtung, Taiwan	See Tables 2-3.	Dr. Chiahsin Lin	chiahsin@nmmba.gov.tw
NUS	Singapore	See Tables 3-8.	Dr. Benjamin Wainwright	ben.wainwright@yale-nus.edu.sg
TORI	Kaohsiung, Taiwan	See Tables 3 & 6-8.	Dr. Chien-Hsun Chen	chienhsun@narlabs.org.tw
<i>United States of America</i>				
Northwestern	Chicago, IL	See Tables 5-6 & 8.	Dr. Luisa Marcelino	l-marcelino@northwestern.edu
RSMAS	Miami, FL	See Tables 4-8.	Dr. Nikki Traylor-Knowles	ntraylorknowles@rsmas.miami.edu
SmithBioRep	Washington, D.C.	See Tables 3-5 & 8-9.	Current lab manager	NMNHBiorepository@si.edu
UMBP	Miami, FL	See Tables 2-3 & 8-9.	Dr. Pei-Ciao Tang Dr. Sanjoy Bhattacharya	pct286@miami.edu sbhattacharya@med.miami.edu

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