

# New Developments in Reef Coral Biotechnology

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The increasing occurrence of marine heatwaves, and the coral bleaching events they elicit, pose a significant threat to coral reef ecosystems [1], affecting marine biodiversity, ecosystem services, and the livelihoods of communities dependent on these lush habitats [2]. The aim of this Special Issue, entitled “New Developments in Reef Coral Biotechnology”, was to compile a series of research projects that employed state-of-the-art technologies or monitoring approaches to (1) understand the implications of climate change on coral reefs, (2) develop tools to identify resilient corals, and (3) conserve and/or biopreserve reef-building corals. With respect to the first topic, Ye et al. [3] provided a comprehensive, multi-year account of a catastrophic bleaching event in southern Taiwan’s Hengchun Peninsula, and by conducting surveys in both natural and artificial reefs exposed to dramatically differing oceanographic conditions, they were able to gain a better grasp on the eco-physiological factors that govern patterns of heterogeneity observed in the coral response to elevated temperatures in situ. The cooling effects of typhoons, which previously mitigated bleaching, were absent, marking the most severe bleaching event in Taiwan’s history [4]. Although some areas in the vicinity were severely impact by this same marine heatwave [5], others were not [6].

Approximately two-thirds of coral tissue was bleached in September, and one-third remained bleached by December 2020. Coral cover in shallow natural reefs failed to recover fully, with non-upwelling and upwelling reefs showing only around 20% cover (compared to pre-bleaching levels of 40%). Even in cases where coral abundance recovered, the most dominant species changed as a result of the bleaching event [7], and some species went virtually extinct at the local scale. Although some reefs weathered the bleaching event better than others, especially those corals at greater depths (which experienced nearly 10-fold less bleaching than those at 3 m), the marine heatwave fundamentally altered the reef community of Nanwan Bay. The silver lining is that it was made abundantly clear that the unique oceanography of the incredibly high-biodiversity reefs of southern Taiwan signifies that this continues to be an ideal “natural laboratory” for those interested in better understanding how changes in the Earth system as a result of climate change culminate in complex ecological modifications of the reef community at smaller (m–km) spatial scales.

It was clear from the work of Ye et al. [3], as well as a plethora of prior research, that, although rising seawater temperatures are certainly the biggest threats to coral reefs, resilient corals do exist; such colonies, genotypes, and/or species should clearly, then, be those cultivated for reef restoration efforts [8]. Current methods for assessing coral climate resilience rely on the retroactive observation of bleaching in stressed individuals during surveys or lab experiments [9,10]. However, by the time resilient corals have been identified in this post hoc matter, many others will have died; would it not be better to grasp an understanding of the stress loads, and environmental tolerance, of corals prior to their demise? Then, perhaps something could be done, such as a “coral reef intervention” or “coral rescue” [11], to improve the odds of survival for stress-susceptible corals.

Towards the building of the capacity for just this sort of “coral reef triage”, my colleague Dr. Anderson Mayfield of the Coral Research and Development Accelerator Plat-



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form and I took a proteomic and machine learning approach to identify biomarker patterns/signatures indicative of high-temperature tolerance in the once-common Caribbean reef-builder *Orbicella faveolata* along the Florida Reef Tract [12]. We sought to leverage breakthroughs in both coral reef diagnostics [13–15] with the thoroughly characterized nature of many reefs of the Florida Keys [16–18] whereby we could use the power of hindsight (i.e., “hindcasting”) to ensure that the artificial intelligence (AI) trained in JMP® Pro (USA) could accurately predict the likelihood of future bleaching in select coral colonies from reefs of varying oceanographic conditions that were tracked over space and time. Concentration levels of a subset of only a handful of proteins involved in lipid trafficking, immunity, and other key cellular pathways could be used to predict the likelihood of bleaching in over 90% of the date-tracked colonies based on having trained with AI with a mix of both laboratory [15] and field coral protein biomarker data.

Although this proteomic and AI approach for proactively identifying resilient Floridian corals was highly accurate, the endeavor was costly, both in terms of human hours and research funding [19]. Such a method would be poor for developing countries, where the vast majority of coral reefs are located. To this end, Mayfield et al. [20] built upon a series of prior research on profiling a molecular–physiological response metric known as the “coral health index” (CHI) in a wide range of coral reef habitats in the grossly understudied nation of the Solomon Islands. Although the aforementioned *O. faveolata* project in Florida was mainly looking to model temperature effects, the Solomon Islands project factored a far wider region of environmental variables that were predicted to play a role in driving variation in the bleaching response, not limited to light, salinity, exposure, type of reef, dinoflagellate community composition, benthic community structure, and others [21–23]. Based on running a large number of simulations by the AI, in which portions of the dataset were randomly “hidden” from the AI, a number of predicted models for the CHI were constructed that were of sufficient power to accurately predict the whereabouts of other pocilloporid corals of the region (i.e., those whose CHI data were not incorporated in initial AI training). This means that scientists can use the resulting GUI (web link cited in the article) to plan their research trips to the country, particularly in instances in which they seek to identify highly resilient corals (i.e., those with high CHI values) for use in restoration initiatives or even ad hoc experimental work.

Profiling coral health across space and time, as outlined in the prior sections, is an important step towards adaptive management in the face of climate change and our growing anthropogenic footprint. However, perhaps we should take a step back and ask: “Are we even aware of all the places where corals and other marine invertebrates are common?” Only relatively recently have mesophotic coral ecosystems (MCEs) been identified (typically those at depths ranging from 30 to 150 m, where corals still depend on light [24]), and, to date, no such characterization of these ecosystems has been undertaken in Taiwan’s islands within the Kuroshio current, namely Lanyu (Orchid Island in English). We sought to close this knowledge gap in our published work herein [25], using remotely operated vehicles (ROVs) off Lanyu’s coastline. We observed abundant soft corals (families Nephtheidae, Ellisellidae, and Nidaliidae), as well as select scleractinian families in the upper zone. The lower zone featured these same soft coral families, with the addition of Octocorallia and Plexauridae; some hexacorallians were also observed. A lipid analysis found that MCE corals possess lower lipid content (specifically neutral lipids and triacylglycerols) compared to their shallow-water counterparts. Additionally, the mesophotic scleractinian samples had higher polyunsaturated fatty acid ratios, suggesting that, unsurprisingly, they rely more on heterotrophy. What these findings signify for the future of corals and coral reefs, and whether such MCEs could represent putative refugia from climate change impacts, remains to be determined.

Until it is clear that there are places where corals may continue to thrive despite the myriad environmental impacts working against them, it will be important to continue to develop coral aquaculture technology [26]. Towards this end, Taiwan’s National Museum of Marine Biology and Aquarium (NMMBA) has established long-term cultures, both in

situ and ex situ, of a number of coral species for purposes of tourism, research, and conservation. Although numerous studies have focused on enhancing the settlement, survival, and growth rates of coral larvae, they frequently overlook the critical post-settlement developmental phase; this encompasses the development of polyps and skeleton, the formation of tentacles, and the generation of new individuals. To better understand these processes, we tracked the growth of newly settled recruits of a number of species [27] and found that some species, such as *Galaxea fascicularis* and *Mycedium elephantotus*, demonstrate “skeleton-over-polyp” growth, whereby the skeletons grow around the polyps. In contrast, *Pocillopora verrucosa* and *Seriatopora caliendrum* exhibit “polyp-over-skeleton” trajectories, in which calcium carbonate is cemented beneath the polyps. It was clear that the presence of Symbiodiniaceae dinoflagellates influenced growth (both their identity and density), as has been documented previously [28,29]. We found, specifically, that there was a relationship between tentacle growth and Symbiodiniaceae density, and this relationship was coral-host-species-dependent. Such studies of coral development and growth will be key to optimizing coral culture protocols in situ and ex situ, and, more generally, will increase our understanding of coral biology.

Aside from conserving coral reefs for their beauty and value as habitat to myriad species, many coral-reef-dwelling organisms are thought to be a wellspring of prospective natural products of value to humankind, including drugs, cosmetics, and even pigments. Regarding the latter, marine pigments are safer and more stable and biocompatible than their synthetic alternatives [30], and well-characterized pigments include chlorophylls, carotenoids, and melanin [31]. During asexual coral propagation, NMMBA researchers noticed an intriguing occurrence whereby contact with certain octocoral species led to the skin of operators turning black [32]. This black pigment was characterized from the soft coral *Simularia flexibilis* and was found to be similar to melanin. The study drew inspiration from methodologies used for melanin purification in other marine organisms, prompting future investigations of marine pigments via spectroscopic analysis. Perhaps the newly identified dye could even be adopted for human use, such as for the textile industry.

Over the last decade, coral cryopreservation research has seen significant advancements driven by an interdisciplinary approach that combines concepts and technologies from cryogenic biology, nano-engineering, and cellular biochemistry [33–35]. We now have cryopreservation methods for coral biomaterials such as gametes, tissues, larvae, and symbiotic dinoflagellates, garnering attention from conservationists worldwide. The recent breakthrough in coral cryopreservation is the production of adult corals from cryopreserved larvae with our customized devices [36,37]. However, achieving successful cryopreservation requires the fine-tuning of freezing methods to minimize cryoinjury and maximize success rates. As cryopreservation gains recognition as a reliable strategy for preserving coral biodiversity amid ongoing environmental challenges, it has become imperative to delve into its effects on molecular parameters that influence post-thaw viability. The consequences of cryopreservation on physiological process, lipid content, molecular expression, and ultrastructure changes within coral biomaterials are evident [38–43]. Transmission electron microscopy (TEM) has proven invaluable in assessing cryoinjury, offering critical insight for refining protocols [44]. Recent investigations employing TEM have illuminated the intricate ultrastructural changes within coral larvae, underscoring the detrimental effects of chilling and the remarkable preservation achieved through vitrification and nanoparticle laser-warming. Notably, the incorporation of lipid supplementation in vitrification solutions has emerged as a promising strategy, capable of maintaining the dimensions of microvilli and flagella. These discoveries, including those discussed in the prior sections, have broad implications for coral and coral reef conservation and the preservation of biodiversity.

**Conflicts of Interest:** The authors declare no conflict of interest.

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