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Conference Report

The 2nd International Symposium on New Frontiers in Reef Coral Biotechnology (12 May 2023, Taiwan)

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Abstract: For the second year in a row, the theme is "reef coral biotechnology", specifically the interface between basic science and conservation. It has never been more important to attempt to leverage what we know about these beautiful, albeit highly imperiled and fragile, ecosystems towards conserving them. Our invited speakers' areas of expertise span all levels of biological organization: from molecules within coral cells, to coral tissues, to entire coral colonies, and then up to reef-scale processes. Our goal is to promote communication not only among local Taiwanese marine biologists, but also those within Southeast Asia and farther afield; we especially encourage participation from early-career researchers, including Master's students, PhD candidates, and post-doctoral researchers. It is our hope that the presentations (and the discussions that follow) will encourage collaboration. As importantly, we envision that the tools and approaches shared amongst us can be tapped into to expedite our collective efforts to better understand, manage, and conserve coral reefs.

Keywords: artificial intelligence; coral restoration; endosymbiosis; health monitoring; cryopreservation; husbandry

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1. Genomic Signatures Underlying the Adaptability and Resilience of Scleractinian Corals

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Abstract

Corals today face increasingly challenging conditions, including rising ocean temperatures brought about by climate change. To gain insight into how phylogenetically divergent species may respond to thermal stress events, we compared the gene complement of four coral species, *Acropora digitifera*, *Favites colemani*, *Montipora digitata*, and *Seriatopora caliendrum*, that were previously shown to have different sensitivities to acute thermal stress. The comparison of gene repertoires in the four scleractinian corals revealed an extensive set of cnidarian stress response genes (SRGs). Antioxidant protein families and chaperones were relatively more abundant in *F. colemani* and *M. digitata*; proteins linked to immunological functions were enriched in *S. caliendrum* and *A. digitifera*; and extracellular matrix components were abundant in *S. caliendrum* and *F. colemani*. The species that showed greatest heat susceptibility, *S. caliendrum*, also exhibited drastic upregulation of SRGs under acute thermal stress. This suggests that differences in SRGs, as well as the mechanisms that control SRG expression response, contribute to the ability of corals to maintain stable physiological functions and survive shifts in seawater temperature.

2. Data-Driven Marine Conservation in the Coral Triangle

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Abstract

Due to the plethora of threats to coral reef ecosystems, marine biologists have been attempting to both document reef decline and devise conservation solutions that could thwart coral extinction or partially restore ecosystem function. In most cases, local-scale stressors are not adequately addressed prior to initiating these projects, not because researchers do not appreciate their impact but because, in many cases, it is not possible to do so on a timescale that is commensurate with coral rescue. What this means, though, is that many coral reef conservation initiatives are doomed to fail from the start, notably many

restoration projects that seek to grow corals for later outplanting on the reef; if temperatures continue to rise, and seawater quality continues to deteriorate, only those projects that stress-harden, genetically modify, or, more generally, modulate the underlying biology of the farmed corals have any hope of success. Ideally, a manager would collect some rudimentary data on the habitat in question, then use statistical tools to determine the conservation approach that has the highest probability of success due to what is known about the habitat in question. This can be achieved with machine learning, a modeling approach based on artificial intelligence (AI), though the marine biology field has generally been slow to leverage breakthroughs in such "big data" analytics that have revolutionized other disciplines, such as advertising. I discuss a "coral rescue" flow chart and show how an AI could be trained to robustly project the conservation approach with the highest probability of success (e.g., maximum coral cover, longest coral colony lifespan, etc.).

3. Rehabilitating Reef Rehabilitation: Applying Scientific Knowledge to Improve Reef Restoration Practice in Indonesia

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Abstract

Indonesia's coral reefs have been severely damaged by global and local stressors, and a range of active restoration techniques are now used in attempts to rebuild degraded reefs. However, it is difficult to summarize Indonesia's restoration efforts due to a lack of consistent reporting. Here, we first discuss Indonesia's legal policy framework concerning reef restoration. We then provide an extensive review of reef restoration projects in Indonesia, documenting 533 records between 1990 and 2020. Most (73%) of these records come from the past 10 years, and many (42%) are reported in online news articles. This review identified 120,483 units of artificial reef installed across Indonesia, along with 53,640 units of coral transplantation (including both coral nurseries and direct out-planting onto reefs); in total, 965,992 fragments of hard coral have been planted across Indonesia. The most favoured restoration materials are concrete (46%) and steel structures (24%). Projects are organized by a diverse range of governmental, NGO, private and community-led organizations. This review demonstrates that Indonesia's policy has encouraged a diverse range of practitioners to implement reef restoration, but projects are often not coordinated with wider networks of restoration practitioners or scientists, and only 16% of the identified projects included a post-installation monitoring framework. Incorporating clear objectives and long-term monitoring programmes in project planning stages, while prioritizing knowledge exchange and engagement with the international scientific community, will substantially improve restoration outcomes in Indonesia. This will allow the country to fulfill its considerable potential as a global leader in rebuilding damaged coral reefs.

4. Importance of Lipid Droplets in Endosymbiotic Marine Dinoflagellate Associated with Corals

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Abstract

The significance of intracellular lipid droplets (LDs) was crucial in the mutualistic endosymbiosis between reef-building corals and the dinoflagellate endosymbiont *Symbiodinium* spp. In this study, the researchers aim to investigate and identify LD proteins present in Symbiodinium residing within the coral host. To achieve this, various methods, including Discontinuous Percoll gradient centrifugation, were utilized to separate Symbiodinium cells from *E. glabrescens* tentacles. Additionally, the use of Nile red, a fluorescent probe, demonstrated the accumulation of lipids in the freshly isolated Symbiodinium cells. We successfully purified stable LDs from the endosymbiotic Symbiodinium cells. The structural integrity of the Symbiodinium LDs was maintained through mechanisms such as electronegative repulsion and steric hindrance, potentially facilitated by their surface proteins. Protein extracts obtained from the purified LDs revealed a prominent protein band referred to as Symbiodinium lipid droplet protein (SLDP). This study illuminates the importance of lipid droplets in the endosymbiotic dinoflagellate and their potential role in the symbiotic relationship between Symbiodinium and reef-building corals.

5. Low-Technology In-Vitro Cultivation of Small Corals Fragments: A Model System for Clone Production towards Developing Quality R & D

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Abstract

Most of the profound findings in the past investigations of corals have focused on understanding its aspects of biology vis-a-vis growth-reproduction-dysfunction where the dynamics of the coexisting host animal and symbiotic algae are crucially challenged by stress and other factors. In experimental studies where several trials are conducted in controlled conditions, it is sometimes necessary to establish a knowledge on how to produce coral cultures as samples to lessen the extraction of healthy colonies from the reef. In such an event, developing a plausible culture technique holds the key towards capability in propagating this highly subtle species. In this study, the method of inducing tissue regeneration in in-vitro systems for scleractinian coral *Pocillopora damicornis* is essentially discussed. Following the procedure that was first established in 2014, numbers of isolated tiny fragments excised from small coral colonies had successfully grown just in glass dishes with raw seawater at room conditions. These regenerating clones were then utilized as model samples in observing the growth, cell division, and dysfunction of coral host and symbiotic cells revealed by light and electron microscopes. With the significant results of the past experiments derived from utilizing these cultures, this study had demonstrated and further promote a low-technology system that may produces mass number of viable clones for various academic and scientific R & Ds, as well as community-based undertakings, including coral biotechnology, reef restoration, husbandry, MPA conservation, and several others. However, there are still uncertainties in the success of propagation for other reef-building species. Hence, this remains a challenging task in future studies.

6. Proteomic Analysis of *Acropora cervicornis* Exposed to Thermal Acclimation

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Abstract

Continuous warming of the oceans has led to increased bleaching events and higher levels of disease susceptibility. Current scientific efforts have predominately focused on utilizing transcriptomics to understand thermal stress responses. These efforts have shown that corals possess plasticity in their response to thermal stress, with certain genotypes responding well and surviving longer after an initial heat acclimation period. To better understand the mechanisms by which acclimation occurs, the proteomic response to thermal acclimation and heat stress of previously studied genotypes was analyzed. The three least and most plastic genotypes from a prior study were utilized. Fragments of each genotype were acclimated at 30 °C for four days, returned to ambient temperatures (27 °C) for five days, and then heat stressed at 32 °C for 2 days. Samples for proteomic analysis were taken on Day 0, before the experiment began, and then at each temperature transition. Data will be shown regarding proteomic changes throughout the acclimation process and subsequent heat stress. Comparisons among genotypes illustrate differences in thermal acclimation abilities.

7. Heat Tolerance and Symbiodiniaceae Profiles of Acroporids in a Philippine Reef

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Abstract

The warming of the oceans is decimating coral reefs worldwide. Yet it has been observed that a proportion of coral populations from the same general reef area, experiencing a similar thermal regime, are able to tolerate heat stress and endure. To understand the mechanisms underlying coral thermal tolerance, we subjected fragments taken from 30 colonies of *Acropora digitifera*, *A. millepora*, and *A. tenuis* to 33 °C (high temperature) versus 29 °C (control) for about a week (~2 DHW). Our results revealed inter-individual differences in heat tolerance in all three coral species, with 70% of *A. digitifera*, 50% of *A. millepora*, and 30% of *A. tenuis* colonies showing high tolerance to elevated temperature (i.e., no bleaching). However, the analysis of associated microalgal symbionts in these corals using ITS2 sequencing did not demonstrate a clear correlation between differences in heat tolerance and Symbiodiniaceae composition, suggesting that host-specific factors should also be examined.

8. The First Cryo-Repository for Coral Larvae: Safeguarding Corals for Future Generations

Kanokpron Loeslakwiboon [1], Wen-Chung Hsieh [2], Cheng-Liang Huang [3], Sujune Tsai [4] and Chiahsin Lin [1,5,*]

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Abstract

Research on the development of innovative cryobanking techniques will aid coral restoration and conservation. To date, there are no published studies on long-term cryo-repository of coral larvae. The aim of this study was to apply our customized freezing device and cryojig together with vitrification and laser warming techniques to create the first cryo-repository for coral larvae. In this study, pelagic phase larvae from the corals *Seriatopora caliendrum*, *Pocillopora verrucosa*, *P. acuta*, and *Stylophora pistillata* were used for cryobanking. Three vitrification solutions were formulated with Ficoll and gold nanoparticles. The results showed that over a thousand coral larvae of *S. caliendrum*, *P. verrucosa*, *P. acuta*, and *St. pistillata* were successfully stored in the cryo-repository. Our customized innovative technology enabled the long-term cryobanking of coral larvae, which has never been accomplished before. We believe the methods applied in this study have the potential to be a critical research and conservation tool for wild reef restoration and reef habitat diversity.

9. Coral Assessment in Selected Areas in the Verde Island Passage

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Abstract

The Verde Island Passage is one of the most biologically diverse ecosystems in the world. It provides food, livelihood, and other benefits to communities in and around its waters. However, previous studies conducted here were fragmentary and site-specific. This study addressed this gap by examining the coral genera and distribution along four (4) provinces in the Verde Island Passage: Batangas, Marinduque, Occidental Mindoro, and Oriental Mindoro using the photo transect method. Data were collected during the northeast monsoon, summer, and southwest monsoon seasons. Each province was assigned (2) sites classified into highly impacted (HI) and less-impacted (LI) sites. For HI and LI sites, results showed an average of 21.77% and 27.78% coral cover, 12.70% and 11.98% macroalgae cover, 24.87% and 30.01% bare rock, 32.06% and 22.89% sand/rubble, respectively. An average of 25 coral genera were found in Batangas but were mostly dominated by Galaxea sp. in the highly impacted site and Porites sp. in the less-impacted site. Marinduque recorded 16 genera, where Porites sp. dominated both highly impacted and less-impacted sites. Occidental Mindoro recorded 18 genera, where Porites sp. also dominated both highly impacted and less impacted sites. Lastly, Oriental Mindoro recorded 14 genera where Galaxea sp. dominated the highly impacted site and Porites sp. dominated the less-impacted site. Porites sp. Dominance in most of the sites may be linked to their ability

to withstand strong wave action and be more tolerant of stressors and environmental extremes, such as sedimentation and temperature increase. In general, most of the sites in all four provinces had good coral cover, including several non-MPA sites. This justifies their protection, conservation, and possibly elevation of their status to Marine-Protected Areas.

10. Differential Symbiodiniaceae Association with Acropora Humilis Coral While Rearing in Hatchery Conditions

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Abstract

Several studies have reported that most coral-Symbiodiniaceae adaptation occurs when coral faces temperature stress. However, little is known about Symbiodiniaceae population, particularly during coral development in ex-situ conditions. This study provides the first investigation on the Symbiodiniaceae diversity and community change of reared Acropora humilis colonies captured in the hatchery system at Samae San Island, Gulf of Thailand. Coral sexual propagation techniques were conducted to produce experimental coral colonies. The gamete of A. humilis showed a high fertilization rate of 98.48 $\pm$ 0.34%. Nevertheless, the survival rate of reared coral from the early stage to 18 month olds is declining to 45.96 $\pm$ 1.31%. Acropora humilis randomly acquired symbionts in early stages (1 to 3 months old) from an environment associated with three genera (Symbiodinium, Cladocopium, and Durusdinium) and several Symbiodiniaceae lineages, whereas the community structure was found stable with almost 100% harbored of Durusdinium D1 at 6 to 18 months old. Reared coral's offspring was significantly different from Symbiodiniaceae species compared to wild parent colonies. These results indicated that coral was shown to change Symbiodiniaceae community composition during development under hatchery conditions. Knowledge of this study is important to understand the corals-Symbiodiniaceae association, the status of coral reef prediction, and coral conservation in Thailand.

11. Coral Restoration Program in the Bicol Region, the Philippines: Experiences and Challenges

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Abstract

Several coral restoration programs in Bicol Region, Philippines were conducted by various sectors in the hope to help improve the ailing coral reefs in the area. In this paper, we discussed the experiences and challenges in the implementation of the program. The program did not only intend to rehabilitate degraded reef areas but also to showcase

to the communities its viability as a community-based endeavor, allowing partnership with different sectors of the society. Various rehabilitation methods were discussed from low-cost to high-cost designs and the possibilities of other economic opportunities. Challenges enumerated from natural calamities to social issues were also pointed out. Overall, coral rehabilitation program in the region showed high acceptability from the community. However, sustainability has been observed as the problem.

12. Spatiotemporal Lipid Profiling during Coral Embryogenesis

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Abstract

To evaluate the variation in the content and composition of lipids and understand their role during the embryonic developmental stages, major lipid classes: sterol ester (SE), wax ester (WE), triacylglycerol (TAG), cholesterol, phosphatidylethanolamine (PE), phosphatidylcholine (PC), lysophosphatidylcholine (LPC), and fatty acids were assessed on the octopus coral *Galaxea fascicularis* in this study. The gametes and embryos were homogenized with a mixture of chloroform and methanol, obtaining a biphasic system in which the chloroform layer contained the lipids. Thin layer chromatography (TLC), transesterification to fatty acid methyl ester (FAME), and gas chromatography mass spectrometry were used to determine the lipid and fatty acids content. The results showed that SE, WE, and TAG, altered in content during the different developmental stages, although not constantly, while cholesterol, PC, PE, and LPC remained unchanged in content throughout the course of the various developmental stages. Cholesterol and PC were the dominant lipids in all stages, followed by WE and LPC, which has medium lipid contents, whereas the contents of TAG, PE, and SE were significantly lower than those of the aforementioned lipid classes. In contrast with the lipid profile of the oocyte, sperm exhibited a much lower content of WE and the non-appearance of SE. However, the rest of the lipid classes showed no statistical difference in content. As concerned the content of fatty acids, there was a peak of concentration in the early stages of embryonic development with the prevalence of saturated fatty acids (SFA). On the other hand, there was a low content of fatty acids at the sperm level. This study revealed that lipid content and composition may fluctuate between embryonic developmental stages. The findings will undoubtedly assist future lipid research in other coral species.

13. Benthic Community Structures with Varying Sedimentation and Water Quality Conditions in Mabini, Batangas

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Abstract

Marine-protected areas have long been used as tools for protecting reef ecosystems in the Philippines. No-take marine zones are established to limit direct human activity, which is one of the primary causes for the degradation of coral reefs and reef-associated organisms. However, Philippine reefs are not only at risk from direct human activities in reef areas but from nonpoint sources such as sedimentation and poor water quality. This

study assessed and compared the hard coral cover with physical and chemical water quality parameters, such as sedimentation rate, TSS, pH, nitrate concentration, and phosphate concentrations or reefs inside and outside a marine-protected area. Samples were obtained through the photo-transect method, use of sediment traps, and standard methods for water quality assessment. Hard corals were significantly higher in the MPA, although no spatial differences were observed for sedimentation rate and water quality. Sedimentation rates, TSS, and nutrient concentrations were significantly higher during the wet season, while no temporal differences were observed for the benthic cover. Principal Component Analysis (PCA) revealed that sites were differentiated through hard coral cover, pH, and TSS concentrations, while seasons are influenced by sedimentation rates and nutrient concentrations. This study highlights the need for a ridge-to-reef approach in conserving coastal ecosystems. The current management practices of the MPA do not entirely inhibit the effects of sedimentation and nutrient pollution in the reef, which occur when run-offs from land-based activities such as infrastructure developments happen along the coast without proper management measures being set in place.

14. Characteristics of Black Pigment Released from the Octocoral *Sinularia flexibilis*

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Abstract

In our previous study, we determined that the octocoral *Sinularia flexibilis* released a black pigment that stained the operator's hands black when it was cut during the process of asexual propagation. This black pigment was water-insoluble, sticky, and constantly entangled with Symbiodiniaceae. At the time, we were unable to identify any solvents that could dissolve the pigment. In the present study, we established a method to purify the pigment and remove the contamination of Symbiodiniaceae to better understand the pigment's nature. We determined that the pigment can be dissolved in a strong basic solution, 1M sodium peroxide (pH value 14). We confirmed that the black pigment is not melanin by using Fourier–Transform Infrared Spectroscopy and the Fontana–Masson melanin stain. By staining the tissue sections, we determined that the black pigment is generated in the endoderm of the coral, then moved to the ectoderm, and finally released outside of the corals. The structure and function of this black pigment merit further study for its potential to become a new black dye for human industries.