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Conference Report

International Symposium on New Frontiers in Reef Coral Biotechnology (5 May 2022, Taiwan) †

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Abstract: Given the global threats towards coral reefs, this conference's central theme, "Reef coral biotechnology", is particularly timely. Our goal is to promote communication and dialogue in this field among marine researchers within and outside of Taiwan, and we have invited experts in the fields of coral reef ecology, physiology, conservation, and biotechnology to discuss their recent findings with a cadre of both local and foreign scientists, as well as students (undergraduate, Master's, and Ph.D. students). We envision that these presentations will segue into discussions and collaborations that stimulate innovation in reef coral biotechnology, and particularly in the development of tools and approaches that improve the odds of conserving coral reefs and biopreserving reef corals.

Keywords: coral biotechnology; genetic conservation; reef restoration; cryopreservation; ocean monitoring; deep sea coral

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Envisioning the Future of Coral Reefs with Machine Learning

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Abstract

Coral reef fate is currently modeled on temperature and coral abundance alone. I hypothesize that we can make more robust predictions by not only considering these benchmarks, but by also factoring in data from the framework-building corals themselves. After all, actuaries do not estimate our own lifespans by looking exclusively at the sizes of the cities in which we live, but instead consider our respective physiologies, as well. To this end, I have spent the better part of 20 years in both the Pacific Rim and Caribbean regions growing the datasets needed to devise more holistic models for coral reef ecosystem forecasting by conducting (1) field surveys, (2) laboratory tank experiments (microcosms and coral reef mesocosms), (3) molecular and cellular biology benchwork ("multi-Omics"), and (4) bioinformatic pipeline development. I then used the resulting "molecules-to-ecosystems" datasets to train incredibly sophisticated machine-learning models capable of accurately predicting coral bleaching susceptibility and reef resilience. With these same datasets, I have been formulating a different series of machine-learning-based decision-making schematics that instead dictate the optimal solution for preventing coral extinction (be it via stress-hardening, cross-breeding, transplantation, or other means). If conditions in the ocean are no longer amenable to target species survival regardless of the intervention measure(s) taken, then we can instead employ the coral biopreservation tools (e.g., ex situ husbandry and cryopreservation) my colleagues and I have developed over the years.

Keywords

coral reefs; coral triangle; global climate change; resilience; diagnostics

Production of Multi-Generations of Stress-Tolerant Corals for Reef Restoration in Pangasinan, Northwestern Philippines

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Abstract

Sexual propagation of corals for reef restoration is more advantageous than asexual propagation as it leads to high genetic diversity and provides access to millions of propagules. Most of the studies on the sexual mode of propagation focus on fast-growing, less stress-tolerant *Acropora* species. Long-term studies on massive corals as material for reef restoration using the sexual mode of propagation are still lacking despite the fact that massive corals are generally more stress-tolerant and slow to recruit naturally to degraded reefs. Here, we report on the survivorship, growth, and reproduction of two sexually propagated, massive coral species (*Favites abdita* and *F. colemani*; F1 generation), outplanted to the reef and monitored until 12 years post-fertilization (2009–2021), and their production of an F2 generation of propagules. In 2009, gravid parent colonies were collected, generating F1 parents which were outplanted in 2011. After 6 years, almost 90% of outplanted colonies were sexually mature, and after 12 years, colony diameter ranged between 3.0–31.6 cm and 3.5–21.0 cm for *F. abdita* and *F. colemani*, respectively. In 2021, gravid F_1 colonies were spawned, generating F_2 gametes. F_2 generation larvae were successfully collected and successfully settled on artificial substrates for both species prior to outplanting on the reef. By far, this is the first study to demonstrate long-term sexual propagation of massive corals. It is highly recommended to use multiple species, with various life history strategies, via sexual propagation to restore and sustain coral populations in degraded reef areas.

Keywords

Favites abdita; *Favites colemani*; F_1 generation; F_2 generation; spawning

Exploring Mesophotic Reefs in Taiwan via Remotely Operated Vehicles and Technical Diving

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Abstract

Mesophotic coral ecosystems (MCEs) are characterized by light-dependent corals and associated with communities typically found at depths ranging from 30~40 m to over 150 m in tropical and subtropical regions. MCEs represent approximately 80% of potential coral reef habitat worldwide, yet very little is known about them in comparison with shallow reefs. In the previous studies, we explored the possible hot spots of the mesophotic coral across the southern waters of Taiwan, Xiao Liuqiu, Hengchun Peninsula and Lanyu island. The diversity and abundance of coral at Lanyu island is the highest. In waters off the western coast of Lanyu island, Scleractinia can be found at depths around 75 m; moreover, Hexacorallia, such as Antipatharia and Stylasteridae, can be found at depths from 40 m to 150 m. Among the three places we surveyed, Lanyu island at 50 m had the best light penetration rate (more than 40%), and the light spectrum was wider than at Xiao Liuqiu, Hengchun Peninsula. The distribution of Scleractinia at different depths is correlated to the benthic substrate and light penetration rate. The light penetration rate is affected by suspended solids including plankton and the nutrients that are influenced by currents, temperature, salinity and terrigenous materials. In the present study, we used underwater remotely operated vehicles (ROVs) to explore the mesophotic coral of southern Taiwan. The preliminary data showed that here in southern Taiwan, while most coral are Nephtheidae, Ellisellidae and Nidaliidae, there are a few Scleractinia (such as Fungiidae or Agariciidae) in the upper MCE zone. In lower MCE zone, the majority were Octocorallia, Nephtheidae, Ellisellidae, Nidaliidae, Plexauridae, and some Hexacorallia (such as Antipatharia and Dendrophylliidae). According to lipid analysis, samples collected from the mesophotic region generally had lower lipid content, and specifically, the contents

of neutral lipids, triacylglycerol, and polar lipids (free fatty acid) in MCEs were lower than those in shallow water. Furthermore, the ratio of poly-unsaturated fatty acids was higher in samples collected from the mesophotic zone than from shallow water. These results indicate that Scleractinia in MCEs are heterotrophic instead of autotrophic.

Keywords

light irradiation; mesophotic zone; Scleractinia; lipids

Exploring Natural Mechanisms and Active Enhancement of Coral Thermal Tolerance

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Abstract

Coral reefs globally are being impacted by ocean warming and marine heatwaves caused by climate change, resulting in the loss and degradation of these ecologically and economically important ecosystems. Although predictions for the fate of corals into the future are relatively grim, some optimism can be found in the range of responses to elevated temperatures exhibited among different coral species and regions. We explored the natural variability in thermal tolerance using three species of scleractinian corals (*Pocillopora acuta*, *Acropora nana*, and *Porties lutea*) sourced from reefs with distinct thermal regimes (variable vs. stable) in southern Taiwan. We monitored the seasonal dynamics of the holobiont lipidome and Symbiodiniaceae genera over the course of 15 months to assess baseline data of primary energy provision sources. This in situ study revealed site-specific Symbiodiniaceae genera associations, whereby a higher proportion of corals from the thermally variable reef site hosted the more heat-resistant genus, *Durusdinium*; no site differences were found for the holobiont lipidome. Building upon this, we conducted a lab-based experiment, using the same species and sites, to assess coral response to chronic moderate warming (30 °C), and then acute high temperature exposure (32 °C). In general, corals showed adequate to good performance under the chronic warming scenario but experienced substantial bleaching under the higher acute temperature. Lastly, we explored the potential capacity for active enhancement of coral thermal tolerance through assisted evolution (via transgenerational acclimation) in *Pocillopora acuta*. We found that thermally conditioned adult colonies reproduced earlier in the lunar cycle and had smaller offspring with lower photosynthetic efficiency than offspring sourced from colonies held at a control temperature. We did not find evidence of improved thermal performance in offspring sourced from heated parent colonies when exposed to elevated temperatures. Collectively, the finite natural upper temperature limits of corals and the lack of evidence for enhanced thermal tolerance via transgenerational acclimation highlight the need for urgent action to mitigate climate change to ensure the persistence of healthy coral reefs into the future.

Keywords

coral; climate change; lipids; algal symbionts; thermal tolerance; assisted evolution

Coral Cryopreservation

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Abstract

Over the past decade, coral cryopreservation research has advanced rapidly due to the field's inter-disciplinary approach, whereby concepts and technologies from cryogenic biology, nano-engineering, and cellular biochemistry have been combined. Cryopreservation of coral gametes, larvae, and their dinoflagellate endosymbionts has been under continuous development, and the resulting publications have gathered the attention of conservationists across the globe. In this talk, I will present the progress in this field, with a particular emphasis on (1) understanding the factors required for successful cryopreservation and (2) optimizing cryopreservation protocols for different cell/tissue types. We advocate, at a minimum, (1) reducing cryo-injury (i.e., ice crystal formation) and (2) optimizing freezing techniques to develop successful cryopreservation protocols for coral gametes, tissues, larvae, and the endosymbiotic dinoflagellates that reside within the tissues of all reef-building corals.

Keywords

cryopreservation; cryobank; coral hospital; ex situ conservation

Scaling-Up Management in Apo Reef Natural Park, Sabalayan, Occidental Mindoro, Philippines

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Abstract

Coral reefs worldwide have shown significant decline in biodiversity and structural complexity in the last 50 years. Hence, it has become an impetus in marine science to identify coral reef areas to protect and conserve for ecological and economic purposes. In the Philippines, threats to corals and their associated communities are more pronounced near population centers and relatively less on offshore oceanic reefs. However, these less accessible offshore reefs remain vulnerable to direct resource extraction, *Acanthaster* predation, bleaching, and typhoons. In Apo Reef Natural Park (ARNP), these perturbations have occurred at short intervals and resulted in significant changes in the reefscape. In the last 13 years, ARNP has experienced two cycles of *Acanthaster* outbreaks that were closely followed by strong typhoons. These consecutive disturbances resulted in notable changes in the reef complex's benthic features, with hard corals showing uneven but consistent decline across established monitoring stations. Thus, it has become a government priority to improve our understanding of the ecological processes that underlie the recovery and resilience of ARNP. Historical data have shown that protection from extractive activities is not sufficient to ensure hard coral recovery and the maintenance of topographic complexity in ARNP. It has become imperative to craft and implement science-based management strategies that are cost-effective in achieving specific conservation targets. In the study, important ecological processes have been elucidated through regular community monitoring activities and the conduct of assisted rehabilitation experiments. The results of the study showed

three important findings. First, the recovery of hard corals after a major disturbance is slow in ARNP. Second, protection from intense fishing pressure is essential in maintaining the community structure of the reef's associated fish and invertebrate assemblages. The relative health of these communities is seen to contribute to coral community recovery in the reef complex. Lastly, assisted substrate stabilization in a high energy reef environment is slow and possible only in certain areas of ARNP. The study supports the continued strict protection of the reef complex from extractive activities. In addition, active substrate rehabilitation activities must be improved and implemented together with other techniques that enhance coral growth and substrate complexity. It is clear that aside from enforcing the no-take policy in the reef complex, investing in adaptive management strategies is essential in ARNP.

Keywords

Apo Reef Natural Park; spatiotemporal monitoring; substrate stabilization

In vitro Regeneration of Small Colony Fragments of *Pocillopora damicornis* Produces Clones to Examine the Effect of Monochromatic Lights on Coral Tissue Growth and the Density of Symbiotic Zooxanthellae

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Abstract

Scleractinian corals predominantly reproduce by means of fragmentation; thus, experimental infliction of tissue lesions is the primary approach to investigate coral growth. However, most of the early investigations were performed from the field, sometimes in difficult conditions and inaccessible areas. In this study, clones of *P. damicornis* produced by inducing tissue regeneration through a simple in vitro system were used to examine the effect of various monochromatic lights on the growth of its tissues and density of in situ symbiotic zooxanthellae using light microscopy. To induce coral regeneration, small apical colony fragments (c.a. = 8–10 cm) were excised, then fixed separately on a cover slip with a drop of silica gel inside a glass dish containing raw seawater. After 1–2 weeks, new tissue layers tended to extend towards the glass surfaces. Healthy clones were exposed independently to intermittent light treatment (14 h light: 10 h dark) every 48 h for 10 days at irradiance levels of 1.0, 1.5 and 2.0 W/m^2, respectively. The length of tissue extension and density of in situ zooxanthellae cells from light-treated and control samples were calculated by examining serial micrographs. Coral tissue growth and populations of zooxanthellae cells changed significantly depending on the coral light and intensity. Among monochromatic lights, blue rays (470 nm) further enhanced tissue regeneration by up to 159.7 μm/day, for a 5.34% growth rate, and increased zooxanthellae density to 35.8 cell/μm^2/day or by 4.42%. In contrast, red 62 (620 nm), far red (737 nm) and UV-A (375 nm) radiation inhibited the proliferation rates of both host and symbiont cells. This study contributes knowledge to further understand coral reproducibility and its viability for clone production that could be utilized as a feasible source of explants for various coral examinations, transplantation, husbandry, biotechnology and microscale analyses.

Keywords

Pocillopora damicornis; in vitro regeneration; monochromatic lights; tissue extension; zooxanthellae density

The Organelle in the Ointment: Cryptic Mitochondria Bias Cross-Species Microbiome Comparisons

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Abstract

The microbiomes of tropical corals are actively studied using 16S rRNA gene amplicons to understand microbial roles in coral health, metabolism, and disease resistance. However, due to the prokaryotic origins of mitochondria, primers targeting bacterial and archaeal 16S rRNA genes may also amplify homologous 12S mitochondrial rRNA genes from the host coral, associated microbial eukaryotes, and encrusting organisms. Standard microbial bioinformatics pipelines attempt to identify and remove these sequences by comparing them to reference taxonomies. However, commonly used tools have severely under-annotated mitochondrial sequences in 1440 coral microbiomes from the Global Coral Microbiome Project, preventing annotation of over 95% of reads in some samples. This issue persists when using Greengenes or SILVA prokaryotic reference taxonomies, and in other hosts, including 16S studies of vertebrates, and of marine sponges. Worse, mitochondrial under-annotation varies between coral families and across coral compartments, biasing comparisons of α- and β-diversity. By supplementing existing reference taxonomies with over 3000 animal mitochondrial rRNA gene sequences, we resolved roughly 97% of unique unclassified sequences as mitochondrial. These additional sequences did not cause a false elevation in mitochondrial annotations in mock communities with known compositions. We recommend using these extended taxonomies for coral microbiome analysis and whenever eukaryotic contamination may be a concern.

Keywords

taxonomy; classification; mitochondria; 16S; contamination

Enhancing the Growth and Color of *Pocillopora acuta* and *Stylophora pistillata* with Different Levels of Blue Light and Heterotrophic Feeding

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Abstract

Recent studies showed that heterotrophic feeding can improve coral growth rates and resilience against environmental stress, while blue light was able to enhance coral calcification. In this study, we designed a 90-day experiment by using *Stylophora pistillata* (SP) and *Pocillopora acuta* (PD), which are common coral species in Taiwan, to investigate the effects of heterotrophic feeding and blue light on both coral species. Each coral species was divided into 72 nubbins and distributed evenly into four treatment groups: the combination of different blue light intensities (high and low) and different feeding concentrations (high and low). All coral nubbins were cultured in a recirculating aquaculture system (RAS) and fed to *Artemia salina* in different concentrations based on treatment twice a week. During the experiment, the Fv/Fm of all groups was higher than 0.6, showing that corals remained

healthy and that there was no mortality in each group. Based on the results, we observed that the specific growth rate and linear growth rate were highest in SP and PD under HLHF treatment, while SP under HLHF treatment showed significantly different rates compared to the other treatments, indicating there was an interaction effect between light intensity and feeding. Additionally, we conducted a coral health analysis based on RGB values. The RGB value was highest in the HL treatment of SP and PD and remarkably distinct compared to other treatments, showing that corals were more healthy under HL treatment. We also observed that RGB values in the tip and tissue parts of coral nubbins were different in each treatment. As a result, we demonstrated that high blue light intensity and heterotrophic feeding could improve the growth rates of both coral species. These results provide clues for further coral cultivation and restoration.

Keywords

Pocillopora acuta; *Stylophora pistillata*; RAS system; heterotrophic; color

Using the Red Sea Upside-Down Jellyfish *Cassiopea* as a Model to Study Cnidarian–Symbiodiniaceae Symbiosis

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Abstract

Tropical coral reefs are considered one of the most diverse and productive ecosystems on the planet. Corals heavily rely on the symbiotic relationships with photosynthetic dinoflagellates of the Symbiodiniaceae family, receiving photosynthates in exchange for inorganic nutrients. The breakdown of this symbiosis leads to coral bleaching, resulting in major coral reef losses. Despite the fact that the environmental stressors causing coral bleaching are well known, the cellular and molecular mechanisms underlying this endosymbiosis are not well understood. The upside-down jellyfish *Cassiopea*, similarly to corals, has a mutualistic relationship with Symbiodiniaceae. However, unlike corals, this upside-down jellyfish has a closed sexual reproductive cycle, can be easily maintained in laboratory conditions, and has a short generation time. The use of *Cassiopea* as a model organism to investigate symbiosis with Symbiodiniaceae would provide us with a better understanding of Cnidarian symbiosis and would bring us a step closer to the development of a cnidarian symbiosis model. *Cassiopea* polyps can stay aposymbiotic and propagate if fed regularly. To understand the molecular mechanisms behind this symbiosis, gene expression profiles between aposymbiotic and symbiotic *Cassiopea* polyps were compared, revealing genes and pathways putatively involved in symbiosis. We identified 1227 differentially expressed genes out of 63,340 transcripts, of which 560 were upregulated and 667 were downregulated in symbiotic polyps. GO enrichment analysis indicated that processes linked to lipids, sterols, cholesterol, and membrane transport were upregulated in symbiotic *Cassiopea* polyps. We also compared GO enriched terms between *Cassiopea* and *Aiptasia* upon symbiosis, revealing shared enrichment in processes related to steroid hormone receptor activity and sterol homeostasis. Sterols are essential cell components that cannot be synthesized by cnidarian hosts, which rely on symbionts or on prey consumption for their acquisition. Symbiodiniaceae can produce and transfer a variety of sterols to the host, including dinosterol, cholesterol, and gorgosterol, among others. Our results show that two genes annotated as NPC intracellular cholesterol transporter 2 were also increased during symbiosis, suggesting that sterol transport via Symbiodiniaceae may potentially play an essential function in *Cassiopea*.

Keywords

Cassiopea; Symbiodiniaceae; symbiosis; bleaching; transcriptomics

Good Coral Cover and High Biodiversity in Non-MPA Reefs of the Verde Island Passage as a Basis for Increased Protection and Conservation

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Abstract

The Verde Island Passage (VIP) is a region in the Philippines with a high index of biodiversity. Baseline coral reef assessments were conducted in four provinces along the VIP (Batangas, Marinduque, Occidental Mindoro, and Oriental Mindoro) to compare coral reef abundance and biodiversity in marine protected area (MPA) and non-MPA sites. The average abundance of coral and other substrates (e.g., macroalgae, sponges, rock, sand/rubble) were measured, and the numbers of coral genera were identified for each site. Batangas showed equal coral cover and coral species richness for MPA and non-MPA sites. Marinduque showed equal coral cover for MPA and non-MPA sites, and the non-MPA site had higher species richness. Occidental Mindoro showed higher coral cover and species richness in the non-MPA site compared to the MPA site. Oriental Mindoro was the only exception, showing higher coral cover and species richness in the MPA site compared to the non-MPA site. The findings indicate that many non-MPA coral reefs in the VIP have potential to become MPAs, thus warranting their protection and conservation. Continued monitoring and assessment should be conducted to build on the growing database of biodiversity data that are being compiled for the VIP.

Keywords

coral reef; biodiversity; Verde Island Passage; marine protected areas (MPA)

Ultrastructural Analysis of Chilled and Cryopreserved Coral Larvae

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Abstract

The cryobanking of marine invertebrates in early developmental stages has been extensively explored, but few ultrastructural studies have investigated the effects of cryopreservation on biomaterials. Transmission electron microscopy (TEM) provides useful information on sites and extent of cryoinjuries. Ultrastructural observations were conducted on larvae of corals *Seriatopora caliendrum* and *Pocillopora verrucosa* after subjecting samples to vitrification and nano-laser warming, with lipid-free vitrification solutions (VSs) or VSs supplemented with erucic acid (EA), phosphatidylethanolamine, or linoleic acid. Samples then underwent TEM, and several ultrastructural parameters were examined. Cryoinjury in larval epithelia manifested as a lack of tissue coherency and affected the chloroplast structure of Symbiodiniaceae. Vitrified and laser-warmed coral larvae exhibited a patchy well-preserved ultrastructure. On average, liposome-supplemented VSs allowed for better preservation of thickness and area occupied by microvilli and flagella. Ultrastructural analysis of vitrified and nanoparticle laser–warmed coral larvae revealed the essential role of nanoparticle dispersion stability in the homogeneous laser thawing of the

sample, as well as the relevance of cellular projection preservation to cryopreservation outcomes. The ultrastructural effects of vitrified and nanoparticle laser–warmed coral larvae were examined for the first time. The findings will aid the development of efficient coral cryopreservation protocols.

Keywords

coral larvae; vitrification; laser-warming; ultrastructure

Phylogenetic Comparisons of Innate Immune Gene Repertoires, Microbiome Structure, and Disease Susceptibility across Coral Diversity

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Abstract

Coral innate immunity plays a key role in regulating coral microbiomes. These microbiomes may contribute to corals' resistance or susceptibility to environmental stress, predation or disease. Changes in immunity over coral evolution may, therefore, have driven changes in microbiome structure, which may in turn contribute to modern cross-species differences in disease susceptibility. We used phylogenetic comparative methods to test for correlations between coral innate immune gene repertoire, microbiome structure, and disease susceptibility across genera representing >240 million years of coral evolution. This analysis drew on 1440 microbiome 16S rRNA samples from coral mucus, tissue and endolithic skeleton from our Global Coral Microbiome Project (GCMP); three long-term regional disease datasets; and innate immune gene repertoires annotated from all publicly available coral genomes. Across sequenced coral genomes, we found that gene family expansions of TIR-domain containing innate immune genes strongly predict reduced microbiome richness (PGLS R^2 = 0.40, p = 0.021), especially within corals' endolithic skeletons (PGLS R^2 = 0.791 p = 0.0003). Indeed, gene copy number expansions of IL-1R genes alone explained an astounding ~83.5% of overall variance in microbiome richness across coral genera in our data (PGLS R^2 = 0.84, $p = 5.13 \times 10^{-15}$). We further found that across 40 coral genera with both microbiome and disease data available, disease susceptibility does not significantly correlate with microbiome richness. Instead, ecological dominance of the most abundant microbial taxon increased overall disease susceptibility (PGLS R^2 = 0.27, p = 0.0006), especially in corals with more γ- than α- proteobacteria (PGLS R^2 = 0.40, p = 0.0003). We are investigating whether a growth/defense tradeoff in symbiotic association with *Endozoicomonas* explains this correlation between ecological dominance in the microbiome and disease susceptibility. Overall, these results demonstrate the utility of consistently collected cross-species datasets for exploring the interactions between coral immunity, microbiome structure, and disease susceptibility.

Keywords

coral microbiome; innate immunity; coral disease

Reproductive Timing of *Neopetrosia compacta* in Bolinao, Northwestern Philippines

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Abstract

Reproduction in marine sponges remains poorly understood despite their known significance as melting pots of important biomedical compounds. Particularly in the Indo-Pacific, with noted high sponge diversity, existing studies in sponge reproduction are limited. In this study, sexual reproductive features, particularly the onset and timing of gametogenesis in relation to temperature, of the cryptic yellow demosponge *Neopetrosia compacta* were investigated. Sponge samples (N = 10, minimum) were collected monthly and dissected from November 2020 to March 2022 in Lucero, Bolinao, Pangasinan from tagged and random individuals. Temperature loggers were also deployed over the course of the study to collect temperature data. Results show that reproductive individuals were detected almost throughout the sampling period with increasing detection as seawater temperature rose. Gametes begin to appear more frequently starting in April 2021, with a peak of gametogenesis recorded in June 2021 with more than 70% of collected individuals presenting reproductive propagules. Coincidentally, this was also the same month with the highest seawater temperature recorded at the site, of 30.6 °C. Moreover, *N. compacta* most likely exhibit a viviparous mode of reproduction. Learning the timing and patterns as well as determining environmental cues that influence reproduction in sponges will further enhance applications that could facilitate technologies that may increase marine sponge biomass for pharmaceutical explorations.

Keywords

cryptic sponge; viviparous; *Neopetrosia compacta;* temperature; gametogenesis

Coral Bioerosion in South Central Vietnam and Some Discussion about the Monitoring Method

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Abstract

The term bioerosion refers to the biological destruction of hard structures, such as coral skeletons. On the reefs, this process is contrary to coral calcification. If rates of bioerosion are higher than calcification, healthy reef habitats can be in danger of ruin. In recent years, bioerosion monitoring units (BMUs) have been used by scientists to assess, monitor, and understand how coral reefs are responding to ocean acidification. According to NOAA's guidelines, BMUs would be deployed at reef monitoring sites, after collection, changes in density, volume, and mass indicate rates of bioerosion. However, maybe due to unclear guidelines or inappropriate conditions/designs, when applied in Vietnam, this method presented some difficulties and limitations in implementation. Even so, we have made some improvements and obtained initial data on coral bioerosion. We compared the histograms of tomography images from a CT scanner (0.6 mm thick slides) to evaluate the bioerosion with the following rule: the whiter the image, the higher the coral density. The results show that the bioerosion trend in coral samples at the southwest Mun Island

site was similar to the average for the whole Nha Trang area (117.5% and 114.2%), while the rate in the Ninh Thuan area was much higher, at 158.8% (in two years). This might be due to environmental factors in the Ninh Thuan area (lower ΩAr value) that could have made corals more vulnerable. In addition, we found that the more hollow/porous the coral sample before deployment, the more susceptible it was to bioerosion. On the basis of the difficulties and limitations that we faced and from the results obtained, we had some discussions and suggested improvements for a more appropriate bioerosion monitoring method (artificial coral, tomography technique, histogram analysis, etc.).

Keywords

coral bioerosion; BMUs; bioerosion monitoring method

Successful Cryopreservation and Nano-Laser Warming of Coral Larvae

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Abstract

Styplophora pistillata is an important scleractinian coral species that dominates the reefs in southern Taiwan. *S. pistillata* is an excellent candidate for cryopreservation due to its low resistance to coral bleaching exacerbated by ocean warming. The coral colonies were collected from the reefs of Houwan bay and kept in flow-through tanks for larval collection. The peak larval release was observed during the first quarter (January–March) of the year. Each coral larva was subjected to vitrification and laser warming (300V, 10 ms pulse width, 2 mm beam diameter) using vitrification solutions (VS) previously selected from suitable cryoprotectants. Two VS were used (VS1: 2M EG, 1M DMSO, and VS2: 2M DMSO and 1M EG), mixed with 40% (w/v) Ficoll and 10% (v/v) gold nano bars (GNBs) in FSW (final concentration of 1.2×10^{18} particles/m^3, ƛ= 530nm). The initial results showed that VS1 yielded a higher vitality (16%), settlement (5%), and post-settlement survival (2.5%) rate than VS2. Thus, *S. pistillata* is a good candidate for rearing corals in captivity, given the higher post-settlement survival (5 months) among the cryopreserved corals to date. The GNBs also played an important role in equalizing heat distribution during laser warming. This study will aid in cryopreservation of corals in the same genera and enhance efforts in the conservation of threatened coral species.

Keywords

coral; cryopreservation; laser nanowarming

Water-Insoluble Black Pigments Released from the Octocoral *Sinularia flexibilis*

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Abstract

Coral reefs are the most diverse and productive marine ecosystems on Earth. The National Museum of Marine Biology and Aquarium has cultured more than 35 species of

corals for research. When we conducted the asexual propagation of corals, the octocoral *Sinularia flexibilis* released black pigments that stained the operator's hands black. We quantified the blackness of the stain by using the RGB value of the color. The longer the coral contacted the skin, the darker the skin became. Incubating the tentacles of *S. flexibilis* in high-salinity filtered seawater increased the amount of the black pigments released. However, collecting 100% of the black pigments was exceedingly challenging because they were very sticky and were constantly entangled with Symbiodiniaceae. Furthermore, we were unable to identify any solvents that could dissolve the pigment. The structure and function of the black pigment merit further study as it has the potential to become a new black dye for human industries.

Keywords

non-polar pigment; soft coral; asexual coral propagation; melanin

Reproductive Biology of the Philippine Blue Sponge *Xestospongia* sp. in Puerto Galera, Oriental Mindoro, Philippines

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Abstract

Sponges are important components of the benthic communities in marine environments, being able to affect various benthic and pelagic processes. Sponges are also important sources of bioactive compounds that can be used for drug development. The Philippine blue sponge *Xestospongia* sp. is known to produce a compound called renieramycin, which has anti-inflammatory and anti-proliferative properties. However, information on different aspects of the biology and ecology of this species is lacking. This study aimed to describe the reproduction of the Philippine blue sponge *Xestospongia* sp. in Puerto Galera, Oriental Mindoro, southwestern Philippines. Sponge tissues were collected monthly from December 2020–December 2021. Additionally, collection was performed weekly for the months of March–May 2021. Tissues were collected and then observed under a dissecting microscope for the presence of reproductive propagules. Some factors that may affect reproduction, such as temperature and the presence of predators, were also observed. HOBO loggers were deployed at the collection site to record temperature data. Results showed that *Xestospongia* sp. are monthly brooders, due to the presence of larvae found in the collected tissues. It was also observed that most individuals of this species reproduce during March to May and September to December. Incidentally, low temperatures were recorded during these months. Furthermore, the reproductive peak of *Xestospongia* sp. preceded the peak occurrence of its predator *Jorunna funebris*, indicating that the presence of the nudibranch may have affected the reproduction of *Xestospongia* sp. The results of this study can provide more insights into the biology of this species that in turn can be used for its conservation. Furthermore, this knowledge can be used to enhance mariculture techniques to bridge the supply gap in sponge tissues used as sources of natural products.

Keywords

porifera; sexual reproduction; *Xestospongia* sp.

Finding Coral Bioactive Ingredients

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Abstract

Marine corals offer great potential for the discovery of new entities that can aid in the prevention and treatment of cancer. Natural products, in general, have been a prime source of compounds for the treatment of many forms of cancer and offer a promising opportunity for evaluation of not only new chemical classes of anticancer agents, but also novel and potentially relevant mechanisms of action. The aim of this study was to isolate bioactive ingredients from coral c63 that have potential anti-cancer activity. After separation using various column chromatography methods, two compounds, 9-2-3-2 and 9-2-3-3, were isolated, and their chemical structures were determined based on NMR spectroscopy. In previous studies, both compounds demonstrated noticeable cytotoxicity against A549 with IC_{50} of 2.2 μM and HepG2 with IC_{50} of 17.7 ± 1.5 μg/mL, respectively. Therefore, further efforts in separation will be continued for c63, and the anti-cancer activity of the isolated compounds can be evaluated in the future.

Keywords

marine natural product; biological activities; soft coral

Challenges to and Perspectives for Conservation and Sustainable Use of Coral Reefs in Nha Trang Bay, Vietnam

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Abstract

Nha Trang bay is considered a part of the most diverse region of coral reefs within the western South China Sea. This south-central city in Vietnam is a well-known tourist destination where coral reefs have been used for diverse services of marine tourism. Rapid economic development has caused a continuous process of reef degradation in recent decades. A review of the literature and analysis of updated data indicated a number of challenges with environmental impacts, including sedimentation from terrestrial run-off, coastal and island infrastructure development, outbreaks of crown of thorn starfish (COT), and coral bleaching due to increased sea surface temperatures. Some reefs were also damaged by tropical typhoons. Efforts to apply technology for coral reef restoration were investigated with environmental changes and demonstrated less success compared with efforts in other reef areas in Vietnam. In terms of management, a portion of the reefs is in the core zone of the marine protected area managed by Nha Trang Bay Management Board, and most of the remaining reefs have been assigned to businesses for their own use, including tourism-related purposes and the collection of swallow's nests. In practical terms, active and responsible participation by businesses and an improved coordination role for the Nha Trang Bay Management Board will be the means to overcome the aforementioned challenges. Further proposed activities include minimizing sediment loading into the marine environment, collection of COT before their spawning season, restoration of hard

corals, sea ranching of reef resources and effective conservation of biodiversity in the core zone.

Keywords

reef degradation; tourism; environment impacts; engagement; conservation

Cryopreservation Impacts on Protein Expression in Coral Larvae

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Abstract

Cryopreservation has been shown to be a reliable method for preserving coral biodiversity in the face of present environmental threats. To be successful in cryopreservation, the effect of cryopreservation on molecular parameters is an important factor that needs to be taken into consideration. The goal of this research was to see how cryopreservation affected protein expression in *Seriatopora caliendrum* larvae. Larvae were subjected to vitrification (2 M ethylene glycol + 1 M propylene glycol, 40% w/v Ficoll and 1.2×10^{18} /m^3 gold nano particles) and laser warming (300 V, 10 ms pulse width, 2 mm laser beam diameter). Successfully vitrified and warmed larvae were maintained for 8 and 24 hours, respectively, before protein extraction. The proteins with various molecular masses were separated using one-dimensional gel electrophoresis, and target proteins were identified using liquid chromatography–tandem mass spectrometry. After vitrification and nano-laser warming, keratin-associated protein 9-2 was found to be reduced, although mitogen-activated protein kinase 13 exhibited the reverse tendency. The reduction in keratin-associated protein 9-2 in coral larvae following vitrification and nano-laser warming is thought to have had a severe impact on the skeletons of the coral larvae, resulting in a poor survival rate. Mitogen-activated protein kinase (MAPK) is a key regulator of cells. Consequently, it is probable that freezing insults for larval development activated MAPK. The existing understanding of the influence of cryopreservation on protein expression was improved by this work.

Keywords

cryopreservation; coral; vitrification; laser warming; conservation