ORIGINAL PAPER

Growth and survival of coral micro-fragment in Chonburi Province, the Upper Gulf of Thailand

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Abstract. Coral reefs are an important ecosystem with their unique characteristics and high biodiversity. They provide great benefits to the coastal communities and national economy. However, coral reefs and their ecosystem services have significantly declined. The advancement of coral restoration is necessary to develop improved techniques and methods, aiming to ensure the success of large-scale coral restoration projects. This study aims to compare the growth and survival rates of coral micro-fragments among four species, i.e, *Diploastrea heliopora*, *Pavona desussata*, *Pavona varians* and *Lithophyllon undulatum* in coral nursery plots at Ko Larn, Chonburi Province, the upper Gulf of Thailand. The results showed that the highest growth rate was observed in the coral micro-fragments with an initial size of 1 cm. *D. heliopora* had the highest growth rate (59.70%) and *P. varians* had the lowest micro-fragment growth rate (50.04%). The survival rate of every micro-fragment with initial sizes between 1 and 3 cm was higher than 95.67%. Among the initial sizes, there were significant differences in the survival rates of coral micro-fragments (One-way ANOVA, $p < 0.05$). The survival rates of *D. heliopora* and *L. undulatum* micro-fragments were higher than those of *P. varians* and *P. decussata* (Tukey's HSD, $p < 0.05$). This study highlights the critical importance of active coral reef restorations through the use of newly developed technologies of coral micro-fragmentation and colony fusion techniques, to enhance ecotourism, community-based tourism, and carbon-neutral tourism, as well as the efficacy and efficiency of efforts to restore coral reefs.

Keywords: Micro-fragmentation, Coral restoration, Growth, Survival, Gulf of Thailand

1. Introduction

Coral reefs are an essential ecosystem with their unique characteristics and high biodiversity. They are distributed in tropical seas, providing great benefits to the well-being of coastal communities and national economy in terms of food sources, employment, tourism, coastal protection, pharmaceutical and cosmetic products and cultural importance. (Pittman and Brown 2011; Mace et al. 2012; Hicks et al. 2013; Yee et al. 2015; Thomas et al. 2017; Robinson et al. 2022). However, during the previous few decades, coral reefs and the ecosystem services they provide have significantly declined. They are the marine ecosystems most vulnerable to human activity, both locally and globally (Ayre and Hughes 2004; Underwood et al. 2009; van der Ven et al. 2021).

The degraded coral reefs are needed to be restored. Active coral restoration, which utilizes human efforts implemented to revitalize the coral ecosystem, such as direct coral transplantation, coral gardening etc., provides a potential coral reef conservation and management (Hein et al. 2021; Quigley et al. 2022). Active coral restoration has been increasingly used as a tool to restore coral reefs at local scales, especially by the tourism industry (Omori et al. 2011; Young et al. 2012). The advancement of coral restoration is necessary to develop improved techniques and methods, aiming to enhance efficiency and ensure the success of both current and future large-scale coral restoration projects. Most importantly, the development of a method with low operational cost and ease of implementation needs to be highlighted. Coral restoration in Thailand has continuously been implemented using both asexual and sexual propagation methods. However, there are still several limitations, including lower genetic diversity of corals in the restoration projects, low survival and growth rates of coral larvae, and high operational costs (Palumbi 2004; Hoegh-Guldberg and Bruno 2010; Thomas et al. 2017; McManus et al. 2021). Therefore, coral reef restoration is an urgent issue in Thailand to enhance the marine and coastal ecosystem conservation, especially, to preserve the natural capital and promote the country's Ocean Health Index (Yeemin et al. 2006; Yeemin et al. 2012; Suraswadi and Yeemin 2013; Chen et al. 2018; Sutthacheep et al. 2022).

One of the potential strategies for active restoration is the use of coral micro-fragmentation, which promotes high growth rates of coral fragments. (Bayraktarov et al. 2019; Knapp et al. 2022; Lock et al. 2022; Page et al. 2023). The main advantage of the coral micro-fragments is that the fusion of small coral colonies to form a larger colony can produce gametes faster. The selection of parent corals for micro-fragmentation is also essential to obtain the genetically desirable coral fragments with high stress-tolerant traits. Moreover, it is important to promote the genetic diversity of coral populations in restoration sites similar to that in natural coral reefs and support a sustainable bioeconomy of marine and coastal resources. (Boström-Einarsoon et al. 2020; Combilet et al. 2022). Sutthacheep et al. (2023) reported the first coral micro-fragmentation study in *Porites lutea* and found that micro-fragments with an initial diameter of 1 cm exhibited a high growth rate of up to 51.32% in 9 months. This study aims to compare the growth and survival rates of coral micro-fragments among four species with the potential for coral breeding in reef restoration efforts in Thailand.

2. Materials and Methods

2.1 Study site

Ko Larn, Chonburi Province, the upper Gulf of Thailand, is selected for this study (Figure 1). The coral nursery plot was constructed at Ao Nuan, southeast of Ko Larn. The nursery plot was made from welded steel into a rectangular frame with 0.5 meter in width, 1.0 meter in length, and 0.7 meter in height. The frame was applied with anti-rust paint and covered with a plastic net, providing a space for coral micro-fragments. The metal frame was placed approximately 50 centimeters above the sandy substrate to prevent sediment accumulation and scraping by macro-benthic invertebrates. The coral nursery plot was located on a coral reef at a suitable water depth, approximately between 3-5 meters (Figures 2 and 3). The environment is favorable for the growth of corals. For example, there are a few macro-benthic invertebrates and reef fishes, which may affect the survival rates of coral micro-fragments. The nursery plot also has the potential to be a destination for ecotourism.

2.2 Coral micro-fragment preparation

Diploastrea heliopora, *Pavona desussata*, *Pavona varians* and *Lithophyllon undulatum* were selected in this study. A set of criteria, including the lack of partial mortality, bleaching, invasive organisms, and coral diseases, was used to select all colonies from these four species (Figure 4). Ten selected colonies were acclimatized in a nursery pond for at least 24 hours. Then, coral fragments of approximately 10x10 cm were cut and transferred to a culture pond equipped with an aerated system and seawater circulation. Micro-fragments were prepared by cutting the coral fragments into 1, 2, and 3 cm using an electric cutter while being cooled in seawater. The rubber gloves were used to avoid direct contact with the coral tissue during handling. The coral micro-fragments were then placed in a container with swirling seawater to reduce stress and mucus.

The coral micro-fragments were adhered to circular cement plates with a radius of 5 cm, which were pre-soaked in seawater for 48 hours to acclimate. The epoxy glue was used to attach four micro-fragments per plate, with 1 cm of space between each micro- fragment. A SCUBA diver then attached the plates with coral micro-fragments to the nursery plot using a plastic net and cable tie. Each nursery plot contained a total of 15 cement plates in five rows, spaced approximately 10 cm apart and numbered for monitoring the growth and survival rates of the coral micro-fragments (Figure 5).

2.3 Data analysis

In the coral nursery plots, seawater temperature, light intensity, salinity, pH, dissolved oxygen (DO), oil or grease, suspended solid content, and sedimentation rate were monitored throughout the study.

The growth and survival rates of coral micro-fragments were monitored from June 2022 to February 2023 by measuring changes in the area of coral micro-fragments. The growth and survival rates of the coral micro-fragments were analyzed using a one-way ANOVA to determine the statistical differences between the groups. To identify which groups differed significantly from each other in terms of growth and survival rates, Tukey's Honestly Significant Difference (Tukey's HSD) test was applied.

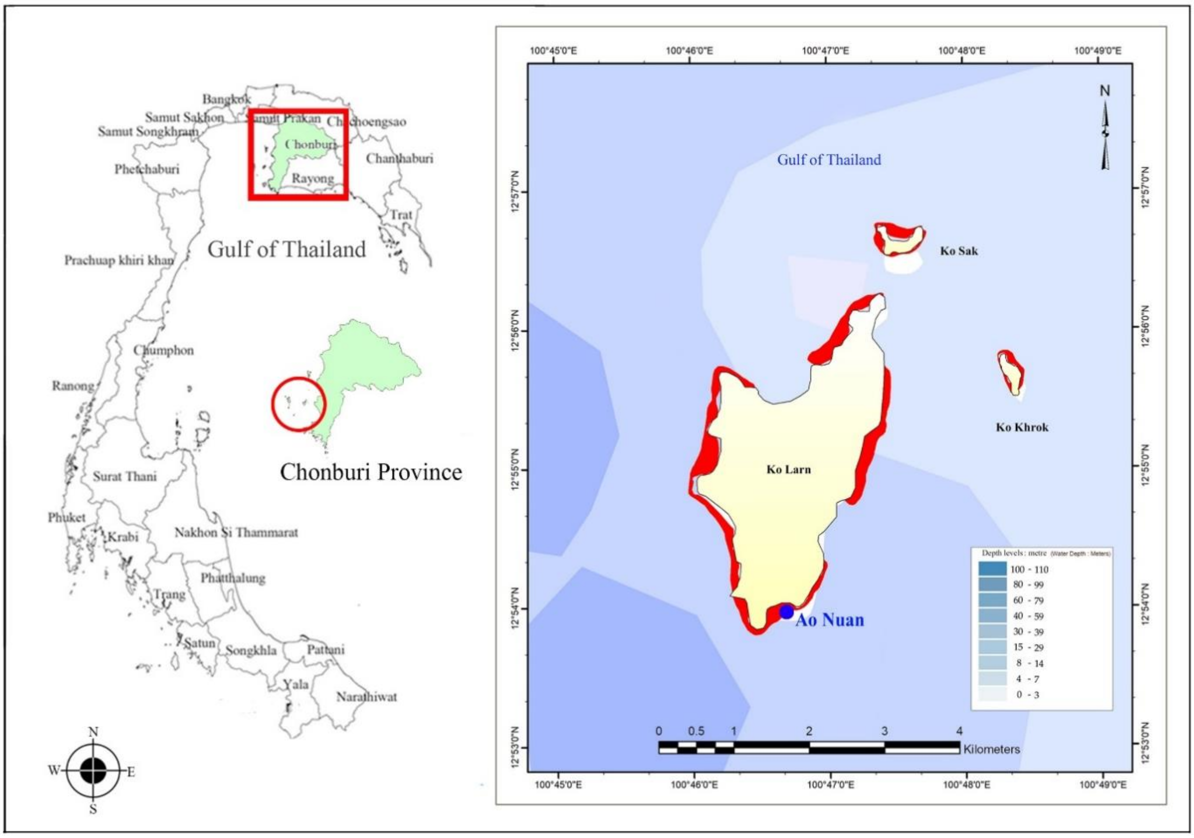


Figure 1. Location of the study site at Ao Nuan, Ko Larn

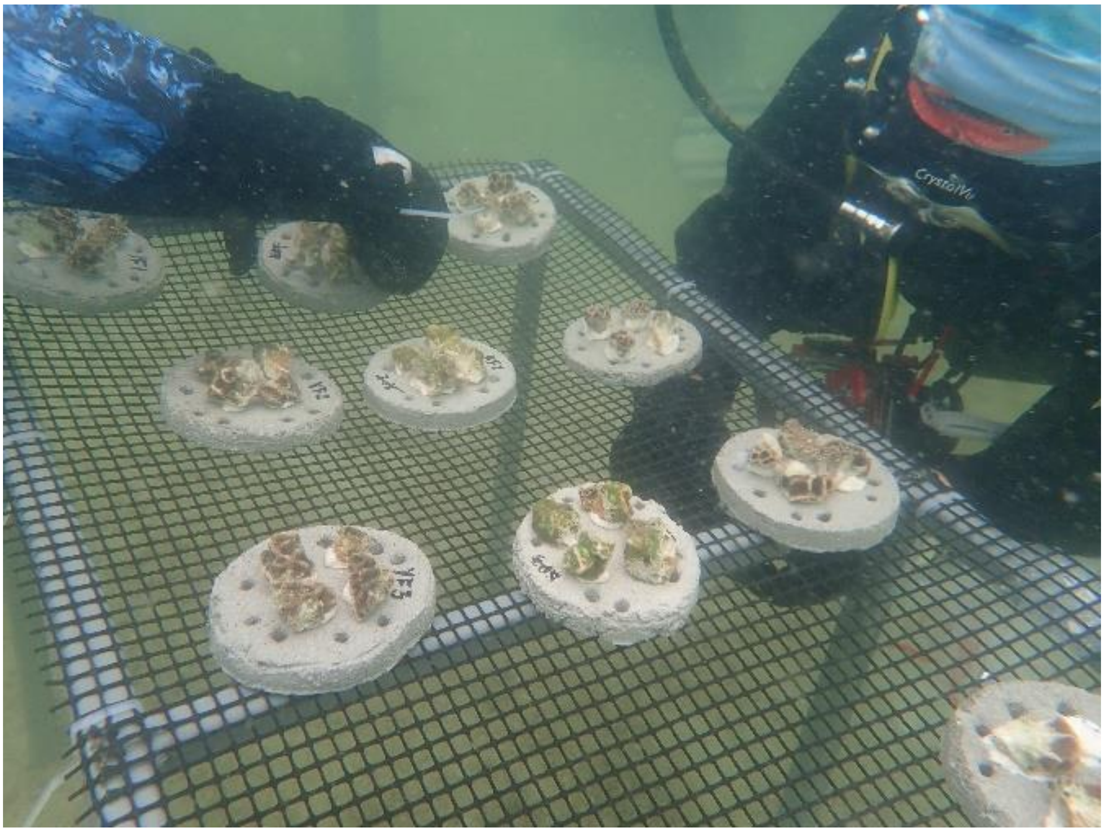


Figure 2. SCUBA divers set up a coral nursery plot at the study site.

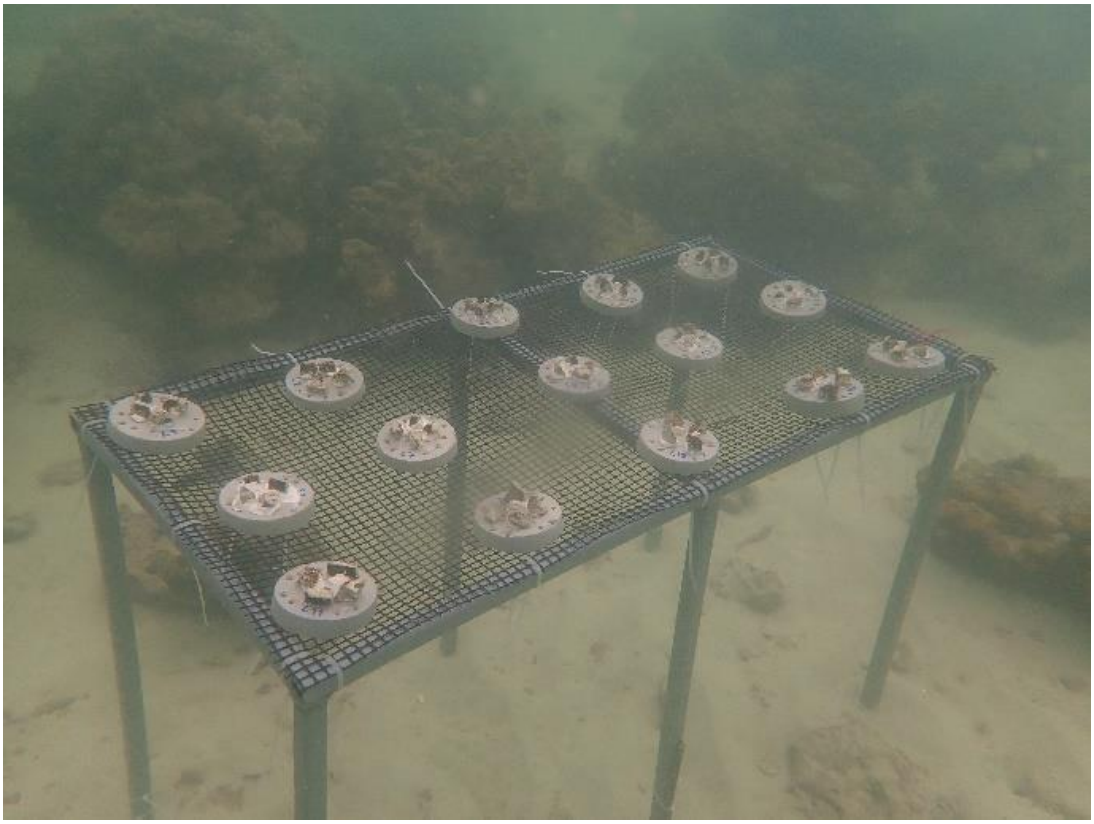


Figure 3. A coral nursery plot on a coral reef, about 3-5 meters in depth, at Ko Larn.

Diploastrea heliopora

Pavona desussata

Pavona varians

Lithophyllon undulatum

Figure 4. Four selected coral species for preparing micro-fragments

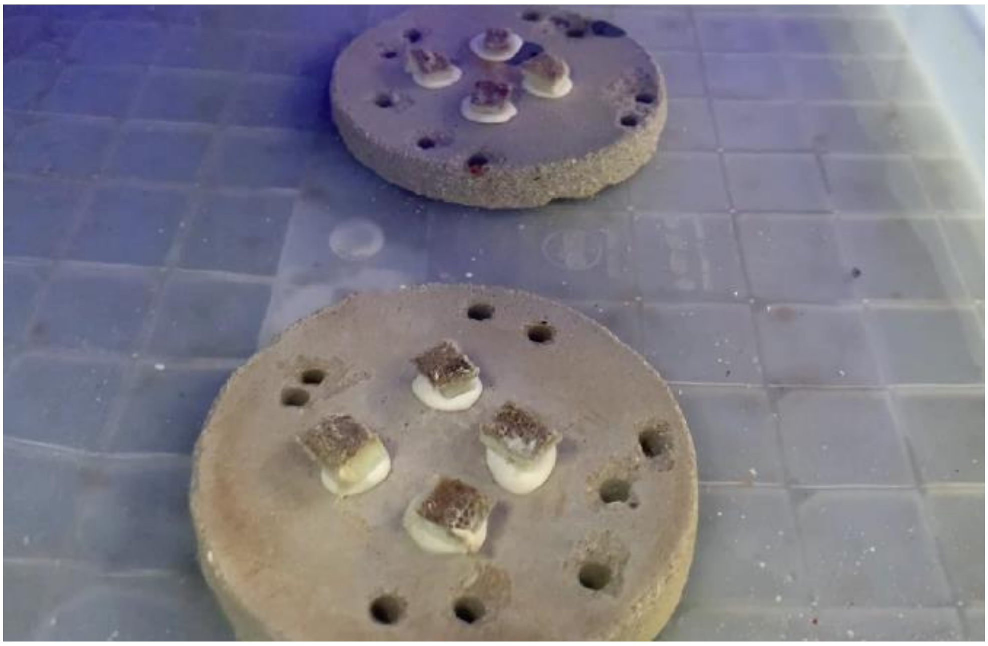


Figure 5. Cement plates for attaching coral micro-fragments.

3. Results

3.1 Measurement of environmental factors

In the coral nursery plots, several environmental conditions were examined with respect to the growth and survival rates of micro-fragments of *Diploastrea heliopora*, *Pavona desussata*, *Pavona varians*, and *Lithophyllon undulatum*. During the study period from June 2022 to September 2023, the environmental factors, i.e. seawater temperature, salinity, pH, dissolved oxygen (DO), oil or grease, suspended solid content, and sedimentation rate were measured. The results indicated that the temperature range of the seawater was 21.95 to 32.81°C, with July 2022 recording the highest temperature and September 2022 recording the lowest (Figure 6). The range of light intensity was 14–11,980 lux, with June 2023 having the highest intensity and August 2022 having the lowest (Figure 7). The salinity varied between 28.76 and 31.10 psu, with July 2022 recording the greatest salinity and August 2022 recording the lowest. The pH was 7.32 to 8.40, with September 2022 recording the highest value and March 2023 recording the lowest. There was a range of 4.58 to 6.95 mg/l of dissolved oxygen concentration; June 2022 had the highest concentration and June 2023 had the lowest. The transparency level varied from 1.0 to 3.5 meters, reaching its maximum in July 2022 and its minimum in September and October of the same year. During the study period neither oil nor grease were found. The range of suspended solid contents was 17.00 to 20.56 mg/l, with August 2022 recording the lowest level and June 2022 recording the highest. The sedimentation rates varied from 5.34 to 15.64 mg/cm^2/day, the maximum rate was observed in June 2022, and the lowest in March 2023.

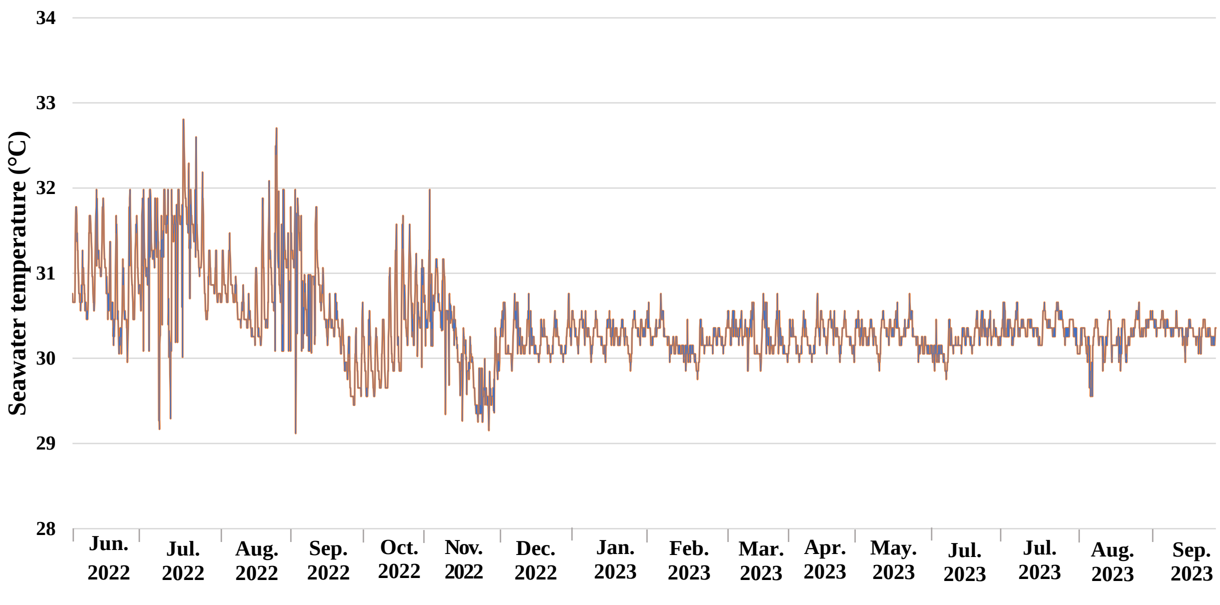


Figure 6. Seawater temperatures between June 2022 and September 2023 as recorded by a data logger

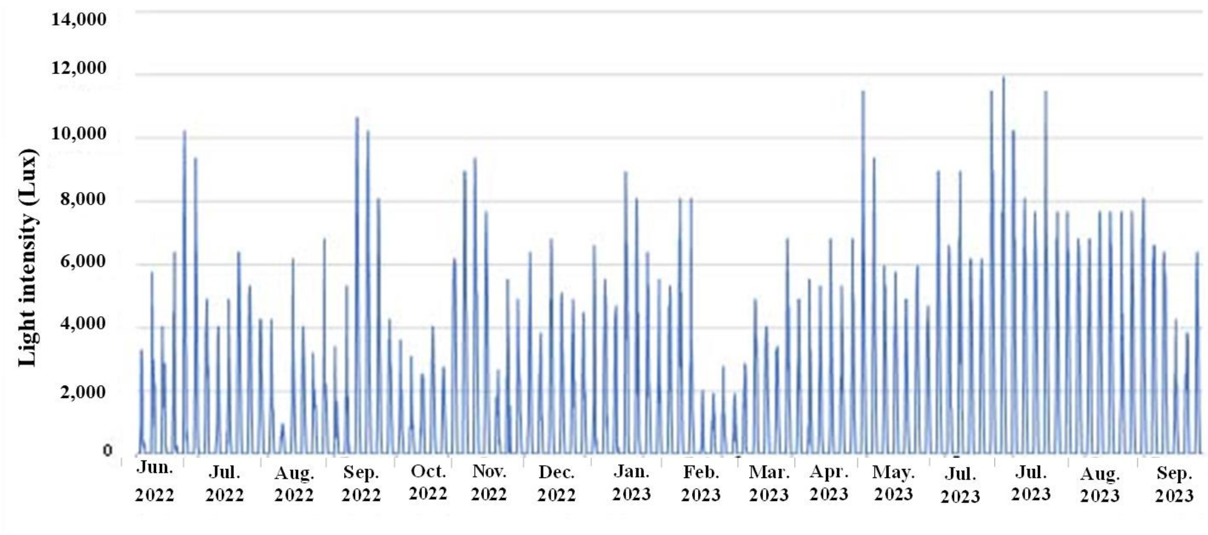


Figure 7. Data logger recording of light intensity from June 2022 to September 2023

3.2 Growth rate of micro-fragments in coral nursery plots

Four coral species were investigated in relation to the growth rates of coral micro-fragments in the coral nursery plots at Ao Nuan, Ko Larn: *D. heliopora*, *P. decussata*, *P. varians*, and *L. undulatum*. The highest growth rate was observed in the coral micro-fragments with an initial size of 1 cm, which were then followed by 2 and 3 cm (One-way ANOVA, $p < 0.05$). However, in *D. heliopora*, the initial size of 2 cm did not differ statistically significantly from the other sizes (Tukey's HSD, $p > 0.05$) (Figure 8).

The results showed that *D. heliopora* had the highest growth rate (59.70%) and *P. varians* had the lowest micro-fragment growth rate (50.04%) (Figure 9). Nevertheless, there was no statistically significant difference in the growth rates of micro-fragments among the coral species (One-way ANOVA, $p > 0.05$).

3.3 Survival rate of coral micro-fragments in coral nursery plots

The result showed that the survival rate of every micro-fragment with initial sizes between 1 and 3 cm was higher than 95.67% (Figure 10). Particularly, *D. heliopora* outperformed (99.25%) in terms of survival rate, and was followed by *P. decussata* (96.78%), *L. undulatum* (97.35%), and *P. varians* (95.67%). Among the initial sizes, there were significant differences in the survival rates of coral micro-fragments (One-way ANOVA, $p < 0.05$). While the micro-fragments of *P. desussata* had a much greater survival rate than its other initial sizes, the micro-fragments of *D. heliopora* with an initial size of 1 cm had a significantly lower survival rate than its other initial sizes. (Tukey's HSD, $p < 0.05$).

The results showed that survival rates of micro-fragments were significantly different among coral species (One-way ANOVA, $p < 0.05$). The survival rates of *D. heliopora* and *L. undulatum* micro-fragments were higher than those of *P. varians* and *P. decussata*. (Tukey's HSD, $p < 0.05$) (Figure 11). The results showed that, within a few months, the coral micro-fragments in the coral nursery plots, which had initially been of varying sizes, gradually fused to form large coral colonies (Figure 12).

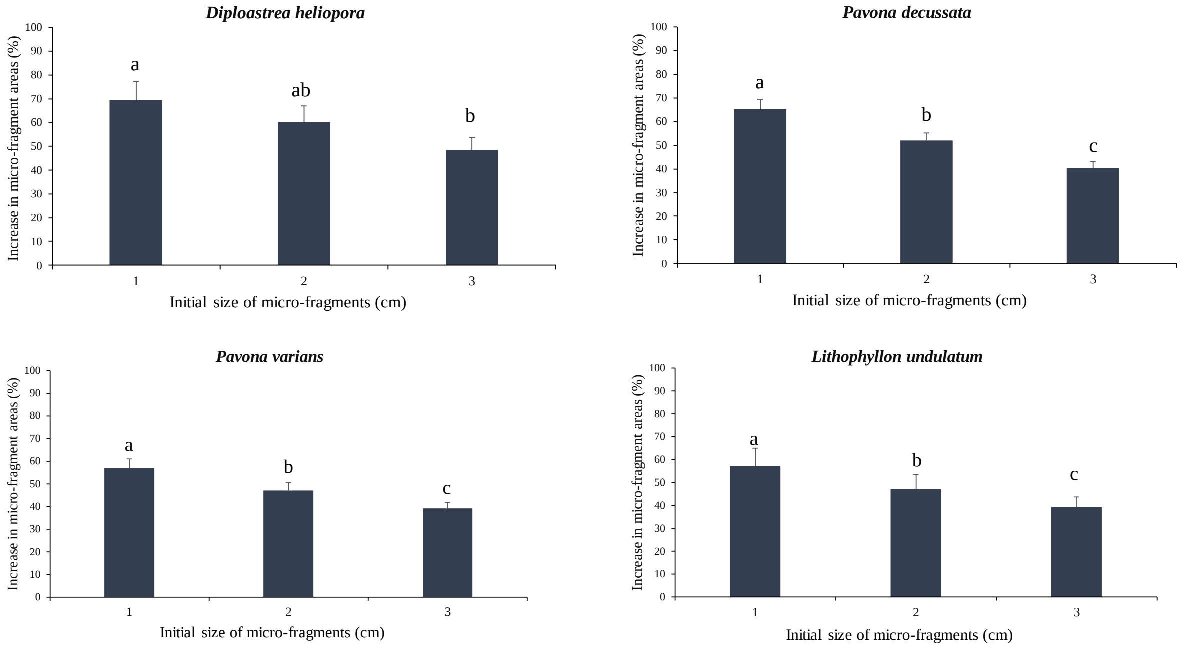


Figure 8. Mean growth rates of coral micro-fragments from coral nursery plots

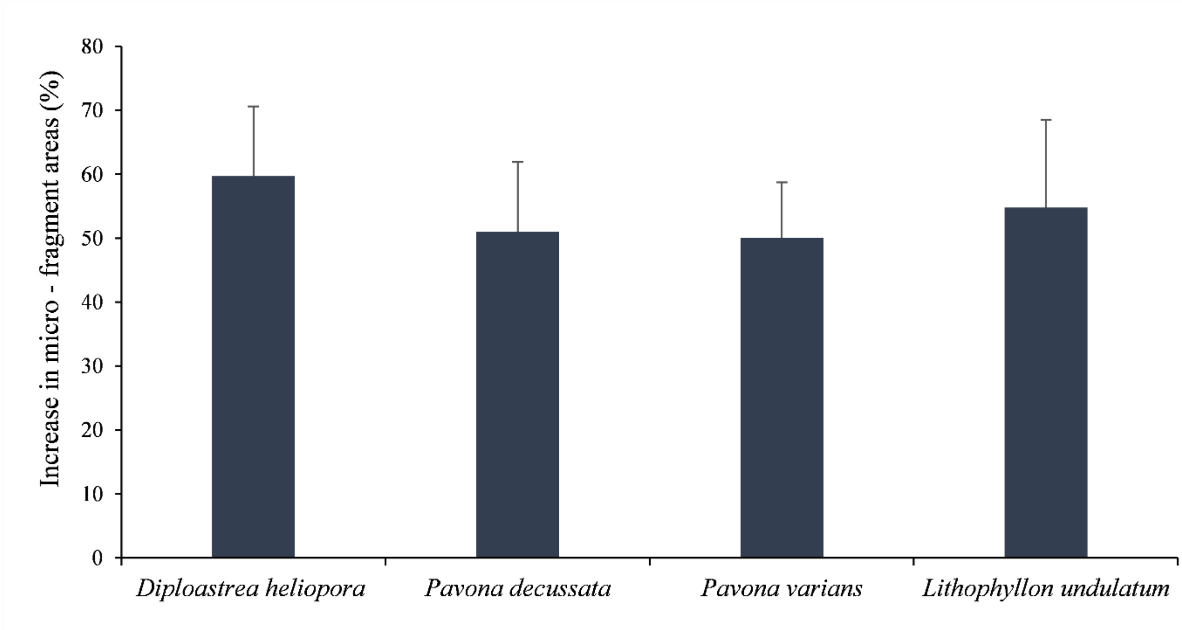


Figure 9. Mean growth rates of four different coral species' micro-fragments from coral nursery plots

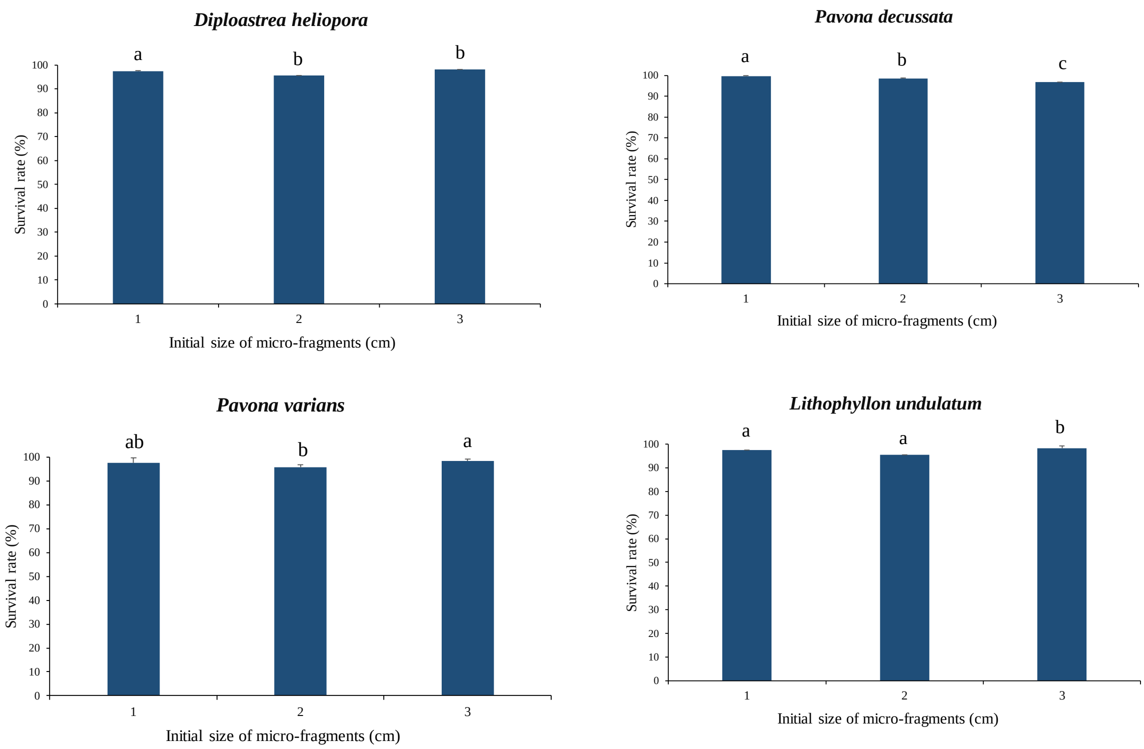


Figure 10. Mean survival rates of coral micro-fragments from coral nursery plots

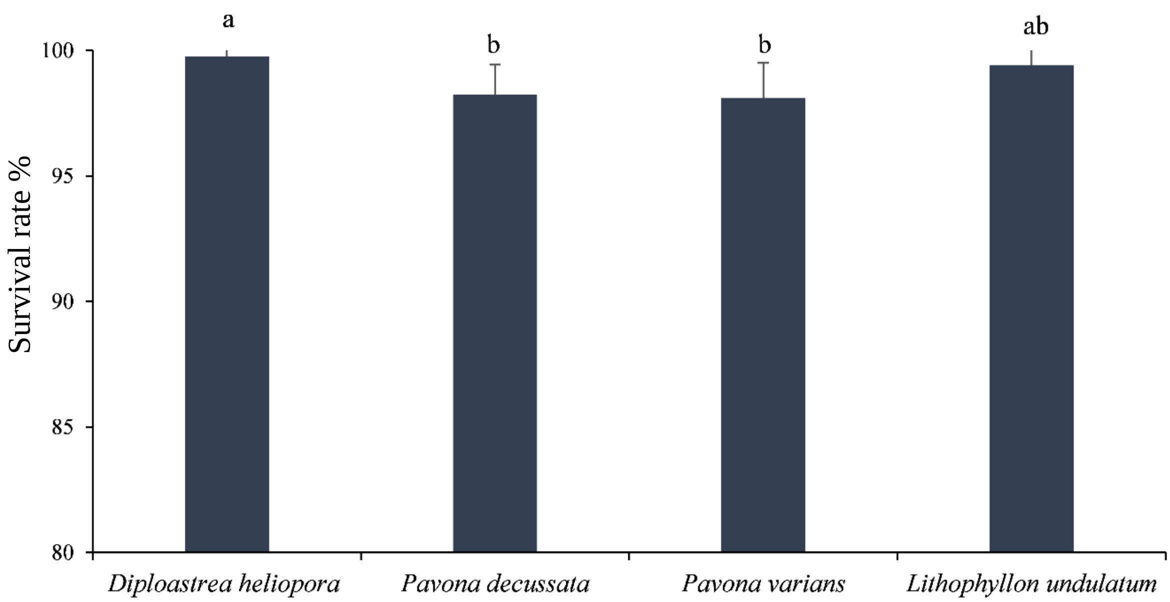
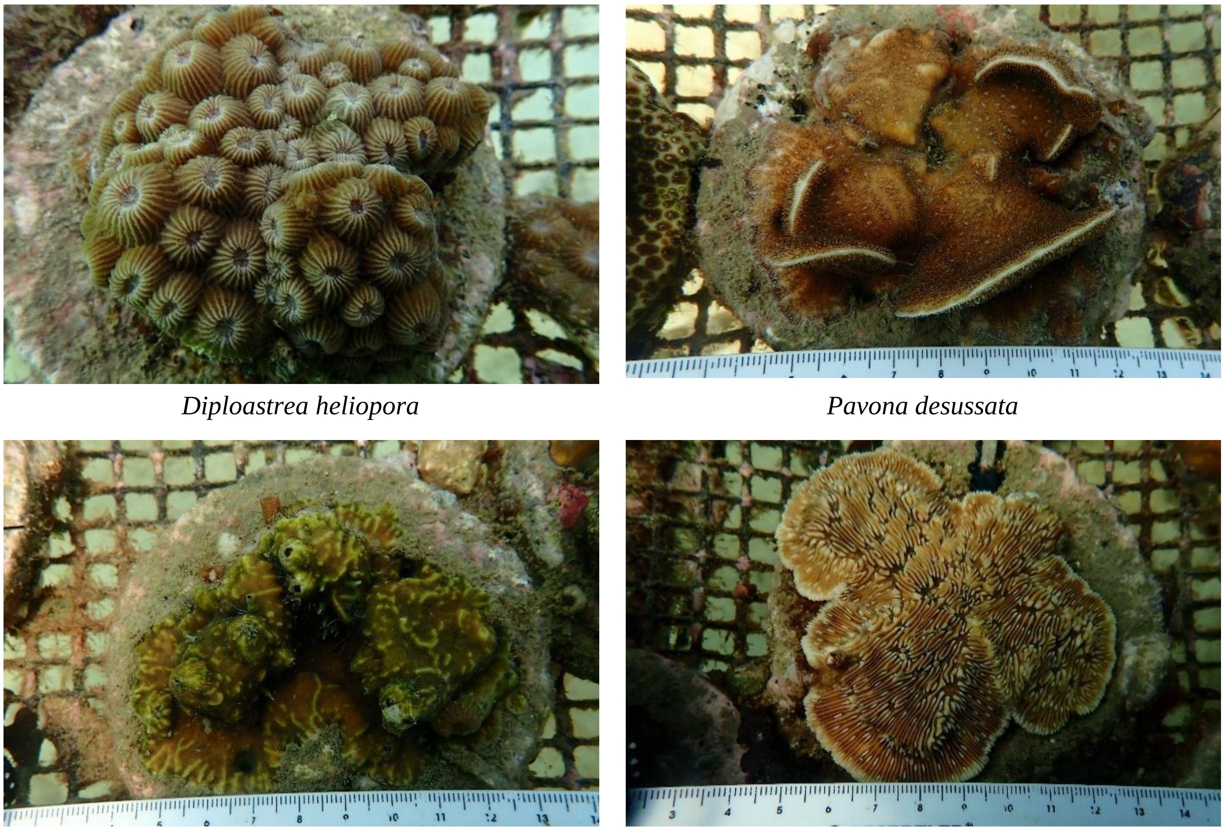


Figure 11. Mean survival rates of four different coral species' micro-fragments from coral nursery plots



Diploastrea heliopora *Pavona desussata*

Pavona varians *Lithophyllon undulatum*

Figure 12. Large coral colonies were formed by the successful fusion of the coral micro-fragments.

4. Discussion

The present study shows that coral tissue may be quickly covered with the micro-fragmentation and fusing process on a range of provided substrates, offering an excellent opportunity for future restoration projects. Additionally, this study supports the earlier coral micro-fragmentation study of *Porites lutea* in the Gulf of Thailand (Sutthacheep et al. 2023) and advances our understanding of coral micro-fragmentation in *Diploastrea heliopora*, *Pavona desussata*, *Pavona varians*, and *Lithophyllon undulatum*.

The research results indicate that coral micro-fragments of varying sizes show diverse growth rates in coral nursery plots. In general, coral micro-fragments with an initial size of 1 cm from the nursery plots at Ko Larn had the highest growth rate. Our results aligned with earlier research that demonstrated faster coral growth rates in smaller coral fragments (Harrington et al. 2004; Lirman and Schopmeyer 2016; Steinberg 2021; Sutthacheep et al. 2023).

The findings of this study demonstrated that micro-fragments of different sizes had varied survival rates. Higher survival rates were observed in coral micro-fragments of *D. heliopora* and *P. varians* from the nursery plots with larger sizes. On the other hand, coral micro-fragments of *P. desussata* and *L. undulatum* from the nursery plots with smaller sizes showed greater survival rates. Simple outplanting experiments of *Porites compressa* and *Montipora capitata* were reported in Hawaii to support active reef restoration of slower growing species and raise the possibility of restoration success (Knapp et al. 2022).

According to their findings, outplanting medium fragments as soon as feasible without the need for an intermediate culture in a nursery may be more economical. The significance of taking geographical variation in coral growth and survival after outplanting into account is emphasized. They also emphasize the importance of evaluating size and location-specific performance using short-term micro-fragmentation assays before outplanting in order to maximize the likelihood of success for active coral restoration projects and optimize the efficiency of active reef restoration efforts (Forsman et al. 2015; Knapp et al. 2022).

It is crucial to understand the physiological mechanisms that enable corals for rapid wound healing and stabilize themselves after physical injury. Lock et al. (2022) showed that when *Porites lobata* fragments are physically injured, they heal in two different ways: first, they regenerate the wound quickly around the incisions, and then they grow more slowly, which binds the colony to the substrate. The coral host responded quickly to acute physical damage and outplanting, resulting in markedly elevated energy production, disruption of calcium homeostasis, and endoplasmic reticulum (ER) stress, which raised rates of protein turnover and antioxidant expression. After a physical injury, phosphoinositide-mediated acute disruption of calcium homeostasis promotes the healing of wounds (Page et al. 2018; Lock et al. 2022).

In addition to *in situ* coral nursery plots, land-based culture can yield a higher rate of coral micro-fragment growth. Page et al. (2023) investigated the possibility of enhancing the development and health indices of micro-fragments of *P. compressa*, the Hawaiian coral, by the use of culture methods utilizing distinct algal fouling communities. They demonstrated that the Green Film approach yielded the greatest tank and plate metric health scores as well as the quickest micro-fragment mean growth during the winter, taking only 28 days to form the first row of new polyps. Large-scale restoration projects will benefit greatly from the use of standardized, time-efficient techniques for land-based culture that are intended to enhance coral fragment growth and production (Sneed et al. 2014; Page et al. 2023).

The results showed that the micro-fragment growth rate in four coral species was comparatively high. After a few months, the coral micro-fragments began to fuse and were then transported to the coral restoration sites. The pilot coral restoration sites may serve as outdoor education centers for children, young people, and ecotourists (Young et al. 2012; Schopmeyer et al. 2017; Sutthacheep et al. 2023). This study emphasizes how crucial it is to actively restore coral reefs using recently developed technologies, especially coral micro-fragmentation and colony fusion techniques, in order to improve community-based tourism, ecotourism, and carbon-neutral tourism, as well as the efficiency and effectiveness of coral restoration projects.

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