ORIGINAL PAPER

Survival and growth rate of coral micro-fragments for coral reef restoration in Chonburi Province, the Upper Gulf of Thailand

Thamasak Yeemin[a], Nilnaj Chaithanavisut [c], Wiphawan Aunkhongthong [a], Charernmee Chamchoy [a], Sittiporn Pengsakun [a], Wanlaya Klinthong [a], Jirasin Limpichat [a], Phiradet Chuabsak [b], Makamas Sutthacheep[a,*]

[a]Marine Biodiversity Research Group, Department of Biology, Faculty of Science, Ramkhamhaeng University, Huamak, Bangkapi, Bangkok, Thailand
[b]Department of Biology, Faculty of Science, Ramkhamhaeng University, Huamak, Bangkapi, Bangkok, Thailand
[c] Koh Sichang Marine Science Research Centre of Chulalongkorn University, Ko Si Chang District, Chonburi Province, Thailand

*Corresponding author: *smakmas@hotmail.com*

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Abstract. Coral reefs provide very important ecosystem services, especially nursery grounds for economically important species, tourism sites and coastal defense. Coral micro-fragmentation is a new technology that allows coral fragments to have a high growth rate. This study aims to compare the survival and growth rates of coral micro-fragments among four species, i.e, *Porites lutea*, *Favites abdita*, *Galaxea fascicularis* and *Leptastrea purpurea* in a coral nursery plot at Ko Khang Khao, Chonburi Province, the Upper Gulf of Thailand. The results showed that all micro-fragment with initial sizes between 1 and 3 cm had survival rates exceeding 90%. The mean survival rate of *F. abdita* was the highest (97.29%), followed by *L. purpurea* (96.89%), *G. fascicularis* (96.14%), and *P. lutea* (94.60%). 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, $p < 0.05$). This study highlights the importance of active coral reef restoration using micro-fragmentation technique as a newly developed technology to elevate the efficiency and effectiveness of coral restoration projects as well as enhancing community based eco-tourism and carbon neutral tourism or net zero tourism.

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

1. Introduction

Coral reefs are the important tropical marine ecosystem, and have suffered significant degradation and loss globally over the past decades (Wilkinson, 2004; De'ath et al., 2012; Hughes et al., 2017; Sutthacheep et al., 2022). Future predictions under moderate climate scenarios indicate a potential loss of up to 99% of the remaining reefs (Bindoff et al., 2019). The increasing frequency and severity of coral bleaching events is driven primarily by global warming and other anthropogenic stressors (Hoegh-Guldberg et al., 2007; Bruno & Selig, 2008), These stressors disrupt essential ecological processes, triggering widespread coral bleaching and mortality, ultimately jeopardizing the future of these critical ecosystems (Hughes et al., 2017). The coral reef degradation has pushed scientists towards exploring more radical interventions for mitigation (Moberg and Folke, 1999). This alarming trend highlights the urgent need for innovative management strategies to safeguard the vital ecological and economic services coral reefs provide, supporting millions of livelihoods.

Under the crisis facing coral reef ecosystems, the coral restoration has emerged as a pivotal strategy in combating their decline. A particularly promising approach for coral reef restoration is micro-fragmentation. This technique involves the asexual propagation of small fragments obtained from healthy coral colonies to establish new coral populations. These fragments are carefully nurtured in controlled environments before being transplanted

onto natural reefs. Micro-fragmentation stands out from traditional restoration methods due to its minimally invasive nature, ability to rapidly propagate corals, and the potential to select resilient genotypes. (Bayraktarov et al. 2019; Knapp et al. 2022; Lock et al. 2022; Page et al. 2023).

Building on this approach, a recent study by Yeemin et al. (2023) in Thailand found that coral micro-fragments sized 1 cm exhibited the fastest growth rate, with *Diploastrea heliopora* growing the most. All fragments 1-3 cm in size had a survival rate exceeding 95.67%, although there were significant variations among species. *D. heliopore* and *Lithophyllon undulatum* showed higher survival rates compared with *Pavona desussata* and *P. varians*.

Despite the promise of coral micro-fragmentation, concerns remain regarding its potential applications in other coral species and reef sites. Additionally, research on the effectiveness of this technique for Thai coral reef restoration is limited. This study aims to address this knowledge gap by investigating the efficiency and optimal methods for restoring coral reefs in the high turbidity areas of the Gulf of Thailand using micro-fragmentation. This research has the potential to significantly contribute to preserve coral reefs and their associated ecosystems

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2. Materials and Methods

2.1 Study site

Ko Khang Khao, Chonburi Province, the upper Gulf of Thailand, is selected for this study (Figure 1). The selected coral reef for this study has spaces in the reef slope zone, in the range of 3-5 meters in depth, suitable environment for coral growth, a few large macro-benthic animals and reef fish that affect survival rates of coral micro-fragments and having the potential to be an eco-tourism destination (Figure 2). 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 suitable area of coral reef in the north of Ko Khang Khao (Figures 3).

2.2 Coral micro-fragment preparation

Porites lutea, *Favites abdita*, *Galaxea fascicularis* and *Leptastrea purpurea* were selected in this study. A set of coral condition 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 colonies that were chosen underwent acclimatization in a nursery pond for a minimum of 24 hours. Then, coral fragments measuring about 10 x 10 cm were cut and moved to a culture pond fitted with an aerated system and seawater circulation. Micro-fragments were created by using an electric cutter to slice the coral fragments into 1 cm, 2 cm, and 3 cm pieces, all while keeping them cooled in seawater. Rubber gloves were utilized to prevent direct contact with the coral tissue during handling. The coral micro-fragments were subsequently placed in a container with gently swirling seawater to minimize stress and mucus production. The coral micro-fragments were attached to circular cement plates with a 5 cm radius, and these plates had been soaked in seawater for 48 hours beforehand to acclimate them. Epoxy glue was employed to affix four micro-fragments onto each plate, leaving a 1 cm gap between them. Subsequently, a SCUBA diver secured the plates, bearing coral micro-fragments, onto the nursery plot using a plastic net and cable tie. There were 15 cement plates arranged in five rows, with around 10 cm of

space between them. These plates were numbered to facilitate monitoring of the growth and survival rates of the coral micro-fragments at the nursery plot.

2.3 Data analysis

Throughout the study period, the coral nursery plots underwent continuous monitoring of seawater temperature, light intensity, salinity, pH, dissolved oxygen (DO), oil or grease levels, suspended solid content, and sedimentation rate.

From June 2022 to September 2023, changes in the area of coral micro-fragments were measured to assess their growth and survival rates. Statistical analysis was conducted using a one-way ANOVA to ascertain differences in these rates among the groups. Tukey's Honestly Significant Difference (Tukey's HSD) test was then employed to detect significant variations in growth and survival rates between different groups.

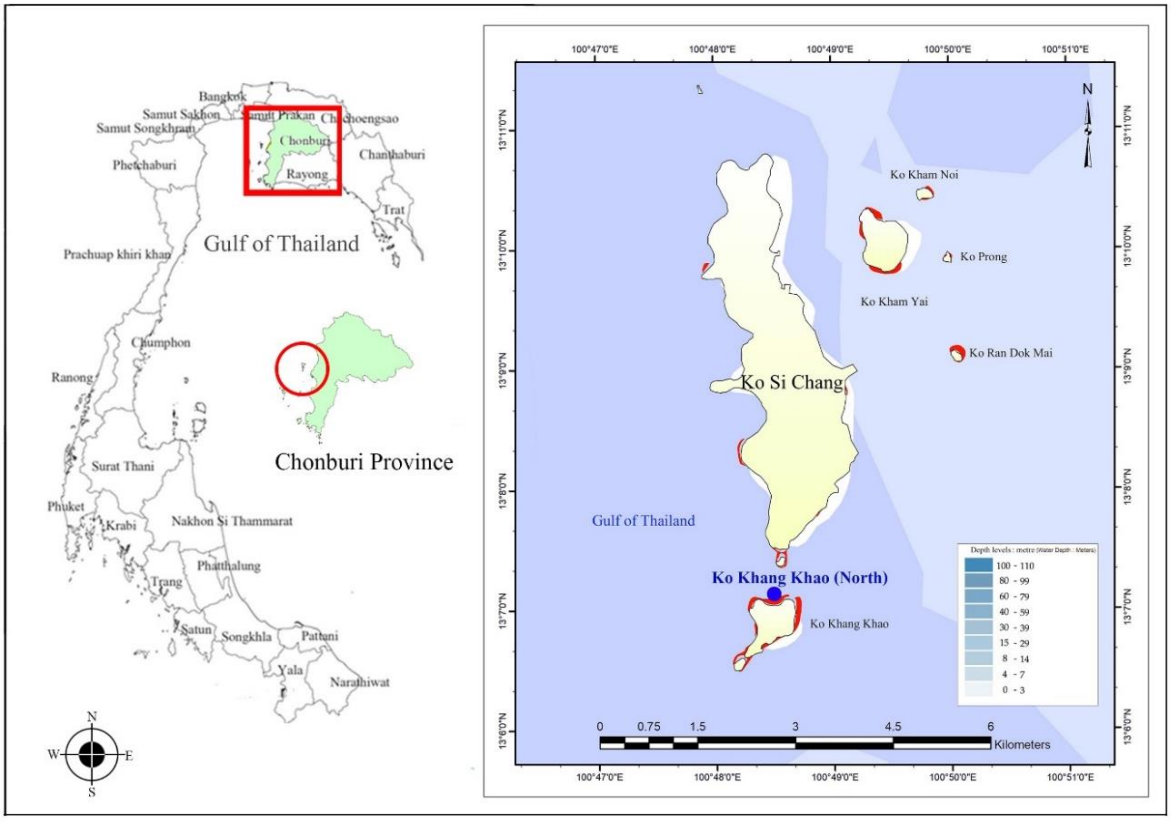
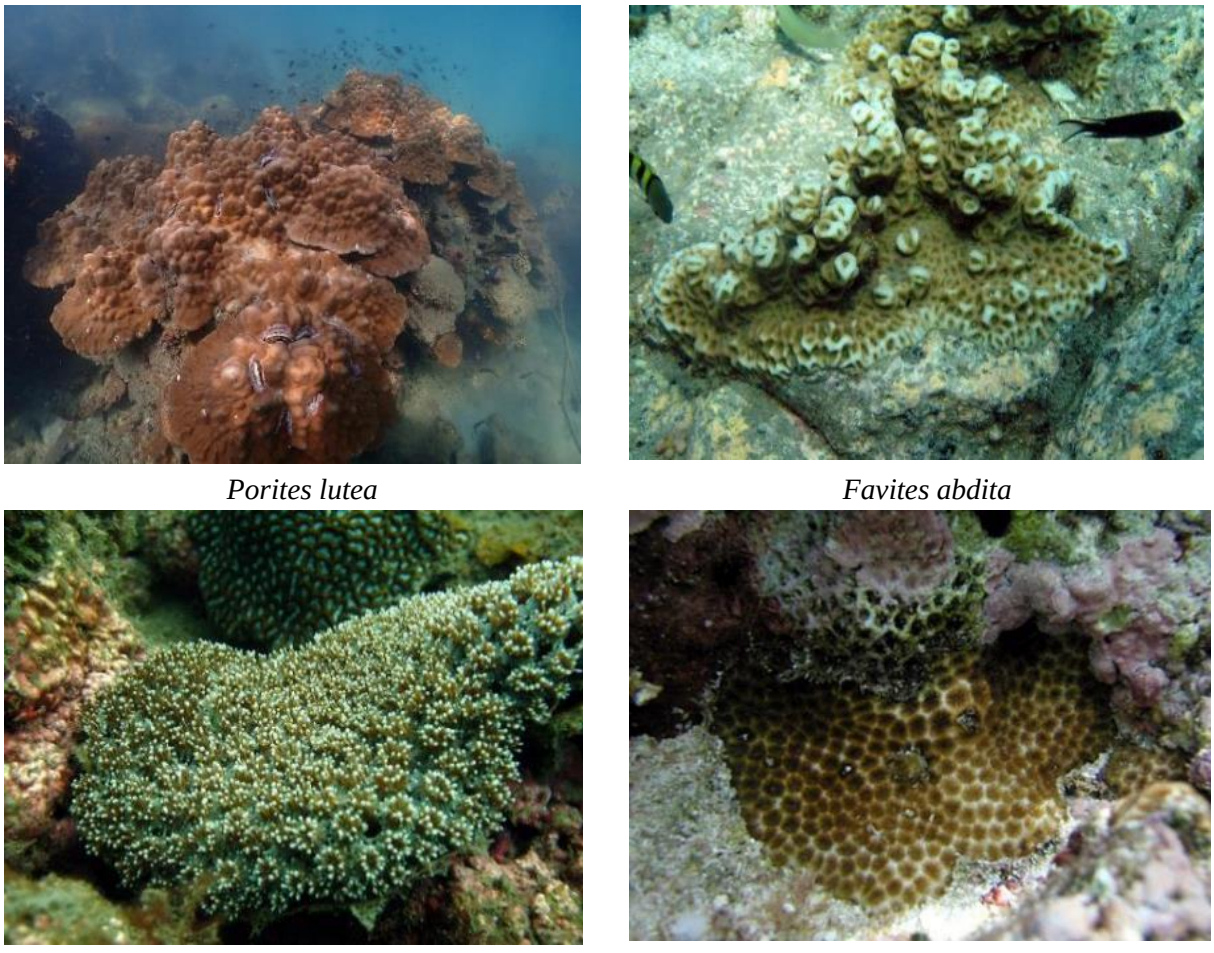


Figure 1. Location of the study site at Ko Khang Khao (North)

Figure 2. A suitable site for establishing a coral nursery.

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



Porites lutea *Favites abdita*

Galaxea fascicularis *Leptastrea purpurea*

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

3. Results

3.1 Measurement of environmental factors

The coral nursery plot was studied to assess various environmental conditions affecting the growth and survival rates of micro-fragments from *Porites lutea*, *Favites abdita*, *Galaxea fascicularis*, and *Leptastrea purpurea*. During June 2022 and September 2023, we measured environmental factors, including seawater temperature, salinity, pH, dissolved oxygen (DO), presence of oil or grease, suspended solid content, and sedimentation rate. The results indicated that the temperature range of the seawater was 22.95 to 32.78°C, with July 2022 recording the highest temperature and February 2022 recording the lowest (Figure 5). The range of light intensity was 60–23,904 lux, with September 2022 having the highest intensity and Jun 2022 having the lowest (Figure 6).

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

The results showed that the survival rates of all micro-fragment with initial sizes between 1 and 3 cm had survival rates exceeding 90%. Specifically, micro-fragments of *Favites abdita* had a mean survival rate 97.29%. The survival rate of micro-fragments of *Leptastrea purpurea* was 96.89%. The micro-fragments of *Galaxea fascicularis* had a mean survival rate 96.14%. *Porites lutea* had the lowest survival rate of micro-fragments 94.60%. (Figure 7).

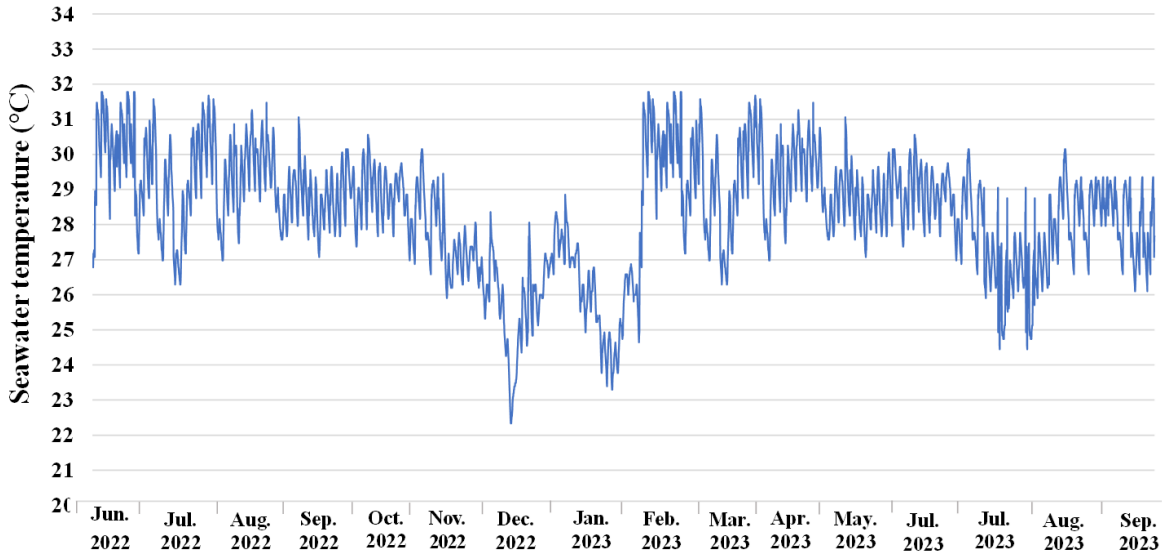


Figure 5. Seawater temperatures between June 2022 and September 2023 as recorded by a data logger

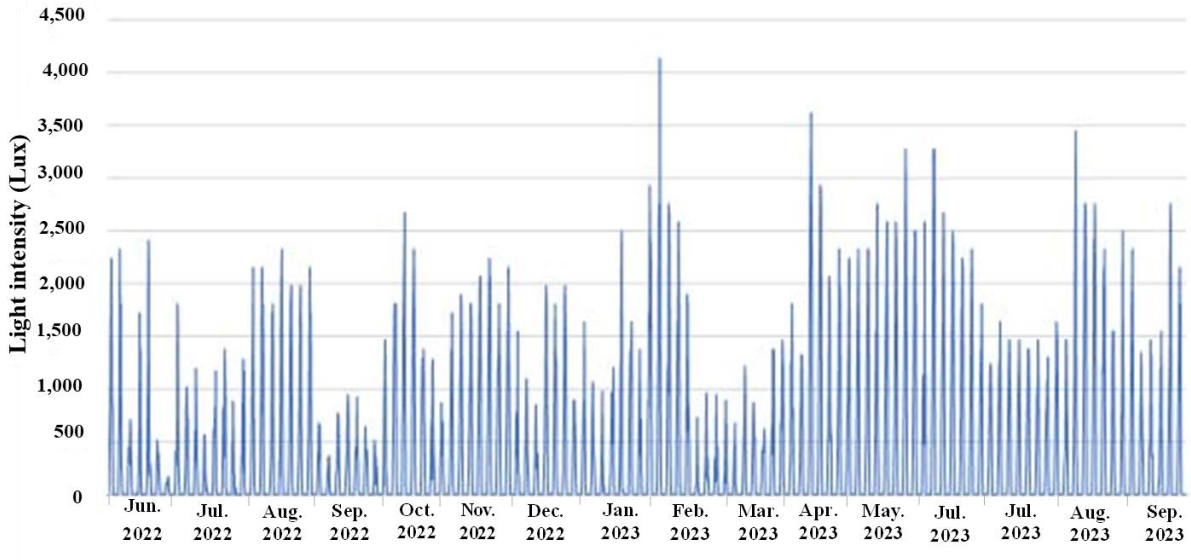


Figure 6. Data logger recording of light intensity from June 2022 to September 2023

3.3 Growth rate of micro-fragments in a coral nursery plot

Four coral species were investigated for the growth rates of coral micro-fragments in the coral nursery plot at Ko Khang Khao: *P. lutea*, *F. abdita*, *G. fascicularis* and *L. purpurea*. 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, $p < 0.05$). (Figure 8).

The results showed that growth rates of micro-fragments were significantly different among coral species (One-way ANOVA, $p < 0.05$). The growth rate of *L. lutea* micro-fragments was higher than that of *G. fascicularis*, *L. purpurea*, and *F. abdita* (Tukey's HSD, $p < 0.05$) (Figure 9).

The results indicated that over a few months, the coral micro-fragments in the nursery plot, varying initial micro-fragment sizes, gradually fused to develop into larger coral colonies. (Figure 10).

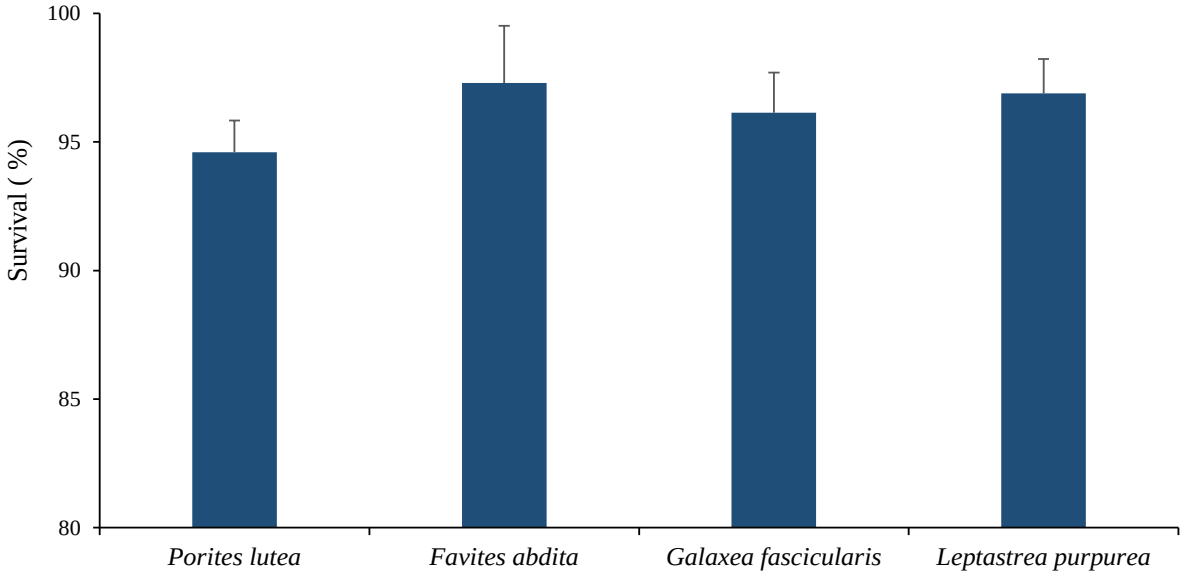


Figure 7. Mean survival rates of micro-fragment of four coral species from the coral nursery plot

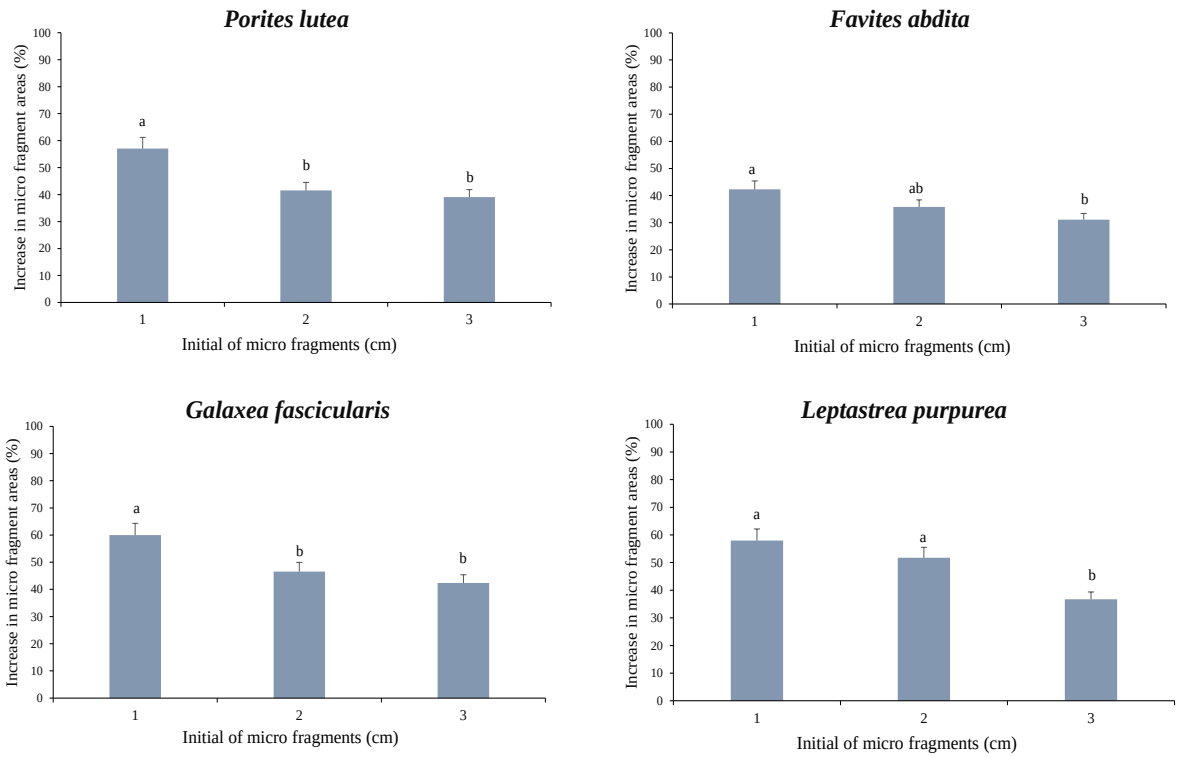


Figure 8. Mean growth rates of coral micro-fragments from coral nursery plots

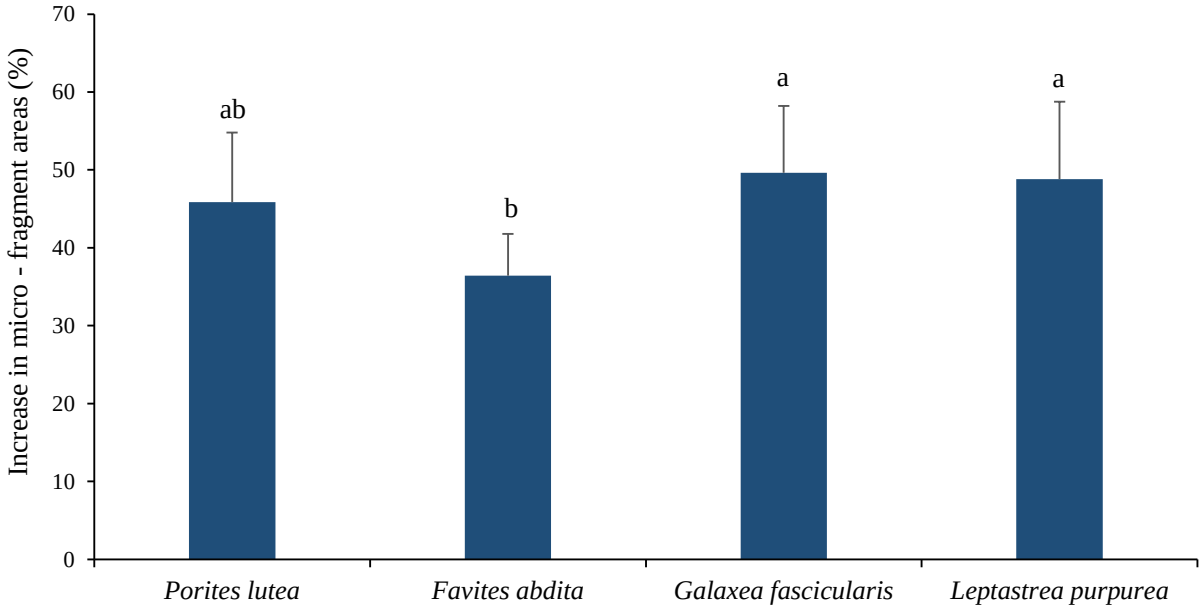


Figure 9. Mean growth rates of micro-fragments of four coral species from the coral nursery plot.

4. Discussion

This study shows the successful of using coral micro-fragments in a coral restoration project at Ko Khang Khao, Chonburi Province, the Upper Gulf of Thailand which is located at the high turbidity area. Our results are the first report of coral micro-fragmentation technique in *P. lutea*, *F. abdita*, *G. fascicularis* and *L. purpurea*. The survival rates of micro-fragments of *P. lutea*, *F. abdita*, *G. fascicularis* and *L. purpurea* in this study ranged between 94.60 to 97.29% which are comparable to those of *Diploastrea heliopora*, *Pavona desussata*, *Pavona variansand*, and *Lithophyllon undulatum* in coral nursery plots at Ko Larn,

Chonburi Province, the upper Gulf of Thailand, ranging 95.67-99.25 % (Yeemin et al, 2023), *Orbicella faveolata* (96.18%) in La Parguera, Puerto Rico (Raker et al, 2023), *Pocillopora* spp. (96.4%) in southern Mexican Pacific (García-Medrano et al, 2023) in table 1.

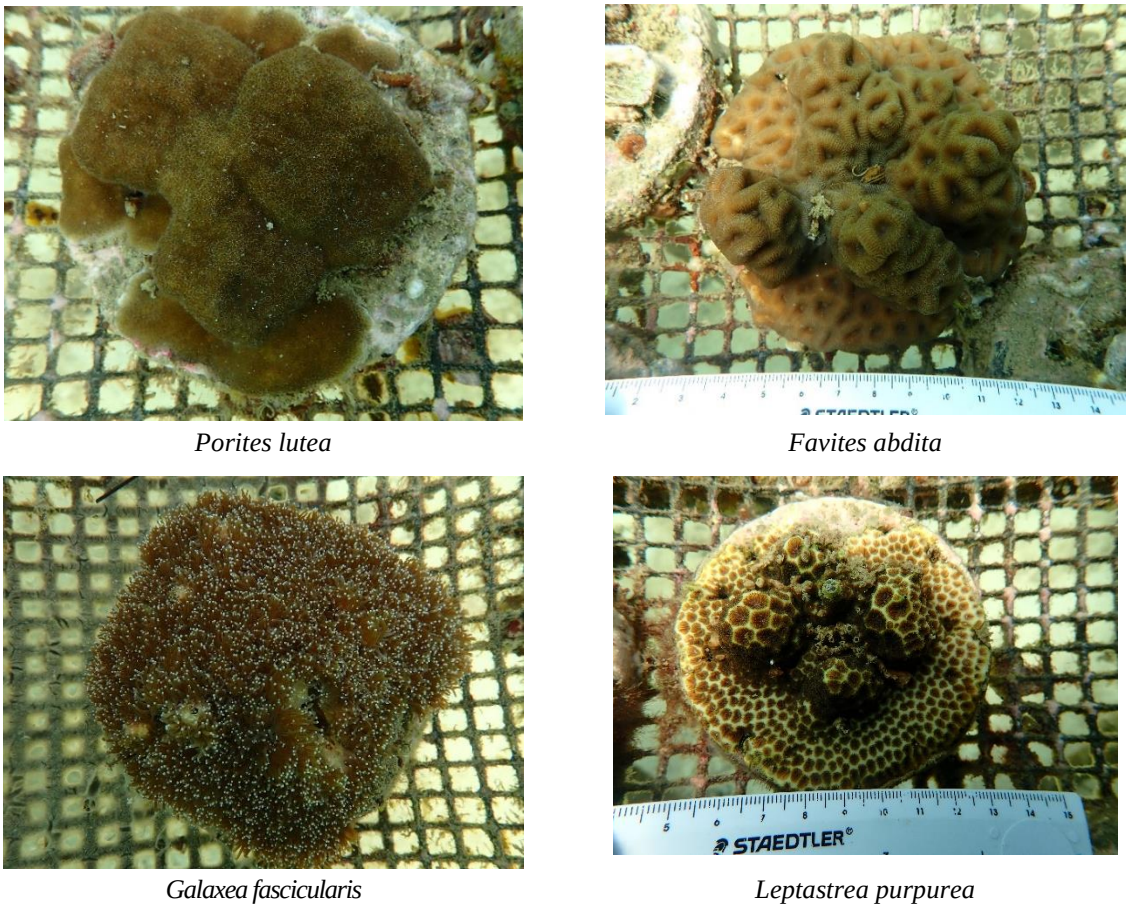
Our results indicate that coral micro-fragment sizes in a range of 1-3 cm showed significant different growth rates in the coral nursery plot. The coral micro-fragments with an initial size of 1 cm from the nursery plot at Ko Khang Khao, Chonburi Province, the Upper Gulf of Thailand had the highest growth rate. Our results are consistent with prior research that has shown higher coral growth rates in smaller coral fragments. (Harrington et al. 2004; Lirman and Schopmeyer 2016; Steinberg 2021; Sutthacheep et al. 2023, Yeemin et al, 2023).

The growth rate of *P. lutea* micro-fragments was higher than that of *G. fascicularis*, *L. purpurea*, and *F. abdita*. Growth rates of coral micro-fragments significantly vary among coral species and reef sites. Tortolero-Langarica et al. (2020) reported the *Pocillopora* growth measurements from the Eastern Tropical Pacific showing a 183% linear extension and a 253% width increase, compared to initial sizes, and with average extension highs of 4.16 ± 1.02 cm while the growth of *Pavona clavus* hemispherical structures increased by 158% and 174% in height and width, respectively. However, a previous study on coral micro-fragments at Ao Nuan, Ko Larn, the Upper Gulf of Thailand revealed that *D. heliopore* had the highest growth rate (59.70%) and *P. varians* had the lowest (50.04%) (Yeemin et al., 2023).

The growth of coral micro-fragments can be explained from the healing processes of coral fragments. After being physically injured, coral fragments undergo two distinct healing processes: initially, they rapidly regenerate the wounded areas around the incisions, followed by a slower growth phase that firmly attaches the colony to the substrate. The coral host swiftly reacted to acute physical damage and transplantation, leading to significantly increased energy production, disturbance in calcium balance, and endoplasmic reticulum (ER) stress. This stress, in turn, heightened rates of protein turnover and the expression of antioxidants. Following a physical injury, the disruption of calcium homeostasis mediated by phosphoinositides facilitates the healing of wounds (Page et al. 2018; Lock et al. 2022, Yeemin et al., 2023).

Establishing coral nursery plots in coral reefs and creating reef restoration areas using coral micro-fragments in each reef site should be considered the appropriate time period, and important environmental factors, such as the amount of sediment, the sea urchin and fish populations that affect coral micro-fragments, in order to increase the survival and growth rates of coral micro-fragments (Yeemin et al., 2023). Raker et al. (2023) showed that using predator exclusion cages to enhance coral micro-fragment survivorship and growth during restoration can boost coral survival during initial stages of restoration by over than 50% and increase the persistence of transplanted coral micro-fragments.

The coral micro-fragmentation technique is highly appropriate for reef restoration in Thai waters, particularly for reviving coral species with limited populations that face the threat of extinction. Moreover, local communities can transform reef restoration sites utilizing coral micro-fragments into eco-tourism hotspots which foster economic growth within the community, generate income for the local area, and pave the way for future developments in community-based carbon neutral tourism or net zero tourism.



Porites lutea *Favites abdita*

Galaxea fascicularis *Leptastrea purpurea*

Figure 10. Large coral colonies of four coral species from successful fusion of coral micro-fragments

Table 1 Survival rates of coral micro-fragments from different locations

Coral species	Study sites	Survival rates	Reference
Favites abdita	Ko Khang Khao, Chonburi province	97.29%	This study
Galaxea fascicularis	Ko Khang Khao, Chonburi province	96.14%	This study
Leptastrea purpurea	Ko Larn, Chonburi Province	96.25%	Yeemin et al, 2023
Leptastrea purpurea	Ko Khang Khao, Chonburi province	96.89%	This study
Lithophyllon undulatum	Ko Larn, Chonburi Province	97.35%	Yeemin et al, 2023
Orbicella faveolata	La Parguera, Puerto Rico	96.18%	Raker et al, 2023
Orbicella faveolata	Hawaii, USA	100%	Forsman et al, 2015
Orbicella faveolata	Big Pine Key, USA	80%	Page et al, 2018
Pavona clavus	Central Mexican Pacific	61%	Tortolero-Langarica et al, 2020
Pavona decussata	Ko Larn, Chonburi Province	96.78%	Yeemin et al, 2023
Pavona varians	Ko Larn, Chonburi Province	95.67%	Yeemin et al, 2023
Pocillopora spp.	Central Mexican Pacific	58%	Tortolero-Langarica et al, 2020
Pocillopora spp.	southern Mexican Pacific	96.4%	García-Medrano et al, 2023
Porites lobata	Hawaii, USA	99%	Forsman et al, 2015

Table 1 Survival rates of coral micro-fragments from different locations (*continued*)

Coral species	Study sites	Survival rates	Reference
Porites lutea	Ko Khang Khao, Chonburi province	94.60%	This study
Porites porites	Driftwood, Barbados	22%	Henriksen, 2020
Porites porites	Folkestone, Barbados	50%	Henriksen, 2020
Pseudodiploria strigose	Driftwood, Barbados	90%	Henriksen, 2020
Pseudodiploria strigose	Folkestone, Barbados	6%	Henriksen, 2020

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