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Transplantation of Acropora in Different Depth: First Report on an Effort for Environmental Sustainability in Gili Ketapang, Probolinggo

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Abstract: The *Acropora* commodity in Gili Ketapang, Probolinggo, is massively deteriorated; thus, a transplantation program for environmental sustainability is commenced. This study is aimed to determine the effect of depth on the *Acropora* transplantation activity as a reference of the *Acropora* rehabilitation program in the Gili Ketapang, Probolinggo. The result showed that transplanted *Acropora* at 5 m and 10 m depths showed different survival rates. The highest survival rate at a depth of 5 m was 94%, while the lowest was 84% at 10 m in depth. Their bright and diverse colors characterized the live corals in this study; meanwhile, the dead corals were pale white and fragile. The average density of zooxanthellae cells at a depth of 5 meters was 4.79 x 10^6 cells/cm^2 and at a depth of 10 meters was 2.75 x 10^6 cells/cm^2. Uniquely, the level of nitrate and phosphate did not comply with the quality standards. Thus, we recommend that transplantation activity of *Acropora* in Gili Ketapang, Probolinggo, was performed at a depth of 5 m for optimal results.

Keywords: Acropora, transplantation, environmental education, depth, marine conservation.

不同深度的鹿角移植：关于在探戈的吉利吉打邦努力实现环境可持续性的第一份报告

摘要：位于探戈的吉利吉打邦的鹿角商品严重恶化；因此，启动了环境可持续性移植计划。本研究旨在确定深度对鹿角移植活动的影响，作为探戈的吉利吉打邦的鹿角康复计划的参考。结果表明，移植后5仪表和10仪表深度的鹿角珊瑚存活率不同。5仪表深度的最高成活率为94%，而10仪表深度的最低成活率为84%。它们鲜艳多样的颜色是本研究中活珊瑚的特征。与此同时，死去的珊瑚呈淡白色且脆弱。5米深处的虫黄藻细胞平均密度为4.79x10^6个细胞/平方厘米，10米深度处的平均密度为2.75x10^6个细胞/平方厘米。独特的是，硝酸盐和磷酸盐的水平不符合质量标准。因此，我们建议在探戈的吉利吉打邦进行鹿角的移植活动，在5仪表的深度进行以获得最佳结果。

关键词：鹿角、移植、环境教育、深度、海洋保护。

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1. Introduction

Coral reefs are one of Indonesia's marine assets. The area covers 2.5 million hectares in Indonesia's water, representing 14% of the world's coral reefs [1]. It is a distinguished marine area designated by a high level of biodiversity, with more than 480 types of stony corals identified [2]. The existence of the coral reef denotes many benefits, a habitat for living and a nursery for marine animals [3]. Coral reefs are also useful in preventing erosion due to waves and wind forces [4]. One of the many coral reefs found in Indonesia is Acropora.

Acropora is the most diverse coral reef genus, with 114 species recognized worldwide and 91 species identified in the Indonesian archipelago [5]. Acropora spread from the red sea through the Indo-Pacific Ocean to the Caribbean [6]. Among shallow coastal zone in Indonesia, Gili Ketapang, Probolinggo is well-known for its diversity of coral reefs, especially genus Acropora [7]. The waters are portrayed with high frequency, sandy, and bright water suitable for Acropora to grow well [8]. Unfortunately, Acropora's condition there is starting to break down.

Previous research in 2019 stated that the coral reefs had begun to deteriorate, and the number of fish had continued to decrease [9]. That is due to overfishing activities and the use of non-environmentally friendly fishing gear in Probolinggo [10]. Acropora is a coral reef sensitive to pollution, sedimentation, and fishing due to human activities [8]. The recovery by nature takes time, and the initial stage of coral's self-planting may be interrupted by an unexpected event. Thus, human intervention is required to keep the progress in check, and transplantation is preferable activity introduced in a coral rehabilitation program.

Transplantation is a coral reef rehabilitation technique by planting coral reefs in growing media [11]. Coral reef transplantation is useful in enhancing coral reef populations, mitigating damaged areas, and protecting beaches from waves. This technique has been successfully carried out on Acropora coral reefs [12] [13]. In the Acropora transplant activity, depth determination is very important. The difference in depth greatly affects the growth of Acropora [14]. The corals would be easily destroyed by waves whenever it transplants at low depth [15]. Opposite, going down in-depth, the coral growth is hampered due to the lack of light for the growth of zooxanthella on the coral [16]. Zooxanthella requires sufficient sunlight for photosynthesis to produce calcium carbonate, which affects coral growth [17]. This study is aimed to determine the effect of depth on the Acropora transplantation activity. Thus it can become a reference in the Acropora rehabilitation program in the Gili Ketapang, Probolinggo.

2. Methods/Materials

2.1. Sampling Site

This research was conducted within 2 months (February - April 2020), and the observation was carried out every 1 week in the waters of Sumberasih District, Gili Ketapang Island, Probolinggo, East Java, Indonesia. Geographically, the research was located at the coordinates of latitude: 07º40'46.7" South and Longitude: 113º15'72.7' East. The location was selected due to massive human activities exploiting the area, such as overfishing and non-environmentally friendly fishing gear. The activities are starting to damage the coral reef ecosystem [7], [10], [18].

2.2. Transplantation Activity

This study used 2 treatments, including P1 and P2, based on the proposed depths. In P1 treatment, Acropora was planted at a depth of 5 meters; meanwhile, P2 treatment planted Acropora at 10 m in depth. The depth was elected based on the probability of survival rate of zooxanthellae lived as mutualistic symbiosis on the coral [2]. Ensuring the reproducibility of the data, we applied 6 replications for each treatment. The transplantation utilized artificial reefs as a medium for growth [19]. The material for the artificial reef was made of concrete blocks for its low-cost, flexible, easy to be shaped, easy to carry, good resistance, and suitable for living microorganisms [1], [20], [21]. The artificial reef designed to have a compact structure, rectangular in shape, 120 cm long of a leg beam structure, 40 cm high, 10 cm thick, and the table beam structure size was 120 cm long, 50 cm wide, and 10 cm thick. The picture of the transplant rack used showed in Fig. 1.

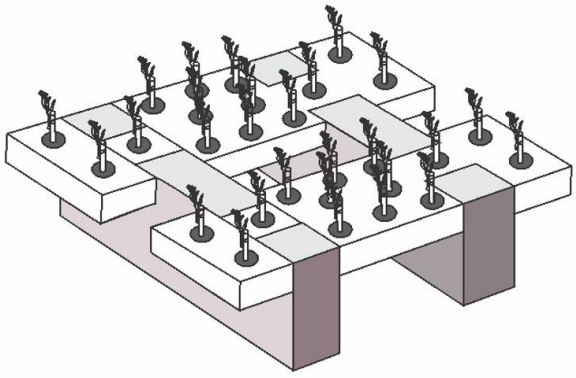

Fig.1 Artificial reef for *Acropora* transplantation activity

The coral fragments used in this study were coral fragments of the *Acropora* species with branching growth from Sumberasih District, Gili Ketapang Island, Probolinggo, East Java, Indonesia. Coral fragments were harvested by cutting coral fragments from the colony, measuring their initial height and width before transplanting, and attaching them to artificial reefs

following previous studies [22]. An artificial reef consisted of 28 *Acropora* fragments, as showed in Figure 1. Following that, the concrete table was slowly submerged at a depth of 5 m and 10 m.

The data retrieved from this study were survival rate, zooxanthella, and water quality. The coral survival rate in this study was carried out once a week for 2 months. Survival of the coral *Acropora* observed and calculated according to the previous research as the following formula [23]:

$$\text{Survival rate} = Nt/No \times 100\% \quad (1)$$

Nt represents the number of living coral fragments at the end of the study. *No* is the number of living coral fragments at the beginning of the study. Meanwhile, the zooxanthellae density was searched and calculated using a modification of the previous research formula [24]:

$$\text{Zooxanthellae density (Cell/cm}^2) = N \times At \times Vt / Ac \times Cs \times As \quad (2)$$

N display counted the number of zooxanthellae (cell), *At* represents glass cover area (mm^2), *Vt* is total sample volume (ml), meanwhile *Ac*, *Vs*, *As* are the area of the scraped sample (cm^2), sample volume used (ml), hemacytometer area (mm^2), respectively.

2.3. Water Quality Measurement

Supporting the survival of the Acropora experiment, the physicochemical parameters of seawater were also monitored, as presented in Table 1. Those parameters are suspected to affect the survival capability of zooxanthellae on the Acropora directly.

Table 1 Measured environmental parameters and tools used [25], [26], [27]

Parameter	Device
Temperature (°C)	Thermometer
Flow velocity (m/s)	Flowball and stopwatch
Brightness (%)	Sacchi disk
Nitrate (NO_3^-) (mg/l)	Spectrophotometer
Phosphate (PO_4^-) (mg/l)	Spectrophotometer
pH	pH paper
Salinity ($^0/_{00}$)	Refractometer

3. Results and Discussion

The experiment on the survival rate of Acropora was carried out within two months in response to the persistence and adaptability of the fragments. Within the proposed time, the Acropora drifts off between two conditions either it successfully grows or death. The measurement and observation to differentiate the living and the dead Acropora were also obvious. The initial fragments of Acropora introduced in the artificial reef were the same in number, *Viz.*168 fragments to standardize the experiment. Within two (2) months of the experiment, the dead of Acropora at depth 10 m (48 fragments) was three times that at 5 m (12 fragments). This mortality designated the different survival rates. The data of transplanted *Acropora* at 5 m and 10 m depths showed significant differences in survival rates ($p<0.01$). The highest survival rate (94%) was at a depth of 5 m, while the lowest (84%) was at a depth of 10 m (Table 2).

Table 2 *Acropora* survival rate data at depths of 5 m and 10 m

Depth	Total live coral	Average live coral	Total dead coral	Average dead coral	Survival Rate (%)
5 m (P1)	156	26	12	2	94^a
10 m (P2)	120	20	48	8	84^b

Note: Different superscript letters in the same column showed a very significant difference ($p < 0.01$)

The detail of survived Acropora was also monitored weekly. The observation provided conspicuous even in the field as the function of time. Both treatments assigned different adaptability and durability of Acropora to live in different depths. The data showed the depth contributed a negative effect to the Acropora. The survival of Acropora was higher in shallower waters. Both experiments, 5 m depth P1 and 10 m depth P2, were started with 168 fragments of Acropora. The survived Acropora was slightly down at depth 5 m (P1) weekly. Up to week 3, the number of survived Acropora was reduced to 164 fragments, and 4 fragments were dead.

Interestingly, it maintained the number of survived Acropora till week 5 (164 fragments). In the following weeks, the survived Acropora lessen at 2-3 fragments. In the 8th week, the survived Acropora were 156 fragments. Meanwhile, at depth 10 m, the number of survived Acropora was cut down progressively. In the 3rd week, the number of survived Acropora decreased to 155 fragments; 13 fragments were dead. As the following week, the dead fragment fell to the number of 5-12. At the end of the experiment, the survived Acropora at 10 m in depth was 120 fragments. Complete data showed in Figure 2.

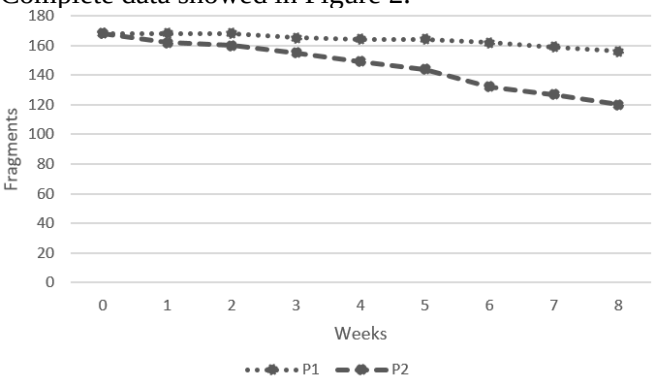

Fig. 2 Weekly coral survival rate data

The field observations showed that live Acropora was characterized by bright and diverse colors, opposite the dead corals were characterized by pale white and fragile. Figure 3 shows the difference between living and dead coral fragments.

Fig. 3 Living *Acropora* sp. (a), dead *Acropora* (b)

Table 3 present the zooxanthellae density data. Statistically, there was a significant difference in zooxanthellae density between the depth of 5 meters and 10 meters ($p < 0.01$). The average density of zooxanthellae cells at a depth of 5 meters was 4.79 x 10^6 cells / cm^2 and at a depth of 10 meters was 2.75 x 10^6 cells/cm^2.

Table 3 Data on zooxanthellae cell density (cells/cm^2)

Depth (m)	Zooxanthellae Density (cell/cm^2)
5 (P1)	4799298 ± 734920[a]
10 (P2)	2759921 ± 631981[b]

Note: Different superscript letters in the same column showed a very significant difference ($p < 0.01$)

Water quality measurements were carried out every week in the waters of Gili Ketapang Island. Based on observations of water conditions, water parameters did not comply with quality standards, namely nitrate and phosphate levels. However, in general, the condition of Gili Ketapang waters was in good condition and supported *Acropora*.

Table 4 Water quality measurement at a depth of 5 m and 10 m

Parameter	Depth 5 m (P1)	Depth 10 m (P2)	Standard
Brightness (%)	6	4	>3 [28]
Flow velocity (m/s)	0.1	0.3	0.15-0.23 [30]
Salinity ($^0/_{00}$)	30	30	33-34 [29]
Temperature (^{0}C)	31	31	18-36 [29]
pH	7	7	6-9 [28]
Nitrate (mg/l)	0.6	2	0.01-0.5 [31]
Phosphate (mg/l)	0.1	0.1	0.015 [31]

Many factors influence the survival rate of Acropora, and one of the most important factors is received light intensity. Sunlight penetration into seawater is a negative function of depth. Shallower waters grant a higher possibility of sunlight penetrating its bottom. Corals that grew at lower depths received more sunlight and had better survival [15], [32]. Light is an important aspect of common coral survival because zooxanthella's growth depends on sunlight [33]. Zooxanthella, a mutualistic symbiosis organism on corals, relies upon sunlight for photosynthesis in its chloroplasts [34]. The mutualistic mechanism is well-studied; 80% of zooxanthella photosynthetic products such as carbohydrates and oxygen were transferred to corals [35]. Thus, coral as the host depends on this transferred photosynthetic product to maintain its life. The success of zooxanthella in producing its photosynthetic product determines the survival of coral. Therefore, the shallower waters with higher sunlight intensity provide vital energy for coral to live.

The field observations showed that live Acropora was characterized by bright and diverse colors, opposite the dead corals were characterized by pale white and fragile. Figure 4 shows the difference between living and dead coral fragments. According to previous research, the living Acropora would be bright while the dead were pale [36], [37]. The bleaching phenomenon caused the death of Acropora. Coral bleaching, the release of zooxanthella from corals, was marked by the fading of the whole coral color to white [38]. Many environmental factors stimulate the bleaching phenomenon, such as increased temperature, light, and sediment [39].

The average density of zooxanthellae cells at a depth of 5 meters was 4.79 x 10^6 cells / cm^2 and at a depth of 10 meters was 2.75 x 10^6 cells/cm^2. That indicated that the number of zooxanthellae in the corals of *Acropora* sp. in the study area decreased with increasing depth. The zooxanthellae density at a depth of 5 meters was higher than at a depth of 10 meters. The ability of light intensity to penetrate is different between the two depths. The level of brightness depicted this crucial factor. Brightness affects the light intensity that enters the water [16]. The brightness level at a depth of 5 meters was higher than at a depth of 10 meters. If the water deeper, the value of brightness became less. This light intensity played a role in the photosynthesis process in zooxanthellae [40]. Zooxanthellae lived in animal tissue cells of corals in the form of cysts. Zooxanthellae utilize carbon dioxide produced from corals during respiration, and as a

return, coral gets nutrients and oxygen from the photosynthesis process [34]. Whenever the light intensity was low, the capability of zooxanthellae to produce oxygen and nutrients was limited. The transfer of those precious supporting live components to coral was then stranded lead to the death of coral. Hence, when the corals die, the zooxanthellae could not contain enough carbon dioxide to carry out photosynthesis; thus, their numbers are reduced. Previous research study stated that the highest density of zooxanthellae in the coral reef zone was at a depth of 2-5 m with an average density of approximately 7.7 x 10^6 cells/cm^2, and the lowest density was at 5-10 m with an average of approximately 3.2 x 10^6 cells/cm^2 [41].

Water quality measurements were carried out every week in the waters of Gili Ketapang Island. Based on observations of water conditions, water parameters did not comply with quality standards, namely nitrate and phosphate levels. However, in general, the condition of Gili Ketapang waters was in good condition and supported *Acropora*.

The transplant method had been widely used for the rehabilitation of Acropora [42]. However, no journals report coral conservation in Gili Ketapang, Probolinggo, even though the corals' condition had been damaged [9]. The new novelty obtained from this research was the reported first attempt to improve environmental sustainability in Gili Ketapang, Probolinggo. In applying transplants with this technique, water depth and quality were considered, especially the brightness, because it affected photosynthetic activity and zooxanthellae.

4. Conclusion

This study indicated that transplanted activity of *Acropora* at 5 m showed better results in survival rate and zooxanthellae density than at 10 m. The highest survival rate at a depth of 5 m was 94%, while the lowest was at a depth of 10 m with 84%. The average density of zooxanthellae cells at a depth of 5 meters was 4.79 x 10^6 cells / cm^2 and at a depth of 10 meters was 2.75 x 10^6 cells/cm^2. Based on observations of water conditions, water parameters did not comply with quality standards, namely nitrate and phosphate levels. From this study, we recommended that transplantation activity of *Acropora* in Gili Ketapang, Probolinggo, was performed at a depth of 5 m for optimal results.

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