<u>Riwayat Korespondensi Karya Ilmiah Syarat Khusus Guru Besar</u>

Penulis Koresponden	: **Munasik**
Judul Artikel	: Coral transplantation on a multilevel substrate of Artificial Patch Reefs: effect of fixing methods on the growth rate of two Acropora species
Terbit (Issue)	: Volume 21, Number 5, **May 2020**: 1816-1822
Nama Jurnal	: **Biodiversitas** Journal of Biological Diversity
Alamat Situs Artikel	: https://smujo.id/biodiv/article/view/5279
Terindeks Scopus	: Q3 (Animal Science and Zoology)
SJR	: 0.22

No.	Aktivitas Korespondensi	Waktu	Halaman
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COVERING LETTER

Dear **Editor-in-Chief,**

I herewith enclosed a research article,

Title:

Coral transplantation on a multilevel substrate of Artificial Patch Reefs: effect of fixing methods on the growth rate of two Acropora species

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Biodiversitas

Novelty:

(state your claimed novelty of the findings versus current knowledge)

Coral transplantation and artificial reef are known as the methods for coral reef rehabilitation. However, the application of these methods in Indonesia waters were apparently not successful, indicated by high mortality of coral fragments in coral transplantation and many artificial reefs that applied damage to natural reefs. In the present study, we firstly combine both method to create new habitat of coral reef called Artificial Patch Reef (APR) and examine the growth rate of Acropora branching with different fixing method on APR.

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Sincerely yours,

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Munasik

Coral transplantation on a multilevel substrate of Artificial Patch Reefs: effect of fixing methods on the growth rate of two Acropora species

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Manuscript received: 21 01 2020 (Date of abstract/manuscript submission). Revision accepted: .... 2020.

Abstract. Acropora branching is generally used in coral transplantation to rehabilitate coral reefs. However, these corals are sensitive to environmental changes. Artificial Patch Reef (APR) is an artificial structure that provides a multilevel hard substrate. The purpose of the study was to investigate the effectiveness of the APR structure to facilitate the growth and survival of Acropora branching. Two species *Acropora aspera* and *Acropora copiosa* were transplanted vertically and horizontally on a modular concrete block in different levels of APR situated in the shallow reef of Panjang Island, Central Java. The results showed that the coral growth rate varied from 96.7 to 346.9 cm^3/month, while survival ranged from 95.8 to 100% after 8 months. Lower survival rate mostly was found in the upper level of APR.. The statistical analyses showed that the growth rate of *A. copiosa* fragment was significantly higher than that of *A. aspera* ($p<0.05$). Moreover, there were also significantly differences on the treatments of transplantation method ($p<0.05$) to enhance the coral growth. However, multilevel substrates were not significantly influence of the coral growth. This study suggested that *A. copiosa* which has high-level complexity in branching pattern will be selected to apply in shallow reef rehabilitation with transplanted vertically.

Key words: coral transplant, *Acropora aspera*, *Acropora copiosa*, artificial patch reef, Panjang Island

Running title: transplantation of two Acropora species on multilevel substrate

INTRODUCTION

Coral reef is one of an important ecosystem on earth, it is most complex and bio-diverse ecosystem that provides the ecological services for humankind. Recently, coral reefs worldwide have been degrading by natural and man-made stress (Wilkinson 2000; Burke et al. 2011). Reef health has been declining apparently by limiting space for natural recruitment and change in physical environmental conditions (Done et al. 2010). Thus, coral reef rehabilitation is considered one of the major reef management strategies that coral reefs may not be able to recover naturally without human intervention.

To rehabilitate damage of natural reefs, artificial reefs and coral transplantation has been applied regardless of environmental condition, cause of decline, or goals. Coral transplantation generally applied by transplanted coral fragments on table cages in shallow water in order to cultivate coral fragments due to transferred and transplanted to rehabilitation reef areas (Heeger and Sotto 2000; Ammar 2013). It seems to be the most widely implemented for coral reef rehabilitation. Many studies dealing with reef rehabilitation by applied coral transplantation (Yap 2000, 2003; Epstein et al. 2001, 2003; Sabater and Yap 2002). Coral transplantation may contribute to enhance the coral population in the reef areas, although natural recovery indicated by coral recruitment (Edward and Clark 1998; Ng et al. 2015). Coral transplantation method potentially has an impact on reef health by loss colonies from the donor area, reducing the growth of transplanted corals, reducing fecundity of transplant due to stress. Alternatively, artificial reefs are considered an efficient rehabilitation tool, it is a suitable method for protection of existing natural reefs, environmental, mitigation for damaged reef areas and shoreline protections (Meester et al. 2012; Ng et al. 2016). Artificial reefs are expected to increase in available substrates for reef organisms, provide structural complexity and natural recruitment. However, the application of these methods in Indonesia waters were apparently not successful, indicated by high mortality of coral fragments in coral transplantation and many artificial reefs that applied damage to natural reefs (Munasik 2009). In order to optimize reef rehabilitation, combining artificial reefs and coral transplantation is recommended (Abelson 2006; Cummings et al. 2015; Ammar et al. 2013).

Artificial Patch Reefs (APR) is an artificial structure which is applied to rehabilitate coral reef in order to develop shallow water habitat (Munasik et al. 2018). APR is a rehabilitation tool that is designed by combination coral transplantation and artificial reefs. Multilevel of the substrate of APR provide the hard substrate to facilitate fragment of coral grows in shallow turbid water. However, the information about the effect of multilevel the structure on survival and growth of transplanted corals is limited. This study aims to address the effectiveness of the APR structure to provide the multilevel substrate to facilitate the growth rate and survival of coral fragment. In more specific, Acropora branching was selected and applied to this study in order to investigate the suitable method and species selection for reef rehabilitation. *Acropora* spp. is generally considered as a good for candidates for use in coral transplantation or population enhancement project due to their high growth rate and high survivorship of fragments (Lirman et al. 2010; Stephanie et al. 2017; Boch and Morse 2012; Mercado-Molina 2016). Acroporid corals are significantly important in the shallow reef of Panjang Island, however the population decline slightly due to the anthropogenic stressor (Munasik et al. 2012). Thus, the application of APR with Acropora transplanted on their substrates is considered contributing to the local conservation of small island reefs in the near future.

MATERIALS AND METHODS

Study area

Rehabilitation of coral reefs program was carried out in shallow reefs of Panjang Island Central Java by deployed 12 (twelve) artificial patch reefs (APR) from 2015 to 2018 at 3 m depth (Figure 1). In order to conduct a coral transplantation experiment, one unit of Artificial Patch Reef (APR) No. 12 was selected to perform the study of the effect of species and coral transplantation method in multilevel of substrates on growth of coral fragments.

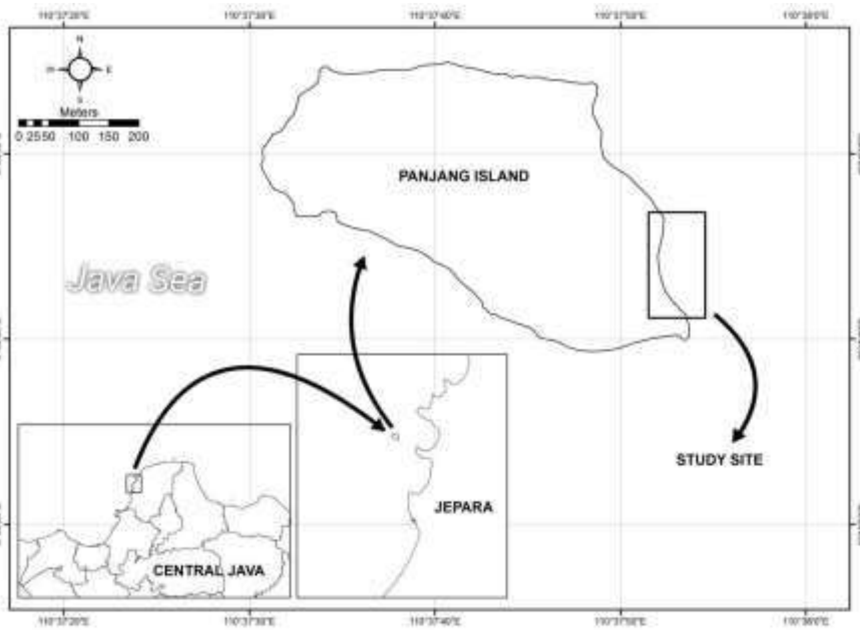

Figure 1. Study site of coral transplantation on Artificial Patch Reef at Panjang Island, Central Java (6º34'30" S; 110º37'44" E)

Procedures

Fragments of Acropora branching, i.e. *Acropora aspera* and *A. copiosa* were transplanted on multilevel substrates of Artificial Patch Reefs which deployed in the shallow reef of Panjang Island, Central Java (Java Sea; Figure 1). Both of *A. aspera* and *A. copiosa* were as a limiting local population of Acroporid in Panjang Island, Central Java. *A. aspera* is generally found in the inner lagoon which colony is defined as a corymbose clump with short thick branches. Veron and Stafford-Smith (2000) described that *A. aspera* has small axial corallites while radial corallites are composed two sizes, crowded and have prominent lower lips giving a scale-like appearance (Veron and Stafford-Smith 2000). Colonies of *A. copiosa* are generally found in front of the reef flat and the species were characterized as arborescent clumps of upright branches. The corals have irregular branching patterns with frequent sub-branches and axial corallites relatively small while radial corallites are crowded, all tubular with unequal height (Veron and Stafford-Smith 2000). Comparing to the previous species, *Acropora copiosa* have more complexity in branching pattern.

Artificial Patch Reefs (APR) is artificial reefs made by concrete blocks which composed as modular circular structures in shape, constructed 5 (five) levels of substrates were deployed from small boats by SCUBA divers, and are suitable near natural reefs in shallow water (Munasik et al. 2018; Figure 2). The total height of the multilevel APR structure is about 80 cm from the bottom of the sea, and the height of each level is 20 cm. In this experiment, coral fragments were transplanted in the upper, middle, and lower level. Coral transplantation experiments were not applied at the top level to prevent physical damaged in coral fragments. The experiments were also not implemented in the coral transplantation in the base of APR (levels 4 and 5) since the surface of the substrate usually covering sediment due to resuspension. At the beginning of November 2018, 120 coral fragments were transplanted on three levels of APR by two fixation methods: vertically and horizontally orientation of the fragments fixing on the surface of substrates and tied to a paired of the nail using cable ties (Figure 3). Fixation of fragments using cable ties is considered the increasing survival of transplanted corals (William and Miller 2010; Okubo et al. 2005).

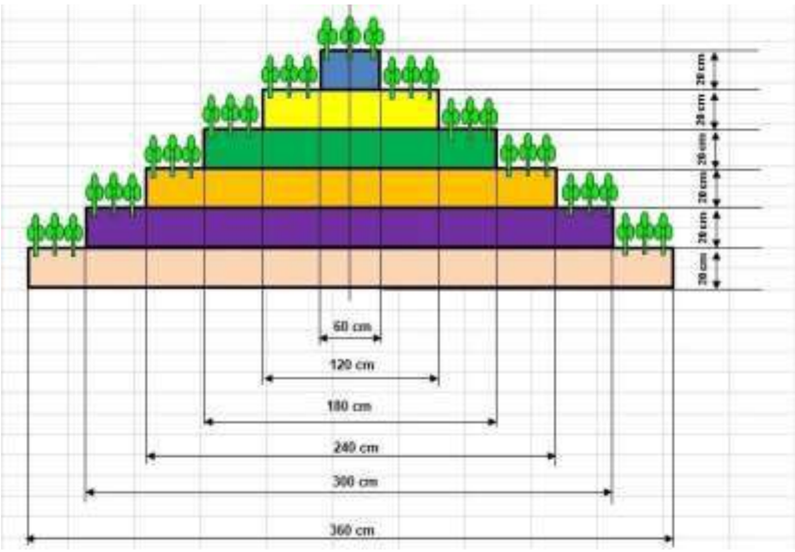

Figure 2. Structure of Artificial Patch Reef (APR) deployed in the shallow reef of Pulau Panjang, Central Java (Munasik et al. 2018)

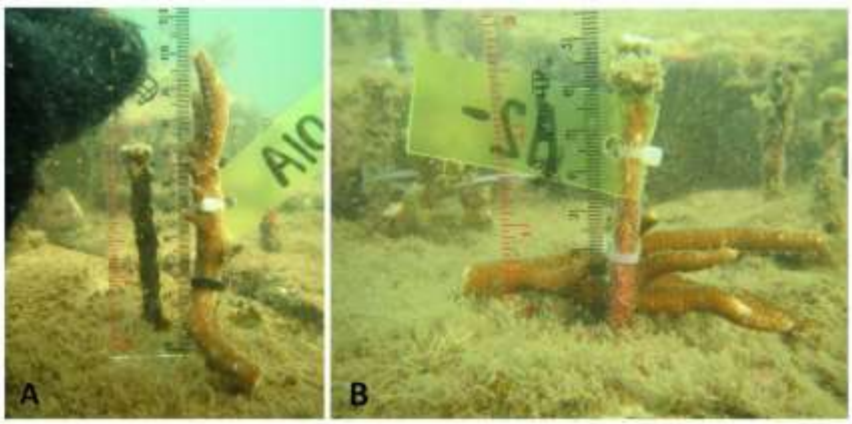

Figure 3. Fixing methods of coral fragments tied to nail by cable ties (A. vertically fixing method, B. horizontally fixing method)

Data analysis

In order to investigate the growth rate of Acropora branching fragments, we used a measurement of corallum size in volume dimension (Buddemeier and Kinzie III 1976). The final measurement of volume (length, wide, and height) of the coral fragments was evaluated in late July 2019. The size of the fragments was measured by taking a picture using an underwater camera and putting the scale beside each the fragment (Mercado-Molina et al. 2016). The size measurement of the fragments was analyzed using image analyses of computer software. Volume of the fragment was determined by ecological volume (EV; de la Cruz et al. 2014), and its calculated following the cylindrical volume formula (Levy et al. 2010) as define, in equation (1)

$$EV = \pi r^2 h, \text{ where } \quad r = \frac{(l+W)}{4} \qquad (1)$$

Growth rate (GR) of the corals (Ecological Volume per month) was calculated using the formula (2)

$$Gr = [EVf - EVi]/m \quad (2)$$

where Gr is the standardized growth rate, EVf and EVi are final and initial Ecological Volume and m is number of months elapsed.

Only the tagged coral fragments alive at 8 (eight) months post transplantation where included in the growth rate determination.

In order to test the effect of different levels of transplant position, and different fixing methods of coral transplantation to the growth of two Acropora species, data of growth rate of the fragments were analyzed using two-way of variance (ANOVA, at 95% confidence level, p<0.05).

RESULTS AND DISCUSSION

Survival rate

The survival rate of Acropora branching fragments which transplanted on multilevel substrates is varied among species, fixing method and level of substrates. The average survival rate of *A. copiosa* which transplanted on the lower level of substrates was higher than *A. aspera* at a similar level. Both species of Acropora branching which transplanted horizontally possess higher survivorship than fragments that transplanted vertically. The lower survival rate of the fragments is found in the upper level of substrates (Figure 4) which located on the top of APR, about 1 m from the bottom of the sea during low tide.

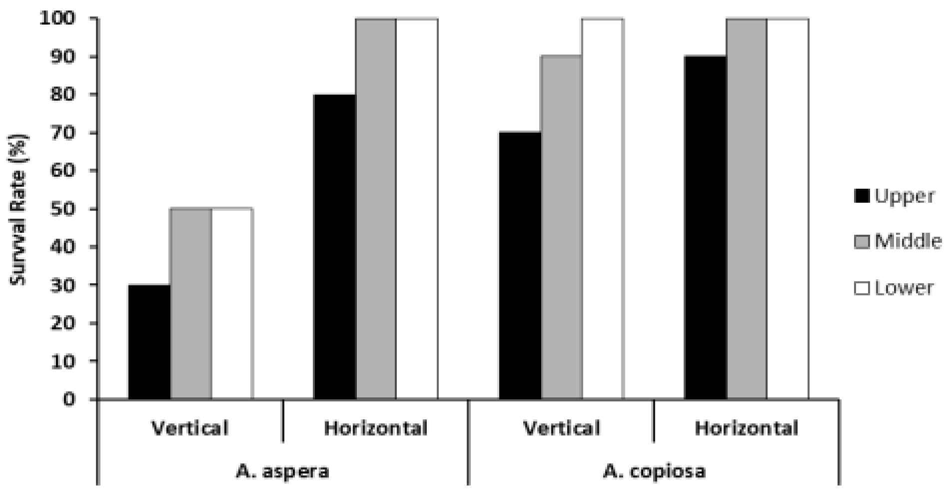

Figure 4. Survival rate (%) of transplanted two Acropora species on multilevel substrates of Artificial Patch Reef after 8 months (November 2018-July 2019)

Growth Rate

The growth rate of Acropora branching which transplanted on multilevel substrates of APR varied from 96.7 to 346.9 cm^3/month. The growth rate of Acropora branching fragments was significantly different between species, substrate levels and fixing method of transplantation (p<0.05) after 8 months. The growth rate of Acropora branching on multilevel substrates of APR after 8 months demonstrated that there were no significant different on species *A aspera.* Whereas, there were significantly different on the *A. copiosa* growth that transplanted either in the upper and the middle levels or in the lower and in the middle levels. However, there were no significantly different on the coral growth transplanted in the upper and in the lower levels (Table 1).

Table 1. Growth rate (cm^3/month) of Acropora branching transplanted on multilevel substrates of Artificial Patch Reef after 8 months (November 2018-July 2019)

Level	*A. aspera*		*A. copiosa*	
Upper	130.05±47.16	a	293.00±76.23	a
Middle	178.75±34.17	a	152.05±95.11	a
Lower	202.75±44.74	a	333.3±64.21	a

The growth rate of Acropora branching transplanted in different fixing method (vertical vs. horizontal) demonstrated significantly different (Table 3). Growth rate of fragments which transplanted in vertical fixing method was higher than the horizontal method. The lowest growth rate was found in *Acropora aspera* which transplanted in horizontal fixing method, while the highest growth rate occurred in *A. copiosa* which transplanted in vertical fixing methods (Figure 4). Fragments of coral *A. copiosa* can grow optimally on all levels by both vertical and horizontal fixing method of coral transplantation. Comparing the species, the growth of transplanted *A. copiosa* is higher than that of *A. aspera* due to the different branching patterns (Figure 5 and 6).

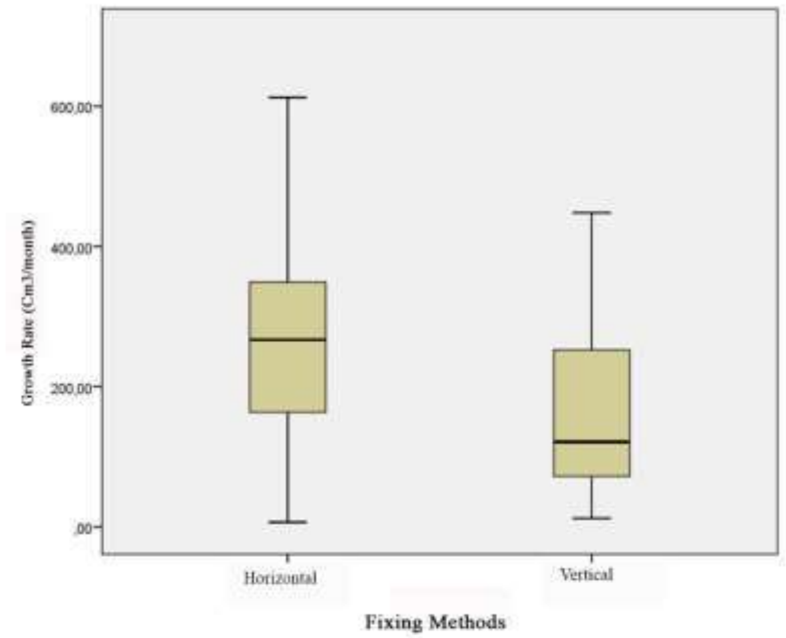

Figure 5. Growth rate (cm^3/month) of two Acropora species transplanted on multilevel substrates of Artificial Patch Reef in different fixing method after 8 months (November 2018-July 2019)

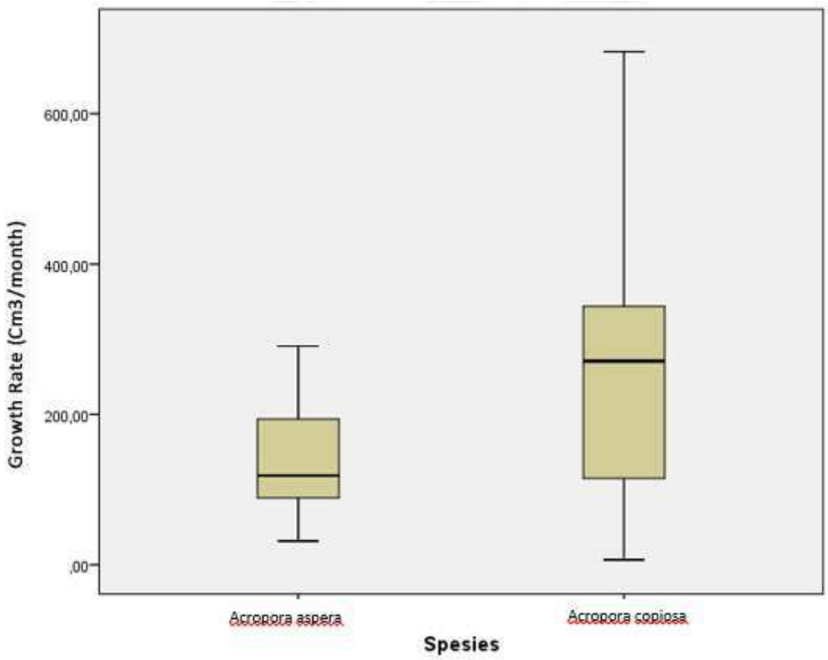

Figure 6. Comparison of growth rate (cm^3/month) *A. aspera* and *A. copiosa* fragments after 8 months transplanted on the multilevel substrates of Artificial Patch Reefs (APR) in the shallow reef.

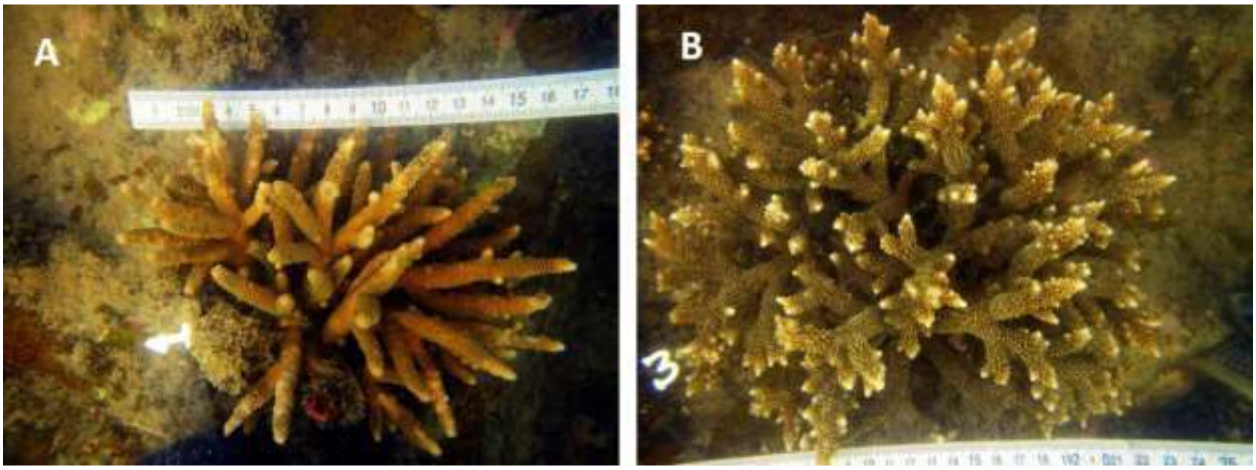

Figure 7. Transplanted coral Acropora on Artificial Patch Reef after 8 months (A. horizontal fixing method of transplanted *Acropora aspera*; B. vertical fixing method of transplanted *Acropora copiosa*)

Discussion

Lower survival rate during the experiment was revealed by *A. aspera* in all levels of substrates particularly in fixing vertically. About 12.5 to 17.5 % of mortality was found during the experiment that occurred in *A. aspera* which fixed on a vertical orientation. Transplanted *A. aspera* is generally found in did not fix on substrates during the early experiment due to the lifeform of the coral is a branching robust (Wallace 2000; Veron and Stafford-Smith 2000), consequently the coral fragments detached from the substrates and died. Lower survival rate mostly was found in the upper level of APR and occurred in the beginning experiment, during the nursery phase, after fixed the coral fragments due to fragment stabilization. Monthly monitoring reveals that coral fragments mortality caused by competition by algae and some of them lost by wave actions. In order to mitigate physical damage, the design strategic placement of nurseries in the substrates was needed by reducing wave exposure (Young et al. 2012).

Acropora branching is one of the important coral in the shallow water and usually applied to coral transplantation. The corals have the competency to grow fast, inversely they are also sensitive responding to the environment. Survival of the corals which have transplanted varied in different location and various in rehabilitation technic. The survival rate of Acropora in nubbin fix to the nursery table was 46% (Nithyanandan et al. 2017), while the high survival rate of the coral was found in *Acropora hyacinthus*. 83.3% (Bongiorni et al. 2011). In the present study, the survival rate of the Acropora branching is high, it may be supported by the stabilization of the substrate of Artificial Patch Reefs (APR) and also the multilevel designed of substrate contribute to enhance the survival of Acropora fragments.

Some previous studies of coral transplantation revealed that the growth rate of Acropora branching was higher than that of other life-form hard corals. Bongiorni et al. (2011) reported that Acropora branching possess relative growth ranged 66.9 to 83.3%, while growth rate of Acropora branching which transplanted on the artificial reef dome-shaped was 1.07 cm/month (Muzaki et al. 2019), Acropora fragments fixed to the dead coral was 7.8 cm/year (Nithyanandan et al. 2017). This result showed that the growth rate of both Acropora branching which transplanted on multilevel substrate possesses a high growth rate. Presumably, the construction of multilevel APR can optimize coral grow by increasing light and preventing sediment coverage. The different branching pattern of the Acropora may affect to the growth of the corals, Two Acropora branching shows a different level of complexity (Mercado-Monila et al. 2016) *A. copiosa* was more complex than *A. aspera*. Veron and Stafford-Smith (2000) identified that *A. copiosa* was clumps of prostrate or upright branches irregular branching patterns with frequent sub-branches, while *A. aspera* which is defined as a corymbose clump with thick branches (Veron and Stafford-Smith 2000). The higher growth rate of vertically fixing method in *Acropora copiosa* indicated that vertical fixing of the fragments was suitable orientation of the natural growth form of the donor colony of Acropora (Okubo et al. 2005). This study suggests that Acropora branching which has high-level complexity such as *A. copiosa* will be selected to apply coral rehabilitation.

ACKNOWLEDGEMENTS

This study was supported by of Applied Research grant (No. 101-170/UN7.P4.3/PP/2018) from Ministry of Research and Technology, Higher Education to M, AS, and SS. Part of the study was supported by research grant No. 1501-26/UN7.5.10/LT/2018 from Faculty of Fisheries and Marine Science, Diponegoro University to M, DPW, I, and RP. We thank Agus Susanto, and staff of Marine Diving Club and Tanjung Jati B Coal-Fired Power Plant to facilitate maintaining the artificial reefs.

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• Colored figures are only used if the information in the text may be losing without those images	X
• Charts (graphs and diagrams) are drawn in black and white images; use shading to differentiate	X

Gmail munasik motawi <munasikmotawi@gmail.com>

[biodiv] Submission Acknowledgement

Ahmad Dwi Setyawan <smujo.id@gmail.com> 21 January 2020 at 11:03
Reply-To: Ahmad Dwi Setyawan <editors@smujo.id>
To: M Munasik <munasikmotawi@gmail.com>

M Munasik:

Thank you for submitting the manuscript, "Coral transplantation on a multilevel substrate of Artificial Patch Reefs: effect of fixing methods on the growth rate of two Acropora species " to Biodiversitas Journal of Biological Diversity. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

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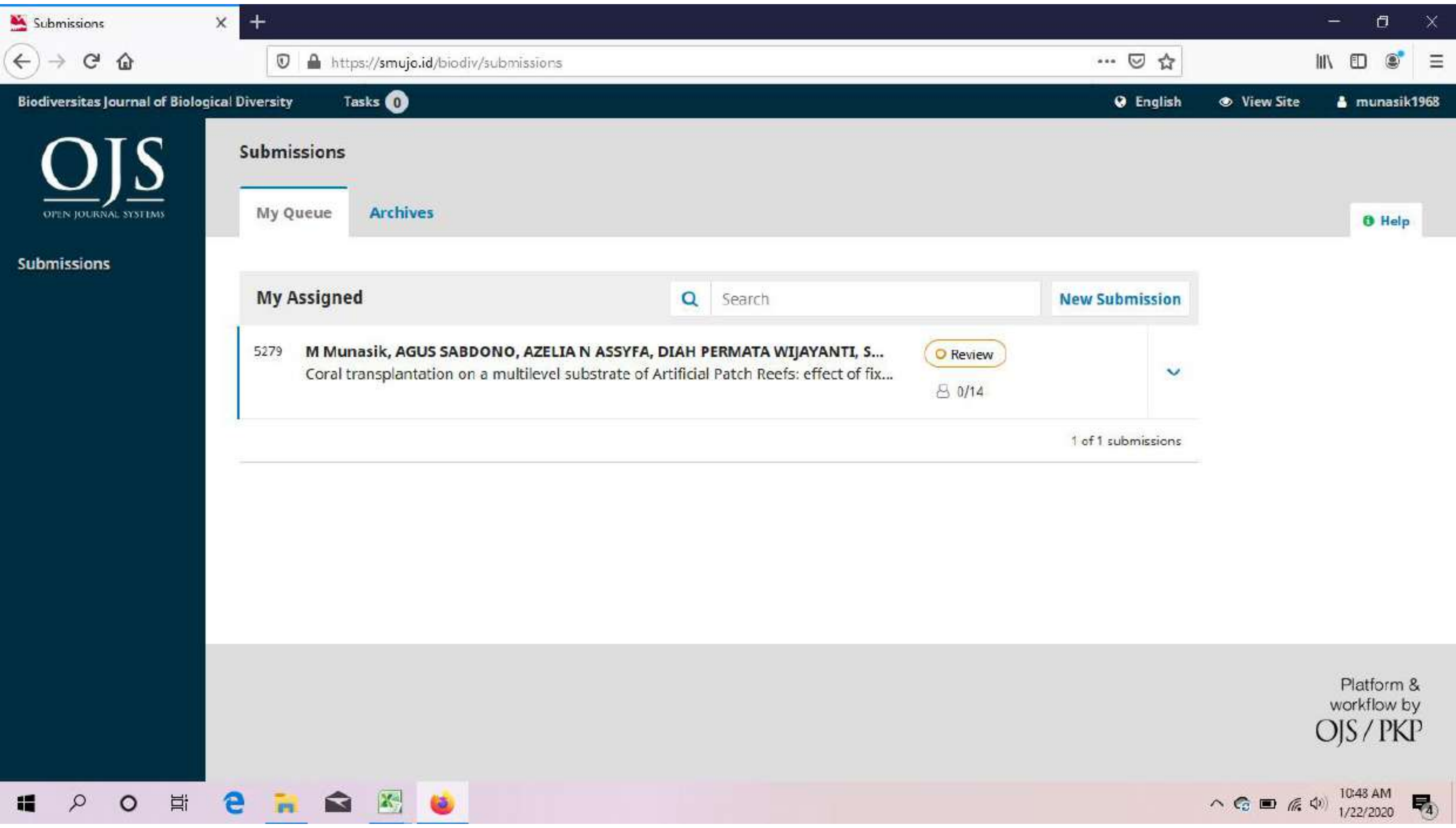
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Coral transplantation on a multilevel substrate of Artificial Patch Reefs: effect of fixing methods on the growth rate of two Acropora species

Abstract. Acropora branching is generally used in coral transplantation to rehabilitate coral reefs. However, these corals are sensitive to environmental changes. Artificial Patch Reef (APR) is an artificial structure that provides a multilevel hard substrate. The purpose of the study was to investigate the effectiveness of the APR structure to facilitate the growth and survival of Acropora branching. Two species *Acropora aspera* and *Acropora copiosa* were transplanted vertically and horizontally on a modular concrete block in different levels of APR situated in the shallow reef of Panjang Island, Central Java. The results showed that the coral growth rate varied from 96.7 to 346.9 cm^3/month, while survival ranged from 95.8 to 100% after 8 months. Lower survival rate mostly was found in the upper level of APR.. The statistical analyses showed that the growth rate of *A. copiosa* fragment was significantly higher than that of *A. aspera* ($p<0.05$). Moreover, there were also significantly differences on the treatments of transplantation method ($p<0.05$) to enhance the coral growth. However, multilevel substrates were not significantly influence of the coral growth. This study suggested that *A. copiosa* which has high-level complexity in branching pattern will be selected to apply in shallow reef rehabilitation with transplanted vertically.

Key words: *Acropora aspera*, *Acropora copiosa*, artificial patch reef, coral transplant, Panjang Island

Running title: transplantation of two Acropora species on multilevel substrate

INTRODUCTION

Coral reef is one of an important ecosystem on earth, it is most complex and bio-diverse ecosystem that provides the ecological services for humankind. Recently, coral reefs worldwide have been degrading by natural and man-made stress (Wilkinson 2000; Burke et al. 2011). Reef health has been declining apparently by limiting space for natural recruitment and change in physical environmental conditions (Done et al. 2010). Thus, coral reef rehabilitation is considered one of the major reef management strategies that coral reefs may not be able to recover naturally without human intervention.

To rehabilitate damage of natural reefs, artificial reefs and coral transplantation has been applied regardless of environmental condition, cause of decline, or goals. Coral transplantation generally applied by transplanted coral fragments on table cages in shallow water in order to cultivate coral fragments due to transferred and transplanted to rehabilitation reef areas (Heeger and Sotto 2000; Ammar 2013). It seems to be the most widely implemented for coral reef rehabilitation. Many studies dealing with reef rehabilitation by applied coral transplantation (Yap 2000, 2003; Epstein et al. 2001, 2003; Sabater and Yap 2002). Coral transplantation may contribute to enhance the coral population in the reef areas, although natural recovery indicated by coral recruitment (Edward and Clark 1998; Ng et al. 2015). Coral transplantation method potentially has an impact on reef health by loss colonies from the donor area, reducing the growth of transplanted corals, reducing fecundity of transplant due to stress. Alternatively, artificial reefs are considered an efficient rehabilitation tool, it is a suitable method for protection of existing natural reefs, environmental, mitigation for damaged reef areas and shoreline protections (Meester et al. 2012; Ng et al. 2016). Artificial reefs are expected to increase in available substrates for reef organisms, provide structural complexity and natural recruitment. However, the application of these methods in Indonesia waters were apparently not successful, indicated by high mortality of coral fragments in coral transplantation and many artificial reefs that applied damage to natural reefs (Munasik 2009). In order to optimize reef rehabilitation, combining artificial reefs and coral transplantation is recommended (Abelson 2006; Cummings et al. 2015; Ammar et al. 2013).

Artificial Patch Reefs (APR) is an artificial structure which is applied to rehabilitate coral reef in order to develop shallow water habitat (Munasik et al. 2018). APR is a rehabilitation tool that is designed by combination coral transplantation and artificial reefs. Multilevel of the substrate of APR provide the hard substrate to facilitate fragment of coral grows in shallow turbid water. However, the information about the effect of multilevel the structure on survival and growth of transplanted corals is limited. This study aims to address the effectiveness of the APR structure to provide the multilevel substrate to facilitate the growth rate and survival of coral fragment. In more specific, Acropora branching was selected and applied to this study in order to investigate the suitable method and species selection for reef rehabilitation. *Acropora* spp. is generally considered as a good for candidates for use in coral transplantation or population enhancement project due to their high growth rate and high survivorship of fragments (Lirman et al. 2010; Stephanie et al. 2017; Boch and Morse 2012; Mercado-Molina 2016). Acroporid corals are significantly important in the shallow reef of Panjang Island, however the population decline slightly due to the anthropogenic stressor (Munasik et al. 2012). Thus, the application of APR with Acropora transplanted on their substrates is considered contributing to the local conservation of small island reefs in the near future.

MATERIALS AND METHODS

Study area

Rehabilitation of coral reefs program was carried out in shallow reefs of Panjang Island Central Java by deployed 12 (twelve) artificial patch reefs (APR) from 2015 to 2018 at 3 m depth (Figure 1). In order to conduct a coral transplantation experiment, one unit of Artificial Patch Reef (APR) No. 12 was selected to perform the study of the effect of species and coral transplantation method in multilevel of substrates on growth of coral fragments.

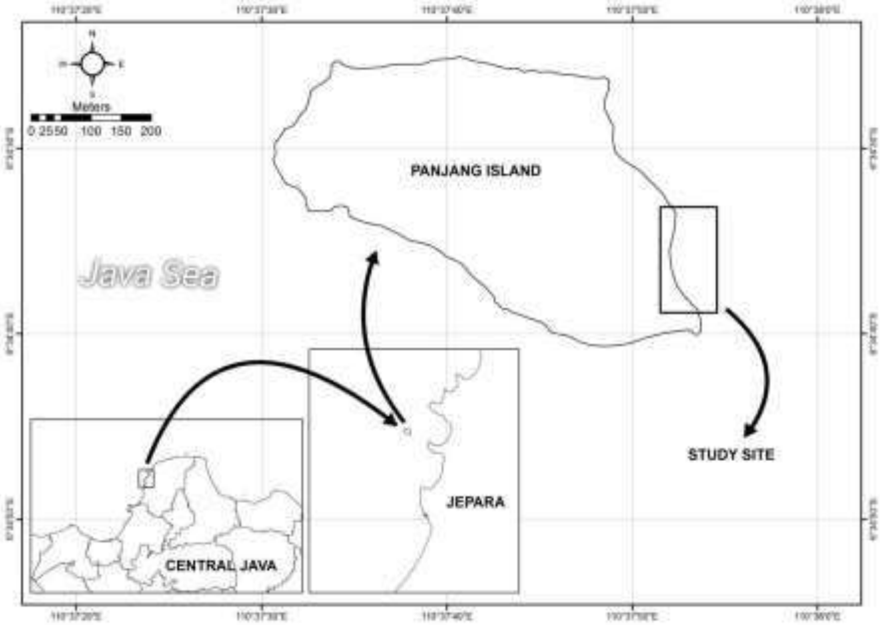

Figure 1. Study site of coral transplantation on Artificial Patch Reef at Panjang Island, Central Java (6º34'30" S; 110º37'44" E)

Procedures

Fragments of Acropora branching, i.e. *Acropora aspera* and *A. copiosa* were transplanted on multilevel substrates of Artificial Patch Reefs which deployed in the shallow reef of Panjang Island, Central Java (Java Sea; Figure 1). Both of *A. aspera* and *A. copiosa* were as a limiting local population of Acroporid in Panjang Island, Central Java. *A. aspera* is generally found in the inner lagoon which colony is defined as a corymbose clump with short thick branches. Veron and Stafford-Smith (2000) described that *A. aspera* has small axial corallites while radial corallites are composed two sizes, crowded and have prominent lower lips giving a scale-like appearance (Veron and Stafford-Smith 2000). Colonies of *A. copiosa* are generally found in front of the reef flat and the species were characterized as arborescent clumps of upright branches. The corals have irregular branching patterns with frequent sub-branches and axial corallites relatively small while radial corallites are crowded, all tubular with unequal height (Veron and Stafford-Smith 2000). Comparing to the previous species, *Acropora copiosa* have more complexity in branching pattern.

Artificial Patch Reefs (APR) is artificial reefs made by concrete blocks which composed as modular circular structures in shape, constructed 5 (five) levels of substrates were deployed from small boats by SCUBA divers, and are suitable near natural reefs in shallow water (Munasik et al. 2018; Figure 2). The total height of the multilevel APR structure is about 80 cm from the bottom of the sea, and the height of each level is 20 cm. In this experiment, coral fragments were transplanted in the upper, middle, and lower level. Coral transplantation experiments were not applied at the top level to prevent physical damaged in coral fragments. The experiments were also not implemented in the coral transplantation in the base of APR (levels 4 and 5) since the surface of the substrate usually covering sediment due to resuspension. At the beginning of November 2018, 120 coral fragments were transplanted on three levels of APR by two fixation methods: vertically and horizontally orientation of the fragments fixing on the surface of substrates and tied to a paired of the nail using cable ties (Figure 3). Fixation of fragments using cable ties is considered the increasing survival of transplanted corals (William and Miller 2010; Okubo et al. 2005).

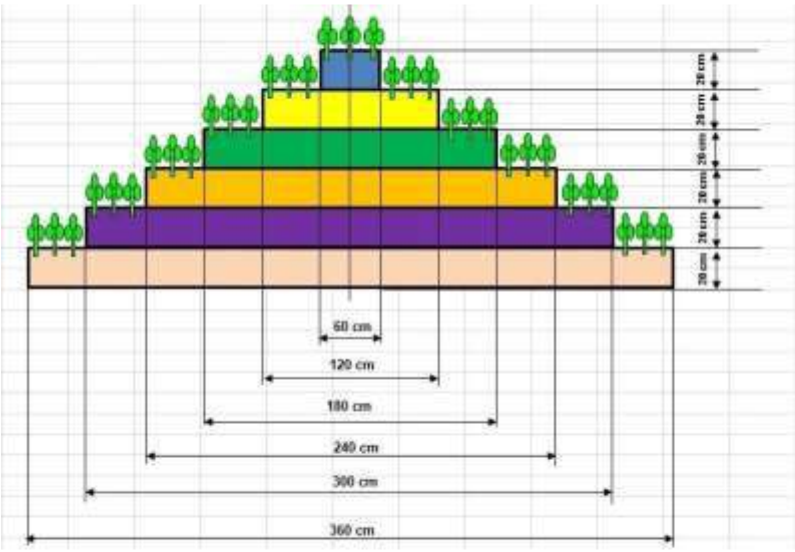

Figure 2. Structure of Artificial Patch Reef (APR) deployed in the shallow reef of Pulau Panjang, Central Java (Munasik et al. 2018)

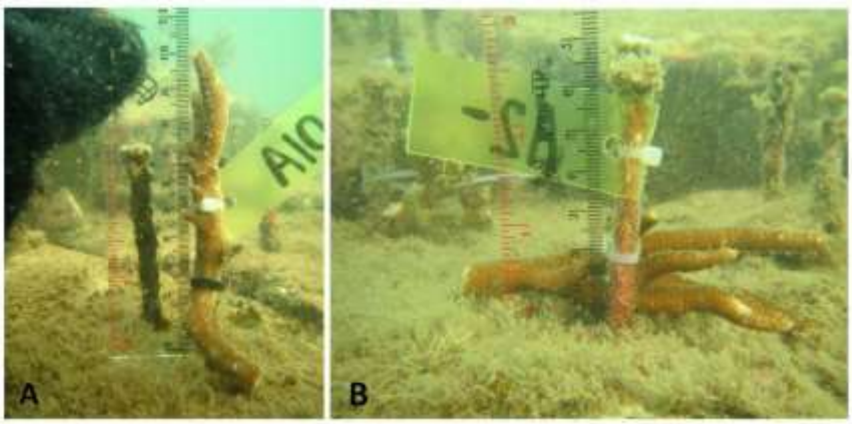

Figure 3. Fixing methods of coral fragments tied to nail by cable ties (A. vertically fixing method, B. horizontally fixing method)

Data analysis

In order to investigate the growth rate of Acropora branching fragments, we used a measurement of corallum size in volume dimension (Buddemeier and Kinzie III 1976). The final measurement of volume (length, wide, and height) of the coral fragments was evaluated in late July 2019. The size of the fragments was measured by taking a picture using an underwater camera and putting the scale beside each the fragment (Mercado-Molina et al. 2016). The size measurement of the fragments was analyzed using image analyses of computer software. Volume of the fragment was determined by ecological volume (EV; de la Cruz et al. 2014), and its calculated following the cylindrical volume formula (Levy et al. 2010) as define, in equation (1)

$$EV = \pi r^2 h, \text{ where } \quad r = \frac{(l+W)}{4} \qquad (1)$$

Growth rate (GR) of the corals (Ecological Volume per month) was calculated using the formula (2)

$$Gr = [EVf - EVi]/m \qquad (2)$$

where Gr is the standardized growth rate, EVf and EVi are final and initial Ecological Volume and m is number of months elapsed.

Only the tagged coral fragments alive at 8 (eight) months post transplantation where included in the growth rate determination.

In order to test the effect of different levels of transplant position, and different fixing methods of coral transplantation to the growth of two Acropora species, data of growth rate of the fragments were analyzed using two-way of variance (ANOVA, at 95% confidence level, $p<0.05$).

RESULTS AND DISCUSSION

Survival rate

The survival rate of Acropora branching fragments which transplanted on multilevel substrates is varied among species, fixing method and level of substrates. The average survival rate of *A. copiosa* which transplanted on the lower level of substrates was higher than *A. aspera* at a similar level. Both species of Acropora branching which transplanted horizontally possess higher survivorship than fragments that transplanted vertically. The lower survival rate of the fragments is found in the upper level of substrates (Figure 4) which located on the top of APR, about 1 m from the bottom of the sea during low tide.

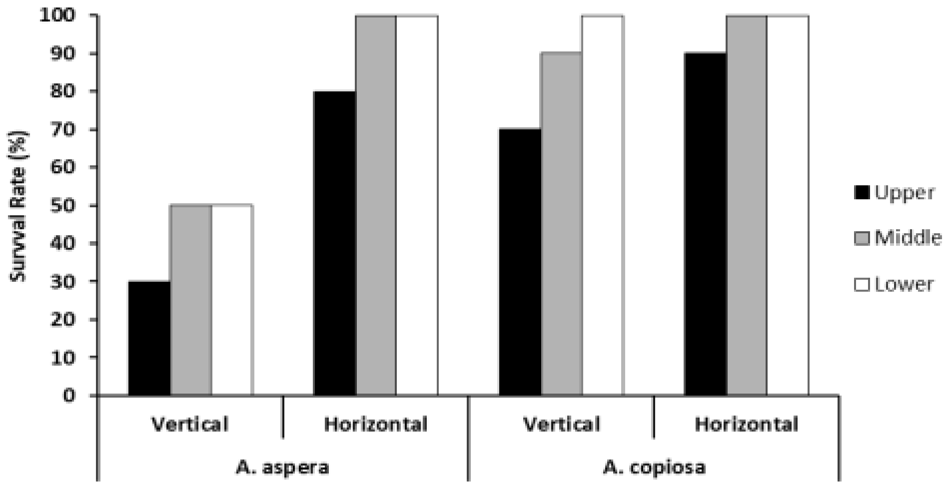

Figure 4. Survival rate (%) of transplanted two Acropora species on multilevel substrates of Artificial Patch Reef after 8 months (November 2018-July 2019)

Growth rate

The growth rate of Acropora branching which transplanted on multilevel substrates of APR varied from 96.7 to 346.9 cm^3/month. The growth rate of Acropora branching fragments was significantly different between species, substrate levels and fixing method of transplantation ($p<0.05$) after 8 months. The growth rate of Acropora branching on multilevel substrates of APR after 8 months demonstrated that there were no significant different on species *A aspera.* Whereas, there were significantly different on the *A. copiosa* growth that transplanted either in the upper and the middle levels or in the lower and in the middle levels. However, there were no significantly different on the coral growth transplanted in the upper and in the lower levels (Table 1).

Table 1. Growth rate (cm^3/month) of Acropora branching transplanted on multilevel substrates of Artificial Patch Reef after 8 months (November 2018-July 2019)

Level	*A. aspera*		*A. copiosa*	
Upper	130.05±47.16	a	293.00±76.23	a
Middle	178.75±34.17	a	152.05±95.11	a
Lower	202.75±44.74	a	333.3±64.21	a

The growth rate of Acropora branching transplanted in different fixing method (vertical vs. horizontal) demonstrated significantly different (Table 3). Growth rate of fragments which transplanted in vertical fixing method was higher than the horizontal method. The lowest growth rate was found in *Acropora aspera* which transplanted in horizontal fixing method, while the highest growth rate occurred in *A. copiosa* which transplanted in vertical fixing methods (Figure 4). Fragments of coral *A. copiosa* can grow optimally on all levels by both vertical and horizontal fixing method of coral transplantation. Comparing the species, the growth of transplanted *A. copiosa* is higher than that of *A. aspera* due to the different branching patterns (Figure 5 and 6).

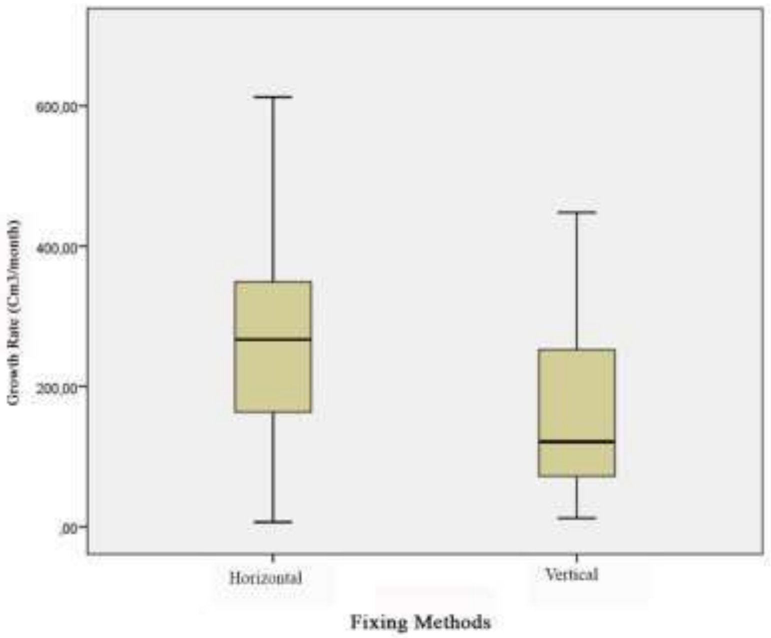

Figure 5. Growth rate (cm^3/month) of two Acropora species transplanted on multilevel substrates of Artificial Patch Reef in different fixing method after 8 months (November 2018-July 2019)

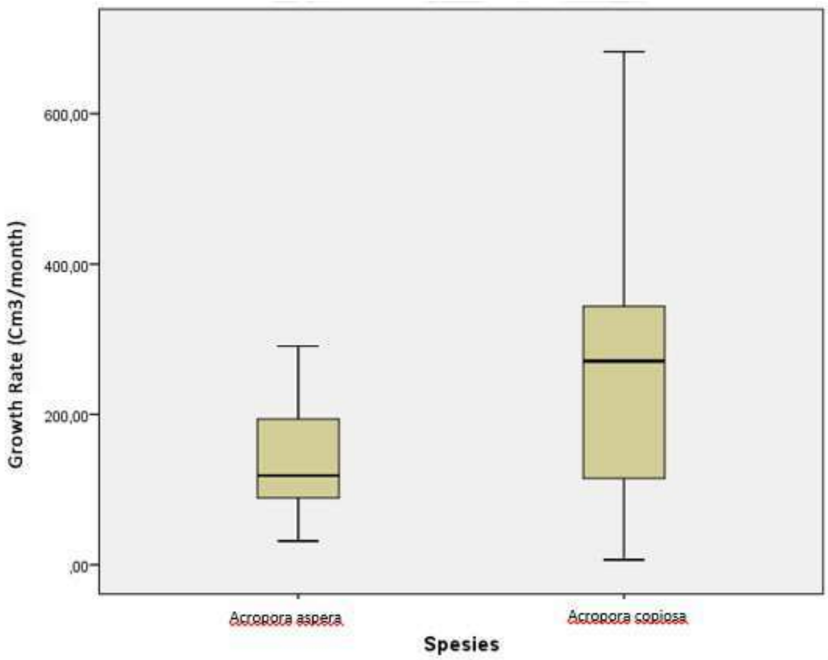

Figure 6. Comparison of growth rate (cm^3/month) *A. aspera* and *A. copiosa* fragments after 8 months transplanted on the multilevel substrates of Artificial Patch Reefs (APR) in the shallow reef.

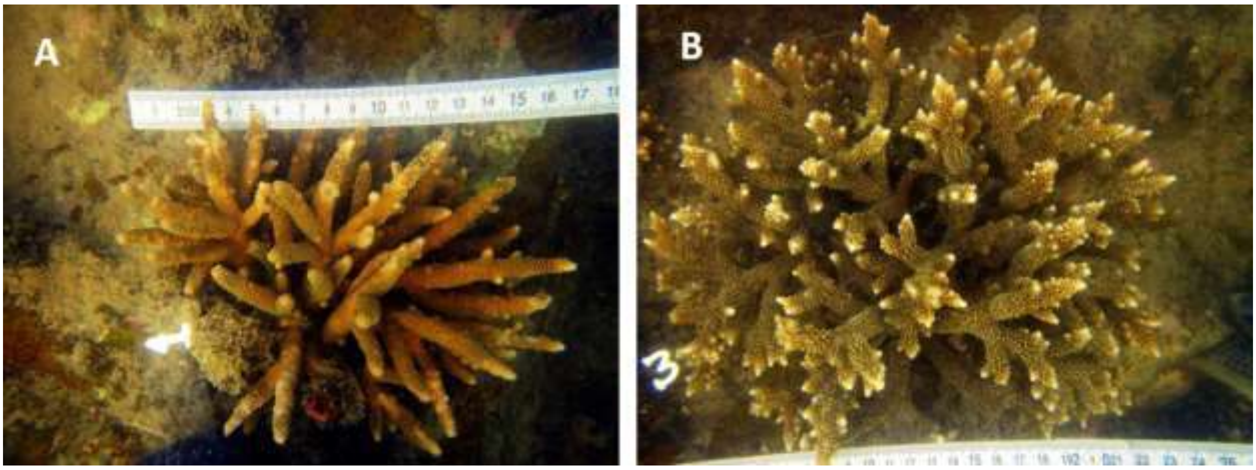

Figure 7. Transplanted coral Acropora on Artificial Patch Reef after 8 months (A. horizontal fixing method of transplanted *Acropora aspera*; B. vertical fixing method of transplanted *Acropora copiosa*)

Discussion

Lower survival rate during the experiment was revealed by *A. aspera* in all levels of substrates particularly in fixing vertically. About 12.5 to 17.5 % of mortality was found during the experiment that occurred in *A. aspera* which fixed on a vertical orientation. Transplanted *A. aspera* is generally found in did not fix on substrates during the early experiment due to the lifeform of the coral is a branching robust (Wallace 2000; Veron and Stafford-Smith 2000), consequently the coral fragments detached from the substrates and died. Lower survival rate mostly was found in the upper level of APR and occurred in the beginning experiment, during the nursery phase, after fixed the coral fragments due to fragment stabilization. Monthly monitoring reveals that coral fragments mortality caused by competition by algae and some of them lost by wave actions. In order to mitigate physical damage, the design strategic placement of nurseries in the substrates was needed by reducing wave exposure (Young et al. 2012).

Acropora branching is one of the important coral in the shallow water and usually applied to coral transplantation. The corals have the competency to grow fast, inversely they are also sensitive responding to the environment. Survival of the corals which have transplanted varied in different location and various in rehabilitation technic. The survival rate of Acropora in nubbin fix to the nursery table was 46% (Nithyanandan et al. 2017), while the high survival rate of the coral was found in *Acropora hyacinthus*. 83.3% (Bongiorni et al. 2011). In the present study, the survival rate of the Acropora branching is high, it may be supported by the stabilization of the substrate of Artificial Patch Reefs (APR) and also the multilevel designed of substrate contribute to enhance the survival of Acropora fragments.

Some previous studies of coral transplantation revealed that the growth rate of Acropora branching was higher than that of other life-form hard corals. Bongiorni et al. (2011) reported that Acropora branching possess relative growth ranged 66.9 to 83.3%, while growth rate of Acropora branching which transplanted on the artificial reef dome-shaped was 1.07 cm/month (Muzaki et al. 2019), Acropora fragments fixed to the dead coral was 7.8 cm/year (Nithyanandan et al. 2017). This result showed that the growth rate of both Acropora branching which transplanted on multilevel substrate possesses a high growth rate. Presumably, the construction of multilevel APR can optimize coral grow by increasing light and preventing sediment coverage. The different branching pattern of the Acropora may affect to the growth of the corals, Two Acropora branching shows a different level of complexity (Mercado-Monila et al. 2016) *A. copiosa* was more complex than *A. aspera*. Veron and Stafford-Smith (2000) identified that *A. copiosa* was clumps of prostrate or upright branches irregular branching patterns with frequent sub-branches, while *A. aspera* which is defined as a corymbose clump with thick branches (Veron and Stafford-Smith 2000). The higher growth rate of vertically fixing method in *Acropora copiosa* indicated that vertical fixing of the fragments was suitable orientation of the natural growth form of the donor colony of Acropora (Okubo et al. 2005). This study suggests that Acropora branching which has high-level complexity such as *A. copiosa* will be selected to apply coral rehabilitation.

ACKNOWLEDGEMENTS

This study was supported by of Applied Research grant (No. 101-170/UN7.P4.3/PP/2018) from Ministry of Research and Technology, Higher Education to M, AS, and SS. Part of the study was supported by research grant No. 1501-26/UN7.5.10/LT/2018 from Faculty of Fisheries and Marine Science, Diponegoro University to M, DPW, I, and RP. We thank Agus Susanto, and staff of Marine Diving Club and Tanjung Jati B Coal-Fired Power Plant to facilitate maintaining the artificial reefs.

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[biodiv] Editor Decision

Smujo Editors <smujo.id@gmail.com> 27 February 2020 at 09:42
To: M Munasik <munasikmotawi@gmail.com>, AGUS SABDONO <agus_sabdono@yahoo.com>, AZELIA N ASSYFA <azeliaasyifa@gmail.com>, DIAH PERMATA WIJAYANTI <diah_permata@mail.com>, SUGIYANTO SUGIYANTO <edt.sugiyanto@gmail.com>, IRWANI IRWANI <irwani.semarang@gmail.com>, RUDHI PRIBADI <rudhi_pribadi@yahoo.uk>

M Munasik, AGUS SABDONO, AZELIA N ASSYFA, DIAH PERMATA WIJAYANTI, SUGIYANTO SUGIYANTO, IRWANI IRWANI, RUDHI PRIBADI:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Coral transplantation on a multilevel substrate of Artificial Patch Reefs: effect of fixing methods on the growth rate of two Acropora species ".

Our decision is: Revisions Required

Smujo Editors
editors@smujo.id

Reviewer E:

This paper reporting a short term result (8 months) of coral transplantation using artificial structure (APR) at Panjang Island, central Java. Considering current lack of information on reef-building corals in Indonesia, the provided information in this paper is important. The accumulation of such information examined and collected in Indonesia will surely become an important body of knowledge for management and conservation of Indonesia coral reefs in the future. Therefore I agree to publish this paper, but before that, authors need to revise some parts of the current manuscript.

1. Acropora need to be italicized all through the text.
2. The description of the two acropora species in the procedure section should be minimized (no point to provide such detailed morphological characters of the species in this paper.)
3. In the procedure section, the text says that the three levels (upper, middle, lower) of APR were used, but exactly where (which height from the bottom) on the figure 2.
4. Can you provide any statistical analyses on the figure 4? Also the statistical results of anova need to be provided clearly in the table 1 and in the text. Only the p value is not enough, and I don't understand the meaning of "a" in the table 1 (no explanation in the legend).
5. Also figures 5 and 6 need statistical analyses to compare the difference statistically.
6. Related to the above two comments, more detailed methodological descriptions on statistical analyses are needed. Current one is just one sentence and I don't understand the meaning of "at 95% confidence level" in the parentheses.

Recommendation: Revisions Required

Reviewer N:

Overall, this is a well-thought out manuscript, which attempted to investigate the growth & survivorship of *Acropora aspera* & *Acropora copiosa* on an artificial patch reef. The results of your study are worthy of publication, but the manuscript in its current form needs some important revisions:

Specifically, the Methods section needs to do give more details.

The Figures & Tables you provided need to be updated to fix spelling and formatting errors.

Your Introduction and Discussion section should be revised slightly to fit your research rationale and your Results section.

Lastly, please read and re-read your manuscript to make sure there are no grammar or spelling errors, which are quite distracting to someone fully proficient in the English language.

Recommendation: Revisions Required

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Coral transplantation on a multilevel substrate of Artificial Patch Reefs: effect of fixing methods on the growth rate of two Acropora species

Commented [DV1]: Overall, this is a well-thought out manuscript, which attempted to investigate the growth & survivorship of *Acropora aspera* & *Acropora copiosa* on an artificial patch reef. The results of your study are worthy of publication, but the manuscript in its current form needs some important revisions:

Specifically, the Methods section needs to do give more details.

The Figures & Tables you provided need to be updated to fix spelling and formatting errors.

Your Introduction and Discussion section should be revised slightly to fit your research rationale and your Results section.

Lastly, please read and re-read your manuscript to make sure there are no grammar or spelling errors, which are quite distracting to someone fully proficient in the English language.

Abstract. Acropora branching is generally used in coral transplantation to rehabilitate coral reefs. However, these corals are sensitive to environmental changes. Artificial Patch Reef (APR) is an artificial structure that provides a multilevel hard substrate. The purpose of the study was to investigate the effectiveness of the APR structure to facilitate the growth and survival of Acropora branching. Two species *Acropora aspera* and *Acropora copiosa* were transplanted vertically and horizontally on a modular concrete block in different levels of APR situated in the shallow reef of Panjang Island, Central Java. The results showed that the coral growth rate varied from 96.7 to 346.9 cm^3/month, while survival ranged from 95.8 to 100% after 8 months. Lower survival rate mostly was found in the upper level of APR.. The statistical analyses showed that the growth rate of *A. copiosa* fragment was significantly higher than that of *A. aspera* ($p<0.05$). Moreover, there were also significantly differences on the treatments of transplantation method ($p<0.05$) to enhance the coral growth. However, multilevel substrates were not significantly influence of the coral growth. This study suggested that *A. copiosa* which has high-level complexity in branching pattern will be selected to apply in shallow reef rehabilitation with transplanted vertically.

Commented [DV2]: Most native English speakers write "branching Acropora" when referring to species of *Acropora* that have a branching morphology.

Key words: *Acropora aspera*, *Acropora copiosa*, artificial patch reef, coral transplant, Panjang Island

Running title: transplantation of two Acropora species on multilevel substrate

INTRODUCTION

Coral reef is one of an important ecosystem on earth, it is most complex and bio-diverse ecosystem that provides the ecological services for humankind. Recently, coral reefs worldwide have been degrading by natural and man-made stress (Wilkinson 2000; Burke et al. 2011). Reef health has been declining apparently by limiting space for natural recruitment and change in physical environmental conditions (Done et al. 2010). Thus, coral reef rehabilitation is considered one of the major reef management strategies that coral reefs may not be able to recover naturally without human intervention.

To rehabilitate damage of natural reefs, artificial reefs and coral transplantation has been applied regardless of environmental condition, cause of decline, or goals. Coral transplantation generally applied by transplanted coral fragments on table cages in shallow water in order to cultivate coral fragments due to transferred and transplanted to rehabilitation reef areas (Heeger and Sotto 2000; Ammar 2013). It seems to be the most widely implemented for coral reef rehabilitation. Many studies dealing with reef rehabilitation by applied coral transplantation (Yap 2000, 2003; Epstein et al. 2001, 2003; Sabater and Yap 2002). Coral transplantation may contribute to enhance the coral population in the reef areas, although natural recovery indicated by coral recruitment (Edward and Clark 1998; Ng et al. 2015). Coral transplantation method potentially has an impact on reef health by loss colonies from the donor area, reducing the growth of transplanted corals, reducing fecundity of transplant due to stress. Alternatively, artificial reefs are considered an efficient rehabilitation tool, it is a suitable method for protection of existing natural reefs, environmental, mitigation for damaged reef areas and shoreline protections (Meester et al. 2012; Ng et al. 2016). Artificial reefs are expected to increase in available substrates for reef organisms, provide structural complexity and natural recruitment. However, the application of these methods in Indonesia waters were apparently not successful, indicated by high mortality of coral fragments in coral transplantation and many artificial reefs that applied damage to natural reefs (Munasik 2009). In order to optimize reef rehabilitation, combining artificial reefs and coral transplantation is recommended (Abelson 2006; Cummings et al. 2015; Ammar et al. 2013).

Artificial Patch Reefs (APR) is an artificial structure which is applied to rehabilitate coral reef in order to develop shallow water habitat (Munasik et al. 2018). APR is a rehabilitation tool that is designed by combination coral transplantation and artificial reefs. Multilevel of the substrate of APR provide the hard substrate to facilitate fragment of coral grows in shallow turbid water. However, the information about the effect of multilevel the structure on survival and growth of transplanted corals is limited. This study aims to address the effectiveness of the APR structure to provide the multilevel substrate to facilitate the growth rate and survival of coral fragment. In more specific, Acropora branching was selected and applied to this study in order to investigate the suitable method and species selection for reef rehabilitation. *Acropora* spp. is generally considered as a good for candidates for use in coral transplantation or population enhancement project due to their high growth rate and high survivorship of fragments (Lirman et al. 2010; Stephanie et al. 2017; Boch and Morse 2012; Mercado-Molina 2016). Acroporid corals are significantly important in the shallow reef of Panjang Island, however the population decline slightly due to the anthropogenic stressor (Munasik et al. 2012). Thus, the application of APR with Acropora transplanted on their substrates is considered contributing to the local conservation of small island reefs in the near future.

Commented [DV3]: Consider moving these sentences to a different part of your Introduction so the whole section reads more succinctly.

MATERIALS AND METHODS

Study area

Rehabilitation of coral reefs program was carried out in shallow reefs of Panjang Island Central Java by deployed 12 (twelve) artificial patch reefs (APR) from 2015 to 2018 at 3 m depth (Figure 1). In order to conduct a coral transplantation experiment, one unit of Artificial Patch Reef (APR) No. 12 was selected to perform the study of the effect of species and coral transplantation method in multilevel of substrates on growth of coral fragments.

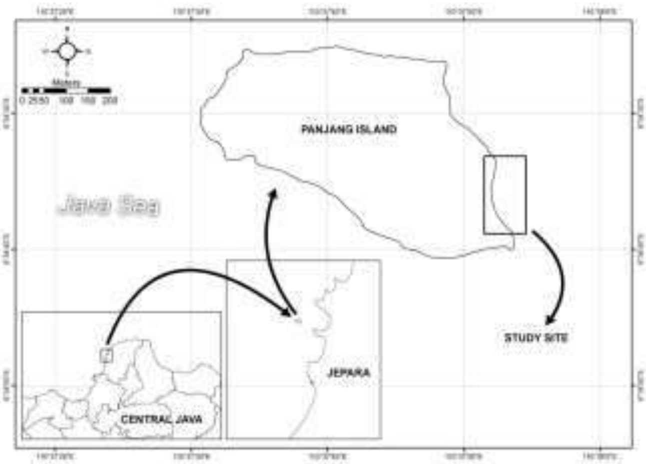

Figure 1. Study site of coral transplantation on Artificial Patch Reef at Panjang Island, Central Java (6º34'30" S; 110º37'44" E)

Procedures

Fragments of Acropora branching, i.e. *Acropora aspera* and *A. copiosa* were transplanted on multilevel substrates of Artificial Patch Reefs which deployed in the shallow reef of Panjang Island, Central Java (Java Sea; Figure 1). Both of *A. aspera* and *A. copiosa* were as a limiting local population of Acroporid in Panjang Island, Central Java. *A. aspera* is generally found in the inner lagoon which colony is defined as a corymbose clump with short thick branches. Veron and Stafford-Smith (2000) described that *A. aspera* has small axial corallites while radial corallites are composed two sizes, crowded and have prominent lower lips giving a scale-like appearance (Veron and Stafford-Smith 2000). Colonies of *A. copiosa* are generally found in front of the reef flat and the species were characterized as arborescent clumps of upright branches. The corals have irregular branching patterns with frequent sub-branches and axial corallites relatively small while radial corallites are crowded, all tubular with unequal height (Veron and Stafford-Smith 2000). Comparing to the previous species, *Acropora copiosa* have more complexity in branching pattern.

Commented [DV4]: How were the fragments made? In other words, was only a single mother colony of *A. aspera* and *A. copiosa* fragmented to produce all 120 transplants?

Additionally, what process did you use to create the fragments (e.g. bone shears, saw)?

Lastly, how big were the individual fragments?

Artificial Patch Reefs (APR) is artificial reefs made by concrete blocks which composed as modular circular structures in shape, constructed 5 (five) levels of substrates were deployed from small boats by SCUBA divers, and are suitable near natural reefs in shallow water (Munasik et al. 2018; Figure 2). The total height of the multilevel APR structure is about 80 cm from the bottom of the sea, and the height of each level is 20 cm. In this experiment, coral fragments were transplanted in the upper, middle, and lower level. Coral transplantation experiments were not applied at the top level to prevent physical damaged in coral fragments. The experiments were also not implemented in the coral transplantation in the base of APR (levels 4 and 5) since the surface of the substrate usually covering sediment due to resuspension. At the beginning of November 2018, 120 coral fragments were transplanted on three levels of APR by two fixation methods: vertically and horizontally orientation of the fragments fixing on the surface of substrates and tied to a paired of the nail using cable ties

(Figure 3). Fixation of fragments using cable ties is considered the increasing survival of transplanted corals (William and Miller 2010; Okubo et al. 2005).

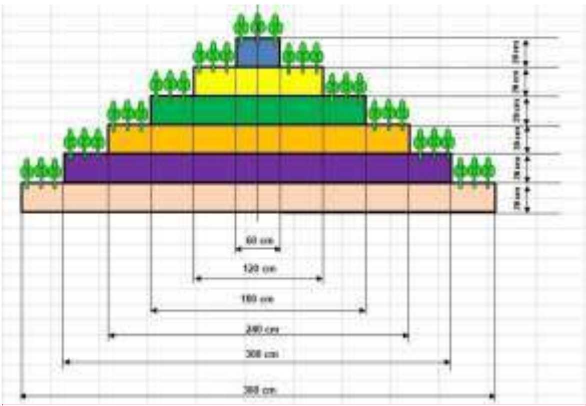

Figure 2. Structure of Artificial Patch Reef (APR) deployed in the shallow reef of Pulau Panjang, Central Java (Munasik et al. 2018)

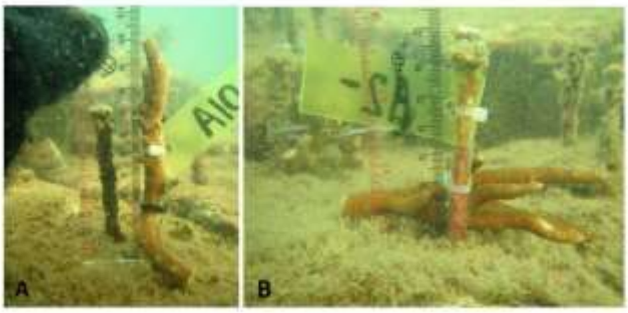

Figure 3. Fixing methods of coral fragments tied to nail by cable ties (A. vertically fixing method, B. horizontally fixing method)

Data analysis

In order to investigate the growth rate of Acropora branching fragments, we used a measurement of corallum size in volume dimension (Buddemeier and Kinzie III 1976). The final measurement of volume (length, wide, and height) of the coral fragments was evaluated in late July 2019. The size of the fragments was measured by taking a picture using an underwater camera and putting the scale beside each the fragment (Mercado-Molina et al. 2016). The size measurement of the fragments was analyzed using image analyses of computer software. Volume of the fragment was determined by ecological volume (EV; de la Cruz et al. 2014), and its calculated following the cylindrical volume formula (Levy et al. 2010) as define, in equation (1)

$$EV = \pi r^2 h, \text{ where } \quad r = \frac{(l+W)}{4} \qquad (1)$$

Growth rate (GR) of the corals (Ecological Volume per month) was calculated using the formula (2)

$$Gr = [EVf - EVi]/m \qquad (2)$$

where Gr is the standardized growth rate, EVf and EVi are final and initial Ecological Volume and m is number of months elapsed.

Only the tagged coral fragments alive at 8 ~~(eight)~~ months post transplantation ~~where~~ were included in the growth rate determination.

Commented [DV5]: Not necessarily true. Though the cable ties are useful in anchoring the transplant to the substrate (i.e. preventing mortality via detachment), there are studies that have shown rapid tissue necrosis at the sites where the cable tie touches the living tissue, which ultimately can lead to the mortality of the transplant. Consider re-writing this sentence to be more specific.

Commented [DV6]: Though this is an informative figure, it does not seem to actually depict what *your* APR looked like. In your Methods section, you mention that you did not add coral transplants to the top and the base of the structure. Additionally, you mention that the whole APR was only 80 cm high, with each level being 20 cm tall. Thus, should this image only show a "pyramid" with 4 levels, with coral transplants depicted only on the middle 2 levels?

Or if this image is indeed an accurate representation of your APR, then the text portion of your methods should be revised (i.e. the APR structure was 120 cm tall).

Commented [DV7]: What computer software was used?

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In order to test the effect of different levels of transplant position, and different fixing methods of coral transplantation to the growth of two Acropora species, data of growth rate of the fragments were analyzed using two-way of variance (ANOVA, at 95% confidence level, $p<0.05$).

RESULTS AND DISCUSSION

Survival rate

The survival rate of Acropora branching fragments which transplanted on multilevel substrates is varied among species, fixing method and level of substrates. The average survival rate of *A. copiosa* which transplanted on the lower level of substrates was higher than *A. aspera* at a similar level. Both species of Acropora branching which transplanted horizontally possess higher survivorship than fragments that transplanted vertically. The lower survival rate of the fragments is found in the upper level of substrates (Figure 4) which located on the top of APR, about 1 m from the bottom of the sea during low tide.

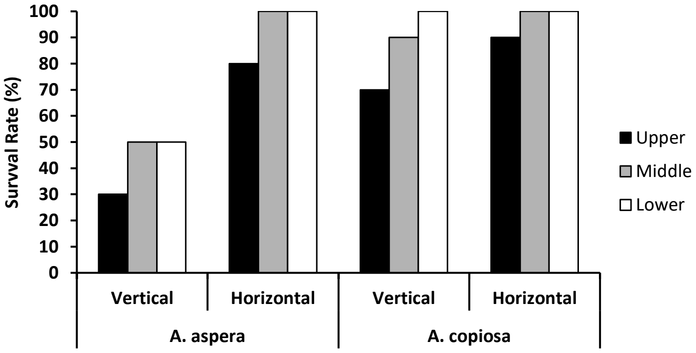

Figure 4. Survival rate (%) of transplanted two Acropora species on multilevel substrates of Artificial Patch Reef after 8 months (November 2018-July 2019)

Growth rate

The growth rate of Acropora branching which transplanted on multilevel substrates of APR varied from 96.7 to 346.9 cm^3/month. The growth rate of Acropora branching fragments was significantly different between species, substrate levels and fixing method of transplantation ($p<0.05$) after 8 months. The growth rate of Acropora branching on multilevel substrates of APR after 8 months demonstrated that there were no significant different on species *A aspera.* Whereas, there were significantly different on the *A. copiosa* growth that transplanted either in the upper and the middle levels or in the lower and in the middle levels. However, there were no significantly different on the coral growth transplanted in the upper and in the lower levels (Table 1).

Table 1. Growth rate (cm^3/month) of Acropora branching transplanted on multilevel substrates of Artificial Patch Reef after 8 months (November 2018-July 2019)

Commented [DV8]: I do not understand what the letter "a" means in column #3 and #5.

Level	*A. aspera*		*A. copiosa*	
Upper	130.05±47.16	a	293.00±76.23	a
Middle	178.75±34.17	a	152.05±95.11	a
Lower	202.75±44.74	a	333.3±64.21	a

The growth rate of Acropora branching transplanted in different fixing method (vertical vs. horizontal) demonstrated significantly different (Table 3). Growth rate of fragments which transplanted in vertical fixing method was higher than the horizontal method. The lowest growth rate was found in *Acropora aspera* which transplanted in horizontal fixing method, while the highest growth rate occurred in *A. copiosa* which transplanted in vertical fixing methods (Figure 4). Fragments of coral *A. copiosa* can grow optimally on all levels by both vertical and horizontal fixing method of coral transplantation. Comparing the species, the growth of transplanted *A. copiosa* is higher than that of *A. aspera* due to the different branching patterns (Figure 5 and 6).

Commented [DV9]: Since I only observed one Table in your whole manuscript, did you mean to say Figure 3?

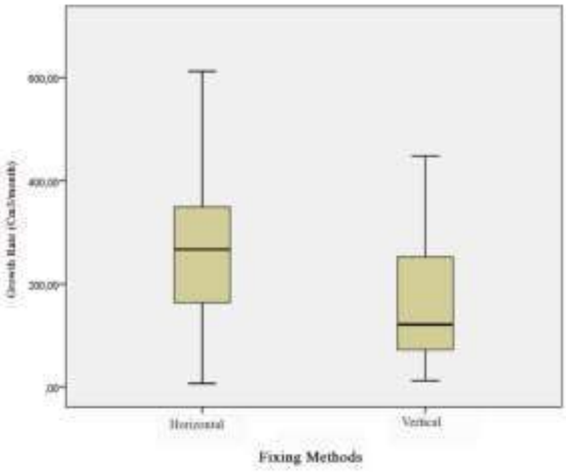

Figure 5. Growth rate (cm^3/month) of two Acropora species transplanted on multilevel substrates of Artificial Patch Reef in different fixing method after 8 months (November 2018-July 2019)

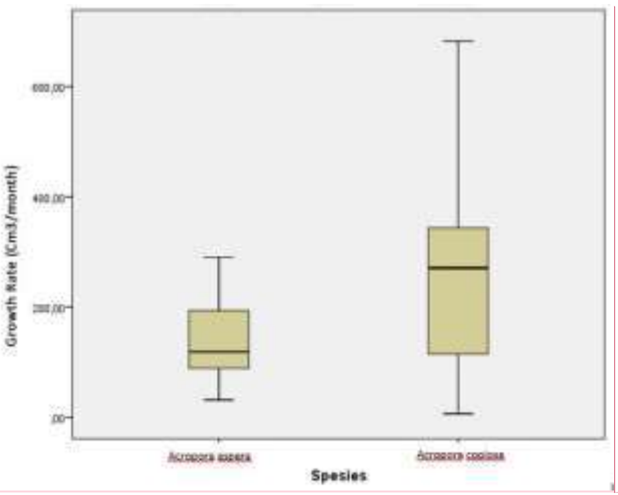

Figure 6. Comparison of growth rate (cm^3/month) *A. aspera* and *A. copiosa* fragments after 8 months transplanted on the multilevel substrates of Artificial Patch Reefs (APR) in the shallow reef.

Commented [DV10]: The title for your X-axis is misspelled. Should be "species."

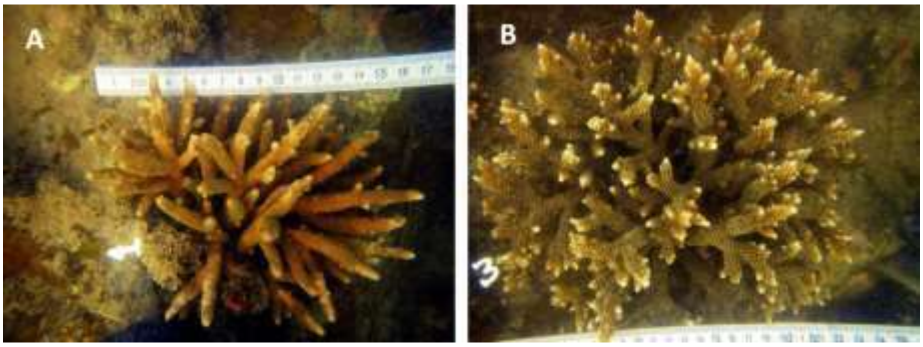

Figure 7. Transplanted coral Acropora on Artificial Patch Reef after 8 months (A. horizontal fixing method of transplanted *Acropora aspera*; B. vertical fixing method of transplanted *Acropora copiosa*)

Commented [DV11]: Though this is a nice figure, I do not see it mentioned anywhere in the main text of your manuscript. Consider including it in the text, or removing it from your manuscript.

Discussion

Lower survival rate during the experiment was revealed by *A. aspera* in all levels of substrates particularly in fixing vertically. About 12.5 to 17.5 % of mortality was found during the experiment that occurred in *A. aspera* which fixed on a vertical orientation. Transplanted *A. aspera* is generally found in did not fix on substrates during the early experiment due to the lifeform of the coral is a branching robust (Wallace 2000; Veron and Stafford-Smith 2000), consequently the coral fragments detached from the substrates and died. Lower survival rate mostly was found in the upper level of APR and occurred in the beginning experiment, during the nursery phase, after fixed the coral fragments due to fragment stabilization. Monthly monitoring reveals that coral fragments mortality caused by competition by algae and some of them lost by wave actions. In order to mitigate physical damage, the design strategic placement of nurseries in the substrates was needed by reducing wave exposure (Young et al. 2012).

Commented [DV12]: Very confusing. Consider re-wording this part for clarity.

Commented [DV13]: Are you saying that halfway through your experiment, you moved to the whole APR with the attached coral fragments? If so, do you think this could have affected your results?

Acropora branching is one of the important coral in the shallow water and usually applied to coral transplantation. The corals have the competency to grow fast, inversely they are also sensitive responding to the environment. Survival of the corals which have transplanted varied in different location and various in rehabilitation technic. The survival rate of Acropora in nubbin fix to the nursery table was 46% (Nithyanandan et al. 2017), while the high survival rate of the coral was found in *Acropora hyacinthus*. 83.3% (Bongiorni et al. 2011). In the present study, the survival rate of the Acropora branching is high, it may be supported by the stabilization of the substrate of Artificial Patch Reefs (APR) and also the multilevel designed of substrate contribute to enhance the survival of Acropora fragments.

Some previous studies of coral transplantation revealed that the growth rate of Acropora branching was higher than that of other ~~life-form hard corals~~ hermatypic corals. Bongiorni et al. (2011) reported that Acropora branching possess relative growth ranged 66.9 to 83.3%, while growth rate of Acropora branching which transplanted on the artificial reef dome-shaped was 1.07 cm/month (Muzaki et al. 2019), Acropora fragments fixed to the dead coral was 7.8 cm/year (Nithyanandan et al. 2017). This result showed that the growth rate of both Acropora branching which transplanted on multilevel substrate possesses a high growth rate. Presumably, the construction of multilevel APR can optimize coral grow by increasing light and preventing sediment coverage. The different branching pattern of the Acropora may affect to the growth of the corals, Two Acropora branching shows a different level of complexity (Mercado-Monila et al. 2016) *A. copiosa* was more complex than *A. aspera*. Veron and Stafford-Smith (2000) identified that *A. copiosa* was clumps of prostrate or upright branches irregular branching patterns with frequent sub-branches, while *A. aspera* which is defined as a corymbose clump with thick branches (Veron and Stafford-Smith 2000). The higher growth rate of vertically fixing method in *Acropora copiosa* indicated that vertical fixing of the fragments was suitable orientation of the natural growth form of the donor colony of Acropora (Okubo et al. 2005). This study suggests that Acropora branching which has high-level complexity such as *A. copiosa* will be selected to apply coral rehabilitation.

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Commented [DV14]: In Figure 4, you show that you had better survivorship rates with horizontal fixation; so, this appears to be a contradictory sentence.

Commented [DV15]: This statement is not really what the study investigated, nor was it completely supported by your results. Consider mentioning that multi-level APRs and horizontal fixation should be used in future coral rehabilitation projects; or something along similar lines.

ACKNOWLEDGEMENTS

This study was supported by of Applied Research grant (No. 101-170/UN7.P4.3/PP/2018) from Ministry of Research and Technology, Higher Education to M, AS, and SS. Part of the study was supported by research grant No. 1501-26/UN7.5.10/LT/2018 from Faculty of Fisheries and Marine Science, Diponegoro University to M, DPW, I, and RP. We thank Agus Susanto, and staff of Marine Diving Club and Tanjung Jati B Coal-Fired Power Plant to facilitate maintaining the artificial reefs.

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Reviewer Comments

This paper reporting a short term result (8 months) of coral transplantation using artificial structure (APR) at Panjang Island, central Java. Considering current lack of information on reef-building corals in Indonesia, the provided information in this paper is important. The accumulation of such information examined and collected in Indonesia will surely become an important body of knowledge for management and conservation of Indonesia coral reefs in the future. Therefore I agree to publish this paper, but before that, authors need to revise some parts of the current manuscript.

1. Acropora need to be italicized all through the text.
2. The description of the two acropora species in the procedure section should be minimized (no point to provide such detailed morphological characters of the species in this paper.)
3. In the procedure section, the text says that the three levels (upper, middle, lower) of APR were used, but exactly where (which height from the bottom) on the figure 2.
4. Can you provide any statistical analyses on the figure 4? Also the statistical results of anova need to be provided clearly in the table 1 and in the text. Only the p value is not enough, and I don't understand the meaning of "a" in the table 1 (no explanation in the legend).
5. Also figures 5 and 6 need statistical analyses to compare the difference statistically.
6. Related to the above two comments, more detailed methodological descriptions on statistical analyses are needed. Current one is just one sentence and I don't understand the meaning of "at 95% confidence level" in the parentheses.

Gmail **munasik motawi <munasikmotawi@gmail.com>**

[biodiv] Editor Decision

2 messages

Smujo Editors <smujo.id@gmail.com> 2 April 2020 at 11:25
Reply-To: Smujo Editors <editors@smujo.id>
To: M Munasik <munasikmotawi@gmail.com>, AGUS SABDONO <agus_sabdono@yahoo.com>, AZELIA N ASSYFA <azeliaasyifa@gmail.com>, DIAH PERMATA WIJAYANTI <diah_permata@mail.com>, SUGIYANTO SUGIYANTO <edt.sugiyanto@gmail.com>, IRWANI IRWANI <irwani.semarang@gmail.com>, RUDHI PRIBADI <rudhi_pribadi@yahoo.uk>

M Munasik, AGUS SABDONO, AZELIA N ASSYFA, DIAH PERMATA WIJAYANTI, SUGIYANTO SUGIYANTO, IRWANI IRWANI, RUDHI PRIBADI:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Coral transplantation on a multilevel substrate of Artificial Patch Reefs: effect of fixing methods on the growth rate of two Acropora species ".

Our decision is: Revisions Required

Smujo Editors
editors@smujo.id

Reviewer E:

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Recommendation: Revisions Required

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Recommendation: Decline Submission

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Lastly, please read and re-read your manuscript to make sure there are no grammar or spelling errors, which are quite distracting to someone fully proficient in the English language.

Recommendation: Revisions Required

Biodiversitas Journal of Biological Diversity

2 attachments

N-Peer_Review_-_5279-Article Text-17107-1-4-20200122.docx
893K

E-Reviewer Comments.docx
13K

munasik motawi <munasikmotawi@gmail.com> 2 April 2020 at 23:23
To: Smujo Editors <editors@smujo.id>

Dear Editors,

We have completed the revision of our manuscript "**Coral transplantation on a multilevel substrate of Artificial Patch Reefs: effect of fixing methods on the growth rate of two *Acropora* species**" by Munasik, Agus Sabdono, Azelia N Assyfa, Diah P Wijayanti, Sugiyanto, Irwani, Rudhi Pribadi. We are also sending the revision manuscript by online submission to the OJS of Biodiversitas Journal of Biological Diversity. Enclosed herewith are a revised article with a listing of specific response to the reviewer.

We very much appreciate your efforts on behalf. If you need any further information, please contact us.

Sincerely,

Munasik
Department of Marine Science
Diponegoro University

Pada tanggal Kam, 2 Apr 2020 pukul 11.25 Smujo Editors <smujo.id@gmail.com> menulis:

> M Munasik, AGUS SABDONO, AZELIA N ASSYFA, DIAH PERMATA WIJAYANTI, SUGIYANTO SUGIYANTO, IRWANI IRWANI, RUDHI PRIBADI:
>
> We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Coral transplantation on a

multilevel substrate of Artificial Patch Reefs: effect of fixing methods on the growth rate of two Acropora species ".

Our decision is: Revisions Required

Smujo Editors
editors@smujo.id

Reviewer E:

This paper reporting a short term result (8 months) of coral transplantation using artificial structure (APR) at Panjang Island, central Java. Considering current lack of information on reef-building corals in Indonesia, the provided information in this paper is important. The accumulation of such information examined and collected in Indonesia will surely become an important body of knowledge for management and conservation of Indonesia coral reefs in the future. Therefore I agree to publish this paper, but before that, authors need to revise some parts of the current manuscript.

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Biodiversitas Journal of Biological Diversity

2 attachments

1-Acropora_Munasik_Biodiversitas_2020_underReviewREVfix0402.doc
1641K

2_List of Modification_for_Biodiversitas-Peer_Review_FixMun0204.docx
300K

COVERING LETTER

Dear **Editor-in-Chief,**

I herewith enclosed a research article,

Title:

Coral transplantation on a multilevel substrate of Artificial Patch Reefs: effect of fixing methods on the growth rate of two *Acropora* species

Author(s) name:

Munasik[1]*, Agus Sabdono[1], Azelia N Assyfa[1], Diah P Wijayanti[1], Sugiyanto[2], Irwani[1], Rudhi Pribadi[1]

Address
(Fill in your institution's name and address, your personal cellular phone and email)

[1]Department of Marine Science, Faculty of Fisheries and Marine Science, Diponegoro University, Jl. Prof. Soedarto SH Tembalang, Semarang 50275, Central Java, Indonesia, Tel/Fax. +62-24-7474698, *e-mail: munasik@lecturer.undip.ac.id

For possibility publication on the journal:
(fill in *Biodiversitas* or *Nusantara Bioscience* or *mention the others*)

Biodiversitas

Novelty:
(state your claimed novelty of the findings versus current knowledge)

Coral transplantation and artificial reef are known as the methods for coral reef rehabilitation. However, the application of these methods in Indonesia waters were apparently not successful, indicated by high mortality of coral fragments in coral transplantation and many artificial reefs that applied damage to natural reefs. In the present study, we firstly combine both method to create new habitat of coral reef called Artificial Patch Reef (APR) and examine the growth rate of two *Acropora* species with different fixing method on APR.

Statements:

This manuscript has not been published and is not under consideration for publication to any other journal or any other type of publication (including web hosting) either by me or any of my co-authors.
Author(s) has been read and agree to the Ethical Guidelines.

List of five potential reviewers
(Fill in names of five potential reviewers **that agree to review your manuscpt** and their **email** addresses. He/she should have Scopus ID and come from different institution with the authors; and from at least three different countries)

1. Prof. Dr. Suharsono (suharsonolipi@gmail.com) Scopus ID: 57201227119
2. Prof. Dr. Jamaluddi Jompa (jamaluddin.jompa@gmail.com) Scopus ID: 6603032552
3. Prof. Dr. Ocky Karna Radjasa (ocky_radjasa@yahoo.com) Scopus ID: 15824260100
4. Prof. Dr. Kazuhiko Sakai (sakaikz@lab.u-ryukyu.ac.jp) Scopus ID: 35722748300
5. Dr. Yoko Nozawa (nozaway@gate.sinica.edu.tw) Scopus ID: 36504637600

Place and date:

Semarang 21 January 2020

Sincerely yours,
(fill in your name, no need scanned autograph)

Munasik

Coral transplantation on a multilevel substrate of Artificial Patch Reefs: effect of fixing methods on the growth rate of two *Acropora* species

MUNASIK[1,♥], AGUS SABDONO[1], AZELIA N ASSYFA[1], DIAH PERMATA WIJAYANTI[1], SUGIYANTO[2], IRWANI[1], RUDHI PRIBADI[1]

[1]Department of Marine Science, Faculty of Fisheries and Marine Science, Diponegoro University,
Jl. Prof. Soedarto SH Tembalang, Semarang 50275, Central Java, Indonesia, Tel/Fax. +62-24-7474698,
[♥]e-mail: munasik@lecturer.undip.ac.id
[2]Department of Mechanical Engineering, Faculty of Engineering, Diponegoro University,
Jl. Prof. Soedarto SH Tembalang, Semarang 50275, Central Java, Indonesia, Tel/Fax. +62-24-7460059
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Manuscript received: 21 01 2020 (Date of abstract/manuscript submission). Revision accepted: .... 2020.

Abstract. Branching *Acropora* is generally used in coral transplantation to rehabilitate coral reefs. However, these corals are sensitive to environmental changes. Artificial Patch Reef (APR) is an artificial structure that provides a multilevel hard substrate. The purpose of the study was to investigate the effectiveness of the APR structure to facilitate the growth and survival of Acropora branching. Two species *Acropora aspera* and *Acropora copiosa* were transplanted vertically and horizontally on a modular concrete block in different levels of APR situated in the shallow reef of Panjang Island, Central Java. The results showed that the coral growth rate varied from 96.7 to 346.9 cm^3/month, while survival ranged from 30 to 100% after 8 months. Lower survival rate mostly was found in the upper level of APR.. The statistical analyses showed that the growth rate of *A. copiosa* fragment was significantly higher than that of *A. aspera* ($p<0.05$). Moreover, there were also significantly differences on the treatments of transplantation method ($p<0.05$) to enhance the coral growth. However, multilevel substrates were not significantly influence of the coral growth. This study suggested that *A. copiosa* which has high-level complexity in branching pattern will be selected to apply in shallow reef rehabilitation with transplanted vertically.

Key words: coral transplant, *Acropora aspera*, *Acropora copiosa*, artificial patch reef, Panjang Island

Running title: transplantation of two *Acropora* species on multilevel substrate

INTRODUCTION

Coral reef is one of an important ecosystem on earth, it is most complex and bio-diverse ecosystem that provides the ecological services for humankind. Recently, coral reefs worldwide have been degrading by natural and man-made stress (Wilkinson 2000; Burke et al. 2011). Reef health has been declining apparently by limiting space for natural recruitment and change in physical environmental conditions (Done et al. 2010). Thus, coral reef rehabilitation is considered one of the major reef management strategies that coral reefs may not be able to recover naturally without human intervention.

To rehabilitate damage of natural reefs, artificial reefs and coral transplantation has been applied regardless of environmental condition, cause of decline, or goals. Coral transplantation generally applied by transplanted coral fragments on table cages in shallow water in order to cultivate coral fragments due to transferred and transplanted to rehabilitation reef areas (Heeger and Sotto 2000; Ammar 2013). It seems to be the most widely implemented for coral reef rehabilitation. Many studies dealing with reef rehabilitation by applied coral transplantation (Yap 2000, 2003; Epstein et al. 2001, 2003; Sabater and Yap 2002). Coral transplantation may contribute to enhance the coral population in the reef areas, although natural recovery indicated by coral recruitment (Edward and Clark 1998; Ng et al. 2015). Coral transplantation method potentially has an impact on reef health by loss colonies from the donor area, reducing the growth of transplanted corals, reducing fecundity of transplant due to stress. Alternatively, artificial reefs are considered an efficient rehabilitation tool, it is a suitable method for protection of existing natural reefs, environmental, mitigation for damaged reef areas and shoreline protections (Meester et al. 2012; Ng et al. 2016). Artificial reefs are expected to increase in available substrates for reef organisms, provide structural complexity and natural recruitment. However, the application of these methods in Indonesia waters were apparently not successful, indicated by high mortality of coral fragments in coral transplantation and many artificial reefs that applied damage to natural reefs (Munasik 2009). In order to optimize reef rehabilitation, combining artificial reefs and coral transplantation is recommended (Abelson 2006; Cummings et al. 2015; Ammar et al. 2013).

Artificial Patch Reefs (APR) is an artificial structure which is applied to rehabilitate coral reef in order to develop shallow water habitat (Munasik et al. 2018). APR is a rehabilitation tool that is designed by multilevel substrates and applied the combination both of coral transplantation and artificial reefs. *Acropora* spp. is generally considered as a good for candidates for use in coral transplantation or population enhancement project due to their high growth rate and high survivorship of fragments (Lirman et al. 2010; Stephanie et al. 2017; Boch and Morse 2012; Mercado-Molina 2016). The application of APR with *Acropora* transplanted on their substrates is considered contributing to the local conservation of the small island reefs in the near future. In this study, two *Acropora* species were selected and applied to investigate the suitable method and species selection for reef rehabilitation. Multilevel substrates of APR may provide the hard substrate to facilitate fragment of coral grows in shallow turbid water. However, the information about the effect of multilevel substrates on survival and growth of transplanted corals is limited. This study aims to address the effectiveness of the APR structure to provide the multilevel substrate to facilitate the growth rate and survival of coral fragment.

MATERIALS AND METHODS

Study area

Acroporid corals are significantly important in the shallow reef of Panjang Island, Central Java however the population decline slightly due to the anthropogenic stressor (Munasik et al. 2012). Two species *Acropora* i.e. *Acropora aspera* and *A. copiosa* were known as a limiting local population in the Island. Colonies of *A. aspera* is common in the inner lagoon and the species was defined as a corymbose clump with short thick branches. Population of *A. copiosa* is generally found in front of the reef flat and colony was characterized as arborescent clumps of upright branches. Comparing to the previous species, *Acropora copiosa* have more complexity in branching pattern. Rehabilitation of coral reefs program was carried out in shallow reefs of Panjang Island Central Java by deployed 12 (twelve) artificial patch reefs (APR) from 2015 to 2018 at 3 m depth. In order to conduct a coral transplantation experiment, a unit of Artificial Patch Reef (APR) No. 12 was selected to perform the study of the effect of species and coral transplantation method in multilevel of substrates on growth of *Acropora* (Figure 1).

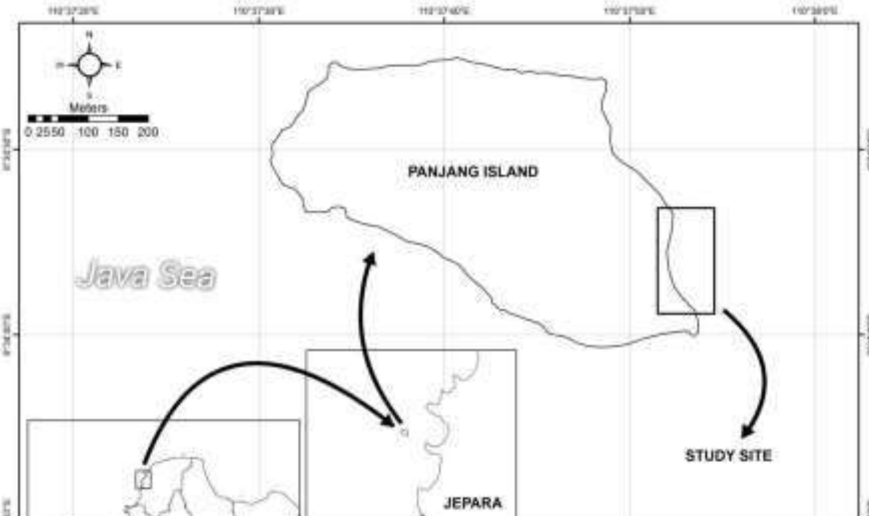

Figure 1. Study site of coral transplantation on Artificial Patch Reef at Panjang Island, Central Java (6º34’30” S; 110º37’44” E)

Procedures

Coral fragments were collected from donor site of two *Acropora* species in the inner lagoon and in front of the reef flat of Panjang Island. Fragments of *A. aspera* were collected by broken off small branches at random mother colonies while *A. copiosa* fragments were chisel off main branches of adult colonies randomly. The small branches of two species (average size was 12.57 cm^3) were transferred into basket and then were transplanted on multilevel substrates of Artificial Patch Reefs (APR) which deployed in the eastern site of Panjang Island.

Artificial Patch Reefs (APR) is artificial reefs made by concrete blocks which composed as modular circular structures in shape, constructed 5 (five) levels of substrates were deployed from small boats by SCUBA divers, and are suitable near natural reefs in shallow water. The total height of the multilevel APR structure is about 120 cm from the bottom of the sea, and the height of each level is 20 cm. In this experiment, coral fragments were transplanted in the upper, middle, and lower level. Coral transplantation experiments were not applied at the top level to prevent physical damaged in coral fragments. The experiments were also not implemented in the coral transplantation in the base of APR (level 5) since the surface of the substrate usually covering sediment due to resuspension (Figure 2). At the beginning of November 2018, 120 coral fragments were transplanted on three levels of APR by two fixation methods: vertically and horizontally orientation of the fragments fixing on the surface of substrates and tied to a paired of the nail using cable ties (Figure 3). Cable ties method of coral transplantation have been widely applied and effective technique for attaching *Acropora* fragments to artificial substrate (William and Miller 2010; Young et al. 2012). Coral fragment stabilization using cable ties was similarly effective to epoxy or cement methods (William and Miller, 2010).

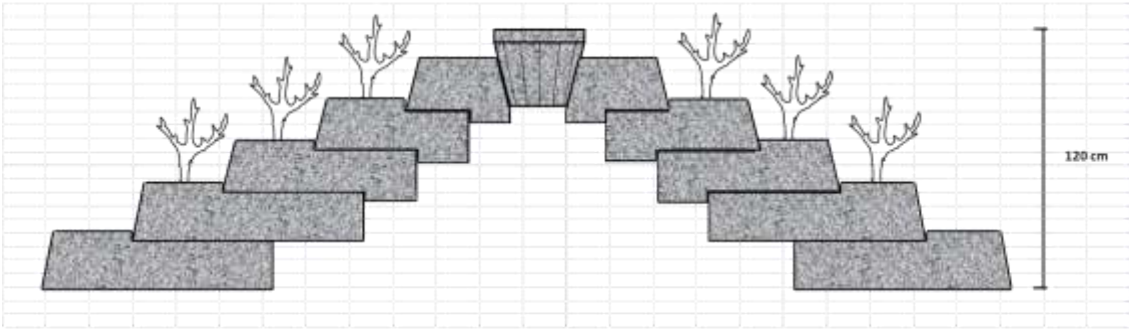

Figure 2. Lay out of coral transplantation experiment, *Acropora* fragment transplanted on the multilevel: in upper, middle and lower of Artificial Patch Reef (APR)

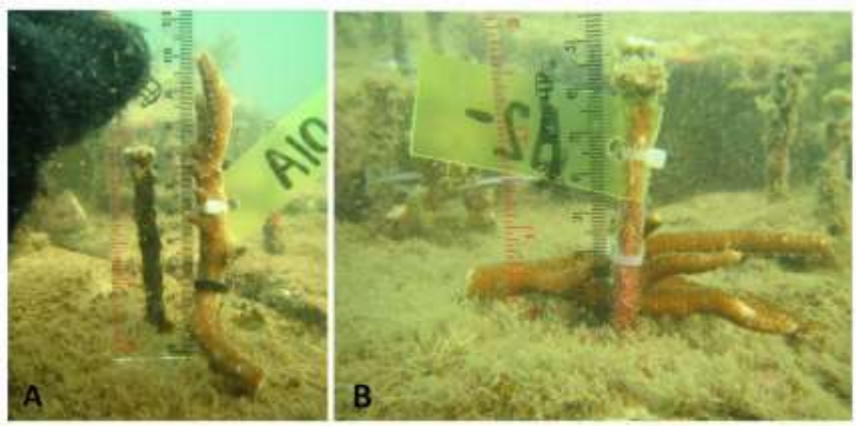

Figure 3. Fixing methods of coral fragments tied to nail by cable ties (A. vertically fixing method, B. horizontally fixing method)

Data analysis

In order to investigate the growth rate of *Acropora* fragments, we used a measurement of corallum size in volume dimension (Buddemeier and Kinzie III 1976). The final measurement of volume (length, wide, and height) of the coral fragments was evaluated in late July 2019. The size of the fragments was measured by taking a picture using an underwater camera and putting the scale beside each the fragment (Mercado-Molina et al. 2016). The size measurement of the fragments was analyzed using image analyses of computer software, Image J. Volume of the fragment was determined by ecological volume (EV; de la Cruz et al. 2014), and its calculated following the cylindrical volume formula (Levy et al. 2010) as define, in equation (1)

$$EV = \pi r^2 h, \text{ where } \quad r = \frac{(l+W)}{4} \tag{1}$$

Growth rate (GR) of the corals (Ecological Volume per month) was calculated using the formula (2)

$$Gr = [EVf - EVi]/m \tag{2}$$

where Gr is the standardized growth rate, EVf and EVi are final and initial Ecological Volume and m is number of months elapsed.

Only the tagged coral fragments alive at 8 (eight) months post transplantation were included in the growth rate determination.

In order to test the effect of different levels of transplant position, and different fixing methods of coral transplantation to the growth of two *Acropora* species, data of growth rate of the fragments were analyzed using two-way of variance (ANOVA, at 95% confidence level, p<0.05).

RESULTS AND DISCUSSION

Survival rate

The survival rate of two *Acropora* species which transplanted on multilevel substrate was varied from 30 to 100%, the average of the survival rate was 80%. Both of *Acropora* species which transplanted horizontally possess higher survivorship (average of survival rate was 95%) than the fragments which transplanted vertically (average of survival rate was 85%). The lower survival rate of the fragments were found in the upper level of substrates (varied from 30 to 50%; Figure 4) which located on the top of APR, about 1 m from the bottom of the sea during low tide. Coral fragment of *A. copiosa* was more survive than *A. aspera,* indicated the lower survival rate coral fragment was found in *A. aspera* which transplanted vertically.

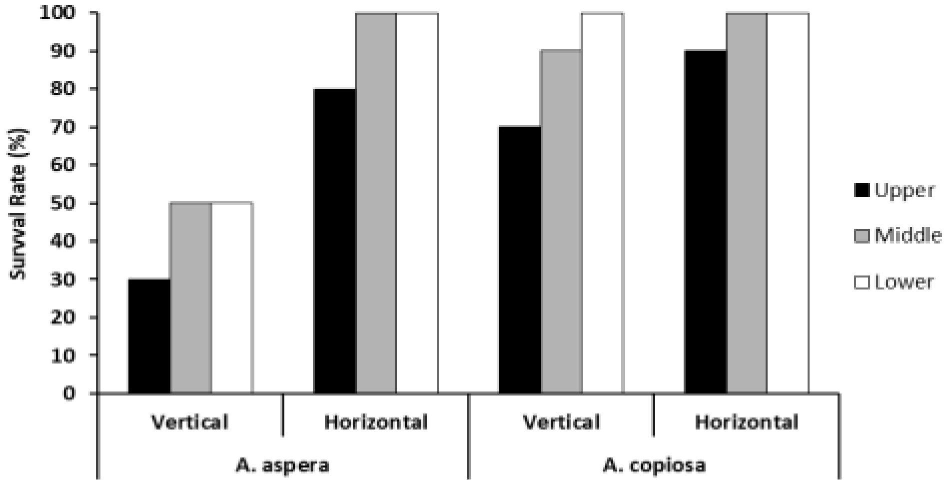

Figure 4. Survival rate (%) of transplanted two Acropora species on multilevel substrate of APR after 8 months (November 2018-July 2019)

Growth Rate

The growth rate of two *Acropora* species which transplanted on multilevel substrate of APR varied from 96.7 to 346.9 cm^3/month. The growth rate of *Acropora* fragments was significantly different among species, substrate levels and fixing method of transplantation (p<0.05) after 8 months. The growth rate of two *Acropora* species on multilevel substrate of APR after 8 months demonstrated that there were no significant different on species *A aspera.* Whereas, there were significantly different on the *A. copiosa* growth that transplanted either in the upper and the middle levels or in the lower and in the middle levels (p<0.05). However, there were no significantly different on the coral growth transplanted in the upper and in the lower levels (Table 1).

Table 1. Growth rate (cm^3/month) of transplanted two species of Acropora on multilevel substrate of APR after 8 months (November 2018-July 2019)

Level	***A. aspera***	***A. copiosa***
Upper	130.05±47.16[a]	293.00±76.23[a]
Middle	178.75±34.17[a]	152.05±95.11[a]
Lower	202.75±44.74[a]	333.30±64.21[a]

Note: All results are expressed as mean ± SD. Values in each column which have the same letters are no significant different (p<0.05)

The growth rate of two Acropora species transplanted in different fixing method (vertical vs. horizontal) demonstrated significantly different (p<0.05; Figure 5). Growth rate of the fragments which transplanted in vertical fixing method was higher than the horizontal method. The lowest growth rate was found in *A. aspera* which transplanted in horizontal fixing method, while the highest growth rate occurred in *A. copiosa* which transplanted in vertical fixing methods. Fragments of coral *A. copiosa* can grow optimally on all levels by both vertical and horizontal fixing method of coral transplantation. Comparing the species, the growth of transplanted *A. copiosa* was higher than that of *A. aspera* due to the different branching patterns ($p<0.05$; Figure 6).

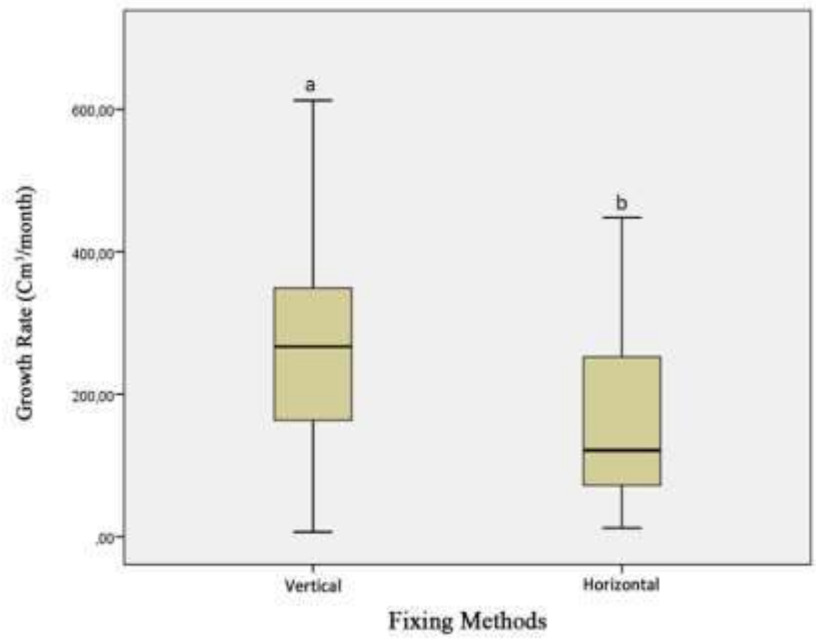

Figure 5. Growth rate (cm^3/month) of transplanted two *Acropora* species on multilevel substrate of APR in different fixing method after 8 months, November 2018-July 2019 (Note: Letter in each bar which have different letters are significant different, $p<0.05$)

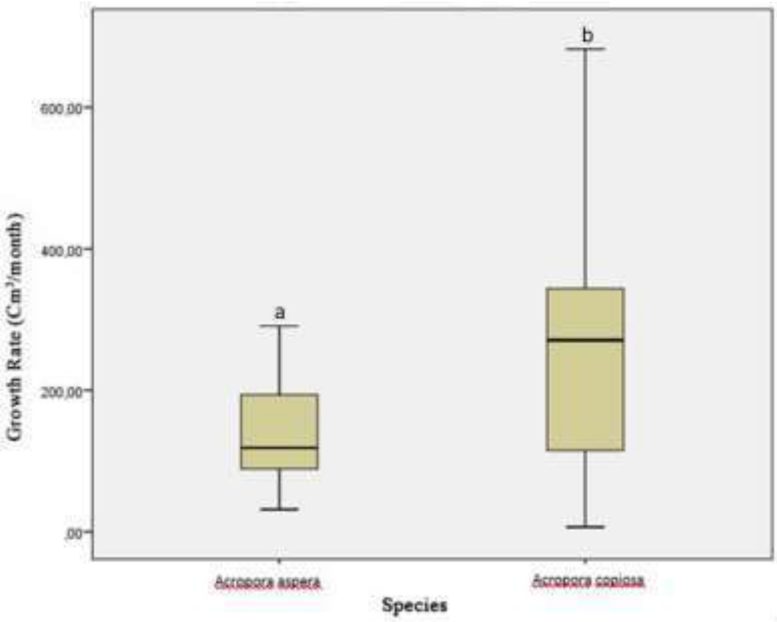

Figure 6. Comparison of growth rate (cm^3/month) transplanted *A. aspera* and *A. copiosa* on multilevel substrate of APR after 8 months, November 2018-July 2019 (Note: Letter in each bar which have different letters are significant different, $p<0.05$)

Discussion

Lower survival rate during the experiment was revealed by *A. aspera* in all levels of substrate particularly in coral transplanted in fixing vertically. Coral fragments mortality were found in *A. aspera* which fixed in vertical orientation during the experiment. Lower survival rate of the coral fragments in vertically fixing method due to minimize of fragment surface attaching to the substrate may affecting the coral expend more energy in repairing the damage (Yap et al. 1992), consequently the coral fragments died and then detached from the substrates. Additionally, lower survival rate of the coral fragments in the upper level may be caused by some of them lost by wave actions occurred in the beginning of experiment, after fixed the coral fragments. Disadvantage of coral transplantation using directly fragment transplantation method on the substrates in shallow water is generally affected by algae competition, sediment accumulation and wave exposure (Young et al. 2012).

Acropora is one of the important coral in the shallow water and usually applied to coral transplantation (Heeger and Sotto, 2000; Edward, 2010; Young et al. 2012).The coral was competens to grow fast, inversely they are also sensitive responding to the environment (Yap et al., 1992). Survival of the corals which have transplanted varied in different location and various in rehabilitation technic. The survival rate of *Acropora* in nubbin fix to the nursery table was 46% (Nithyanandan et al. 2017), while the high survival rate of the coral was found in *Acropora hyacinthus*, 83.3% (Bongiorni et al. 2011). In the present study, the survival rate of the *Acropora* is high due to the fragment stabilization using cable ties method and removing sediment accumulation caused by applying the multilevel designed of substrate. Thus, application of Artificial Patch Reef (APR) in shallow reef rehabilitation can contribute to enhance the survival of *Acropora* fragments.

Some previous studies of coral transplantation revealed that the growth rate of *Acropora* was higher than that of other hermatypic corals. Bongiorni et al. (2011) reported that *Acropora* possess relative growth ranged 66.9 to 83.3%, while growth rate of *Acropora* which transplanted on the artificial reef dome-shaped was 1.07 cm/month (Muzaki et al. 2019), Acropora fragments fixed to the dead coral was 7.8 cm/year (Nithyanandan et al. 2017). This result showed that the growth rate of both Acropora which transplanted on multilevel substrate possesses a high growth rate. Presumably, the construction of multilevel APR can optimize coral grow by increasing light and preventing sediment coverage. The different branching pattern of the Acropora may affect to the growth of the corals, two Acropora shows a different level of complexity (Mercado-Monila et al. 2016) *A. copiosa* was more complex than *A. aspera* (Figure 7). Veron and Stafford-Smith (2000) identified that *A. copiosa* was clumps of prostrate or upright branches irregular branching patterns with frequent sub-branches, while *A. aspera* which is defined as a corymbose clump with thick branches (Veron and Stafford-Smith 2000). The higher growth rate of vertically fixing method in *Acropora copiosa* indicated that vertical fixing of the fragments was suitable orientation of the natural growth form of the donor colony of *Acropora* (Okubo et al. 2005). This study suggests that multilevel APR using vertical fixation method of selected *Acropora* which has high-level complexity should be applied in future coral rehabilitation project.

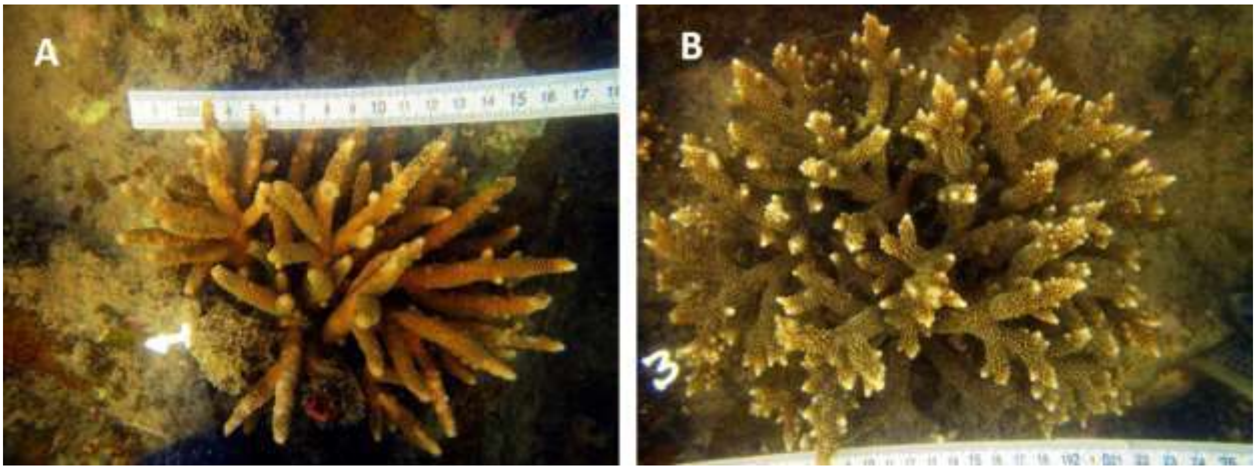

Figure 7. Transplanted of two species Acropora on multilevel substrate of APR after 8 months (A. horizontal fixing method of transplanted *Acropora aspera*; B. vertical fixing method of transplanted *Acropora copiosa*)

ACKNOWLEDGEMENTS

This study was supported by of Applied Research grant (No. 101-170/UN7.P4.3/PP/2018) from Ministry of Research and Technology, Higher Education to M, AS, and SS. Part of the study was supported by research grant No. 1501-26/UN7.5.10/LT/2018 from Faculty of Fisheries and Marine Science, Diponegoro University to M, DPW, I, and RP. We thank Agus Susanto, and staff of Marine Diving Club and Tanjung Jati B Coal-Fired Power Plant to facilitate maintaining the artificial reefs.

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SUBMISSION CHECKLIST

Ensure that the following items are present:

The first corresponding author must be accompanied with contact details:	**Give mark (X)**
• E-mail address	X
• Full postal address (incl street name and number (location), city, postal code, state/province, country)	X
• Phone and facsimile numbers (incl country phone code)	X

All necessary files have been uploaded, and contain:

• Keywords	X
• Running titles	X
• All figure captions	X
• All tables (incl title and note/description)	X

Further considerations

• Manuscript has been "spell & grammar-checked" Better, if it is revised by a professional science editor or a native English speaker	X
• References are in the correct format for this journal	X
• All references mentioned in the Reference list are cited in the text, and vice versa	
• Colored figures are only used if the information in the text may be losing without those images	X
• Charts (graphs and diagrams) are drawn in black and white images; use shading to differentiate	X

List of Modification from reviewer on Feb 27:

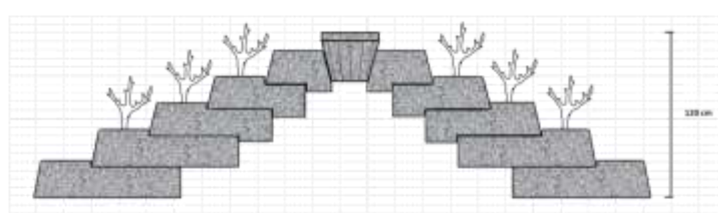
Coral transplantation on a multilevel substrate of Artificial Patch Reefs: effect of fixing methods on the growth rate of two *Acropora* species

Munasik[1]*, Agus Sabdono[1], Azelia N Assyfa[1], Diah P Wijayanti[1], Sugiyanto[2], Irwani[1], Rudhi Pribadi[1]

I. List of Modification and responses from N-Peer Review:

No.	Comments N-Peer Review	Responses
1.	Overall, this is a well-thought out manuscript, which attempted to investigate the growth & survivorship of *Acropora aspera* & *Acropora copiosa* on an artificial patch reef. The results of your study are worthy of publication, but the manuscript in its current form needs some important revisions: Specifically, the Methods section needs to do give more details. The Figures & Tables you provided need to be updated to fix spelling and formatting errors. Your Introduction and Discussion section should be revised slightly to fit your research rationale and your Results section. Lastly, please read and re-read your manuscript to make sure there are no grammar or spelling errors, which are quite distracting to someone fully proficient in the English language.	According to the N- reviewer comments, we have made the following changes in the revised manuscript. After rechecking the experimental results and analyses methods, we found error in the Figure 4 (replaced to Figure 5), which illustrated the effect of fixation methods to the growth rate of *Acropora*, originally the vertical fixation is higher than horizontally method. The figure has been modified accordingly in (L163-166; P5)
2.	Most native English speakers write "branching Acropora" when referring to species of *Acropora* that have a branching morphology	We have replaced Acropora Branching to Branching *Acropora* and also changed two *Acropora* species Branching *Acropora* is generally used in coral transplantation to rehabilitate coral reefs. However, these corals are sensitive to environmental changes. Artificial Patch Reef (APR) is an artificial structure that provides a multilevel hard substrate. (L13; P2) The survival rate of two *Acropora* species which transplanted on multilevel substrate was varied from 30 to 100%, the average of the survival rate was 80%. (L130; P5) The growth rate of two Acropora species transplanted in different fixing method (vertical vs. horizontal) demonstrated

		significantly different ($p<0.05$; Figure 5). (L157; P5)
3.	Consider moving these sentences to a different part of your Introduction so the whole section reads more succinctly.	We have changed the sentences and re-write the paragraph as below: Artificial Patch Reefs (APR) is an artificial structure which is applied to rehabilitate coral reef in order to develop shallow water habitat (Munasik et al. 2018). APR is a rehabilitation tool that is designed by multilevel substrates and applied the combination both of coral transplantation and artificial reefs. *Acropora* spp. is generally considered as a good for candidates for use in coral transplantation or population enhancement project due to their high growth rate and high survivorship of fragments (Lirman et al. 2010; Stephanie et al. 2017; Boch and Morse 2012; Mercado-Molina 2016). The application of APR with *Acropora* transplanted on their substrates is considered contributing to the local conservation of the small island reefs in the near future. In this study, two *Acropora* species were selected and applied to investigate the suitable method and species selection for reef rehabilitation. Multilevel substrates of APR may provide the hard substrate to facilitate fragment of coral grows in shallow turbid water. However, the information about the effect of multilevel substrates on survival and growth of transplanted corals is limited. This study aims to address the effectiveness of the APR structure to provide the multilevel substrate to facilitate the growth rate and survival of coral fragment (L48-54; P3). Some sentences were moved to the materials and methods section: **Study area** Acroporid corals are significantly important in the shallow reef of Panjang Island, Central Java however the population decline slightly due to the anthropogenic stressor (Munasik et al. 2012). Two species *Acropora* i.e. *Acropora aspera* and *A. copiosa* were known as a limiting local population in the Island. Colonies of *A. aspera* is common in the inner lagoon and the species was defined as a corymbose clump with short thick branches. Population of *A. copiosa* is generally found in front of the reef flat and colony was characterized as arborescent clumps of upright branches. Comparing to the previous species, *Acropora copiosa* have more complexity in branching pattern (L60-65; P3).
4.	How were the fragments made? In other words, was only a single mother colony of *A. aspera* and *A. copiosa* fragmented to produce all 120 transplants?	The total of 120 coral transplants were made by broken off the branches of several mother coral colonies that collected randomly from the flat reefs around Panjang Island. Each single fragment was approximately 12.57 cm^3 in size.

	Additionally, what process did you use to create the fragments (e.g. bone shears, saw)? Lastly, how big were the individual fragments?	We have made the following changes in the text. **Procedures** Coral fragments were collected from donor site of two *Acropora* species in the inner lagoon and in front of the reef flat of Panjang Island. Fragments of *A. aspera* were collected by broken off small branches at random mother colonies while *A. copiosa* fragments were chisel off main branches of adult colonies randomly. The small branches of two species (average size was 12.57 cm^3) were transferred into basket and then were transplanted on multilevel substrates of Artificial Patch Reefs (APR) which deployed in the eastern site of Panjang Island (L75-79: P3).
5.	Not necessarily true. Though the cable ties are useful in anchoring the transplant to the substrate (i.e. preventing mortality via detachment), there are studies that have shown rapid tissue necrosis at the sites where the cable tie touches the living tissue, which ultimately can lead to the mortality of the transplant. Consider re-writing this sentence to be more specific.	We agree to your opinion however in our result because of cable ties made the fragments fixed in substrate and then finally survivorship of the fragments were increase. We have made the following changes in the text. Cable ties method of coral transplantation have been widely applied and effective technique for attaching *Acropora* fragments to artificial substrate (William and Miller 2010; Young et al. 2012). Coral fragment stabilization using cable ties was similarly effective to epoxy or cement methods (William and Miller, 2010) (L88-91; P4).
6.	Though this is an informative figure, it does not seem to actually depict what *your* APR looked like. In your Methods section, you mention that you did not add coral transplants to the top and the base of the structure. Additionally, you mention that the whole APR was only 80 cm high, with each level being 20 cm tall. Thus, should this image only show a “pyramid” with 4 levels, with coral transplants depicted only on the middle 2 levels? Or if this image is indeed an accurate representation of your APR, then the text portion of your methods should be revised (i.e. the APR structure was 120 cm tall).	After rechecking the layout of our experimental, we found error in the Figure 2. The total height of the multilevel APR structure is about 120 cm from the bottom of the sea, and the height of each level is 20 cm. In this experiment, coral fragments were transplanted in the upper, middle, and lower level. Coral transplantation experiments were not applied at the top level and were also not implemented in the coral transplantation in the base of APR (level 5). The image has been modified accordingly in (L163-166; P5)  Figure 2. Lay out of coral transplantation experiment, *Acropora* fragment transplanted on the multilevel: in upper, middle and lower of Artificial Patch Reef (APR) (L92-94; P4)
7.	What computer software was used?	

<table>
<tr>
<td></td>
<td></td>
<td>The size measurement of the fragments was analyzed using image analyses of computer software, Image J, so we modified the sentence in (L115; P4)</td>
</tr>
<tr>
<td>8.</td>
<td>I do not understand what the letter “a” means in column #3 and #5.</td>
<td>Some statisticians use letters to show significant differences. Means denoted by a different letter indicate significant differences between treatments ($p<0.05$). For all variables with the same letter, the difference between the means is not statistically significant. If two variables have different letters, they are significantly different. In table 1 means that there were no significant difference among treatments (upper, middle and lower level) in both coral A. aspera and A. copiosa

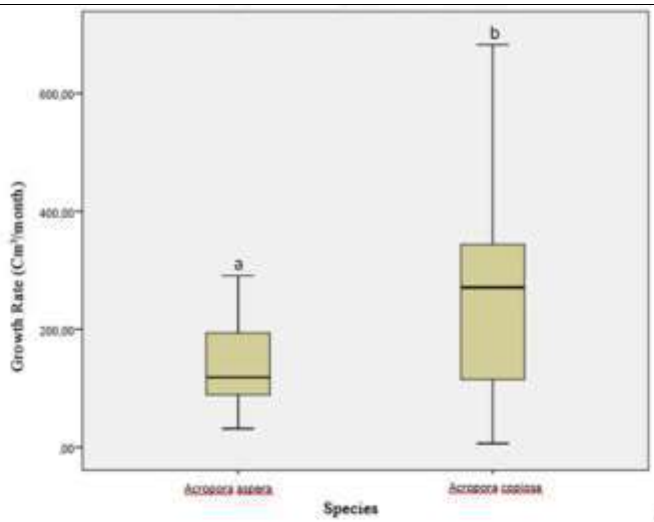
Table 1. Growth rate (cm^3/month) of transplanted two species of Acropora on multilevel substrate of APR after 8 months (November 2018-July 2019)

<table>
<tr><th>Level</th><th>A. aspera</th><th>A. copiosa</th></tr>
<tr><td>Upper</td><td>130.05±47.16^{a}</td><td>293.00±76.23^{a}</td></tr>
<tr><td>Middle</td><td>178.75±34.17^{a}</td><td>152.05±95.11^{a}</td></tr>
<tr><td>Lower</td><td>202.75±44.74^{a}</td><td>333.30±64.21^{a}</td></tr>
</table>
Note: All results are expressed as mean ± Sd. Values in each column which have the same letters are no significant different ($p<0.05$)
(L152-155; P5)</td>
</tr>
<tr>
<td>9.</td>
<td>Since I only observed one Table in your whole manuscript, did you mean to say Figure 3?</td>
<td>In previous manuscript, we prepared the results in the Table 3. However after revised by the author 2, we changed the results in the Figure 5. Unfortunately we did not change this sentence, so we modified the sentences in (L158; P5) and (L162-163; P6).

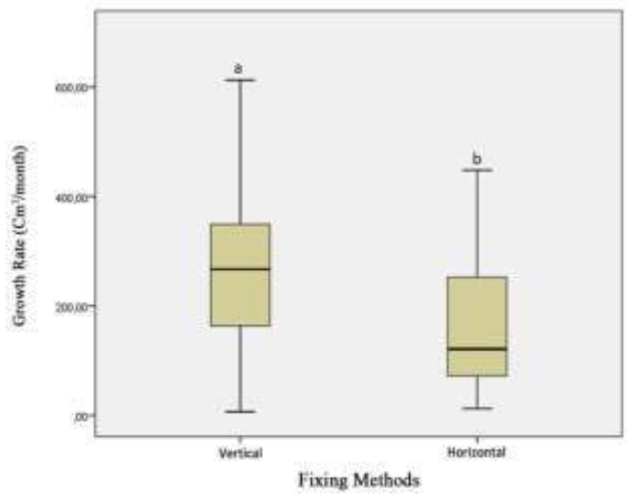
The growth rate of two Acropora species transplanted in different fixing method (vertical vs. horizontal) demonstrated significantly different ($p<0.05$; Figure 5). (L158; P5)

Comparing the species, the growth of transplanted A. copiosa was higher than that of A. aspera due to the different branching patterns ($p<0.05$; Figure 6).

(L162-163; P6)</td>
</tr>
<tr>
<td>10.</td>
<td>The title for your X-axis is misspelled. Should be “species.”</td>
<td>We have replaced the title of X-axis: Spesies to Species and we modified in figure 6.</td>
</tr>
</table>

		 Figure 6. Comparison of growth rate (cm^3/month) transplanted *A. aspera* and *A. copiosa* on multilevel substrate of APR after 8 months, November 2018-July 2019 (Note: Letter in each bar which have different letters are significant different, $p<0.05$) (L167-169; P6)
11.	Though this is a nice figure, I do not see it mentioned anywhere in the main text of your manuscript. Consider including it in the text, or removing it from your manuscript.	The reviewer's point is valid. We put the nice figure (Figure 7) to support the following paragraph in Discussion section, (L196; P7): This result showed that the growth rate of both Acropora which transplanted on multilevel substrate possesses a high growth rate. Presumably, the construction of multilevel APR can optimize coral grow by increasing light and preventing sediment coverage. The different branching pattern of the Acropora may affect to the growth of the corals, two Acropora shows a different level of complexity (Mercado-Monila et al. 2016) *A. copiosa* was more complex than *A. aspera* (Figure 7).
12.	Very confusing. Consider re-wording this part for clarity.	We have changed the sentences and re-write the paragraph as below (L173-180; P6): Lower survival rate during the experiment was revealed by *A. aspera* in all levels of substrate particularly in coral transplanted in fixing vertically. Coral fragments mortality were found in *A. aspera* which fixed in vertical orientation during the experiment. Lower survival rate of the coral fragments in vertically fixing method due to minimize of fragment surface attaching to the substrate may affecting the coral expend more energy in repairing the damage (Yap et al. 1992), consequently the coral fragments died and then detached from the substrates. Additionally, lower survival rate of the coral fragments in the upper level may be caused by some of them lost by wave actions occurred in the

		beginning of experiment, after fixed the coral fragments. Disadvantage of coral transplantation using directly fragment transplantation method on the substrates in shallow water is generally affected by algae competition, sediment accumulation and wave exposure (Young et al. 2012).
13.	Are you saying that halfway through your experiment, you moved to the whole APR with the attached coral fragments? If so, do you think this could have affected your results?	The whole experiment of all transplanted coral and fixed on the multi levels substrate of APR. It means the fragments did not transfer to other substrate. We have changed the sentences and re-write the paragraph as below (L186-188; P7): The survival rate of *Acropora* in nubbin fix to the nursery table was 46% (Nithyanandan et al. 2017), while the high survival rate of the coral was found in *Acropora hyacinthus*, 83.3% (Bongiorni et al. 2011). In the present study, the survival rate of the *Acropora* is high due to the fragment stabilization using cable ties method and removing sediment accumulation caused by applying the multilevel designed of substrate. Thus, application of Artificial Patch Reef (APR) in shallow reef rehabilitation can contribute to enhance the survival of *Acropora* fragments.
14.	In Figure 4, you show that you had better survivorship rates with horizontal fixation; so, this appears to be a contradictory sentence.	Our result, indicate that survivorship in horizontal fixation was better than vertical fixation, inversely the growth rate of coral in vertical fixation was higher than horizontally. After rechecking the experimental results and analyses methods, we found error in the Figure 4 (replaced to Figure 5), which illustrated the effect of fixation methods to the growth rate of *Acropora*, originally the vertical fixation is higher than horizontally method. The figure has been modified accordingly in (L163-166; P6) and we revised the sentence in L134-136; P5) as below: Coral fragment of *A. copiosa* was more survive than *A. aspera*, indicated the lower survival rate coral fragment was found in *A. aspera* which transplanted vertically.

		 Figure 5. Growth rate (cm^3/month) of transplanted two *Acropora* species on multilevel substrate of APR in different fixing method after 8 months, November 2018-July 2019; (Note: Letter in each bar which have different letters are significant different, $p<0.05$)
15.	This statement is not really what the study investigated, nor was it completely supported by your results. Consider mentioning that multi-level APRs and horizontal fixation should be used in future coral rehabilitation projects; or something along similar lines.	We have changed the sentences and re-write the paragraph as below: This study suggests that multilevel APR using vertical fixation method of selected *Acropora* which has high-level complexity should be applied in future coral rehabilitation project. (L200-202; P7)

II. List of Modification and responses from E-Reviewer:

No.	Comments E-Reviewer	Responses
1.	Acropora need to be italicized all through the text.	We have replaced "Acropora" with "*Acropora*" through-out the manuscript.
2.	The description of the two Acropora species in the procedure section should be minimized (no point to provide such detailed morphological characters of the species in this paper.)	We have changed the sentences and rewrite the paragraph and move the sentence to the study area section as below: Acroporid corals are significantly important in the shallow reef of Panjang Island, Central Java however the population decline slightly due to the anthropogenic stressor (Munasik et al. 2012). Two species *Acropora* i.e. *Acropora aspera* and *A. copiosa* were known as a limiting local population in the Island. Colonies of *A. aspera* is common in the inner lagoon and the species was defined as a corymbose clump with

<table>
<tr><td></td><td></td><td>short thick branches. Population of A. copiosa is generally found in front of the reef flat and colony was characterized as arborescent clumps of upright branches. Comparing to the previous species, Acropora copiosa have more complexity in branching pattern.
(L59-L65; P3)</td></tr>
<tr><td>3.</td><td>In the procedure section, the text says that the three levels (upper, middle, lower) of APR were used, but exactly where (which height from the bottom) on the figure 2.</td><td>Totally the height of the APR is 120 cm from bottom of the sea.

We have changed the figure 2 as below:

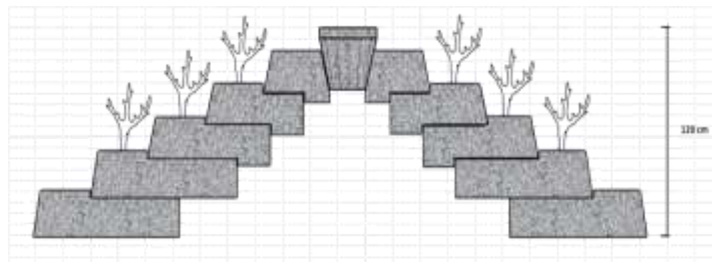


Figure 2. Lay out of coral transplantation experiment, Acropora fragment transplanted on the multilevel: in upper, middle and lower of Artificial Patch Reef (APR)
(L92-94; P4)</td></tr>
<tr><td>4.</td><td>Can you provide any statistical analyses on the figure 4? Also the statistical results of anova need to be provided clearly in the table 1 and in the text. Only the p value is not enough, and I don’t understand the meaning of “a” in the table 1 (no explanation in the legend).</td><td>Some statisticians use letters to show significant differences. Means denoted by a different letter indicate significant differences between treatments ($p<0.05$). For all variables with the same letter, the difference between the means is not statistically significant. If two variables have different letters, they are significantly different. In Table 1 means that there were no significantly difference among treatments (upper, middle and lower level) in both coral A. aspera and A. copiosa. The newly modified Table 1 accordingly in (L152-155; P5).

Table 1. Growth rate (cm^3/month) of transplanted two species of Acropora on multilevel substrate of APR after 8 months (November 2018-July 2019)

<table>
<tr><th>Level</th><th>A. aspera</th><th>A. copiosa</th></tr>
<tr><td>Upper</td><td>130.05±47.16[a]</td><td>293.00±76.23[a]</td></tr>
<tr><td>Middle</td><td>178.75±34.17[a]</td><td>152.05±95.11[a]</td></tr>
<tr><td>Lower</td><td>202.75±44.74[a]</td><td>333.30±64.21[a]</td></tr>
</table>
Note: All results are expressed as mean ± Sd. Values in each column which have the same letters are no significant different ($p<0.05$)
(L152-155; P6)</td></tr>
<tr><td>5.</td><td>Also figures 5 and 6 need statistical analyses to compare the difference</td><td>We modified the image and put the different letters on Figure 5 and 6 accordingly in (L163-166; P6) and (L166-167; P6)</td></tr>
</table>

	statistically.	Note: Letter in each bar which have different letters are significant different ($p<0.05$)
6.	Related to the above two comments, more detailed methodological descriptions on statistical analyses are needed. Current one is just one sentence and I don't understand the meaning of "at 95% confidence level" in the parentheses.	In general, the higher the coefficient, the more certain we are that our results are accurate. For example, a 0.99 coefficient is more accurate than a coefficient of 0.95. So, if our significance level is 0.05, the corresponding confidence level is 95%. If the P value is less than our significance (alpha) level, the hypothesis test is statistically significant.

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Biodiversitas Journal of Biological Diversity

BIODIVERSITAS ISSN: 1412-033X
Volume 21, Number 5, May 2020 E-ISSN: 2085-4722
Pages: xxxx DOI: 10.13057/biodiv/d2105xx

Coral transplantation on a multilevel substrate of Artificial Patch Reefs: effect of fixing methods on the growth rate of two *Acropora* species

MUNASIK[1,♥], AGUS SABDONO[1], AZELIA N ASSYFA[1], DIAH PERMATA WIJAYANTI[1], SUGIYANTO[2], IRWANI[1], RUDHI PRIBADI[1]

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Manuscript received: xxx. Revision accepted: xxx April 2020.

Abstract. *Munasik, Sabdono A, Assyfa AN, Wijayanti DP, Sugiyanto, Irwani, Pribadi R. 2020. Coral transplantation on a multilevel substrate of Artificial Patch Reefs: effect of fixing methods on the growth rate of two* Acropora *species. Biodiversitas 21: xxxx.* Branching *Acropora* is generally used in coral transplantation to rehabilitate coral reefs. However, these corals are sensitive to environmental changes. Artificial Patch Reef (APR) is an artificial structure that provides a multilevel hard substrate. The purpose of the study was to investigate the effectiveness of the APR structure to facilitate the growth and survival of Acropora branching. Two species *Acropora aspera* and *Acropora copiosa* were transplanted vertically and horizontally on a modular concrete block in different levels of APR situated in the shallow reef of Panjang Island, Central Java. The results showed that the coral growth rate varied from 96.7 to 346.9 cm^3/month, while survival ranged from 30 to 100% after 8 months. Lower survival rate mostly was found in the upper level of APR.. The statistical analyses showed that the growth rate of *A. copiosa* fragment was significantly higher than that of *A. aspera* ($p<0.05$). Moreover, there were also significantly differences on the treatments of transplantation method ($p<0.05$) to enhance the coral growth. However, multilevel substrates were not significantly influence of the coral growth. This study suggested that *A. copiosa* which has high-level complexity in branching pattern will be selected to apply in shallow reef rehabilitation with transplanted vertically.

Keywords: *Acropora aspera*, *Acropora copiosa*, artificial patch reef, coral transplant, Panjang Island

INTRODUCTION

Coral reef is one of an important ecosystem on earth, it is most complex and bio-diverse ecosystem that provides the ecological services for humankind. Recently, coral reefs worldwide have been degrading by natural and man-made stress (Wilkinson 2000; Burke et al. 2011). Reef health has been declining apparently by limiting space for natural recruitment and change in physical environmental conditions (Done et al. 2010). Thus, coral reef rehabilitation is considered one of the major reef management strategies that coral reefs may not be able to recover naturally without human intervention.

To rehabilitate damage of natural reefs, artificial reefs and coral transplantation has been applied regardless of environmental condition, cause of decline, or goals. Coral transplantation generally applied by transplanted coral fragments on table cages in shallow water in order to cultivate coral fragments due to transferred and transplanted to rehabilitation reef areas (Heeger and Sotto 2000; Ammar 2013). It seems to be the most widely implemented for coral reef rehabilitation. Many studies dealing with reef rehabilitation by applied coral transplantation (Yap 2000, 2004; Epstein et al. 2001, 2003; Sabater and Yap 2002). Coral transplantation may contribute to enhance the coral population in the reef areas, although natural recovery indicated by coral recruitment (Edwards and Clark 1998; Ng et al. 2015). Coral transplantation method potentially has an impact on reef health by loss colonies from the donor area, reducing the growth of transplanted corals, reducing fecundity of transplant due to stress. Alternatively, artificial reefs are considered an efficient rehabilitation tool, it is a suitable method for protection of existing natural reefs, environmental, mitigation for damaged reef areas and shoreline protections (Meester et al. 2012; Ng et al. 2016). Artificial reefs are expected to increase in available substrates for reef organisms, provide structural complexity and natural recruitment. However, the application of these methods in Indonesia waters were apparently not successful, indicated by high mortality of coral fragments in coral transplantation and many artificial reefs that applied damage to natural reefs (Munasik 2009). In order to optimize reef rehabilitation, combining artificial reefs and coral transplantation is recommended (Abelson 2006; Ammar et al. 2013; Cummings et al. 2015).

Artificial Patch Reefs (APR) is an artificial structure which is applied to rehabilitate coral reef in order to develop shallow water habitat (Munasik et al. 2018). APR is a rehabilitation tool that is designed by multilevel substrates and applied the combination both of coral transplantation and artificial reefs. *Acropora* spp. is

generally considered as a good for candidates for use in coral transplantation or population enhancement project due to their high growth rate and high survivorship of fragments (Lirman et al. 2010; Boch and Morse 2012; Mercado-Molina 2016; Stephanie et al. 2017). The application of APR with *Acropora* transplanted on their substrates is considered contributing to the local conservation of the small island reefs in the near future. In this study, two *Acropora* species were selected and applied to investigate the suitable method and species selection for reef rehabilitation. Multilevel substrates of APR may provide the hard substrate to facilitate fragment of coral grows in shallow turbid water. However, the information about the effect of multilevel substrates on survival and growth of transplanted corals is limited. This study aims to address the effectiveness of the APR structure to provide the multilevel substrate to facilitate the growth rate and survival of coral fragment.

MATERIALS AND METHODS

Study area

Acroporid corals are significantly important in the shallow reef of Panjang Island, Central Java however the population decline slightly due to the anthropogenic stressor (Munasik et al. 2012). Two species *Acropora* i.e. *Acropora aspera* and *A. copiosa* were known as a limiting local population in the Island. Colonies of *A. aspera* is common in the inner lagoon and the species was defined as a corymbose clump with short thick branches. Population of *A. copiosa* is generally found in front of the reef flat and colony was characterized as arborescent clumps of upright branches. Comparing to the previous species, *Acropora copiosa* have more complexity in branching pattern. Rehabilitation of coral reefs program was carried out in shallow reefs of Panjang Island Central Java by deployed 12 (twelve) artificial patch reefs (APR) from 2015 to 2018 at 3 m depth. In order to conduct a coral transplantation experiment, a unit of Artificial Patch Reef (APR) No. 12 was selected to perform the study of the effect of species and coral transplantation method in multilevel of substrates on growth of *Acropora* (Figure 1).

Procedures

Coral fragments were collected from donor site of two *Acropora* species in the inner lagoon and in front of the reef flat of Panjang Island. Fragments of *A. aspera* were collected by broken off small branches at random mother colonies while *A. copiosa* fragments were chisel off main branches of adult colonies randomly. The small branches of two species (average size was 12.57 cm^3) were transferred into basket and then were transplanted on multilevel substrates of Artificial Patch Reefs (APR) which deployed in the eastern site of Panjang Island.

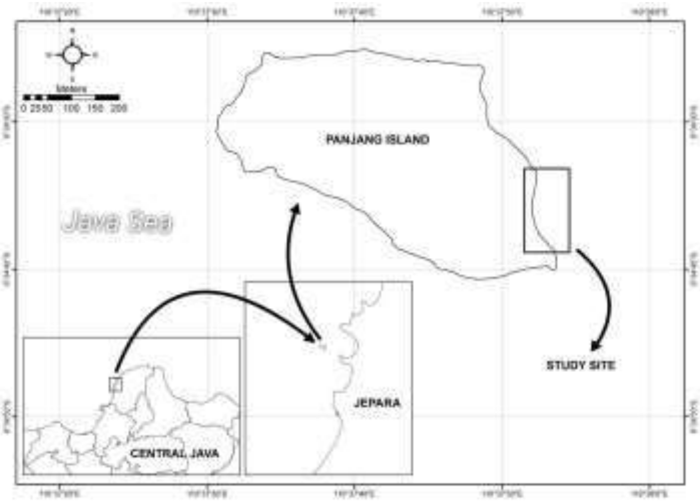

Figure 1. Study site of coral transplantation on Artificial Patch Reef at Panjang Island, Central Java (6º34’30” S; 110º37’44” E)

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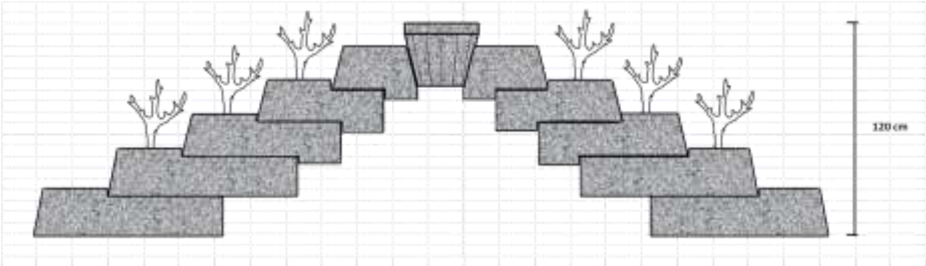

Figure 2. Lay out of coral transplantation experiment, *Acropora* fragment transplanted on the multilevel: in upper, middle and lower of Artificial Patch Reef (APR)

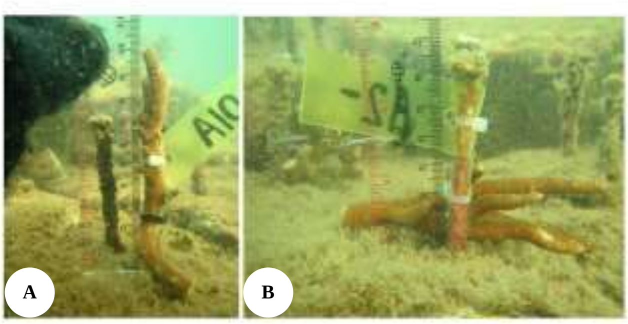

Figure 3. Fixing methods of coral fragments tied to nail by cable ties (A. vertically fixing method, B. horizontally fixing method)

Artificial Patch Reefs (APR) is artificial reefs made by concrete blocks which composed as modular circular structures in shape, constructed 5 (five) levels of substrates were deployed from small boats by SCUBA divers, and are suitable near natural reefs in shallow water. The total height of the multilevel APR structure is about 120 cm from the bottom of the sea, and the height of each level is 20 cm. In this experiment, coral fragments were transplanted in the upper, middle, and lower level. Coral transplantation experiments were not applied at the top level to prevent physical damaged in coral fragments. The experiments were also not implemented in the coral transplantation in the base of APR (level 5) since the surface of the substrate usually covering sediment due to resuspension (Figure 2). At the beginning of November 2018, 120 coral fragments were transplanted on three levels of APR by two fixation methods: vertically and horizontally orientation of the fragments fixing on the surface of substrates and tied to a paired of the nail using cable ties (Figure 3). Cable ties method of coral transplantation have been widely applied and effective technique for attaching *Acropora* fragments to artificial substrate (William and Miller 2010; Young et al. 2012). Coral fragment stabilization using cable ties was similarly effective to epoxy or cement methods (William and Miller 2010).

Data analysis

In order to investigate the growth rate of *Acropora* fragments, we used a measurement of corallum size in volume dimension (Buddemeier and Kinzie III 1976). The final measurement of volume (length, wide, and height) of the coral fragments was evaluated in late July 2019. The size of the fragments was measured by taking a picture using an underwater camera and putting the scale beside each the fragment (Mercado-Molina et al. 2016). The size measurement of the fragments was analyzed using image analyses of computer software, Image J. Volume of the fragment was determined by ecological volume (EV; dela Cruz et al. 2015), and its calculated following the cylindrical volume formula (Levy et al. 2010) as define, in equation (1)

$$EV = \pi r^2 h, \text{ where } \quad r = \frac{(l+W)}{4} \qquad (1)$$

Growth rate (GR) of the corals (Ecological Volume per month) was calculated using the formula (2)

$$Gr = [EVf - EVi]/m \qquad (2)$$

where Gr is the standardized growth rate, EVf and EVi are final and initial Ecological Volume and m is number of months elapsed.

Only the tagged coral fragments alive at 8 (eight) months post transplantation were included in the growth rate determination.

In order to test the effect of different levels of transplant position, and different fixing methods of coral transplantation to the growth of two *Acropora* species, data of growth rate of the fragments were analyzed using two-way of variance (ANOVA, at 95% confidence level, $p<0.05$).

RESULTS AND DISCUSSION

Survival rate

The survival rate of two *Acropora* species which transplanted on multilevel substrate was varied from 30 to 100%, the average of the survival rate was 80%. Both of *Acropora* species which transplanted horizontally possess higher survivorship (average of survival rate was 95%) than the fragments which transplanted vertically (average of survival rate was 85%). The lower survival rate of the fragments were found in the upper level of substrates (varied from 30 to 50%; Figure 4) which located on the top of APR, about 1 m from the bottom of the sea during low tide. Coral fragment of *A. copiosa* was more survive than *A. aspera*, indicated the lower survival rate coral fragment was found in *A. aspera* which transplanted vertically.

Growth rate

The growth rate of two *Acropora* species which transplanted on multilevel substrate of APR varied from 96.7 to 346.9 cm^3/month. The growth rate of *Acropora* fragments was significantly different among species, substrate levels and fixing method of transplantation ($p<0.05$) after 8 months. The growth rate of two *Acropora* species on multilevel substrate of APR after 8 months demonstrated that there were no significant different on species *A aspera*. Whereas, there were significantly different on the *A. copiosa* growth that transplanted either in the upper and the middle levels or in the lower and in the middle levels ($p<0.05$). However, there were no significantly different on the coral growth transplanted in the upper and in the lower levels (Table 1).

The growth rate of two Acropora species transplanted in different fixing method (vertical vs. horizontal) demonstrated significantly different ($p<0.05$; Figure 5). Growth rate of the fragments which transplanted in vertical fixing method was higher than the horizontal method. The lowest growth rate was found in *A. aspera* which transplanted in horizontal fixing method, while the highest growth rate occurred in *A. copiosa* which transplanted in vertical fixing methods. Fragments of coral *A. copiosa* can grow optimally on all levels by both vertical and horizontal fixing method of coral transplantation. Comparing the species, the growth of transplanted *A. copiosa* was higher than that of *A. aspera* due to the different branching patterns ($p<0.05$; Figure 6).

Table 1. Growth rate (cm^3/month) of transplanted two species of Acropora on multilevel substrate of APR after 8 months (November 2018-July 2019)

Level	*A. aspera*	*A. copiosa*
Upper	130.05±47.16[a]	293.00±76.23[a]
Middle	178.75±34.17[a]	152.05±95.11[a]
Lower	202.75±44.74[a]	333.30±64.21[a]

Note: All results are expressed as mean ± SD. Values in each column which have the same letters are no significant different ($p<0.05$)

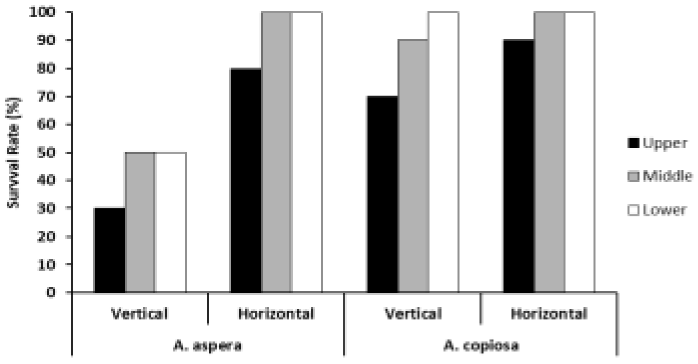

Figure 4. Survival rate (%) of transplanted two Acropora species on multilevel substrate of APR after 8 months (November 2018-July 2019)

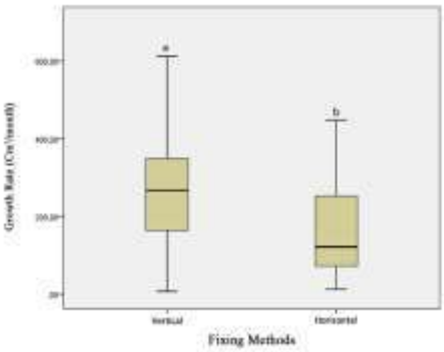

Figure 5. Growth rate (cm^3/month) of transplanted two *Acropora* species on multilevel substrate of APR in different fixing method after 8 months, November 2018-July 2019 (Note: Letter in each bar which have different letters are significant different, p<0.05)

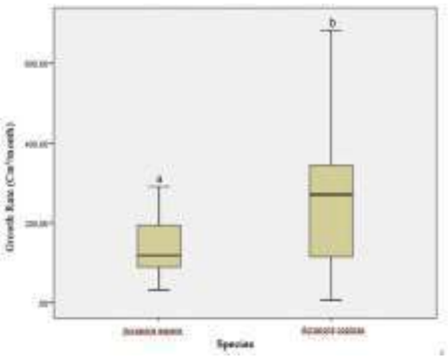

Figure 6. Comparison of growth rate (cm^3/month) transplanted *A. aspera* and *A. copiosa* on multilevel substrate of APR after 8 months, November 2018-July 2019 (Note: Letter in each bar which have different letters are significant different, p<0.05)

Discussion

Lower survival rate during the experiment was revealed by *A. aspera* in all levels of substrate particularly in coral transplanted in fixing vertically. Coral fragments mortality were found in *A. aspera* which fixed in vertical orientation during the experiment. Lower survival rate of the coral fragments in vertically fixing method due to minimize of fragment surface attaching to the substrate may affecting the coral expend more energy in repairing the damage (Yap et al. 1992), consequently the coral fragments died and then detached from the substrates. Additionally, lower survival rate of the coral fragments in the upper level may be caused by some of them lost by wave actions occurred in the beginning of experiment, after fixed the coral fragments. Disadvantage of coral transplantation using directly fragment transplantation method on the substrates in shallow water is generally affected by algae competition, sediment accumulation and wave exposure (Young et al. 2012).

Acropora is one of the important coral in the shallow water and usually applied to coral transplantation (Heeger and Sotto 2000; Edward 2010; Young et al. 2012).The coral was competens to grow fast, inversely they are also sensitive responding to the environment (Yap et al. 1992). Survival of the corals which have transplanted varied in different location and various in rehabilitation technic. The survival rate of *Acropora* in nubbin fix to the nursery table was 46% (Nithyanandan et al. 2018), while the high survival rate of the coral was found in *Acropora hyacinthus*, 83.3% (Bongiorni et al. 2011). In the present study, the survival rate of the *Acropora* is high due to the fragment stabilization using cable ties method and removing sediment accumulation caused by applying the multilevel designed of substrate. Thus, application of Artificial Patch Reef (APR) in shallow reef rehabilitation can contribute to enhance the survival of *Acropora* fragments.

Some previous studies of coral transplantation revealed that the growth rate of *Acropora* was higher than that of other hermatypic corals. Bongiorni et al. (2011) reported that *Acropora* possess relative growth ranged 66.9 to 83.3%, while growth rate of *Acropora* which transplanted on the artificial reef dome-shaped was 1.07 cm/month (Muzaki et al. 2019), Acropora fragments fixed to the dead coral was 7.8 cm/year (Nithyanandan et al. 2018). This result showed that the growth rate of both Acropora which transplanted on multilevel substrate possesses a high growth rate. Presumably, the construction of multilevel APR can optimize coral grow by increasing light and preventing sediment coverage. The different branching pattern of the Acropora may affect to the growth of the corals, two Acropora shows a different level of complexity (Mercado-Monila et al. 2016) *A. copiosa* was more complex than *A. aspera* (Figure 7). Veron and Stafford-Smith (2000) identified that *A. copiosa* was clumps of prostrate or upright branches irregular branching patterns with frequent sub-branches, while *A. aspera* which is defined as a corymbose clump with thick branches (Veron and Stafford-Smith 2000). The higher growth rate of vertically fixing method in *Acropora copiosa* indicated that vertical fixing of the fragments was suitable orientation of the natural growth form of the donor colony of *Acropora* (Okubo et al. 2005). This study suggests that multilevel APR using vertical fixation method of selected *Acropora* which has high-level complexity should be applied in future coral rehabilitation project.

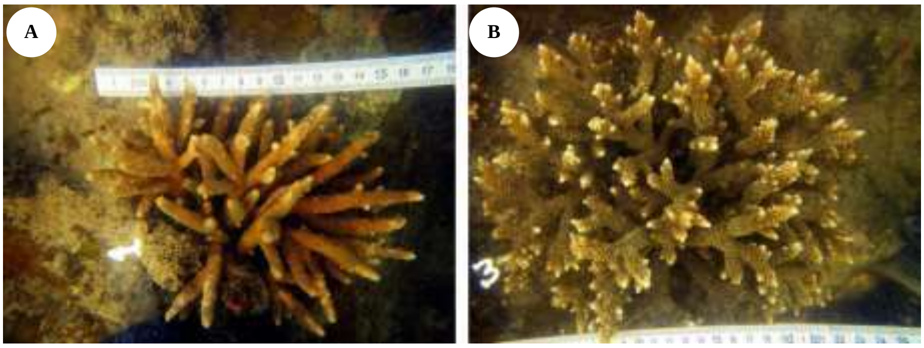

Figure 7. Transplanted of two species Acropora on multilevel substrate of APR after 8 months (A. horizontal fixing method of transplanted *Acropora aspera*; B. vertical fixing method of transplanted *Acropora copiosa*)

ACKNOWLEDGEMENTS

This study was supported by of Applied Research grant (No. 101-170/UN7.P4.3/PP/2018) from Ministry of Research and Technology, Higher Education to M, AS, and SS. Part of the study was supported by research grant No. 1501-26/UN7.5.10/LT/2018 from Faculty of Fisheries and Marine Science, Diponegoro University to M, DPW, I, and RP. We thank Agus Susanto, and staff of Marine Diving Club and Tanjung Jati B Coal-Fired Power Plant to facilitate maintaining the artificial reefs.

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munasik motawi <munasikmotawi@gmail.com> 4 April 2020 at 22:47
To: Smujo Editors <editors@smujo.id>

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5279-Article Text-20577-1-18-20200403_Rev.doc
2846K

BIODIVERSITAS ISSN: 1412-033X
Volume 21, Number 5, May 2020 E-ISSN: 2085-4722
Pages: xxxx DOI: 10.13057/biodiv/d2105xx

Coral transplantation on a multilevel substrate of Artificial Patch Reefs: effect of fixing methods on the growth rate of two *Acropora* species

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Manuscript received: 21 January 2020. Revision accepted: 04 April 2020.

Abstract. *Munasik, Sabdono A, Assyfa AN, Wijayanti DP, Sugiyanto, Irwani, Pribadi R. 2020. Coral transplantation on a multilevel substrate of Artificial Patch Reefs: effect of fixing methods on the growth rate of two* Acropora *species. Biodiversitas 21: xxxx.* Branching *Acropora* is generally used in coral transplantation to rehabilitate coral reefs. However, these corals are sensitive to environmental changes. Artificial Patch Reef (APR) is an artificial structure that provides a multilevel hard substrate. The purpose of the study was to investigate the effectiveness of the APR structure to facilitate the growth and survival of Acropora branching. Two species *Acropora aspera* and *Acropora copiosa* were transplanted vertically and horizontally on a modular concrete block in different levels of APR situated in the shallow reef of Panjang Island, Central Java. The results showed that the coral growth rate varied from 96.7 to 346.9 cm^3/month, while survival ranged from 30 to 100% after 8 months. Lower survival rate mostly was found in the upper level of APR.. The statistical analyses showed that the growth rate of *A. copiosa* fragment was significantly higher than that of *A. aspera* ($p<0.05$). Moreover, there were also significantly differences on the treatments of transplantation method ($p<0.05$) to enhance the coral growth. However, multilevel substrates were not significantly influence of the coral growth. This study suggested that *A. copiosa* which has high-level complexity in branching pattern will be selected to apply in shallow reef rehabilitation with transplanted vertically.

Keywords: *Acropora aspera*, *Acropora copiosa*, artificial patch reef, coral transplant, Panjang Island

INTRODUCTION

Coral reef is one of an important ecosystem on earth, it is most complex and bio-diverse ecosystem that provides the ecological services for humankind. Recently, coral reefs worldwide have been degrading by natural and man-made stress (Wilkinson 2000; Burke et al. 2011). Reef health has been declining apparently by limiting space for natural recruitment and change in physical environmental conditions (Done et al. 2010). Thus, coral reef rehabilitation is considered one of the major reef management strategies that coral reefs may not be able to recover naturally without human intervention.

To rehabilitate damage of natural reefs, artificial reefs and coral transplantation has been applied regardless of environmental condition, cause of decline, or goals. Coral transplantation generally applied by transplanted coral fragments on table cages in shallow water in order to cultivate coral fragments due to transferred and transplanted to rehabilitation reef areas (Heeger and Sotto 2000; Ammar 2013). It seems to be the most widely implemented for coral reef rehabilitation. Many studies dealing with reef rehabilitation by applied coral transplantation (Yap 2000, 2004; Epstein et al. 2001, 2003; Sabater and Yap 2002). Coral transplantation may contribute to enhance the coral population in the reef areas, although natural recovery indicated by coral recruitment (Edwards and Clark 1998; Ng et al. 2015). Coral transplantation method potentially has an impact on reef health by loss colonies from the donor area, reducing the growth of transplanted corals, reducing fecundity of transplant due to stress. Alternatively, artificial reefs are considered an efficient rehabilitation tool, it is a suitable method for protection of existing natural reefs, environmental, mitigation for damaged reef areas and shoreline protections (Meester et al. 2015; Ng et al. 2016). Artificial reefs are expected to increase in available substrates for reef organisms, provide structural complexity and natural recruitment. However, the application of these methods in Indonesia waters were apparently not successful, indicated by high mortality of coral fragments in coral transplantation and many artificial reefs that applied damage to natural reefs (Munasik 2009). In order to optimize reef rehabilitation, combining artificial reefs and coral transplantation is recommended (Abelson 2006; Ammar et al. 2013; Cummings et al. 2015).

Artificial Patch Reefs (APR) is an artificial structure which is applied to rehabilitate coral reef in order to develop shallow water habitat (Munasik et al. 2018). APR is a rehabilitation tool that is designed by multilevel substrates and applied the combination both of coral transplantation and artificial reefs. *Acropora* spp. is

generally considered as a good for candidates for use in coral transplantation or population enhancement project due to their high growth rate and high survivorship of fragments (Lirman et al. 2010; Boch and Morse 2012; Mercado-Molina 2016; Schopmeyer et al. 2017). The application of APR with *Acropora* transplanted on their substrates is considered contributing to the local conservation of the small island reefs in the near future. In this study, two *Acropora* species were selected and applied to investigate the suitable method and species selection for reef rehabilitation. Multilevel substrates of APR may provide the hard substrate to facilitate fragment of coral grows in shallow turbid water. However, the information about the effect of multilevel substrates on survival and growth of transplanted corals is limited. This study aims to address the effectiveness of the APR structure to provide the multilevel substrate to facilitate the growth rate and survival of coral fragment.

MATERIALS AND METHODS

Study area

Acroporid corals are significantly important in the shallow reef of Panjang Island, Central Java however the population decline slightly due to the anthropogenic stressor (Munasik et al. 2012). Two species *Acropora* i.e. *Acropora aspera* and *A. copiosa* were known as a limiting local population in the Island. Colonies of *A. aspera* is common in the inner lagoon and the species was defined as a corymbose clump with short thick branches. Population of *A. copiosa* is generally found in front of the reef flat and colony was characterized as arborescent clumps of upright branches. Comparing to the previous species, *Acropora copiosa* have more complexity in branching pattern. Rehabilitation of coral reefs program was carried out in shallow reefs of Panjang Island Central Java by deployed 12 (twelve) artificial patch reefs (APR) from 2015 to 2018 at 3 m depth. In order to conduct a coral transplantation experiment, a unit of Artificial Patch Reef (APR) No. 12 was selected to perform the study of the effect of species and coral transplantation method in multilevel of substrates on growth of *Acropora* (Figure 1).

Procedures

Coral fragments were collected from donor site of two *Acropora* species in the inner lagoon and in front of the reef flat of Panjang Island. Fragments of *A. aspera* were collected by broken off small branches at random mother colonies while *A. copiosa* fragments were chisel off main branches of adult colonies randomly. The small branches of two species (average size was 12.57 cm^3) were transferred into basket and then were transplanted on multilevel substrates of Artificial Patch Reefs (APR) which deployed in the eastern site of Panjang Island.

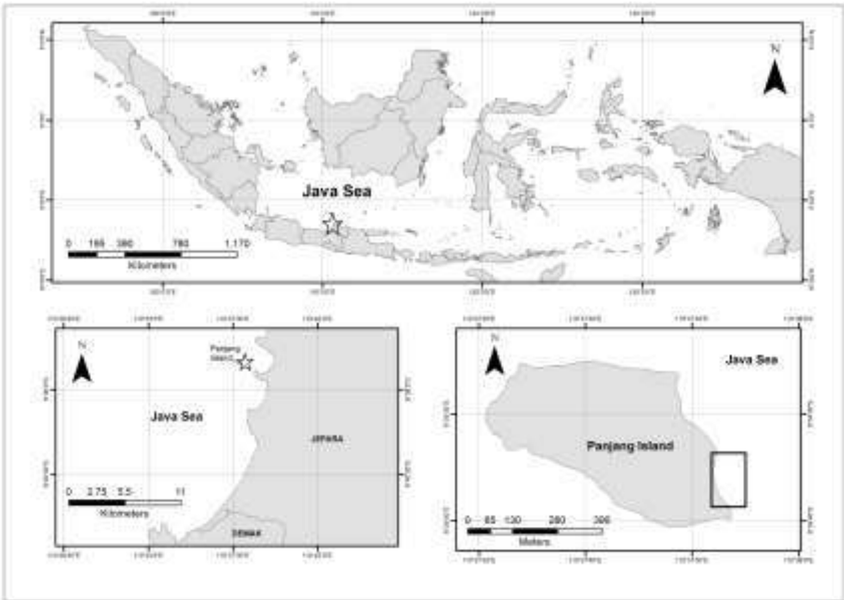

Figure 1. Study site of coral transplantation on Artificial Patch Reef at Panjang Island, Central Java (6º34'30" S; 110º37'44" E)

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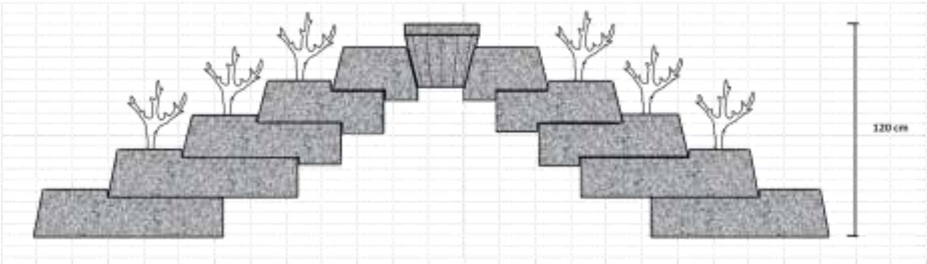

Figure 2. Lay out of coral transplantation experiment, *Acropora* fragment transplanted on the multilevel: in upper, middle and lower of Artificial Patch Reef (APR)

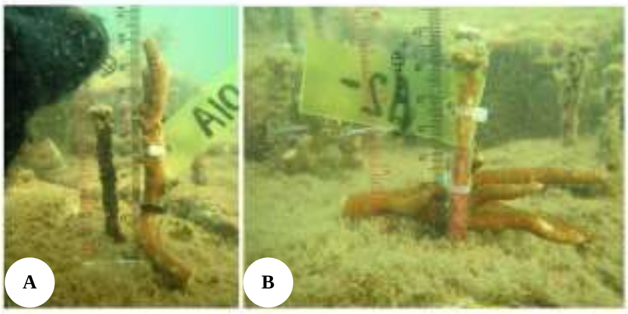

Figure 3. Fixing methods of coral fragments tied to nail by cable ties (A. vertically fixing method, B. horizontally fixing method)

Artificial Patch Reefs (APR) is artificial reefs made by concrete blocks which composed as modular circular structures in shape, constructed 5 (five) levels of substrates were deployed from small boats by SCUBA divers, and are suitable near natural reefs in shallow water. The total height of the multilevel APR structure is about 120 cm from the bottom of the sea, and the height of each level is 20 cm. In this experiment, coral fragments were transplanted in the upper, middle, and lower level. Coral transplantation experiments were not applied at the top level to prevent physical damaged in coral fragments. The experiments were also not implemented in the coral transplantation in the base of APR (level 5) since the surface of the substrate usually covering sediment due to resuspension (Figure 2). At the beginning of November 2018, 120 coral fragments were transplanted on three levels of APR by two fixation methods: vertically and horizontally orientation of the fragments fixing on the surface of substrates and tied to a paired of the nail using cable ties (Figure 3). Cable ties method of coral transplantation have been widely applied and effective technique for attaching *Acropora* fragments to artificial substrate (William and Miller 2010; Young et al. 2012). Coral fragment stabilization using cable ties was similarly effective to epoxy or cement methods (William and Miller 2010).

Data analysis

In order to investigate the growth rate of *Acropora* fragments, we used a measurement of corallum size in volume dimension (Buddemeier and Kinzie III 1976). The final measurement of volume (length, wide, and height) of the coral fragments was evaluated in late July 2019. The size of the fragments was measured by taking a picture using an underwater camera and putting the scale beside each the fragment (Mercado-Molina et al. 2016). The size measurement of the fragments was analyzed using image analyses of computer software, Image J. Volume of the fragment was determined by ecological volume (EV; dela Cruz et al. 2015), and its calculated following the cylindrical volume formula (Levy et al. 2010) as define, in equation (1)

$$EV = \pi r^2 h, \text{ where } \quad r = \frac{(l+W)}{4} \quad (1)$$

Growth rate (GR) of the corals (Ecological Volume per month) was calculated using the formula (2)

$$Gr = [EVf - EVi]/m \quad (2)$$

where Gr is the standardized growth rate, EVf and EVi are final and initial Ecological Volume and m is number of months elapsed.

Only the tagged coral fragments alive at 8 (eight) months post transplantation were included in the growth rate determination.

In order to test the effect of different levels of transplant position, and different fixing methods of coral transplantation to the growth of two *Acropora* species, data of growth rate of the fragments were analyzed using two-way of variance (ANOVA, at 95% confidence level, $p<0.05$).

RESULTS AND DISCUSSION

Survival rate

The survival rate of two *Acropora* species which transplanted on multilevel substrate was varied from 30 to 100%, the average of the survival rate was 80%. Both of *Acropora* species which transplanted horizontally possess higher survivorship (average of survival rate was 95%) than the fragments which transplanted vertically (average of survival rate was 85%). The lower survival rate of the fragments were found in the upper level of substrates (varied from 30 to 50%; Figure 4) which located on the top of APR, about 1 m from the bottom of the sea during low tide. Coral fragment of *A. copiosa* was more survive than *A. aspera*, indicated the lower survival rate coral fragment was found in *A. aspera* which transplanted vertically.

Growth rate

The growth rate of two *Acropora* species which transplanted on multilevel substrate of APR varied from 96.7 to 346.9 cm^3/month. The growth rate of *Acropora* fragments was significantly different among species, substrate levels and fixing method of transplantation ($p<0.05$) after 8 months. The growth rate of two *Acropora* species on multilevel substrate of APR after 8 months demonstrated that there were no significant different on species *A aspera*. Whereas, there were significantly different on the *A. copiosa* growth that transplanted either in the upper and the middle levels or in the lower and in the middle levels ($p<0.05$). However, there were no significantly different on the coral growth transplanted in the upper and in the lower levels (Table 1).

The growth rate of two Acropora species transplanted in different fixing method (vertical vs. horizontal) demonstrated significantly different ($p<0.05$; Figure 5). Growth rate of the fragments which transplanted in vertical fixing method was higher than the horizontal method. The lowest growth rate was found in *A. aspera* which transplanted in horizontal fixing method, while the highest growth rate occurred in *A. copiosa* which transplanted in vertical fixing methods. Fragments of coral *A. copiosa* can grow optimally on all levels by both vertical and horizontal fixing method of coral transplantation. Comparing the species, the growth of transplanted *A. copiosa* was higher than that of *A. aspera* due to the different branching patterns ($p<0.05$; Figure 6).

Table 1. Growth rate (cm^3/month) of transplanted two species of Acropora on multilevel substrate of APR after 8 months (November 2018-July 2019)

Level	*A. aspera*	*A. copiosa*
Upper	130.05±47.16[a]	293.00±76.23[a]
Middle	178.75±34.17[a]	152.05±95.11[a]
Lower	202.75±44.74[a]	333.30±64.21[a]

Note: All results are expressed as mean ± SD. Values in each column which have the same letters are no significant different ($p<0.05$)

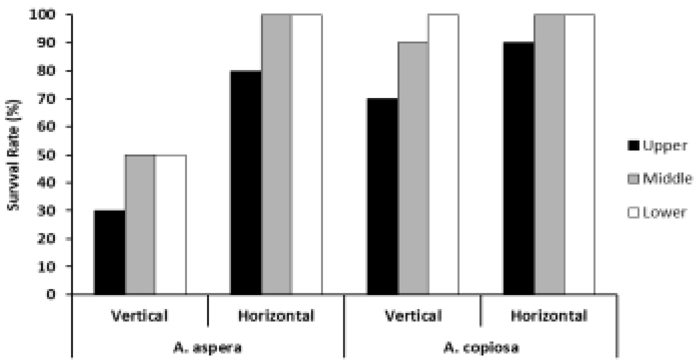

Figure 4. Survival rate (%) of transplanted two Acropora species on multilevel substrate of APR after 8 months (November 2018-July 2019)

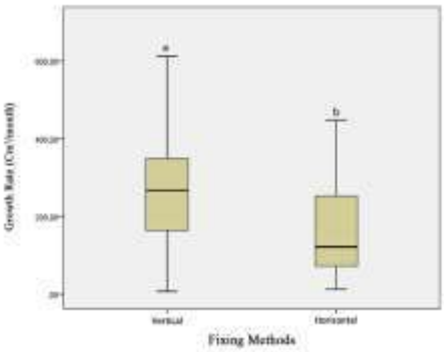

Figure 5. Growth rate (cm³/month) of transplanted two *Acropora* species on multilevel substrate of APR in different fixing method after 8 months, November 2018-July 2019 (Note: Letter in each bar which have different letters are significant different, p<0.05)

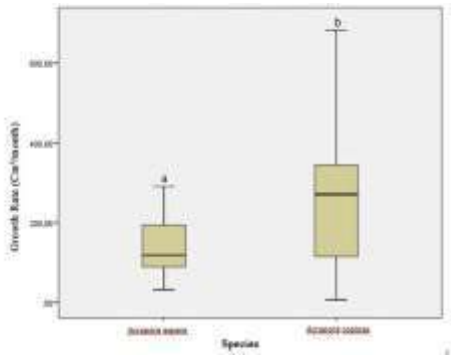

Figure 6. Comparison of growth rate (cm³/month) transplanted *A. aspera* and *A. copiosa* on multilevel substrate of APR after 8 months, November 2018-July 2019 (Note: Letter in each bar which have different letters are significant different, p<0.05)

Discussion

Lower survival rate during the experiment was revealed by *A. aspera* in all levels of substrate particularly in coral transplanted in fixing vertically. Coral fragments mortality were found in *A. aspera* which fixed in vertical orientation during the experiment. Lower survival rate of the coral fragments in vertically fixing method due to minimize of fragment surface attaching to the substrate may affecting the coral expend more energy in repairing the damage (Yap et al. 1992), consequently the coral fragments died and then detached from the substrates. Additionally, lower survival rate of the coral fragments in the upper level may be caused by some of them lost by wave actions occurred in the beginning of experiment, after fixed the coral fragments. Disadvantage of coral transplantation using directly fragment transplantation method on the substrates in shallow water is generally affected by algae competition, sediment accumulation and wave exposure (Young et al. 2012).

Acropora is one of the important coral in the shallow water and usually applied to coral transplantation (Heeger and Sotto 2000; Edwards 2010; Young et al. 2012).The coral was competens to grow fast, inversely they are also sensitive responding to the environment (Yap et al. 1992). Survival of the corals which have transplanted varied in different location and various in rehabilitation technic. The survival rate of *Acropora* in nubbin fix to the nursery table was 46% (Nithyanandan et al. 2018), while the high survival rate of the coral was found in *Acropora hyacinthus*, 83.3% (Bongiorni et al. 2011). In the present study, the survival rate of the *Acropora* is high due to the fragment stabilization using cable ties method and removing sediment accumulation caused by applying the multilevel designed of substrate. Thus, application of Artificial Patch Reef (APR) in shallow reef rehabilitation can contribute to enhance the survival of *Acropora* fragments.

Some previous studies of coral transplantation revealed that the growth rate of *Acropora* was higher than that of other hermatypic corals. Bongiorni et al. (2011) reported that *Acropora* possess relative growth ranged 66.9 to 83.3%, while growth rate of *Acropora* which transplanted on the artificial reef dome-shaped was 1.07 cm/month (Muzaki et al. 2019), Acropora fragments fixed to the dead coral was 7.8 cm/year (Nithyanandan et al. 2018). This result showed that the growth rate of both Acropora which transplanted on multilevel substrate possesses a high growth rate. Presumably, the construction of multilevel APR can optimize coral grow by increasing light and preventing sediment coverage. The different branching pattern of the Acropora may affect to the growth of the corals, two Acropora shows a different level of complexity (Mercado-Monila et al. 2016) *A. copiosa* was more complex than *A. aspera* (Figure 7). Veron and Stafford-Smith (2000) identified that *A. copiosa* was clumps of prostrate or upright branches irregular branching patterns with frequent sub-branches, while *A. aspera* which is defined as a corymbose clump with thick branches (Veron and Stafford-Smith 2000). The higher growth rate of vertically fixing method in *Acropora copiosa* indicated that vertical fixing of the fragments was suitable orientation of the natural growth form of the donor colony of *Acropora* (Okubo et al. 2005). This study suggests that multilevel APR using vertical fixation method of selected *Acropora* which has high-level complexity should be applied in future coral rehabilitation project.

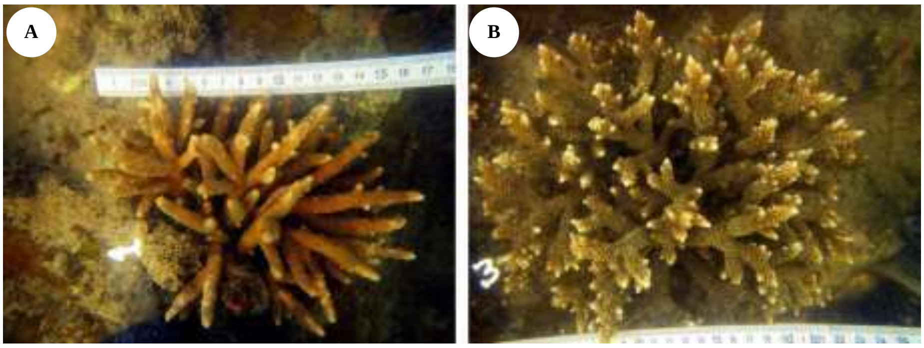

Figure 7. Transplanted of two species Acropora on multilevel substrate of APR after 8 months (A. horizontal fixing method of transplanted *Acropora aspera*; B. vertical fixing method of transplanted *Acropora copiosa*)

ACKNOWLEDGEMENTS

This study was supported by of Applied Research grant (No. 101-170/UN7.P4.3/PP/2018) from Ministry of Research and Technology, Higher Education to M, AS, and S. Part of the study was supported by research grant No. 1501-26/UN7.5.10/LT/2018 from Faculty of Fisheries and Marine Science, Diponegoro University to M, DPW, I, and RP. We thank Andy Ahmad for preparing coral measurement, Agus Susanto, and the staffs of Marine Diving Club for field assistance and Tanjung Jati B Coal-Fired Power Plant to facilitate maintaining the artificial reefs. We appreciate two anonymous reviewers offered constructive comments to improve our manuscript.

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BIODIVERSITAS ISSN: 1412-033X
Volume 21, Number 5, May 2020 E-ISSN: 2085-4722
Pages: 1816-1822 DOI: 10.13057/biodiv/d210507

Coral transplantation on a multilevel substrate of Artificial Patch Reefs: effect of fixing methods on the growth rate of two *Acropora* species

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[1]Department of Marine Science, Faculty of Fisheries and Marine Science, Universitas Diponegoro. Jl. Prof. Soedarto SH, Tembalang, Semarang 50275, Central Java, Indonesia, Tel/fax.: +62-24-7474698, ♥email: munasik@lecturer.undip.ac.id

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Manuscript received: 21 January 2020. Revision accepted: 6 April 2020.

Abstract. *Munasik, Sabdono A, Assyfa AN, Wijayanti DP, Sugiyanto, Irwani, Pribadi R. 2020. Coral transplantation on a multilevel substrate of Artificial Patch Reefs: effect of fixing methods on the growth rate of two* Acropora *species. Biodiversitas 21: 1816-1822.* Branching *Acropora* is generally used in coral transplantation to rehabilitate coral reefs. However, these corals are sensitive to environmental changes. Artificial Patch Reef (APR) is an artificial structure that provides a multilevel hard substrate. The purpose of the study was to investigate the effectiveness of the APR structure to facilitate the growth and survival of Acropora branching. Two species *Acropora aspera* and *Acropora copiosa* were transplanted vertically and horizontally on a modular concrete block in different levels of APR situated in the shallow reef of Panjang Island, Central Java. The results showed that the coral growth rate varied from 96.7 to 346.9 cm^3/month, while survival ranged from 30 to 100% after 8 months. Lower survival rate mostly was found in the upper level of APR. The statistical analyses showed that the growth rate of *A. copiosa* fragment was significantly higher than that of *A. aspera* ($p<0.05$). Moreover, there were also significant differences in the treatments of transplantation method ($p<0.05$) to enhance coral growth. However, multilevel substrates were not significantly influenced by coral growth. This study suggested that *A. copiosa* which has high-level complexity in branching pattern will be selected to apply in shallow reef rehabilitation with transplanted vertically.

Keywords: *Acropora aspera*, *Acropora copiosa*, artificial patch reef, coral transplant, Panjang Island

INTRODUCTION

Coral reef is one of an important ecosystem on earth, it is most complex and biodiverse ecosystem that provides the ecological services for humankind. Recently, coral reefs worldwide have been degrading by natural and man-made stress (Wilkinson 2000; Burke et al. 2011). Reef health has been declining apparently by limiting space for natural recruitment and change in physical environmental conditions (Done et al. 2010). Thus, coral reef rehabilitation is considered one of the major reef management strategies that coral reefs may not be able to recover naturally without human intervention.

To rehabilitate damage of natural reefs, artificial reefs and coral transplantation has been applied regardless of environmental condition, cause of decline, or goals. Coral transplantation generally applied by transplanted coral fragments on table cages in shallow water in order to cultivate coral fragments due to transferred and transplanted to rehabilitation reef areas (Heeger and Sotto 2000; Ammar 2013). It seems to be the most widely implemented for coral reef rehabilitation. Many studies dealing with reef rehabilitation by applied coral transplantation (Yap 2000, 2004; Epstein et al. 2001, 2003; Sabater and Yap 2002). Coral transplantation may contribute to enhance the coral population in the reef areas, although natural recovery indicated by coral recruitment (Edwards and Clark 1998; Ng et al. 2015). Coral transplantation method potentially has an impact on reef health by losing colonies from the donor area, reducing the growth of transplanted corals, reducing fecundity of transplant due to stress. Alternatively, artificial reefs are considered an efficient rehabilitation tool, it is a suitable method for protection of existing natural reefs, environmental, mitigation for damaged reef areas and shoreline protections (Meester et al. 2015; Ng et al. 2016). Artificial reefs are expected to increase in available substrates for reef organisms, provide structural complexity and natural recruitment. However, the application of these methods in Indonesia waters was apparently not successful, indicated by high mortality of coral fragments in coral transplantation and many artificial reefs that applied damage to natural reefs (Munasik 2009). In order to optimize reef rehabilitation, combining artificial reefs and coral transplantation is recommended (Abelson 2006; Ammar et al. 2013; Cummings et al. 2015).

Artificial Patch Reefs (APR) is an artificial structure which is applied to rehabilitate coral reef in order to develop shallow water habitat (Munasik et al. 2018). APR is a rehabilitation tool that is designed by multilevel substrates and applied the combination both of coral transplantation and artificial reefs. *Acropora* spp. is

generally considered as a good for candidates for use in coral transplantation or population enhancement project due to their high growth rate and high survivorship of fragments (Lirman et al. 2010; Boch and Morse 2012; Mercado-Molina 2016; Schopmeyer et al. 2017). The application of APR with *Acropora* transplanted on their substrates is considered contributing to the local conservation of the small island reefs in the near future. In this study, two *Acropora* species were selected and applied to investigate the suitable method and species selection for reef rehabilitation. Multilevel substrates of APR may provide the hard substrate to facilitate fragment of coral grows in shallow turbid water. However, the information about the effect of multilevel substrates on survival and growth of transplanted corals is limited. This study aims to address the effectiveness of the APR structure to provide the multilevel substrate to facilitate the growth rate and survival of coral fragment.

MATERIALS AND METHODS

Study area

Acroporid corals are significantly important in the shallow reef of Panjang Island, Central Java however the population decline slightly due to the anthropogenic stressor (Munasik et al. 2012). Two species *Acropora* i.e. *Acropora aspera* and *A. copiosa* were known as limiting local population on the island. Colonies of *A. aspera* is common in the inner lagoon and the species was defined as a corymbose clump with short thick branches. Population of *A. copiosa* is generally found in front of the reef flat and colony was characterized as arborescent clumps of upright branches. Comparing to the previous species, *Acropora copiosa* have more complexity in branching patterns. Rehabilitation of coral reefs program was carried out in shallow reefs of Panjang Island Central Java by deployed 12 (twelve) artificial patch reefs (APR) from 2015 to 2018 at 3 m depth. In order to conduct a coral transplantation experiment, a unit of Artificial Patch Reef (APR) No. 12 was selected to perform the study of the effect of species and coral transplantation method in multilevel of substrates on growth of *Acropora* (Figure 1).

Procedures

Coral fragments were collected from donor site of two *Acropora* species in the inner lagoon and in front of the reef flat of Panjang Island. Fragments of *A. aspera* were collected by broken off small branches at random mother colonies while *A. copiosa* fragments were chisel off main branches of adult colonies randomly. The small branches of two species (average size was 12.57 cm^3) were transferred into basket and then were transplanted on multilevel substrates of Artificial Patch Reefs (APR) which deployed in the eastern site of Panjang Island.

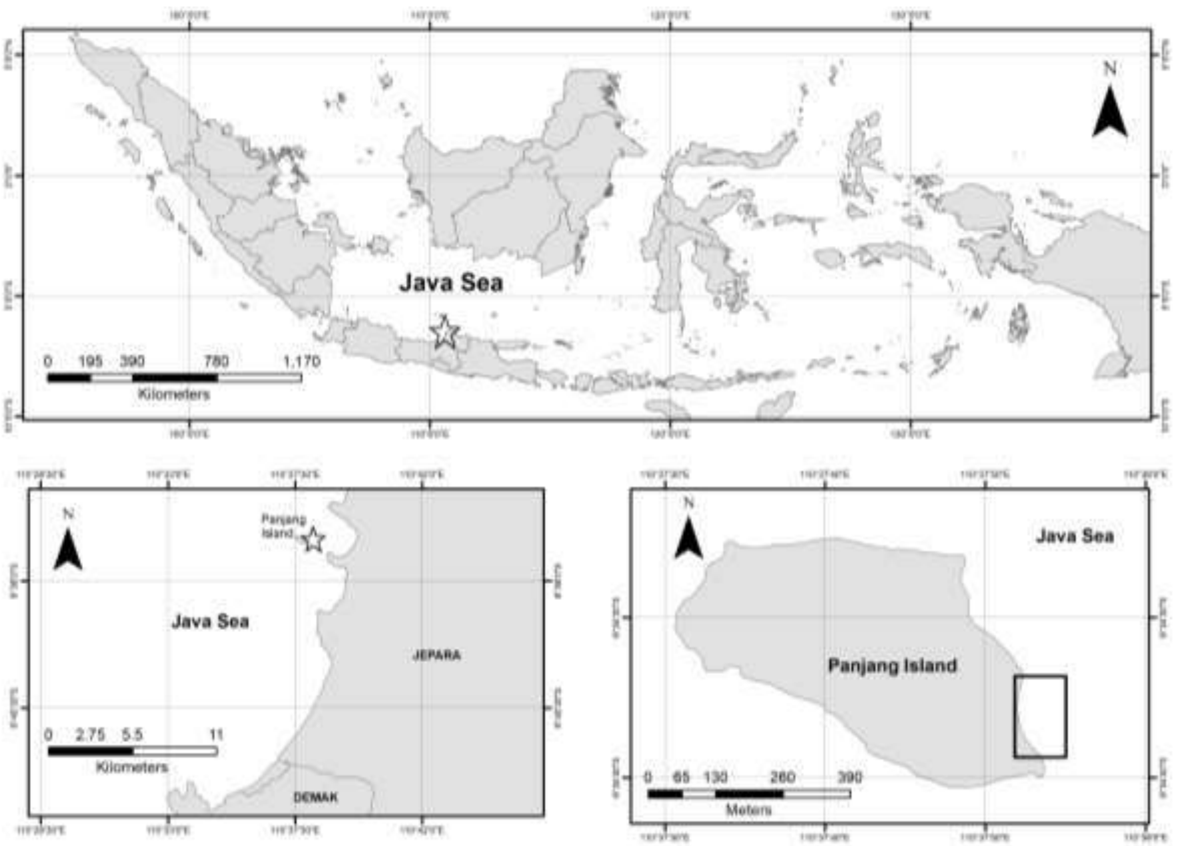

Figure 1. Study site of coral transplantation on Artificial Patch Reef at Panjang Island, Central Java, Indonesia (6º34'30" S; 110º37'44" E)

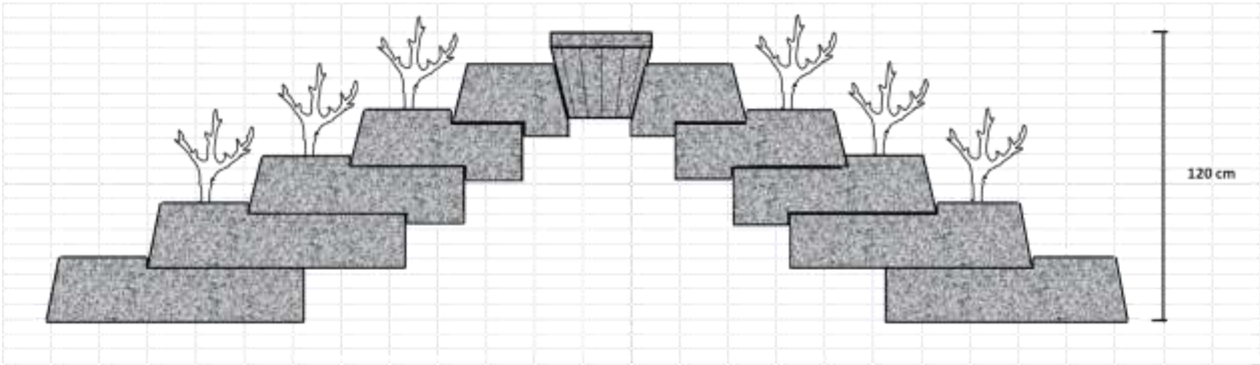

Figure 2. Layout of coral transplantation experiment, *Acropora* fragment transplanted on the multilevel: in upper, middle and lower of Artificial Patch Reef (APR)

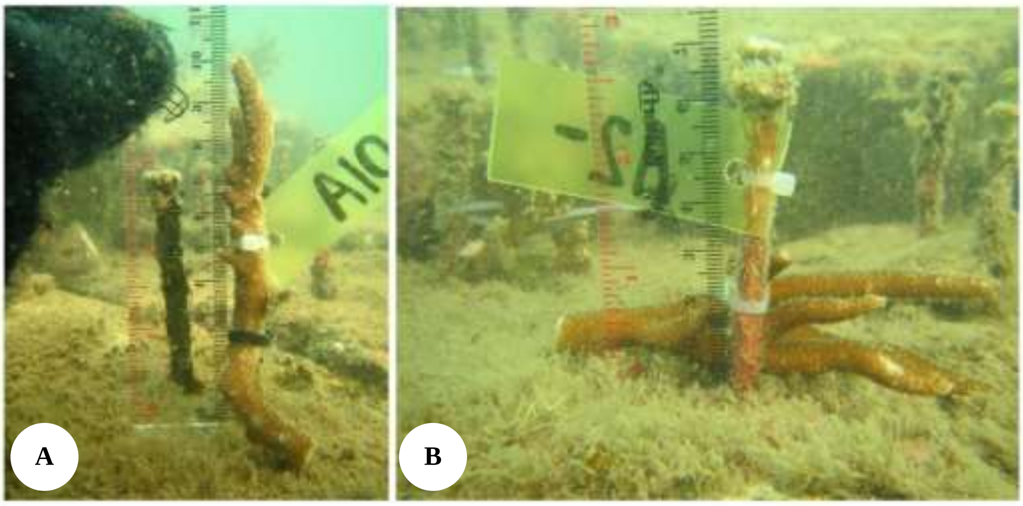

Figure 3. Fixing methods of coral fragments tied to nail by cable ties. A. vertically fixing method, B. horizontally fixing method

Artificial Patch Reefs (APR) is artificial reefs made by concrete blocks composed as modular circular structures in shape, constructed 5 (five) levels of substrates were deployed from small boats by SCUBA divers, and are suitable near natural reefs in shallow water. The total height of the multilevel APR structure is about 120 cm from the bottom of the sea, and the height of each level is 20 cm. In this experiment, coral fragments were transplanted in the upper, middle, and lower level. Coral transplantation experiments were not applied at the top level to prevent physical damage in coral fragments. The experiments were also not implemented in the coral transplantation in the base of APR (level 5) since the surface of the substrate usually covering sediment due to resuspension (Figure 2). At the beginning of November 2018, 120 coral fragments were transplanted on three levels of APR by two fixation methods: vertically and horizontally orientation of the fragments fixing on the surface of substrates and tied to a paired of the nail using cable ties (Figure 3). Cable ties method of coral transplantation has been widely applied and effective technique for attaching *Acropora* fragments to artificial substrate (William and Miller 2010; Young et al. 2012). Coral fragment stabilization using cable ties was similarly effective to epoxy or cement methods (William and Miller 2010).

Data analysis

In order to investigate the growth rate of *Acropora* fragments, we used a measurement of corallum size in volume dimension (Buddemeier and Kinzie III 1976). The final measurement of volume (length, wide, and height) of the coral fragments were evaluated in late July 2019. The size of the fragments was measured by taking a picture using an underwater camera and putting the scale beside each the fragment (Mercado-Molina et al. 2016). The size measurement of the fragments was analyzed using image analyses of computer software, Image J. Volume of the fragment was determined by ecological volume (EV; de la Cruz et al. 2015), and its calculated following the cylindrical volume formula (Levy et al. 2010) as define, in equation (1)

$$EV = \pi r^2 h, \text{ where } \quad r = \frac{(l+W)}{4} \qquad (1)$$

Growth rate (GR) of the corals (Ecological Volume per month) was calculated using the formula (2)

$$Gr = [EVf - EVi]/m \quad (2)$$

Where: Gr is the standardized growth rate, EVf and EVi are final and initial Ecological Volume and m is number of months elapsed.

Only the tagged coral fragments alive at 8 (eight) months post-transplantation were included in the growth rate determination.

In order to test the effect of different levels of transplant position, and different fixing methods of coral transplantation to the growth of two *Acropora* species, data of growth rate of the fragments were analyzed using two-way of variance (ANOVA, at 95% confidence level, $p<0.05$).

RESULTS AND DISCUSSION

Survival rate

The survival rate of two *Acropora* species which transplanted on multilevel substrate was varied from 30 to 100%, the average of the survival rate was 80%. Both *Acropora* species which transplanted horizontally possess higher survivorship (average of survival rate was 95%) than the fragments which transplanted vertically (average of survival rate was 85%). The lower survival rate of the fragments was found in the upper level of substrates (varied from 30 to 50%; Figure 4) which located on the top of APR, about 1 m from the bottom of the sea during low tide. Coral fragment of *A. copiosa* was more survive than *A. aspera,* indicated the lower survival rate coral fragment was found in *A. aspera* which transplanted vertically.

Growth rate

The growth rate of two *Acropora* species which transplanted on multilevel substrate of APR varied from 96.7 to 346.9 cm^3/month. The growth rate of *Acropora* fragments was significantly different among species, substrate levels and fixing method of transplantation ($p<0.05$) after 8 months. The growth rate of two *Acropora* species on multilevel substrate of APR after 8 months demonstrated that there were no significant differences in species *A aspera*. Whereas, there were significantly different on the *A. copiosa* growth that transplanted either in the upper and the middle levels or in the lower and in the middle levels ($p<0.05$). However, there were no significantly different on the coral growth transplanted in the upper and in the lower levels (Table 1).

The growth rate of two Acropora species transplanted in different fixing methods (vertical vs. horizontal) demonstrated significantly different ($p<0.05$; Figure 5). Growth rate of the fragments which transplanted in vertical fixing method was higher than the horizontal method. The lowest growth rate was found in *A. aspera* which transplanted in horizontal fixing method, while the highest growth rate occurred in *A. copiosa* which transplanted in vertical fixing methods. Fragments of coral *A. copiosa* can grow optimally on all levels by both vertical and horizontal fixing methods of coral transplantation. Comparing the species, the growth of transplanted *A. copiosa* was higher than that of *A. aspera* due to the different branching patterns ($p<0.05$; Figure 6).

Table 1. Growth rate (cm^3/month) of transplanted two species of Acropora on multilevel substrate of APR after 8 months (November 2018-July 2019)

Level	*A. aspera*	*A. copiosa*
Upper	130.05±47.16[a]	293.00±76.23[a]
Middle	178.75±34.17[a]	152.05±95.11[a]
Lower	202.75±44.74[a]	333.30±64.21[a]

Note: All results are expressed as mean ± SD. Values in each column which have the same letters are no significant different ($p<0.05$)

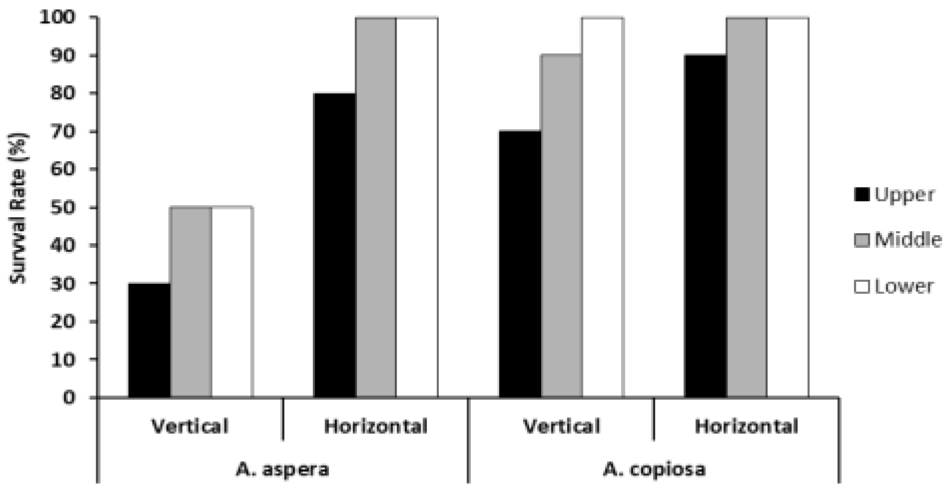

Figure 4. Survival rate (%) of transplanted two Acropora species on multilevel substrate of APR after 8 months (November 2018-July 2019)

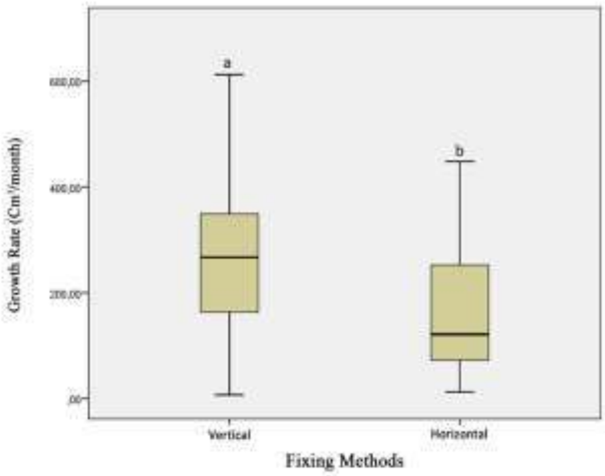

Figure 5. Growth rate (cm^3/month) of transplanted two *Acropora* species on multilevel substrate of APR in different fixing method after 8 months, November 2018-July 2019 (Note: Letter in each bar which has different letters are significantly different, p<0.05)

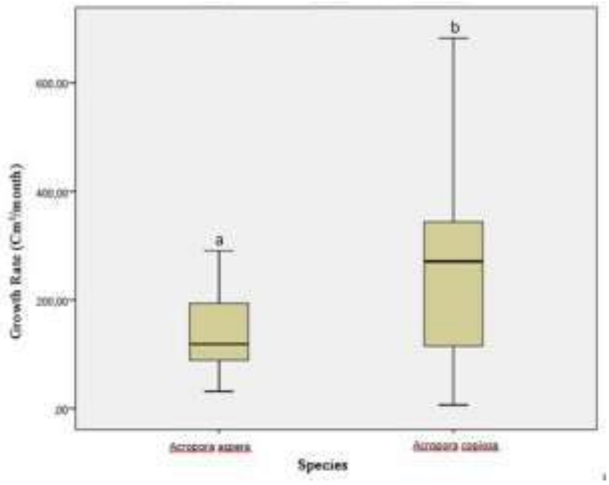

Figure 6. Comparison of growth rate (cm^3/month) transplanted *A. aspera* and *A. copiosa* on multilevel substrate of APR after 8 months, November 2018-July 2019 (Note: Letter in each bar which has different letters are significantly different, p<0.05)

Discussion

Lower survival rate during the experiment was revealed by *A. aspera* in all levels of substrate particularly in coral transplanted in fixing vertically. Coral fragments mortality was found in *A. aspera* which fixed in vertical orientation during the experiment. Lower survival rate of the coral fragments in vertically fixing method due to minimize of fragment surface attaching to the substrate may affecting the coral expend more energy in repairing the damage (Yap et al. 1992), consequently, the coral fragments died and then detached from the substrates. Additionally, lower survival rate of the coral fragments in the upper level may be caused by some of them lost by wave actions that occurred at the beginning of experiment, after fixed the coral fragments. Disadvantage of coral transplantation using directly fragment transplantation method on the substrates in shallow water is generally affected by algae competition, sediment accumulation and wave exposure (Young et al. 2012).

Acropora is one of the important coral in the shallow water and usually applied to coral transplantation (Heeger and Sotto 2000; Edwards 2010; Young et al. 2012). The coral was competent to grow fast, inversely they are also sensitive responding to the environment (Yap et al. 1992). Survival of the corals which have transplanted varied in different location and various in rehabilitation technic. The survival rate of *Acropora* in nubbin fix to the nursery table was 46% (Nithyanandan et al. 2018), while the high survival rate of the coral was found in *Acropora hyacinthus*, 83.3% (Bongiorni et al. 2011). In the present study, the survival rate of the *Acropora* is high due to the fragment stabilization using cable ties method and removing sediment accumulation caused by applying the multilevel designed of substrate. Thus, application of Artificial Patch Reef (APR) in shallow reef rehabilitation can contribute to enhance the survival of *Acropora* fragments.

Some previous studies of coral transplantation revealed that the growth rate of *Acropora* was higher than that of other hermatypic corals. Bongiorni et al. (2011) reported that *Acropora* possesses relative growth ranged 66.9 to 83.3%, while growth rate of *Acropora* which transplanted on the artificial reef dome-shaped was 1.07 cm/month (Muzaki et al. 2019), Acropora fragments fixed to the dead coral was 7.8 cm/year (Nithyanandan et al. 2018). This result showed that the growth rate of both Acropora which transplanted on multilevel substrate possesses a high growth rate. Presumably, the construction of multilevel APR can optimize coral growth by increasing light and preventing sediment coverage. The different branching pattern of the Acropora may affect to the growth of the corals, two Acropora shows a different level of complexity (Mercado-Monila et al. 2016) *A. copiosa* was more complex than *A. aspera* (Figure 7). Veron and Stafford-Smith (2000) identified that *A. copiosa* was clumps of prostrate or upright branches irregular branching patterns with frequent sub-branches, while *A. aspera* which is defined as a corymbose clump with thick branches (Veron and Stafford-Smith 2000). The higher growth rate of vertically fixing method in *Acropora copiosa* indicated that vertical fixing of the fragments was suitable orientation of the natural growth form of the donor colony of *Acropora* (Okubo et al. 2005). This study suggests that multilevel APR using vertical fixation method of selected *Acropora* which has high-level complexity should be applied in future coral rehabilitation projects.

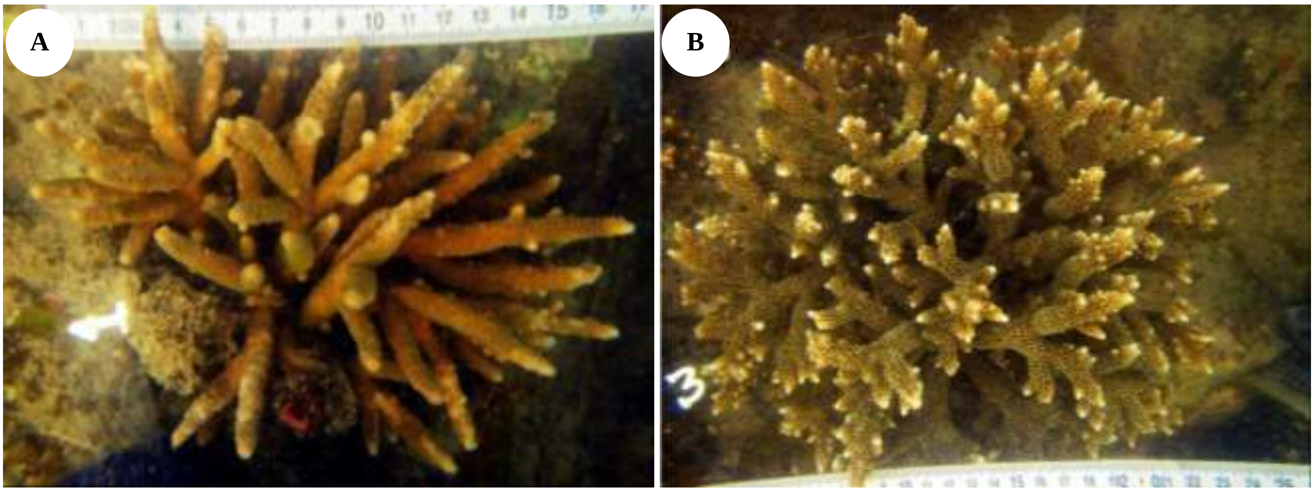

Figure 7. Transplanted of two species Acropora on multilevel substrate of APR after 8 months (A. horizontal fixing method of transplanted *Acropora aspera*; B. vertical fixing method of transplanted *Acropora copiosa*)

ACKNOWLEDGEMENTS

This study was supported by of Applied Research grant (No. 101-170/UN7.P4.3/PP/2018) from Ministry of Research and Technology, Higher Education to M, AS, and S. Part of the study was supported by research grant No. 1501-26/UN7.5.10/LT/2018 from Faculty of Fisheries and Marine Science, Diponegoro University to M, DPW, I, and RP. We thank Andy Ahmad for preparing coral measurement, Agus Susanto, and the staff of Marine Diving Club for field assistance and Tanjung Jati B Coal-Fired Power Plant to facilitate maintaining the artificial reefs. We appreciate two anonymous reviewers offered constructive comments to improve our manuscript.

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