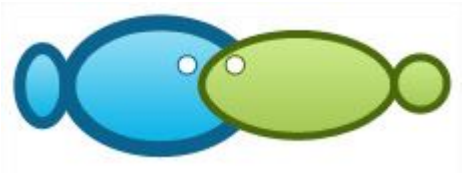

Characteristics of *Acropora divaricata* and *Acropora nobilis* on different transplantation depths based on growth rate and zooxanthellae density

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Abstract. Coral reefs are aquatic ecosystems with the highest biodiversity and a complex structure of interconnections between invertebrates and photosynthetic dinoflagellates. While coral reefs have many ecological and economic functions, the condition keeps declining due to many factors. One of the efforts made to improve the condition is transplantation activities. We transplanted two species of *Acropora*, namely *A. divaricata* and *A. nobilis* using the PVC rack method in water depths of 5, 10 and 15 m. This study was conducted from January to October 2018. The growth rate and zooxanthellae densities from each depth were analyzed using ANOVA and Tukey's studentized rang statistical analysis (HSD) to determine the best treatment. The growth of both species showed significant differences ($P < 0.05$). The highest growth rates of *A. divaricata* and *A. nobilis* were obtained at 5 m depth of 0.539±0.7 and 0.500±0.16 (mean±SD) cm month^{-1}, and the lowest growth at 15 m depth was 0.21 ±0.02 and 0.162±0.14 (mean±SD) cm month^{-1}. Zooxanthellae density also showed differences at each depth. The highest zooxanthellae density of *A. divaricata* and *A. nobilis* at 5 m depth was 1.372±0.25 x 10^6 (mean±SD) cells cm^{-2} and 0.603±0.455 x 10^6 (mean±SD) cells cm^{-2}, respectively. Pearson correlation analysis showed that the correlation between growth and zooxanthellae densities on *A. divaricata* was highly significant ($r^2 = 0.331$ and $p < 0.01$). Meanwhile, it was not significant at *A. nobilis* ($r^2 = 0.011$ and $p > 0.05$). The highest growth rate and zooxanthellae densities were found at a lower depth and decreased with the increasing depth. The correlation between growth rate and zooxanthellae densities is positive, despite different values according to species.
Keywords: coral transplant, optimum depth, algal symbionts, decalcification.

Introduction. Coral reefs are aquatic ecosystems with the highest biodiversity and a complex structure of interconnections (Al-Hammady 2013; Mumby & Steneck 2008), where the symbiosis between invertebrates and photosynthetic dinoflagellates occurs (Obura 2009). Coral reefs have many functions such as breaker of currents and waves, reef fish protection area, feeding and nursery ground for aquatic organisms (Giyanto 2017), and tourist attraction for diving and snorkeling (Baine 2001). The world's coral reef population is declining, due to anthropogenic pressures such as pollution (Bellwood et al 2012), massive development of coastal areas (De'ath et al 2012), irresponsible reef fishing (Flores et al 2017), ghost net fishing (Ballesteros et al 2018), unsustainable tourism such as direct contact while diving and snorkeling (Ong & Musa 2012), waste sewage, dropped anchor, and coral mining (Al-Hammady 2013; Bryant et al 1999). Approximately 19% of the world's coral reefs have been lost and 35% are endangered (Wilkinson 2004; Carpenter et al 2008), due to the decrease in pH and dissolved oxygen (Ainsworth et al 2011), as well as coral reefs bleaching (Hoegh-Guldberg et al 2008). Coral reef ecosystems can recover naturally, but that takes a long time (Soong & Chen 2003). One of the most popular and effective methods in Indonesia for coral reef recovery is transplantation (Edwards et al 2010). The success of coral transplantation is determined by the size of the fragment and species used (Soong & Chen 2003; Shafir et al 2006) as well as aquatic ecological parameters such as temperature, CO_2, turbidity,

salinity, sedimentation, and terrestrial runoff (Fabricius 2005; Veron et al 2009). The representation of coral growth is increasing in length and fragment diameter, which is formed by deposits in the form of lime ($CaCO_3$) produced by coral animals together with algae (Al-Hammady 2013). Coral animals obtain sugar and amino acids from the results of zooxanthellae photosynthesis, while zooxanthellae get ammonia and phosphate from the metabolism of coral animals (Trench 1979; Bhagooli & Hidaka 2004). Zooxanthellae density is affected by the intensity of sunlight, temperature increase, brightness, climate change and ocean acidification (Fabricius 2005; Carpenter et al 2008; Hoegh-Guldberg et al 2008). As Al-Hammady's (2013) research indicates, the density of zooxanthellae was inversely proportional to the depth of the water, the deeper the waters the lower the density of zooxanthellae. In this study, we worked with the species *Acropora divaricata* and *Acropora nobilis*. The study aimed to determine the growth of coral transplants at different depths, the densities of zooxanthellae on each branching coral transplant, and the correlations between growth rate and the density of zooxanthellae at different depths.

Material and Method. The study was carried out in Banyuwangi East Java, Indonesia (Figure 1), S 8°02'07.3" and E 114°26'15.6", the study site is easily accessible, near Bali island. This site is a conservation area managed by a local non-governmental organization in collaboration with the Department of Fisheries and Maritime Affairs, East Java Province, Indonesia. The site has slow - wave energy and sloping topography, the most dominant species are *Millepora* spp. and *Acropora* spp. (Suciyono et al 2019).

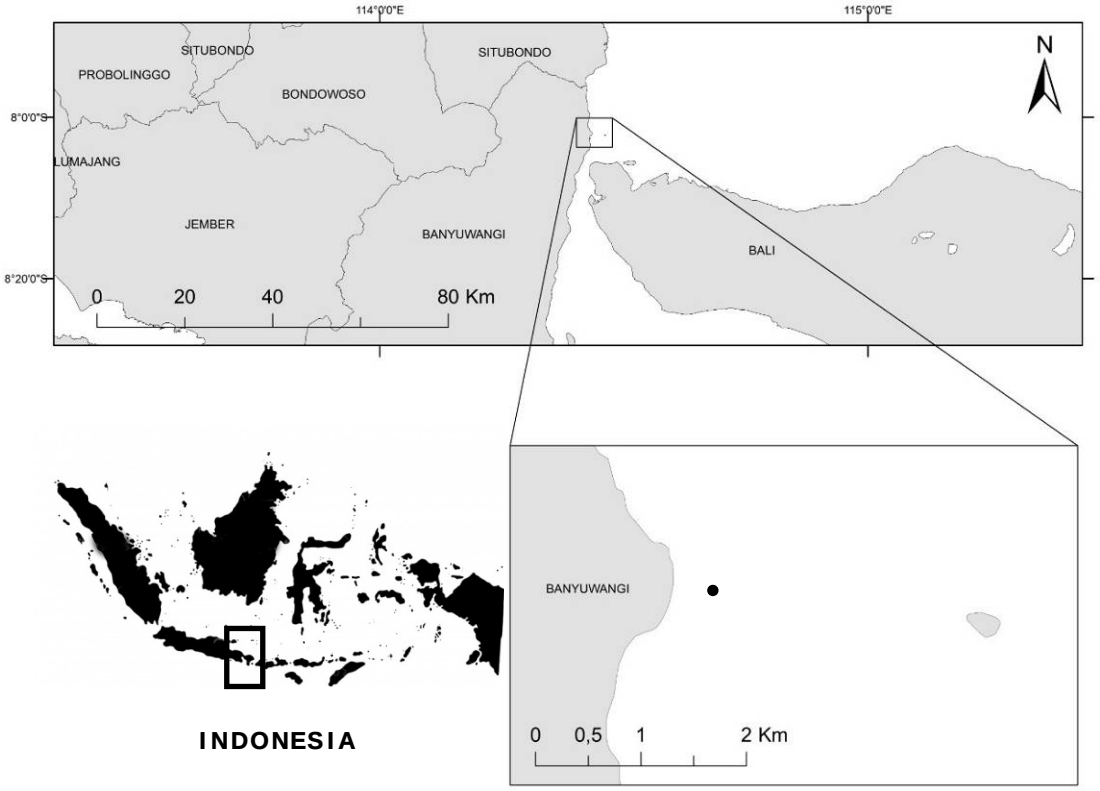

Figure 1. Map showing the study site (black spot on the bottom map), Banyuwangi, East Java, Indonesia.

Fragment origin and transplantation design. Transplantations of *A. divaricata* and *A. nobilis* were carried out at depths of 5, 10 and 15 meters, during January-October 2018. All fragments were marked with plastic cable ties to the fragment. The stem of the transplant module followed Mercado-Molina et al (2015). The fragment origin was obtained from around the transplantation areas with a length of 8-12 cm. The transplant model used cube-shaped planting racks made from, PVC pipes with dimensions of 100 x

75 x 25 cm^3. Each transplant rack has 15 places to bind coral fragments positioned higher than the bottom to prevent the fragments from being close to the sediment (Figure 2).

Figure 2. Transplant racks design (a); *A. nobilis* (b); and *A. divaricata* - origin fragment (c).

Collecting survival data. Survival (coral fragment) was observed every three months, starting from the second month after the transplantation for ten months. The formula below was used to specify the survival of fragments at each depth:

Survival = initial size of fragment/final size of fragment x 100

Water quality measurement. Water quality measurements were carried out every month. Water temperature was measured by a thermometer; pH - pH meter (Horiba™ serial B-711), brightness - Secchi disk; turbidity - portable turbidity meter kit (LaMotte™ model smart3); and salinity - refractometer (Atago™). Water samples for turbidity, nitrate, and phosphate were taken from the same depth as the transplant module. Physical parameters measurements were carried out *in situ*, while chemical parameters were carried out *ex-situ*. Nitrate analysis was carried out using the Brucine Sulfate method. Meanwhile, phosphate analysis used the Ascorbic Acid method (Baird et al 2017).

Growth measurement. The growth rate of fragments was measured in monthly linear extensions (final length - initial length / total month) and expressed in cm $month^{-1}$. Measurements were made on coral fragments bound in the transplant racks, measurements of growth were carried out in the water so that the corals were not stressed. The initial and final sizes of the fragment were measured on the entire fragment using calipers with an accuracy of 0.05 mm.

Biomass measurements. The fragment samples were taken from each colony by 1-2 cm with a cutter, then preserved with 10% formalin for 24 hours. Furthermore, a solution of 10% acetic acid and formalin was used for decalcification in the sample bottle. After the tissue was separated from the limestone skeleton, it was soaked with distilled water for 24 hours and then destroyed by mortar and pestle, thereafter diluted with 5 mL dilution of distilling water. Observation of zooxanthellae was done using a binocular microscope (Nikon E 100), with the magnification of 100-400, and the amount was calculated directly using Haemocytometer (Asisstant™). Furthermore, densities of zooxanthellae were calculated by the formula of Al-Hammady (2013):

Zooxanthellae cm^2 = (counted cells / cells surface area) x cells depth x dilution

Statistical analysis. Different growth rates and zooxanthellae densities were determined using analysis of variance (ANOVA, $p < 0.05$) from each depth, followed by the Tukey's studentized rang statistical analysis (HSD). Thus, the relationship between growth rate and the density of zooxanthellae was obtained through Pearson correlation analysis using SPSS version 2.4.

Results

Survival rate. The survival of both coral species showed similarities in patterns at every depth. At a depth of 5 and 10 meters, the survival rate is 92%, while at a depth of 15 meters, the rate is 84%. Transplanted coral deaths occur at the beginning of planting, influenced by the process of adaptation and disruption of coral predators.

Growth performances. The growth rate of *A. divaricata* and *A. nobilis* in the transplantation showed significant differences ($p < 0.05$) from each depth treatment. The highest growth rates of transplantation of *A. divaricata* (Figure 3a) and *A. nobilis* (Figure 3b) were obtained at 5 m depth of 0.539±0.7 and 0.500±0.16 (±SD) cm month^{-1} respectively. Meanwhile, at a depth of 10 m, the growth rate was 0.431±0.18 and 0.327±0.14 (±SD) cm month^{-1} respectively. Furthermore, the lowest growth at 15 m depth was 0.21±0.02 and 0.162±0.14 (±SD) cm month^{-1} respectively. The growth rate of both species at a depth of 5 meters is significantly different from that of 10 m and 15 m depth (p-value; 0.011 and 0.032) respectively. The best growth rate of both species was in the depth of 5 m.

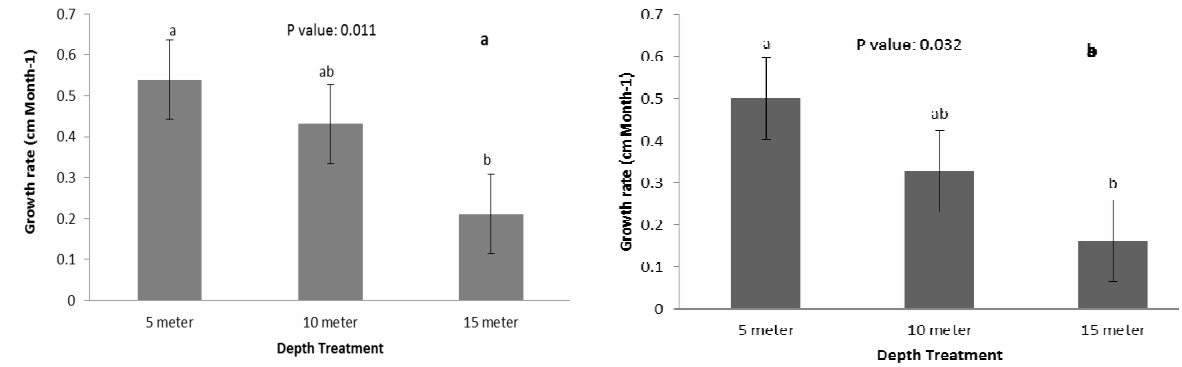

Figure 3. The growth rate of *A. divaricata* (a) and *A. nobilis* (b) (cm month^{-1}) on different depth; superscript above the bar was showing significant differences ($p < 0.05$).

Zooxanthellae densities. Zooxanthellae density of *A. divaricata* and *A. nobilis* in the transplantation showed differences at each depth. The density of both species was inversely proportional to the level of water depth. The zooxanthellae density of *A. divaricata* at a depth of 5 m significantly differs from that at the depths of 10 and 15 m. The density at 10 m depth is also significantly different from that at 15 m depth (P value 0.008). The density of zooxanthellae at 5 m depth was 1.372±0.25x10^6 (±SD) cells cm^{-2}, while at 10 and 15 m depth, the densities were 0.734±0.18x10^6 and 0.701±0.07x10^6 (±SD) cells cm^{-2}, respectively (Figure 4). The highest zooxanthellae density of *A. divaricata* at 5 m depth was 1.372±0.25x10^6 (±SD) cells cm^{-2}. On the other hand, the density of zooxanthellae in *A. nobilis* was also significantly differed at each depth ($p < 0.05$). The density of zooxanthellae at 5 m was significantly different from that at 15 m depth and 10 m depth (P value; 0.009). The highest density of *A. nobilis* at 5 m depth was 0.603±0.455x10^6 (±SD) cells cm^{-2}. At the same time, zooxanthellae densities between 10 and 15 m depth were 0.56 ±0.38x10^6 and 0.452±0.37x10^6(±SD) cells cm^{-2} respectively. The correlation between growth and zooxanthellae densities of both species was highly significant ($r^2 = 0.331$; $p < 0.05$) and ($r^2 = 0.011$; $p < 0.05$).

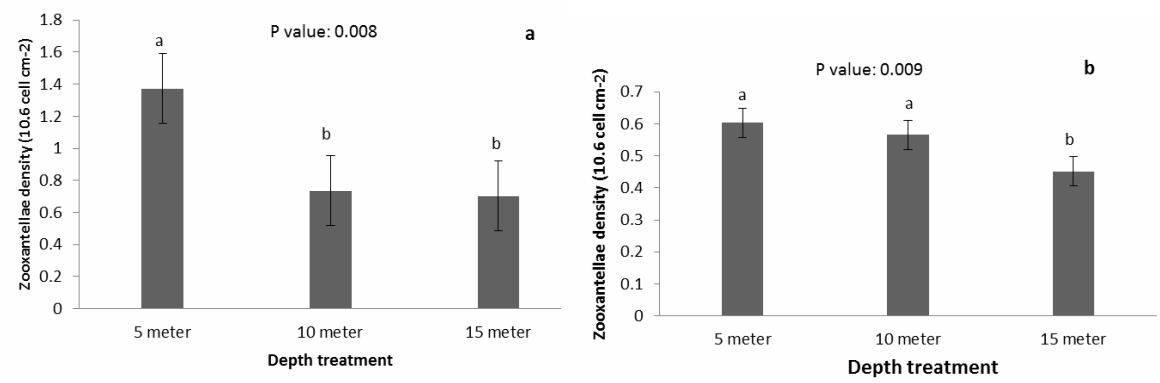

Figure 4. Zooxanthellae densities of *A. divaricata* (a), and *A. nobilis* (b) (10^6 cell cm^{-2}) on differed depth; superscript above the bar was showing significant differences ($p < 0.05$).

Discussion. The study revealed that the growth of *A. divaricata* and *A. nobilis* was affected by depth which is related to the intensity of sun exposure. The highest growth of both corals produced at a depth of 5 m while the lowest growth found at a depth of 15 m. Falkowski et al (1984) mentioned that the sun intensity was higher at a depth of less than 10 m. The sun exposure in location of coral transplantation is an important factor that influenced the coral growth. The growth of coral can be indentified by the increasing of the length and diameter of fragment which is formed by lime ($CaCO_3$) deposits. This process was called as calcification. The deposit of lime was accumulated as a result of the mutualism symbiosis between chlorophyll organisms (zooxanthellae) and polyps (Smith & Hughes 1999; Obura 2009; Al-Hammady 2013). Zooxanthellae use the sunlight to produce some nutrients, such as amino acid and sugar, for polyps through photosynthesis (Al-Hammady 2013; Mongin & Baird 2014).

Meanwhile, polyps generate ammonia and phosphate from its metabolism activity that can be used as nutrients for zooxanthellae (Furla et al 2000). The reduced intensity of sun exposure into the waters can inhibit photosynthesis so that it can inhibit the calcification (Kubicek & Reuter 2016). The calcification rate in dark water that has low intensity of sunlight is slower than in bright water which has high intensity of sunlight (Furla et al 2000; Gattuso et al 2007). It reached four times slower (Houlbrèque et al 2003). Therefore, both corals showed the best growth at a depth of less than 10 m. The previous study conducted by Al-Hammady (2013) also presented a similar result. *Acropora hemprichii* obtained the best growth at 10 m and the growth decreased along with the increasing of depth.

According to this study, the different depth of coral transplantation influenced the density of the zooxanthellae. The highest density of zooxanthellae of both corals was at 5 m of depth while the lowest density was at 15 m of depth. The density of zooxanthellae decreased along with the deeper of transplanted coral location.

The growth rate and zooxanthellae density of *A. divaricata* and *A. nobilis* showed a significant correlation. The growth rate of the hermatypic coral is influenced by the presence of zooxanthellae (Chalker & Barnes 1990; Furla et al 2000; Houlbrèque et al 2003). Zooxanthellae provide energy and nutrients for polyps up to 95% of photosynthetic production (Dubinsky et al 1990; Dubinsky & Jokiel 1994).

In this study, the death of coral fragments occurred at the beginning of transplantation. It was caused by stress due to relocation and adaptation to the new environment and interference by coral predators (Forrester et al 2012). The survival of coral fragments depends on the size and initial species (Smith & Hughes 1999; Soong & Chen 2003). The small fragments have a high-risk mortality rate (Hughes 1984). On the other hand, extreme changes in the environment, such as temperature and salinity fluctuations, lead to bleaching, stress and death (Obura 2009). This condition often occurs in sub-tropical waters where temperature fluctuations can be reached more than 10^oC due to the upwelling phenomenon (El-Mashjary & Ali 2010). Nevertheless, the water quality in this study was in the optimal range for coral reef growth (Figure 5).

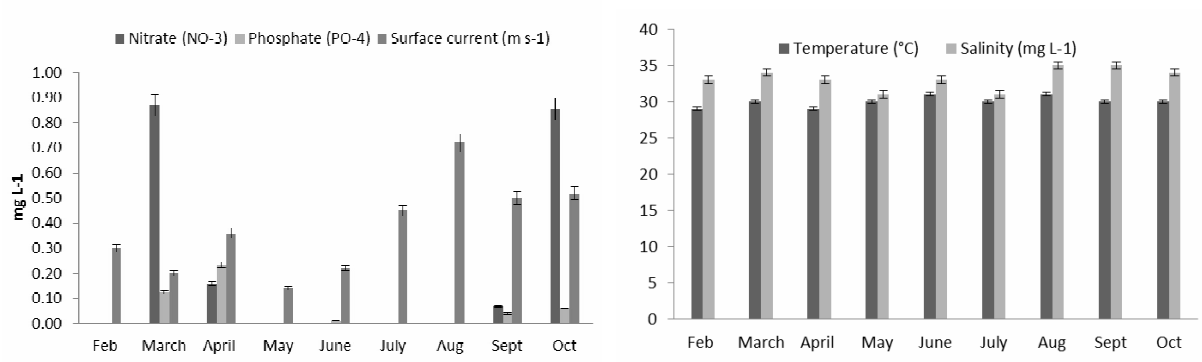

Figure 5. Periodic water quality measurement results on the study site.

The presence of coral predator, such as *Drupella* sp., and *Acanthaster planci* parrotfish also affected the mortality rate of coral (Cumming 1999; Bellwood et al 2004; Nanami 2016). *Drupella cornus* can become a predator by eating the juvenile of coral reef, and *Acropora* sp. is its favorite prey (Mcclanahan 1997; Cumming 1999; Shafir et al 2008). Based on the evidence found in location, it was supposed that the presence of parrotfish disrupted the coral growth by eating the branches (Figure 6). Parrotfish (*Sparisoma viride*) was found eating all branches of living corals, especially *Montastraea annularis* (Bruggemann et al 1994). Hoey & Bellwood (2008) reported that there was a significant interaction between parrotfish abundance and coral abundance. Moreover, Miller & Hay (1998) reported that parrotfish could reduce about 50% of the population of *Porites divaricata* within 48 hours. This recent study has contributed to the evidence that an appropriate depth of coral transplantation location was an important factor on the total abundance of zooxanthellae that related to the growth of coral reefs.

Figure 6. Coral fragments condition affected by coral predators on the transplantation.

Conclusions. The highest growth and density of zooxanthellae from *A divaricata* and *A nobilis* were found at a lower depth (5 m) and decreased with increasing depth. The growth and density of zooxanthellae have a positive correlation, with different values according to species.

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