TIK-67 The survival and growth rate of three species of sea anemones from asexsual reproduction in pulau kerumputan and pulau karayaan, Indonesia

by - Turnitin

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Article in Ecology, Environment and Conservation · January 2016

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Article-80

Eco. Env. & Cons. 22 (3) : 2016; pp. (531-539)
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ISSN 0971–765X

The survival and growth rate of three species of sea Anemones from Asexsual reproduction in Pulau Kerumputan and Pulau Karayaan, Indonesia

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(Received 17 March, 2016; accepted 19 Oktober, 2016)

ABSTRACT

A sea anemone is one kind of marine biota which habitat is found in the coral reef area. Sea anemones have quite high ecological and economical value. Currently, the population keeps on being degraded due to the high intensity of fish catching to meet the demand from domestic market and exports. Therefore, it is necessary of having restocking efforts and environment-friendly aquaculture using the seeds of asexual reproduction results in the hatchery. The purpose of this study is to find out the survival and growth rate of three species of sea anemones, namely *Stichodactyla gigantea*, *Entacmaea quadricolor*, and *Macrodactyla doreensis* are cultured in the water of the islands of Kerumputan and Kerayaan, Indonesia, for 4 months, from June - September 2015. The seeds used are from the result of asexual reproduction of a sea anemone with longitudinal body cleavage techniques, from all three parents. The results showed that asexual technology of seeds reproduction are able to produce the survival and growth rate quite well when they are cultivated in natural water. The three species of anemones had different survival and growth rate. The anemones, *S. gigantea* and *E. quadricolor,* had the highest survival and growth rate, whereas anemone of *M. doreensis* had the lowest survival and growth rate. The survival and growth rate of anemones among cultivation sites had no difference at all species of anemones.

***Key words** : Sea anemone, Survival rate, Growth rate, Pulau Kerumputan, and Pulau Kerayaan.*

Introduction

A sea anemone is one type of marine biota which habitat is found in the coral reef area. This biota is usually found in less fertile coral reef areas, attaches to hard objects, dead coral, crushed coral, and sand (Chia, 1976; Dunn, 1981; Shick, 1991; Fautin and Allen, 1997; Rifa'i and Kudsiah, 2007).

The population of sea anemones in Indonesia continues to decline because of high intensity of fishing to meet the demand of the anemone market for domestic and export market (Rifa'i, 2009; Rifa'i *et al.*, 2013). Some species of sea anemones, such as *actiniaria equima, anemonia sulcata, bunodactis verrucosa,*

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redianthusmalu, and *stoichactis keuti* have been exported to Singapore, Europe, United States, and Canada as an ornamental anemones for sea aquarium(Suwignyo *et al.*, 2005). If this condition continues, then the population of sea anemones will continue to be degraded and someday will become extinct. To preserve the resources, the efforts to increase the population of anemones must be done through the efforts of their breeding and cultivating in nature so that the seedstock does not fully rely on the result of fishing.

Naturally, sea anemones can reproduce sexually and asexually (Barnes, 1963; Russel and Hunter, 1979; McConnaughey and Zottoli, 1983; and Nybakken, 1992). Naturally, asexual reproduction is done by cutting off legs, parts of the pedal discs which were abandoned when the anemones move. A part of this abandoned anemone will grow into small anemones (Barnes, 1963). According to McConaughey and Zottoli (1983), a sea anemone reproduces asexually by crawling slowly to the opposite direction until its body breaks into two parts. The parts then become round and are alive into new anemones. Three types of sea anemones from the family of *Stichodactylidae* can reproduce asexually by longitudinal and transversal cleavage. The three types of sea anemones are s*tichodactyla helianthus*, *entacmaea quadricolor*, dan h*eteractis maginifica* (Dunn, 1981).

Information about the asexual reproduction and culture of sea anemones is still very little in Indonesia. A few publications reported on this information is limited to the species of *Stichodactyla gigantea* (Rifa'i, 1998; Rifa'i and Kudsiah 2007; Rifa'i *et al.*, 2008; Rifa'i 2011; Rifa'i *et al.*, 2013; and Rifa'i, 2013). Therefore, the information about the other anemone species becomes very important. This study wants to find out and to compare the survival rate and the growth rate of the three species of sea anemones, namely *Stichodactyla gigantea*, *Entacmaea quadricolor*, and *Macrodactyla doreensis anemones* using asexual reproduction seeds that are cultured in the waters of Pulau Kerumputan and Pulau Kerayaan, Indonesia.

Materials and Methods

Preparation of seeds: The parents of anemones used in this study are the anemone species of *Stichodactyla gigantea*, *Entacmaea quadricolor*, and *Macrodactyla doreensis* that were collected from the waters around the islands of Pulau Kerayaan and Pulau Kerumputan during the months of January - May

Fig. 1. A Map showing the location and site (red dots) of the study in the islands of Pulau Kerayaan and Pulau Kerumputan

2015. Both islands are in the district of Pulau Laut Kepulauan, Kotabaru regency, South Kalimantan (Fig. 1). The parents of anemones were brought to the mini hatchery for acclimatization for 2 weeks before asexual reproduction process. Concurrently, as the parents' acclimatization process, the acclimation cages and the culture cages were also prepared for the seeds resulting from asexual reproduction. The size of each was about 5 x 4 meters . Cages were made of wire with a framework of 2-inch PVC pipe. At the bottom of the cages, rubble or dead coral was spread evenly with the intention that the *substrate* provided a place for anemone seeds to stick when they were placed in the acclimatization cages. The acclimatization cages were placed at the shore near the mini hatchery to reduce the death rate of seeds.

Then, parents of anemones that have been through a period of acclimatization were cleaved longitudinally into 2 or 4 parts depending on the size of the parent (Rifa'i *et al*., 2013) (Fig. 2). The body parts, then, were put into acclimatization cages for 7 days in order that new seeds of anemones were able to attach on the substrate that has been provided and stress from post-cleavage can be gradually recovered. After that, the seeds can be transferred into the culture cages.

The Culture and Data Collection: The culture sites are the coral reef areas which are categorized from bad category (0 - 25%) to very bad category (26 - 50%) (Gomez *et al*., 1981). At each site, 3 units of cages were placed with the breeding density of 30 anemones respectively. The cages were placed on a flat reef which depth ranged from 3-5 meters so that they can still receive sunlight. The distance between the cages ranged from 8 to10 meters. All seeds spread had attached to the substrate. This attachment occurred when seeds were in the acclimatization cages. Two (2) days after spreading, the two-part cleavage (C) and four-part cleavage of anemones were measured (Rifa'i, *et al*., 2013). The diameters of the tested anemone bodies were measured

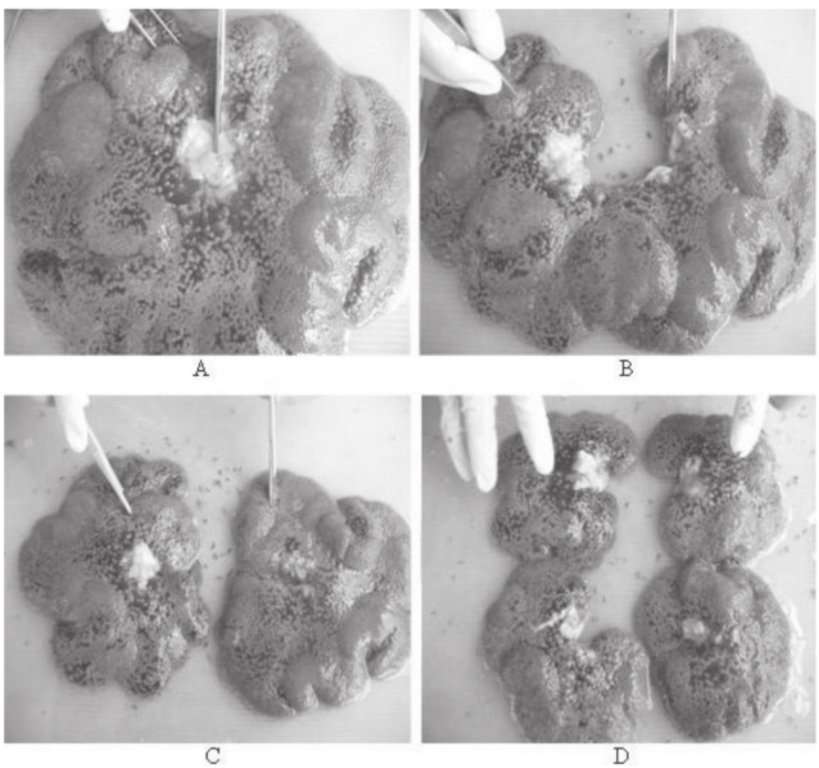

Fig. 2. Longitudinal Cleavage process of the body (A and B); The new seeds of anemones, the results of two parts of cleavage (C); The seeds of new anemones, the results of 4 parts of cleavage (D) (Rifa'i, *et al*., 2013)

by using callipers. The body diameters measured were the the upper parts of the bodies, above the baseldisc. Four (4) months after the culture, the number of living anemones were observed to determine the survival rate, and the diameters of the bodies were measured to determine their growth.

Survival and Growth Rate: The living sea anemones up to the end of the culture period were analyzed using the formula Richer (1975), as follows:

$$SR = \frac{Nt}{No.} \times 100\%$$

Note: SR = Survival Rate (%)

N_t = The number of anemones at the end of the study (tails)

N_o = The number of anemones at the beginning of the study (tails)

The measurement result of the growth of the body diameters were analyzed using a formula Richer (1975) as follows:

$$H = H_t - H_0$$

Note: H = The average of the absolute growth (mm)

H_t = The average width of the diameter of the anemone's body at the end of the study (mm)

H_0 = The average width of the diameter of the anemone's body at the beginning of the study (mm)

Statistical Analysis: Statistical analysis used SPSS program, version 17.0. To determine differences in survival rate (%) and growth rate (cm) among sea anemone species, K-Independent Samples analysis was used by using Kruskal Wallis test, and 2-Independent Samples analysis was used to analyze every species between culture sites by using Mann-Whitney test.

Results

Survival Rate (%)

The results showed the average survival rates (%) of sea anemones of *S. gigantea* was 93,67±5.85 (Mean±SE), of *E. quadricolor* was 89,00±5,48, and *M. doreensis* was 18,67±7,23 (Fig. 3). The survival rates (%) among the three anemones in 4 months of culture showed a significant difference (Sig = 0.002, $p < 0.05$). Similarly, It also occured between *S. gigantean* and *M. doreensis* (Sig=0,004, p<0,05), between *E. Quadricolor* and *M. doreensis* (Sig=0,004, p<0,05). Whereas the survival rates between *S. gigantean* and *E. quadcolor* did not have a significant difference (Sig=0,147, p>0,05).

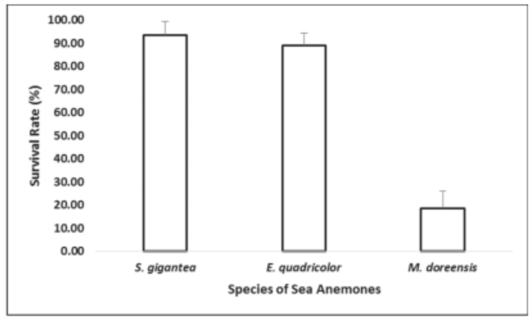

Fig. 3. The average of survival rate (%) of sea anemones for 4 months culture

The average survival rate (%) of *S. gigantean* anemones cultured in waters of Pulau Karumputan was 89.33 ± 5.03 and Pulau Kerayaan was 98.00 ± 2.00. The average survival rate (%) of *E. quadricolor* anemones cultured in waters of Pulau Karumputan was 84,67±3,06 and Pulau Kerayaan was 93,33±3,06. The average survival rate (%) of *M. doreensis* anemones cultured in waters of Pulau Karumputan was 14,67±5,03 and Pulau Kerayaan was 22,67±7,57 (Fig. 4). Sea anemones that were cultured in Pulau Kerumputan and Pulau Kerayaan did not have a significant difference (Sig = 0,093, p>0,05) in their average survival rates (%). A similar condition occured to each species. The average survival rate (%) of *S. gigantea* in between Pulau Kerumputan and Pulau Kerayaan showed no significant difference (Sig = 0,050, p> 0.05). Similarly, *E. quadricolor* (Sig = 0,050, p> 0.05) and *M. doreensis* (Sig = 0.184, p> 0.05) showed no significant difference in their average survival rates (%) too.

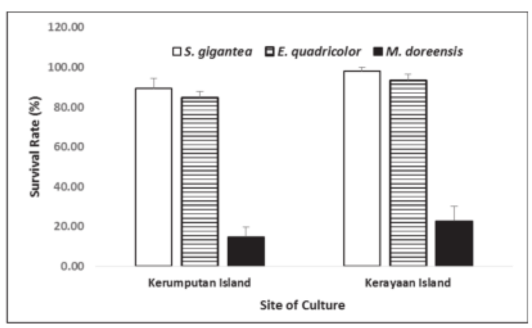

Fig. 4. The average of survival rate (%) of sea anemones in different culture sites

Growth Rate (mm)

The results showed the average growth rate (mm) of *S. gigantea* sea anemones was 54,17±4.36 (Mean±SE), *E. quadricolor* was 46,17±3,66, and *M. doreensis* was 32,83±5,98 (Fig 5). The growth rate (mm) among the three anemones for 4 months of culture showed a significant difference (Sig = 0.001, p <0.05). Similarly, between *S. gigantea* and *M. doreensis* (Sig=0,004, p<0,05), between *E. quadricolor* and *M. doreensis* (Sig=0,005, p<0,05) showed a significance difference. Whereas, the growth rate (mm) between *S. gigantea* and *E. quadcolor* did not have a significant difference (Sig=0,013, p>0,05).

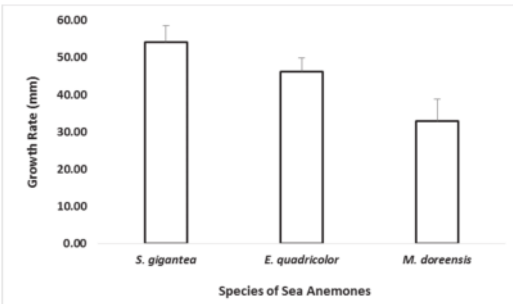

Fig. 5. The average of growth rate (mm) of sea anemones for 4 months culture

The average growth rate (mm) of *S. gigantea* anemones cultured in waters of Pulau Karumputan was 52,33±4,04 and Pulau Kerayaan was 56,00±4,58. The average growth rate (mm) *E. quadricolor* anemones cultured in waters of Pulau Karumputan was 44,67±4,51 and Pulau Kerayaan was 47,67±2,52. The average growth rate (mm) of *M. doreensis* anemones cultured in waters of Pulau Karumputan was 30,67±5,13 and Pulau Kerayaan was 35,00±7,00 (Fig. 6).

The average growth rate (mm) of sea anemones cultured in Pulau Kerumputan and Pulau Kerayaan did not show a significant difference (Sig=0,426, p>0,05). A similar condition occured to each species.

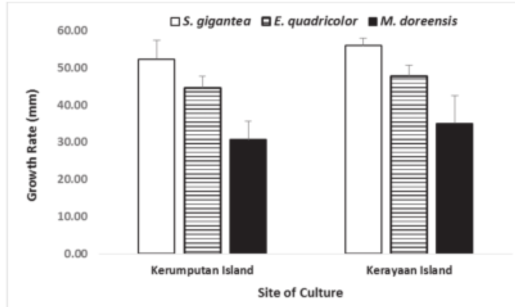

Fig. 6. The average of growth rate (mm) of sea anemones in different culture sites

The average growth rate (mm) of *S. gigantea* cultured in between Pulau Kerumputan and Pulau Kerayaan showed no significant difference (Sig=0,275, p>0,05). Similarly, *E. quadricolor* (Sig=0,376, p>0,05) and *M. doreensis* (Sig=0,275, p>0,05) showed no significant differeence too.

Water Quality

The results of water quality measurements at the study sites in waters of Pulau Kerayaan and Pulau Kerumputan during the four months of culture presented in Table 1.

Discussion

Survival Rate (%)

The results showed the average survival rate (%) of sea anemones had significant differences. The highest survival rate was found from *S. gigantea* and *E. quadricolor* anemones, and the lowest was *M. doreensis* (Fig. 7) anemones. The survival rate (%) of *S. gigantea* was not different from the survival rate of *E. quadricolor*, but their survival rates were different from *M. Doreensis*.

The high survival rate (%) of *S. gigantea* and *E. quadricolor* anemones showed that both types of anemones were able to recover more quickly after splitting the body (asexual reproduction) when cul-

Table 1. Water quality in the study site for 4 months of culture, from June to September 2015

Study site	Water Quality Parameter				
	Salinity (ppt)	Temperature (°C)	Transparency (mm)	DO (ppm)	Current speed (m/sec)
Kerayaan I.	32.5–35.0	27.8 – 32.5	225 – 304	6.3 – 7.0	0.175 – 0.183
Kerumputan I.	32.6 – 34.3	28.1 – 32.2	206 – 295	6.2 – 6.9	0.163 – 0.185

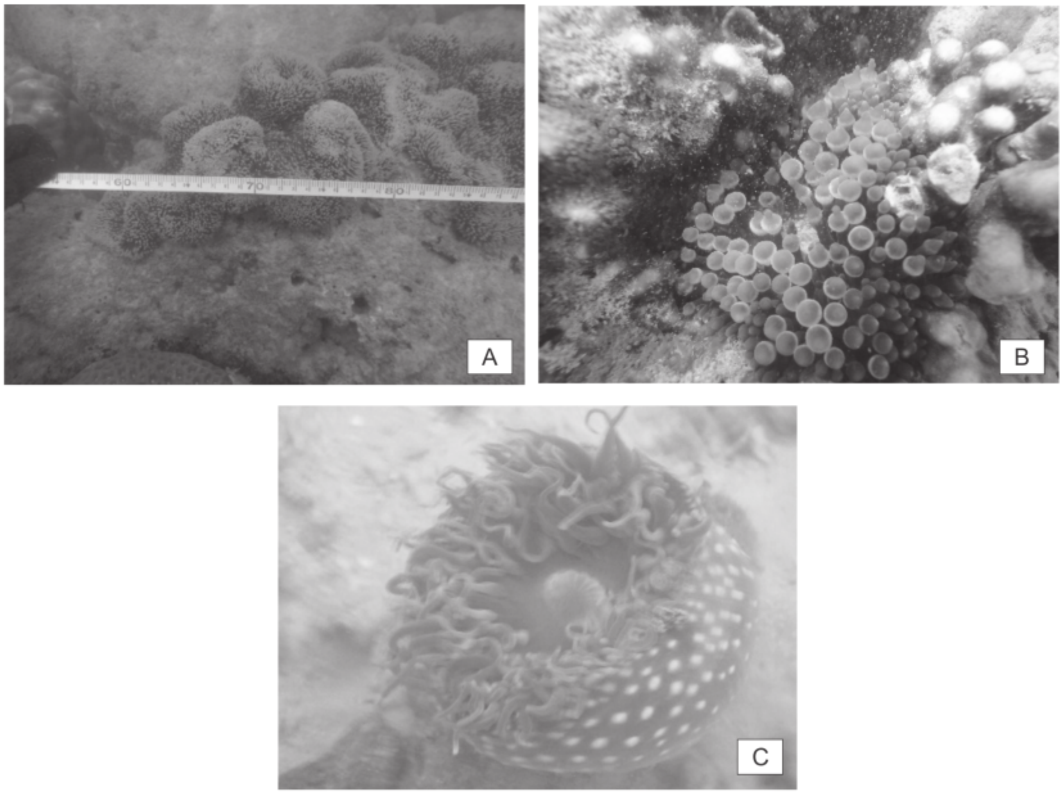

Fig. 7. Anemones *S. gigantea* (A), *E.quadricolor* (B), and *M. doreensis* (C) at culture site

tured in natural waters compared with *M. doreensis* anemones. The ability to recover of both parents of anemones was due to the size and morphology of the pedal disc and column they had were not much different. Parents of *S. gigantea* anemones had 478 mm of the average column height with 401 mm of the average oral disc diameter and 436 mm of the average pedal disc diameter. Whereas the anemones of *E quadricolor* had 435 mm of the average column height with 388 mm of the average oral disc diameter, and 402 mm of the average pedal disc.

The size and morphology similar to these two types of anemones found in the Moreton Bay, Australia with well-developed pedal disc, 400 mm of oral disc diameter, and 500 mm of a column height (Fautin *et al.*, 2008). Pedal disc is an organ that has an important role in the stability of anemones in nature. Pedal disc has a function as a foot for its body to attach to the substrate which is found at the bottom of waters. Because of the pedal disc shape which is quite wide and sturdy, it is possible that the two types of anemones are able to recover more quickly because they settle faster in waters even though their bodies have been injured after splitting. Similarly, the wide column shape causes every anemone having larger body parts and a greater amount of tentacles. A large number of tentacles is correlated positively to the ability to capture preys and defense. According to Dunn (1981) and Shimek (2006), one function of the tentacles of anemones is to actively capture food and then put into their mouths.

Besides, it is related to the presence of zooxanthella algae. The anemones which have less tentacles will automatically also have less amount of zooxanthellae algae that affects the ability of anemones in conducting recovery from stress due to the splitting of the body (Rifa'i *et al.*, 2008 and Rifa'i, 2009). Thus, the high survival rates of both anemones were caused by such matters, and this is proven by the abundance of two types of these anemones in Indonesia (Dunn 1981) and in this research location (Anhar 2013; Firdaus, 2014). *M. Doreensis* anemones have a smaller wide pedal disk and bottle-shaped column with a number of tentacles much less than *S. gigantea* and *E. Quadricolor* anemones. The parents of *M. doreensis* anemones used in this study had 325 mm for their average column height, 156 mm for the diameter of their average of oral disc, and 237 mm for the diameter of an average of pedal disc is 237 mm. The low survival rate of these anemones also was due to their sand living habitats. They like sands for immersing their pedal disks so that they are not able to settle permanently in their habitat and they become vulnerable to move to one place to another due to currents and waves. In contrast to S. *gigantea* and *E. Quadricolor* anemones, they have strong basel disks and they are capable of clinging strongly on hard substrate at the bottom of waters, such as dead coral, crushed coral, and other hard substrates.

In fact, the survival rates of all anemones tested did not show any difference between the two study sites, either cultured in Pulau Kerayaan or Pulau Kerumputan. No difference was caused by the condition of the water quality parameter and the substrate of coral reef that were not much different between the two cultured locations. The result of the measurement of some important water quality parameters showed that Pulau Kerayaan had the salinity of 32.5 – 35.0 ppt, the temperature of 27.8 – 32.5°C, the transparency of 225 – 304 mm, the dissolved oxygen of 6.3 – 7.0 ppm, and the current speed of 0.175 - 0.183 m/sec. Meanwhile, Pulau Kerumputan had the salinity of 32.6 – 34.3 ‰, the temperature of 28.1 – 32.2°C, the transparency of 206 – 295 mm, the dissolved oxygen of 6.2 – 6.9 ppm, and the current speed of 0.163 - 0.185 m/sec. Parameters of salinity was still in the normal range for the lives of anemones and zooxanthellae living associated with endodermis tissues. Tropical coral and anemones can exist in the range of salinity of 32-40 ppt (Hoegh-Guldberg, 1999).

During the study, a drastic decrease of salinity never occured because the location of this study was around the sea waters far from the river estuary so that the salinity is quite conducive to the lives of sea anemones. According to Hoegh-Guldberg (1999), salinity fluctuation plays an important role in limiting the distribution of reef-building corals. The temperature parameter was still in the normal range for the lives of the anemones. Temperature is an important environmental parameter in the life survival of sea anemones (Muscatine *et al.*, 1991 and Hoegh-Guldberg, 1999). Response of cnidarian photosynthesis to temperature showed higher environmental effect on photosynthetic speed (Davison, 1991). An increase and a decrease of sea surface temperature can cause an increase and a decrease of the level of photosynthesis and respiration (Howe and Marshall, 2001 dan Nakamura *et al.*, 2003).

High temperature can lead to the disintegration of enzymatic flow in photosynthesis activities that causes a biochemical and metabolic dysfunction. This stress level depends on the length of exposure and the synergism action of various environment variables (such as light and salinity) (Cossins and Bowler, 1987 *in* Fitt and Cook, 2001). During the study, the temperature fluctuation was very stable so that the activities of photosynthesis and respiration can run normally and conducively to the lives of the cultured sea anemones. The parameter of transparency was still in the normal range for the life of the anemones. Sunlight is one of the main parameter in the lives of sea anemones.

Penetration of sunlight can stimulate the process of photosynthesis by zooxanthellae algae at the tissues of sea anemones. The transparency value is limited by weather, time measurement, turbidity and suspended solids (Effendi, 2003). Parameter of dissolved oxygen was still in the normal range for the lives of anemones. Dissolved oxygen in water is very important to the lives of aquatic organisms. High or low levels of oxygen in waters is very dependent on currents and waves, temperature, salinity, depth, and potential aquatic biotics (Odum, 1971). The parameter of the current speed is still in the normal range for the lives of anemones. Currents are very necessary in the growing process of anemones. Currents can supply food in the form of microplankton. Currents also play a role in the cleaning process of the deposits of materials and the supply of oxygen that comes from the open sea. Therefore, the current circulation is very important in the process of energy transfer.

Growth Rate (mm)

The research results showed that the trend of growth (mm) was similar to the trend of survival (%). The average growth (mm) between sea anemones showed significant differences. The highest growth was found at *S. gigantea* and *E. quadricolor* anemones, and the lowest was found in *M. doreensis* anemones. The growth (mm) of *S. gigantea* was no different from *E. quadricolor*, but both are different from *M. doreensis*. Therefore, anemones of *S. gigantea* and *E. quadricolor* from asexual reproduction cultured in coral reef areas are able to live and grow well. This condition is not apart from the anatomical structure of the body that is more supportive to the lives of both species, compared with *M. doreensis anemones* as described above. Additionally, anemones of the *S. gigantea* and *E. Quadricolor* species are several species of anemones that are widely found in the waters of South Kalimantan, especially in Indonesia. Several previous studies have shown that the anemones of the species of *S. gigantea* and *E. Quadricolor* were widely found in the waters of Indonesia, Malaysia, Thailand, and the Philippines (Dunn, 1981; Fautin and Allen, 1997; and Rifa'i, *et al*., 2014).

The low growth of anemone species of *M. doreensis* was assumed due to the high stress after their body cleavage during the process of asexual reproduction, so that the energy that should be used for the growth and metabolic activities of the body is more widely used to fix the injured tissues as the result of cleavage. According to Byrne (1985), the body tissue damaged by the injury requires additional energy to return to its normal body condition. According to Rifa'i *et al*., (2013) and Rifa'i (2013), at the time of asexual reproduction, the anemone body was split into several parts. As a result, the body of anemone experiences a very massive wound due to its whole body was split longitudinally from the top (oral disk) to the bottom (basel disk). As invertebrate animals, sea anemones naturally begin to regenerate to repair tissues or organs that have been lost.

The regeneration process will cause the metabolism activities of the host concentrated on the basal metabolism to repair body cells damaged due to wounds after cleavage. During the recovery process, there is a physiological dysfunction of the body, especially the dysfunctions of proteins and enzymes which disrupt the distribution of nutrients from the host to the zooxanthellae algae. These dysfunctions cause protein breakdown and formation of mucus on epidermal layers excessively, and also the dysfunction of cell-cell adhesion. This physiological dysfunction causes the anemone host to be stress which causes the reduction of zooxanthellae algae on the endothermic tissues of the host through various mechanisms of excretion, such as exocytosis, apoptosis, necrosis, pinching off, and detachment. As a result, the growth of sea anemones becomes slow.

Unlike the natural anemones which do not have body cleavage, mutualism symbiotic relationship between zooxanthellae algae and their sea anemone hosts runs normally as their respective functions. Zooxanthellae algae living in the host endodermis tissues, is active in photosynthetic activities. By the help of the sunlight energy, algae binds bicarbonate and carbon dioxide into carbohydrate in the form of glycerol, glucose, and amino acid alanine. This activity requires specific nutrients, especially nitrogen in the form of ammonia and phosphate, which is produced by the metabolism of anemones. Zooxanthellae algae provides approximately 60-80% of its photosynthetic product to hosts. Alanine produced by zooxanthellae algae is used by the hosts to form a protein complex, while carbohydrate is used to provide energy for the work and growth of tissues.

Acknowledgement

The authors would like to thank the Directorate General of Strengthening for Research and Development, Ministry of Research and Technology, and Higher Education, Republic of Indonesia for funding through the National Strategic Research Scheme in 2015. Thanks also to the village community of the islands of Pulau Kerumputan and Pulau Kerayaan who helped during the field research.

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