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Translocation Study of Some Zooxanthellae Clade to the Survival and Growth of *Goniastrea aspera* after Bleaching

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ABSTRACT

Inter-host translocation technique of zooxanthellae was attempted to prove Buddemier and Futin's (1993) theory on adaptation. The recent trend of coral products trading must be anticipated by its mass production through artificial techniques, the alternation of natural resources. Translocation bio-technique of zooxanthellae on coral was expected to resolve the problem and the translocation study should provide fundamental answer to coral recovery. The study of zooxanthellae translocation was proposed to: a) Evaluate the effect of zooxanthellae enrichment on its translocation on coral polyp tissue efter optimum bleaching and b) Investigate the effect of translocation on coral growth. The research was experimental, involving coral species *Goneastrea aspera*, and purified zooxanthellae clade A, B and C with circulating incubation condition in BPBBAP Jepara indoor area. The experiment took place for 30 weeks in both model environment waters and natural environment waters of Jepara Panjang Island coral area from March to August 2008. The result showed that: a) In the artificial waters, translocation zooxanthellae to polyp tissue of *Goneastrea aspera* occured at day 17 and more fast in the natural waters; b) In the controlling of temperature environment on translocation provided positive response of Goneastrea aspera's normal life, relocation and growth rate of zooxanthellae as in nature and c) recognition, resettlement, and growth process of zooxanthellae made it possible for *Goneastrea aspera* to grow normally in natural waters.

Key words: *Clade*, bleaching, recognition, resettlement, growth, translocation, *zooxanthellae*, *Goneastrea aspera*

INTRODUCTION

Symbiosis of zooxanthellae and coral in the sea is an occasion that preceded the merging of zooxanthellae and coral. According to Hoegh-Guldberg and Hinde (1986) the mechanisms of symbiosis occur in 5 ways: the first is from planula larvae (Baker, 2003; Pochon *et al*., 2001). The second through a chemosensory mechanism, which is a process of infection that is predicated on the attractant such as ammonium and nitrate that stimulates zooxanthellae to perform symbiotic with coral. The third is called intermediate host. The fourth is through predator feces. The fifth is a random contact that encounters due to the planktonic nature of zooxanthellae. Random contacts resumed the process of Endocytosis if they in harmony.

DNA diversity of zooxanthellae in the coral polyp's tissues is hinged to the infection process. Infection of zooxanthellae can occur in vertical and horizontal mechanism (Coffort and Santos, 2005). The vertical mechanism for infections mention early is through derivative (La Jeunesse *et al*., 2003). In this case of zooxanthellae C17 type only found on *Montipora Spp*; C22 type only found on *Turbinaria Spp* and C27 type found on the *Pavona* variants at a depth of 10 meters. Pochon *et al.* (2001) found that Foraminifera is infected especially by *Symbiodinium* clade type F. Besides that, it is also found the nature of horizontal infection which is a phenomenon of symbiotic relationship to the host that happens from the external environment (Coffort and Santos, 2005). It states that initially infected by a certain clade probably evolved symbion until adulthood. But in its development when the infection occurs openly, then a new clade will devise a new form to enrich the former clade.

The phenomenon of coral bleaching as an early indication of environmental pressures to the reefs and potential of the enriched clade in the host is alive or survived due to the pressure are being the trigger ideas of coral reefs recovery process as a result of degradation. If such limitations either natural or anthropogenic pressures that occur is associated with the evidence of such a recovery have been reported by Suharsono (1998) in the global after bleaching coral reefs of the Seribu island, then questions about the reefs form and structure are still not informed can be supported by the expected results of this research. Study of zooxanthellae translocations aims to: (a) evaluate the effects of optimum enriched zooxanthellae to translocation on the coral polyp tissues after bleaching and (b) examine the effect of translocation to the survival and growth of coral.

MATERIAL AND METHOD

The material is zooxanthellae A, B and C clades, as well as coral *Gonistrea aspera*.

Setup Equipment

Incubation container which irrigated with the results of mass growth and genetic diversity of zooxanthellae type A, B and C clades. The maintained media are temperature less than 25 °C and green lighting with the mass growth technique following the way of Purnomo (*in progress*). 1 ton mass zooxanthellae tank that has been purified is connected to the coral incubation tank of 5 tons. In the coral incubation tank is made a collecting tab of a half ton. From the collecting tab is pumped back to the mass tank.

Execution of the Translocations Research

The research starts with acclimatization, subsequently shock temperature application, zooxanthellae enrichment and growth experiment. Acclimatization is done in 10 tons tank for 2 days. The acclimatized number of *Goneastreaa aspera* colonies as many as 67. Shock temperatures impose to the specimen of *Goneastrea aspera on* 36 °C for 6 hours (Purnomo *et al.,* 2010). Initial zooxanthellae used as an enrichment factor are: (a) A Clade of 18.26 x 10^5 ind L^{-1}, (b) B Clade of 21.19 x 10^5 ind l^{-1}, and (c) C Clade of 19,84 x 10^5 ind l^{-1}. In the estimated zooxanthellae infection to the coral polyp tissues has happened (phase I in 10 days and phase II in 17 days), separation be conducted for 5 coral sample of *Goneastrea aspera* from the incubation media into natural environment of the southern of Panjang Island Jepara to examine its growth. The growth study was done using pigmentation the technique of *Alyzarin Red* (Sya'rani, 1993).

The Independent Test

Measurement of the colony growth was done by measuring the length of $CaCO_3$ deposit on each corralite of 15 specimen *Goniastrea aspera.*

Measurement of Zooxanthellae consentration was conducted in three weeks period. Initially decalcification was performed by taking some specimen to be dissolved in a solution of 5% HCl concentrate for 48 hours (Nordemar *et al.,* 2003). After decalcification, the tissue rinsed and homogenized in 10 mL distillated water for 10-15 minutes with speed 3,000 rpm. Supernatant containing zooxanthellae then analyzed using Sedgwick rafter.

DNA Diversity

Test of zooxanthellae DNA diversity using PCR–RFLP (*Restriction Fragment Length Poloymorphisme*) method. Each clade of Zooxanthellae is bred up to 1 liter with high density and be filtrated gradually with the speed of 3,000 rpm for 10 minutes. Deposits of zooxanthellae are separated from the extract liquid with pipette, then be resuspention with zooxanthellae isolation buffers solution {0.4 M NaCl; 40 mM $MgSO_4$; 10 mM EDTA; 20 mM Tris-HCl; pH 7.6; 8 mM dithiothreitol; 0.05% (v/v) and Tween-20 (pltyoxyethylene-sorbitan); Sigma Chemical Co}, of 10 mL. Zooxanthellae cells be rinsed again with a DNA isolation buffers (DNAB : 0.4 M NaCl; 50 mM EDTA, pH 8.0) in a microcentrifuge tubes and be resuspention in 0.4 ml DNAB with sodium dodecyl sulfat (SDS) as final consentration 1% (v/v). The liquid is then heated at 65 °C for 60 minutes, then be incubated by adding Proteinase K (Boehringer Mannheim Biochemicals) 0.5 mg ml^{-1} on the temperature of 37-45 °C for 6 hours. Nucleic acids of such material be precipitated with the addition of 100 µL 0.3 M sodium asetat, and be precipitated again by ethanol. The residue is resuspention with 50 µl of pure water and stored in temperature -20 °C. After that DNA amplification is done according to techniques of Rowan dan Power (1991) using universal primer ss5 (5'–GGTTGATCCTGCCAGTAGT CATATGCT TG-3') and ss3 (5'–GCAGTTATA-ATTTATTTGATGGTCACTGCTAC- 3'). Reagent to analise PCR containing 4 µl DNA template (target), 10 µL 10 x buffer solution PCR (1 M Tris-HCl, pH : 8.3); 6 µL of 25 mM $MgCl_2$; 1.5 mM total dNTPs, 30 pmol from each primary and 0.5 µL *Taq*polymerase (5 unit μL^{-1}); overall is 100 µL. The process of amplification using the DNA thermal cycler (express PCR, Hybaid) follows the setting temperature profile: 94 °C for 1 minute; 65 °C for 2 minutes and 72 °C for 3 minutes. Overall, the treatment was implemented in 30 cycles. The enzyme used in bond termination of base on the tested sample using Haelll types. The result of restriction using Haelll enzyme obtained DNA ribbons with polymorphic DNA size to measure the base pair (bp) value of tested Zooxanthellae. The value of base pair is then tested its similarity to the base pair value obtained from GenBank through phylogenic analysis using SAS 9.1 program.

Water quality Analysis

Water quality variables measured are temperature, salinity, dissolved oxygen and pH which measured on a daily based for the incubation tank and a weekly based at the sea, while ammonia and nitrite are measured either in the tank or at the sea weekly.

Data Evaluation

Evaluation of zooxanthellae translocation in the coral polyp tissue are tracing by the growth, survival, density development of zooxanthellae, infection of zooxanthellae, replacement profile of zooxanthellae hystologically. Synergisme among collected data will be a benchmarks for the success of translocation.

RESULT AND DISCUSSION

Results

Zooxanthellae Diversity and its Replacement on Polyp' tissues

Zooxanthellae DNA testing is done in two incubation stages, i.e. 10 days incubation (Test phase 1) and 5 days (Test phase II). The analysis of DNA diversity in 10 days incubation period show that at the beginning of bleaching, quality of *Goneastrea aspera* DNA is homogeneous i.e. clade A; in the enrichment media of clade A occurs natural infection of clade B and C; in the enrichment media of clade B occurs natural infection with clade D and in the enrichment media of clade C

occurs natural infection with clade B; on the managed media (laboratory) does not occur infection; there is consistency on clade A and natural infection happens in the depth of 5 meters but not in the depth of 3 meters. The 10 days incubation has not shown artificial infection potency.

Table 1. DNA Diversity of *Goneastrea aspera* Polip after *Bleaching* in the Zooxanthellae enrichment media within 17 days incubation period

Incubation time	Media based on Clade type	Incubation Location	DNA Diversity DNA in the sample : 1	2	3	4
Initial	After bleaching	Natural waters	A	A	A	A
weeks III	**A**	Natural waters	A/D	A	A/B	A
		Laboratory	A	A	A	A
	B	Natural waters	A/B	A/B	A/B/C	A/B
		Laboratory	A/B	A/B	A/B	A
	C	Natural waters	A/C	A	A/B/C	A/B/C/D
		Laboratory	A	A/C	A/C	A/C
weeks VI	**A**	Natural waters	A	A	A/C/D	A/B
		Laboratory	A	A	A	A
	B	Natural waters	A/B	A/B	A/B/D	A/B/C
		Laboratory	A/B	A/B	A/B	A/B
	C	Natural waters	A/C	A/B/C	A/B/C/ D	A/B/C
		Laboratory	A/C	A/C	A/C	A/C

Description : sample is incubated in the coral reefs natural waters at the Southern of Panjang Island, Jepara at the depth of 3-5 meters

The 17 days incubation result in translocation pattern. The phylogenic analysis toward bp diverse is shown in illustration 1. Extension of incubation time is based on the results of phase 1 that natural infection indicate potential infection time in the managed environment, and give flexible time for coral to make internal regeneration process. In these conditions is expected the recognition process happen longer to provide a more random chance for its external clade to translocate. Natural incubation at this stage is done at a depth of 5 meters in consideration of in that depth indicate a natural infection as obtained in the stage I test. The natural infection indicated that prospect of recovery is higher, so give a bigger survival of the tested animal. The recapitulation of infected symbion diversity can be seen on the table as follows.

Based on the results of research in two phases as presented above, the research object is *Goneastrea aspera* coral, a type that not has specific infections preference to a certain symbion clade. The tiered identification against *Goneastrea aspera* as informed on the study of clade diversity as on the growing test of zooxanthellae, a clade type found as zooxanthellae symbion are clade A and B. After a bleaching process, then obtained mono symbion i.e. A that traced from the 4 examples. The translocation process gave an explanation that positive translocation process occurs both in the natural environment and laboratory. Infection from the natural environment characterized by non incubation symbion type.

Development of Zooxanthellae Concentration Level after Translocation

Development of zooxanthellae in the host *Goneastrea aspera* during incubation in natural waters is depicting in illustration 2. A statistical analysis of zooxanthellae density in the week 15 and 17 showed that zooxanthellae development do not differ between the enriched clade resources ($\acute{\alpha} < 0.01$). These developments indicated that after bleaching *Goneastrea aspera* can still survive and are capable of carrying out the physiologic activities. It is also supported by the survival rate examples during incubation.

The observations indicate that all samples that incubated in natural waters are survive (the survival rate is 100%). with its ability to survive and the presence of physiological activities during the incubation period then after bleaching of the *Goneastrea aspera* specimen, the process of cells recovery is possible happen followed by significant growth of zooxanthellae.

The Growth of Goneastrea aspera

The growth measurements of *Goneastrea aspera* after incubation within 17 weeks at 15 corallites in every five specimen on average show that enrichment source of clade A is 5.663 mm; Clade B 5.3 mm and Clade C is 5.563 mm. Based on the one way test toward the growth phenomenon of a clade show that there is no difference on the *Goneastrea aspera* growth ($\sigma < 0.01$). Its means that difference infection of zooxanthellae has no influence to the coral growth.

.Discussion

In some cases, the host and symbion partner occurs in varying forms as response from the environment. Two environmental variables that are correlated with the distribution of specific symbion-host partner are temperature and is light. Rowan and Powers (1991) found symbion relationship variation in *Montastrea* on several temperature and light gradients. Endosymbion clade A and B found in the shallow waters < 6 m, whereas symbion clade C in the deep waters. A

study on *Symbiodinium* diversity at Great Barrier Reef (LaJeunesse *et al*., 2003) identify 9 host species have varied symbion in the depth of 10 m and 3 m. *Stylopora pistillata* have simbiose with clade C1 (depth < 3 m), with clade C27 (depth 10 m). Iglesias-Prieto and Trench (1997) informed that coral reefs in the South Pasific on the shallow waters 0-6 m is dominated by *Pocillopora verrucosa,* while in the deep waters (6-14 m) dominated by *Pavona gigantean.* Symbiodinium *clade D1* was found in *Pocillopora verrucosa*; wgereas clade C1 in *Pavona gigantean.*

Another studi explained that coral colony symbion in shallow waters only happened in single host colony. This is emphasized the argument that symbion distribution is a response of changing the light rate (Rowan *et al*., 1997). In single colony of *Montastrea anularis* and *M. faveolala*, clade A and B are infected with a high light, whereas Clade C is found in a covered area (Rowan *et al*., 1997). Van Oppen *et al*. (2001) also investigated colony structure on symbion population using diverse light intensity. They notice that in *Acropora tenuis* type C2 founded in an exposure to the light, while C1 founded in a covered area.

Correlation of the environment parameters to the symbion distribution prompt to the physiological difference among symbion type. This condition will affect diversity of the host – symbion partner. Characteristics of physiological response from various *Symbiodinium* taxis to the differences of environmental parameter is growth stage, but it appears that this physiological variation persists and got a response at least in some host – symbion partner. Changing type of *Synbiodinium* in its' respond to the environmental parameter is varied (Iglesias-Preto and Trench, 1997; Kinzie *et al*., 2001; Warner *et al*., 1996) as its' tolerance to temperature (Kinzie *et al*., 2001; Perez *et al*., 2001; Rowan, 2004). *Montipora digitata* which resistant to *bleaching* contain symbion type C15 (based on ITS2); while another *Montipora bleach* faster with clade C (LaJeunesse *et al*., 2003). Some reseachers reported that *Symbiodinium* clade D tolerant to heat (Fabricus *et al*., 2004; Rowan, 2004). Those studies report that symbion clade D have dominant simbiose in the field with a routine high temperature (Fabricus *et al*., 2004) and in the former bleaching reefs (Glynn *et al*., 1984).

Study of Gleason (1993) shown that after *bleaching* in Karibia on 1987, the biomass tissues of *Montastrea anularis* recover slowly compared to the growth of symbion algae zooxanthellae. Whilst Szmant and Gassman (1990) discovered that most coral in Carysfort Reef have normal density zooxanthellae of 1-3 x 10^6 indv cm^{-2} within 10 months period after *bleaching*. Within 1 year periods, the variation of coral bleaching cause in coral growth fluctuation. The circumstance where coral-reef can survive in high temperature are characterized by: (a) parsial *bleaching*, in which zooxanthellae live normally in polip tissues, in term of position and its number (Porter *et al*., 1989); (b) zooxanthellae repopulation go on rapidly (Jaap, 1985). Whilst total bleaching that occurred in the experiment will significantly causing corals death. The incident also happened during this research.

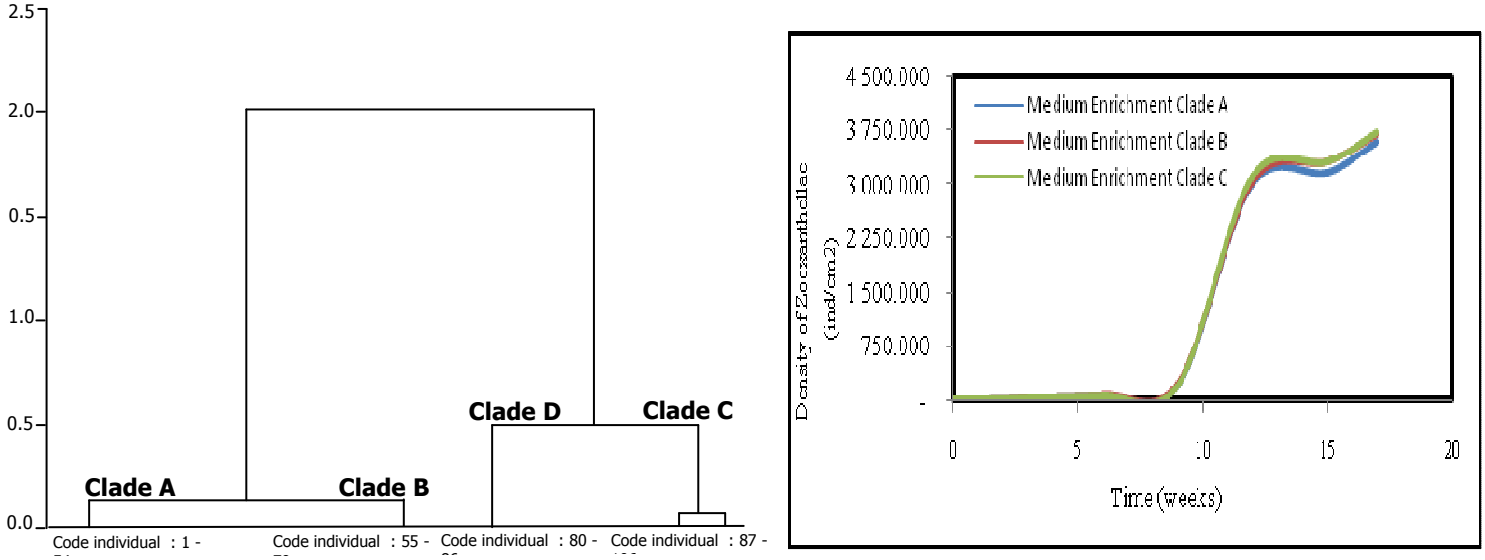

Figure 1. Zooxanthellae growth of *Goneastrea asprea* within incubation period at natural waters

Imimmediately after coral bleaching, symbiotic invertebrate perform photosynthetic and nutritional activities normally (Cole and Jokiel, 1978; Glynn *et al*., 1984; Hoegh-Guldberg and Smith, 1989). On the contrary it's growth will stop when bleaching or influence factors goes on. Other information stated that bleaching can cause selective mortality and give pressures on an affected species. Gleason (1993) reported that coral colonies that suffer complete *bleaching* komplit on April 1991 at Moorea Island are not experiencing growth and all die on August 1991. Coral colonies that suffer parsial *bleaching* has a full recover ability. If immense *bleaching* occurs in high frequency, this will firmly determine species composition and be the source for space provision for a new recruitment. This will cause the occurrence of new space that had low competition between species, particularly a resistant species toward bleaching Gleason (1993). In more specific level, when the bleaching level varied greatly among nearby species, there are great opportunities to affect genotype or vulnerability variations. *Bleaching* can affect coral community structure if secondary influence happen, as an increasing algae abundance

and sea urchin, that resulting in recovery of a fragile coral. Hoegh-Guldberg and Smith (1989) found that coral *recovery* after *bleaching* from temperature influence of 32 °C for 7 hours is 23 days.

Code of individual :

No	Code of Individual	No	Code of Individual	No	Code of Individual	No	Code of Individual	No	Code of Individual
1	T2:O1	23	T2 : 3-Bmb7	45	T2 : 6-Bpa6	67	T2 : 6-Cpa11	89	T2 : 3-Cmb5
2	T2:O2	24	T2 : 3-Cpa1	46	T2 : 6-Bmb7	68	T2 : 6-Apa7	90	T2 : 6-Apa4
3	T2 : 6-Apa1	25	T2 : 3-Bmb1	47	T2 : 6-Bpa8	69	T2 : 6-Bpa2	91	T2 : 6-Bpa10
4	T2 : 6-Apa2	26	T2 : 3-Bmb3	48	T2 : 6-Bpa3	70	T2 : 6-Bmb2	92	T2 : 6-Cpa8
5	T2 : 6-Apa3	27	T2 : 3-Bmb5	49	T2 : 6-Apa6	71	T2 : 6-Bmb6	93	T2 : 6-Cmb2
6	T2 : 3-Apa6	28	T2:O3	50	T2 : 6-Bmb1	72	T2 : 6-Cpa4	94	T2 : 6-Cmb6
7	T2 : 3-Bpa1	29	T2:O4	51	T2 : 6-Cpa3	73	T2 : 6-Cpa7	95	Clade C : EU333740
8	T2 : 6-Amb1	30	T2 : 3-Apa1	52	T2 : 6-Bmb3	74	Clade B : EU449079	96	T2 : 6-Cpa2
9	T2 : 6-Amb2	31	T2 : 3-Cpa7	53	T2 : 6-Cpa1	75	T2 : 3-Bpa4	97	T2 : 3-Cpa2
10	T2 : 6-Amb3	32	T2 : 3-Cpa3	54	T2 : 6-Bmb5	76	T2 : 3-Bmb2	98	T2 : 3-Cmb3
11	T2 : 6-Amb4	33	T2 : 3-Cpa4	55	T2 : 3-Apa5	77	Clade B : EU449072	99	T2 : 3-Cmb7
12	T2 : 6-Bpa1	34	T2 : 3-Cmb1	56	T2 : 3-Bpa6	78	T2 : 6-Cpa6	100	T2 : 6-Cpa5
13	T2 : 3-Bpa3	35	T2 : 3-Cmb2	57	T2 : 3-Bmb6	79	Clade B : EU449066	101	T2 : 6-Cmb4
14	T2 : 3-Bpa4	36	T2 : 6-Cmb1	58	T2 : 3-Cpa5	80	T2 : 3-Apa2	102	T2 : 6-Cpa12
15	T2 : 3-Bpa1	37	Clade A : EU449053	59	T2 : 3-Bpa2	81	T2 : 3-Cpa10	103	T2 : 6-Cmb8
16	T2 : 3-Bpa2	38	T2 : 6-Cmb7	60	T2 : 3-Bpa9	82	Clade D : EU333707	104	Clade C : EU333735
17	T2 : 3-Bpa3	39	T2 : 6-Cmb3	61	T2 : 6-Bmb8	83	T2 : 6-Bpa7	105	T2 : 3-Cpa6
18	T2 : 3-Bpa5	40	Clade A : EU449026	62	T2 : 6-Bpa9	84	T2 : 6-Apa5	106	Clade C : EU333737
19	T2 : 3-Apa3	41	T2 : 6-Cmb5	63	T2 : 3-Bmb4	85	T2 : 6-Cpa9		
20	T2 : 3-Apa4	42	T2 : 6-Cpa10	64	T2 : 3-Cpa8	86	Clade D : EU333708		
21	T2 : 3-Cmb4	43	T2 : 3-Cmb6	65	T2 : 6-Bpa4	87	T2 : 3-Bpa7		
22	T2 : 3-Bpa8	44	T2 : 6-Bpa5	66	T2 : 6-Bmb4	88	T2 : 3-Cpa9		

Illustration 1. DNA connection link of several zooxanthellae types based on Cluster Analysis DNA at diversity test phase of the *Goneastrea aspera* zooxanthellae polyp tissues after bleaching in 17 days incubation time on the zooxanthellae enrichment media. Clade A accessed from the GenBank by code EU449053 and EU449026; Clade B are EU449070, EU449072 and EU449066; Clade C are EU333740, EU333737 and EU333735; Clade D are EU333707 and EU333708. T2 : the 2nd test of DNA diversity; 0 is initial test l; 3 : 3rd weeks test and 6 : 6th weeks test. A, B and C are enrichment media of Clade A, Clade B and Clade C toward *Goneastrea aspera* after recovery; pa is incubation at the natural waters; bm is incubation at the laboratory, pa and 1, 2 is number of tested samples

Gleason (1993) stated that the rest of zooxanthellae after *bleaching* in intraselluler which still rich in nutrients will relatively grow rapidly compared to the reef in big density. In spite of this, it is still depend on the conditional status of zooxanthellae itself. As stated by Glynn's (1984), coral die after suffering parsial *bleaching* may survive in assumption that zooxanthellae has not extremely by the temperature and seluler polyp condition. Nevertheless if temperature or other external factors influence constantly that resulting in uncontrollable environmental conditions of the coral polyps, eventhough physiologically zooxanthellae still tolerant, this will cause zooxanthellae leave away from the polyps and coral will die (Darius *et al*., 1998).

The natural incubation condition showed the maintain level of homogeneity is in low deviation. Temperature is 25.4-26.4 °C a little bit above the laboratory range of 20.3-23.8 °C, salinity is 32.0-33.2 ppt, the respective highest levels of ammonia and nitrite are 0.0014 mg L^{-1} and 0.0005 mg L^{-1} and dissolved oxygen is 4.5-5.66 mg L^{-1}. The level of homogeneity in some environmental variables allows zooxanthellae to grow and do physiological repairmen to fill in coral polyps tissues structures of *Goneastreas aspera*.

Zooxanthellae in the polyp basically have a stable number, although keep on changing due to individuals exchange. This phenomen will occur when variation of the environmental conditions is too wide, same as to the ongoing monitoring during the study. Zooxanthellae density in nature is fluctuated because of the environmental factors effect. The study on the process of zooxanthellae placement in the polyp tissue, followed by its growth reflect the recovery mechanism of the tested sample from the pressure on temperature. Synergism of both consistently supported by two sequence aspects, which are some type of clade found within the maintenance time span on two incubation types and coral ability to grow.

As has been known that every hermatyphic corals colony containing zooxanthellae that live as symbion to the coral colony. Zooxanthellae that live in coral colonies is producing carbon as well as calcium carbonate (lime) or coral that can build lime coral. Sya'rani (1993) stated that zooxanthellae is an essensial factor in the calcification process or lime production for *hermatypic corals* or *reef building corals*. calcification rate is differ in each spesies. Some spesies growth very fast, up to > 2cm $month^{-1}$ (on *branching corals*), however some species (on *massive corals*) has slow growth, > 1 cm $year^{-1}$.

CONCLUSION

Translocations of *Goneastrea aspera* zooxanthellae after bleaching in the laboratory occurred on the day of 17, while in natural waters may occur earlier; Translocations is restrained by environmental parameter, particularly temperature that give positive response to the relocation process and the growth of zooxanthellae in *Goneastrea aspera* polyp tissues and recognition processes, resettlement and zooxanthellae growth in *Goneastrea aspera* polyp tissues allows to grow normally in natural media.

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