Are Zooxanthellae Really Sensitive? Response of Zooxanthellae Size Exposed to Several Pollutants

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Abstract

Pollution is one of the important issues faced by marine resources including zooxanthellae, which is known to be very sensitive to environmental changes. Some pollutants have been reported to have adverse effects on zooxanthellae, however, their sensitivity in regards to changes on cell size of these algae has not been widely explored. This study examined the effects of pollutants on the sensitivity of zooxanthellae through changes in size. Zooxanthellae were isolated from corals Porites lutea, Acropora aspera, and Montipora digitata collected from Panjang Island, Jepara, Indonesia. These algae were exposed to pollutants i.e. heavy metals (Cu, Cd, Pb) and nutrients (ammonium and phosphate) at concentrations of 5,10,15 ppb and 5,10,15 µM, respectively. Zooxanthellae size were measured five hours after pollutants exposure. The results showed that all treatments reduced the size of zooxanthellae. Algae isolated from P. lutea are the least affected by pollutants and the highest percentage cell size reduction was found in phosphate treatment. However, reduction on the size of algae were not statistically significant. These results indicate that in relation to reduction in the size, zooxanthellae are not sensitive to pollutants.

Keywords: *Zooxanthellae, pollutants, sensitivity, size reduction*

Introduction

Zooxanthellae are known as single cell algae that live in association with various invertebrates including mollusks and corals. These algae are capable of supplying energy from the results of photosynthesis that has a very important role for the life of the host (Dubinsky & Jokiel, 1994). Therefore, the survival of the host is highly dependent on the conditions of their endo-symbiotic zooxanthellae (Fitt, 1985; Fitt *et al*., 1986; Hill, 1996). In addition, zooxanthellae are also sensitive to changes in water quality, including changes in water temperature that caused by temperature changes and the presence of pollutants (Hoegh-Guldberg & Smith, 1989; Jones & Belkermans 2010; Ambariyanto, 2011; Pantaleo *et al.,* 2016).

Pollution can be cause by both natural and anthropogenic factors and is a known problem faced by coastal and marine areas of the world. It can be in the form of rise in temperature, nutrients, hydrocarbons, surfactants, and heavy metals (Edinger *et al*., 1998; Birch, 2000; Williams *et al*., 2000; Becker *et al*., 2008; Ahmad, 2012, Suryono and Rochaddi, 2013). These pollutants affect either directly or indirectly to a variety of organisms, both individually, population or community (Gray, 1992; Edinger *et al*., 1998; Meyer-Reil and Köster, 2000), The impact is highly dependent on the sensitivity of the organism to pollutants (Ambariyanto and Hoegh-Guldberg, 1996; Borja *et al*., 2000). Several scientists have reported changes in zooxanthellae which include decreasing the cell density, chlorophyll, mitotic index, and cell damage (Mercier *et al*., 1997; Ferrier-Pages *et al*., 2001; Owen *et al*., 2002; Cervino *et al*., 2003; Ambariyanto, 2011; Stoner *et al.,* 2016).

Various changes in the zooxanthellae, will affect the rate of photosynthesis of algae (Elfwing *et al*., 2002; Owen *et al*., 2002). In the event of a decrease in the rate of photosynthesis, it will also decrease the amount of energy that is translocated by zooxanthellae to the host (Edmunds and Davies, 1986) and affect the growth rate of the host. The amount of energy translocation is also directly dependent on the density of zooxanthellae. Jones

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and Yellowlees (1997) concluded that the cell size and space will determine the density of zooxanthellae within the host. Although many researchers are reporting sensitivity of zooxanthellae to changes in water quality, the response of various pollutants on the cell size of these algae has not been explored.

Materials and Methods

Zooxanthellae Isolation

Zooxanthellae were isolated from three different corals including *Acropora aspera*, *Porites lutea*, and *Montipora digitata* collected from Panjang Island, Central Java, Indonesia. Isolations of zooxanthellae were done by filtered seawater spray method (Berkelmans *et al.*, 2006). Each coral was sprayed with high water pressure sprayer to let the zooxanthellae expelled from coral tissue within a plastic bag. Zooxanthellae solution from the bag was collected in separate beaker glass for each corals. In order to get clean zooxanthellae, the solution from each corals then was filtered by using 15μm plankton net.

Pollutant treatments

Several pollutants used in this experiment were heavy metals (Pb, Cd, and Cu) with concentration of 5, 10, 15 ppb and nutrient (ammonium, phosphate) with concentration of 5, 10, and 15 μM. Zooxanthellae solutions without any pollutants were used as control. Each treatment was done in 50 ml beaker glass filled with 15 ml filtered seawater with three replications. The density of zooxanthellae used in this experiment was 20 cells.ml^{-1}. These experiments were done in ambient water temperature of 29^{o}C.

Size measurement

Sampling of zooxanthellae were done 5 hours after the start of pollutants exposure. A total of ten zooxanthellae were randomly sampled for each beaker. Measurement of these zooxanthellae size was done by a binocular microscope (x400) equipped with micrometer (Wilkerson *et al.*, 1988). Data of zooxanthellae size were analysed using analysis of variance (ANOVA; SPSS).

Results and Discussion

One of the important problems occurring in coastal and marine areas is the increased in pollution (Islam and Tanaka, 2004). Marine ecosystem is heavily influenced by human activities (Halpern *et al.*, 2008) including pollution that has a negative impact on marine resources. Impacts from pollution can occur at the cellular, individual, population or community level (Gray, 1992). Even an ecosystem can be totally damaged as a result of environmental pollution.

The results showed that all types of pollutants causing a decrease in the size of zooxanthellae in line with the increasing concentration of pollutants. See Table 1 and 2. These results confirmed that zooxanthellae are sensitive to changes in the quality of surrounding waters. Therefore, changes to any of the water quality parameters will likely affect the zooxanthellae. Ambariyanto and Hoegh Guldberg (1996) reported that zooxanthellae are sensitive to and response faster to changes in the surrounding water quality than their host animals.

However, ANOVA test results on zooxanthellae size showed no significant difference (P>0.05) between the control and the treatments. This shows that statistically the size of the zooxanthellae isolated from all corals is not affected by pollutants. A possible explanation is the fact that in this study the exposure period of zooxanthellae to pollutants is relatively short (5 h). It is also possible that higher concentrations of pollutants will result in a significant effect on zooxanthellae as has been reported in various waters of the world (Beiras *et al.*, 2003; Fatoki and Mathabatha, 2004).

Zooxanthellae size also affects the amount of chlorophyll *a* in each cell. This will affect the ability of zooxanthellae to perform photosynthesis. Some reports indicate a decline in the rate of photosynthesis as a result of changes in water quality parameters (Elfwing *et al.*, 2002; Owen *et al.*, 2002). This condition will affect the corals due to the decreasing amount of energy that can be translocated.

The sensitivity of the organism strongly influences the impact of pollutants. Based on the percentage of cell size reduction (Tables 3 and 4), the zooxanthellae of *P. lutea* showed minimal response compared to those isolated from *A. aspera* and *M. digitata*. However, this does not necessarily mean that zooxanthellae from this coral are more resistant to environmental changes than zooxanthellae from other corals. Ambariyanto (2012) reported that zooxanthellae isolated from *A. aspera* were the most resistant to hydrocarbon and surfactant. It is possible that zooxanthellae will respond in different ways to different forms and intensities of different pollutants. Some studies showed sensitivity variations of massive corals, particularly on the rate of calcification, to temperature changes (Carricart-Ganivet *et al.*, 2012), or to ocean acidification (Comeau *et al.*, 2014).

Although Figure 1 showed that phosphate gave higher percentage size reduction of zooxanthellae compared with other treatments, it is not statistically significant (*P*=0.93). Reports showed that nutrients, both phosphate and ammonium, have important roles in the photosynthetic processes of algae. Theodorou *et al.* (1991) found that phosphorus limitation will significantly reduce photosynthetic rate of algae. While Li *et al.* (2008) found that availability of external nitrogen source increased chlorophyll content within algae. However, when external nitrogen source is already in use, chlorophyll will be utilized as nitrogen source.

Many reports showed that zooxanthellae are sensitive to environmental changes. For example, changes in water temperature will induced bleaching (Hoegh-Guldberg and Smith, 1989), hydrocarbon and surfactant affects the density of algae (Ambariyanto, 2012); cyanide affects the density and mitotic index of zooxanthellae (Cervino *et al*., 2003); and herbicide affects the photosynthesis process (Owen *et al.*, 2002). These results really suggest that although zooxanthellae are known to be sensitive in response to environmental changes, however, size response is not showing this sensitivity. It must also be considered that this study was conducted on isolated zooxanthellae from its host. Nevertheless, this information is very important to be taken into account, especially in calculating the response of zooxanthellae *in hospite* to environmental changes.

Table 1. Size of zooxanthellae (µm: mean+SD) exposed to different concentration of heavy metal for 5 h

Pollutants	Host of Zooxanthellae	Treatments			
		Control	5 ppb	10 ppb	15 ppb
Cu	*Porites lutea*	9.11±0.93	9.00±0.71	8.60±0.55	8.60±0.89
	Acropora aspera	9.33±0.87	8.80±0.84	8.20±0.45	8.20±0.45
	Montipora digitata	9.44±0.88	8.60±0.89	8.20±0.45	8.20±0.45
Cd	*Porites lutea*	9.11±0.93	9.00±0.71	8.80±0.45	8.20±0.45
	Acropora aspera	9.33±0.87	8.60±0.55	8.20±0.45	8.20±0.45
	Montipora digitata	9.44±0.88	9.00±0.71	8.40±0.55	8.40±0.55
Pb	*Porites lutea*	9.11±0.93	9.20±0.45	8.80±0.84	8.20±0.45
	Acropora aspera	9.33±0.87	8.80±0.84	8.40±0.55	8.40±0.55
	Montipora digitata	9.44±0.88	9.00±0.71	8.60±0.55	8.20±0.45

Table 2. Size of zooxanthellae (µm: mean+SD) exposed to different concentration of nutrients for 5 h

Pollutants	Host of Zooxanthellae	Treatments			
		Control	5µM	10µM	15 µM
PO_4	*Porites lutea*	9.11±0.93	8.60±0.89	8.60±0.55	8.40±0.55
	Acropora aspera	9.33±0.87	8.40±0.55	8.20±0.45	8.40±0.55
	Montipora digitata	9.44±0.88	8.60±0.55	8.20±0.45	8.20±0.45
NH_3	*Porites lutea*	9.11±0.93	9.20±0.84	8.60±0.55	8.20±0.45
	Acropora aspera	9.33±0.87	8.80±0.84	8.20±0.45	8.20±0.45
	Montipora digitata	9.44±0.88	8.60±0.55	8.40±0.55	8.40±0.89

Table 3. Size reduction of zooxanthellae (%) exposed to different concentration of heavy metal for 5 h

Pollutants	Host of Zooxanthellae	Treatments			
		Control	5 ppb	10 ppb	15 ppb
Cu	*Porites lutea*	0	1.22	5.61	5.61
	Acropora aspera	0	5.71	12.14	12.14
	Montipora digitata	0	8.94	13.18	13.18
Cd	*Porites lutea*	0	1.22	3.41	10.00
	Acropora aspera	0	7.86	12.14	12.14
	Montipora digitata	0	4.71	11.06	11.06
Pb	*Porites lutea*	0	1.22	3.41	10.00
	Acropora aspera	0	5.71	10.00	10.00
	Montipora digitata	0	4.71	8.94	13.18

Table 4. Size reduction of zooxanthellae (%) exposed to different concentration of nutrients for 5 h

Pollutants	Host of Zooxanthellae	Treatments			
		Control	5μM	10μM	15 μM
PO_4	*Porites lutea*	0	5.61	5.61	7.80
	Acropora aspera	0	10.00	12.14	10.00
	Montipora digitata	0	8.94	13.18	13.18
NH_3	*Porites lutea*	0	1.22	5.61	10.00
	Acropora aspera	0	5.71	12.14	12.14
	Montipora digitata	0	8.94	11.06	11.06

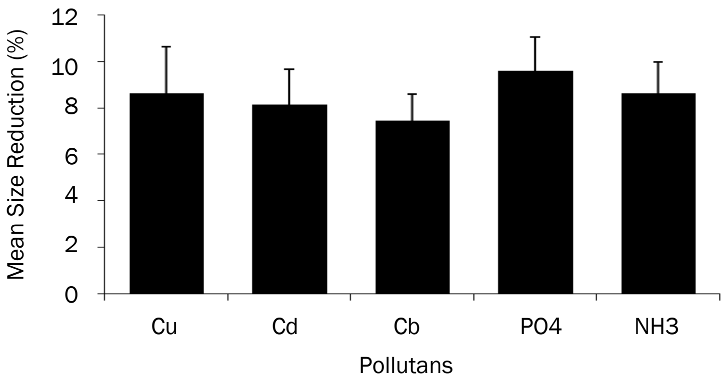

Figure 1. Mean size reduction (%) of zooxanthellae isolated from corals exposed to several pollutants for 5 h

Conclusion

This study confirms that zooxanthellae are insensitive, particularly with respect to changes in the size, to the types of pollutants given. The question whether zooxanthellae are really sensitive to environmental changes depends on the types of response given by symbiont, types of pollutants and their concentration level, and the period of exposure.

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