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## Zooxantella of Giant Clam as a sentinel organism to detect toxicity of lead

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**Abstract.** Zooxantellae is living symbionts in the body of giant clams. This research was conducted to determine the sensitivity of the loss of zooxanthellae (bleaching) from the body of giant clam as a tool to detect metal pollutants. Juvenile of giant clam was exposed to lead in concentrations of 0, 0.004, 0.008, 0.016, 0.032 mg/l for 24 hours. Zooxanthellae density data were analyzed with parametric anova. The zooxanthellae densities at treatments 0, 0.004, 0.008, 0.016 and 0.032 mg / respectively were 32,322,222.2, 32,488,888.89, 24,977,777.78, 17,676,666.67, and 14,542,222.22 cell/g mantle. Statistical analysis showed that there were significant differences in zooxanthellae densities of control with 0.016 and 0.032 mg/l treatments. There was no mortality of the juvenile of giant clams during the experiment. Therefore, it was concluded that the loss of zooxanthellae from giant clam's body could be used as a sensitive biomarker to detect lead metal toxicity. Zooxanthella of *Tridacna derasa* is also considered as a sentinel organism that is potential for ecotoxicological studies.

### 1. Introduction

Giant clams have a symbiotic relationship with one of the phytoplankton from the dinoflagellate group known as zooxanthellae. Zooxanthellae live in the extracellular mantle of giant clam tissue [1,2]. The giant clams provide a place to live for zooxanthellae and supply carbon dioxide and other compounds, which are metabolic products of the giant clams. Meanwhile, zooxanthellae, as photosynthetic organisms, give the results of photosynthesis such as glycerol and oxygen to the giant clam [3]. This symbiotic relationship has occurred since the giant clams were still in the veliger phase [3,4]

The giant clams inhabit in a coral reef environment in the tropic area and have a significant ecological role in coral reef habitats [5]. Coral reef ecosystems are stable environments that are vulnerable to environmental changes, such as the influx of pollutants that can damage the life of organisms on coral reefs, both directly and indirectly. For example, if there is an increase in temperature, zooxanthellae in the body of the giant clams can die or expelled into the environment [6,7] This process is known as bleaching [8]. In invertebrate, bleaching will result in a decrease in the rate of formation of tissue and skeleton, fecundity and survival [9]. The bleaching giant clam has decreased glucose concentration [9], which is a photosynthetic product of zooxanthellae, which will ultimately reduce growth.

In corals, Meehan & Ostrander, (1997) have examined the potential for bleaching as a biomarker from the occurrence of stress on corals [10]. *Galaxea fascicularis*, experienced bleaching, which was then followed by death after being exposed to Cu 0.1 mg/l for 24 hours [11]. Jones, (1997) concluded that the loss of zooxanthellae or bleaching could be used as a bioassay or biomarker to predict stress conditions on corals that are symbiotic with zooxanthella [8].



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To date, research on bleaching of the giant clams has rarely been carried out for the purposes of studies in the field of ecotoxicology, especially using the juvenile of giant clams. This paper will discuss the results of research on the bleaching of giant clam *Tridacna derasa* as a biomarker of lead exposure. Accordingly, in the future, we will be able to develop an ecotoxicological test using zooxanthellae of the giant clam, *T. derasa*, as sentinel organism based on this current research.

## 2. Materials and methods

### 2.1. Exposure experiment

This research was conducted at hatchery of the Faculty of Marine Science and Fisheries, Hasanuddin University in Barranglompo Island, Makassar. The test animal used in this study was juvenile of giant clams, *Tridacna derasa* with a shell length of 2 cm, obtained from the hatchery of PT Dinar Darum Lestari on Barrang Lompo island. Twenty giant clams were placed in a jar with one liter of seawater media. Test animals were exposed to series of lead concentration (Pb) 0 (control), 0.004, 0.008, 0.016 and 0.032 mg/l with three replications. The exposure experiment was performed for 24 hours. Water quality parameters and juvenile of giant clam were observed every four hours. The observed water quality parameters are temperature, pH, and salinity.

### 2.2. Survival rate

Survival rate is calculated using the formula below :

$$SR(\%) = \frac{N_t}{N_o} \times 100 \quad (1)$$

SR = Survival rate .

No = Number of giant clams at the start of the experiment.

Nt = Number of giant clams at the end of the experiment.

### 2.3. Density of Zooxanthellae

After being exposed for 24 hours, the juvenile of giant clam was sacrificed, and the mantle organ was dissected out. Before being crushed, the mantle organ was weighed to determine its weight as a basis for calculating the density of zooxanthellae. Thereafter, the fine mantle tissue was diluted with three milliliters of distilled water. After homogenizing, the solution of the mantle organ in distilled water was filtered using a 100-micron filter to separate the mantle tissue and zooxanthellae. Filtered media containing zooxanthellae were observed under a microscope with a magnification of 100 x using a haemocytometer. To estimate the density of zooxanthellae Hansen's formula is used [12].

$D = N \times \text{dilution} \times 10^4$ .

D = Jumlah zooxanthellae.

N = average number of Zooxanthellae counted by hemocytometer.

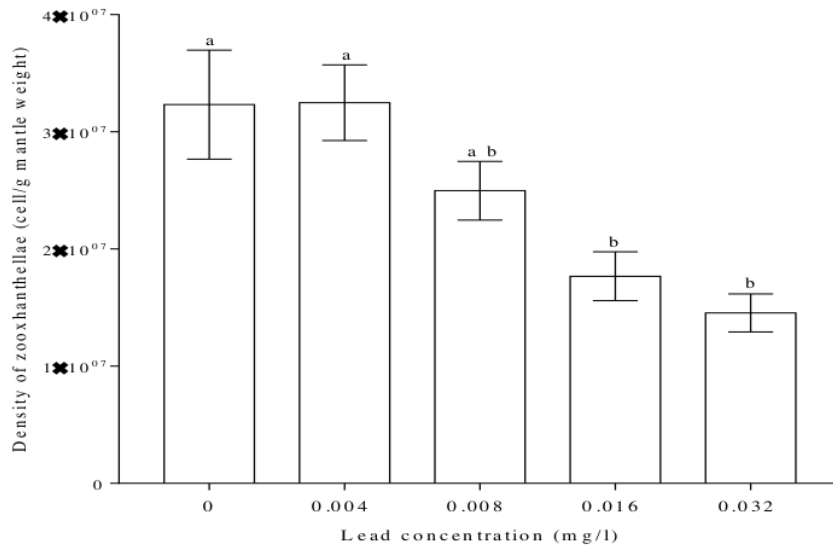
After that, the number of zooxanthellae was divided by the weight of the mantle tissue to obtain the density of zooxanthellae per weight of the mantle tissue.

### 2.4. Data analysis

Statistical data analysis was conducted on zooxanthellae density data in the juvenile of the giant clam mantle. In this experiment, no single juvenile of giant clam died. Hence this data was not analyzed by anova. Zooxanthellae density data were analyzed by parametric anova, since it was normally distributed and homogeneous. To analysis, the average difference between treatments, the Bonferroni test was used. Furthermore, the dose-response dependent tendency was determined using a linear regression test.

### 3. Results and discussion

From 24 hours of observation, no single dead of the giant clam juveniles were found in all treatments. LC<sub>50</sub> (24 hours) of Pb for *Modiolus philippinarum* is 13.545 mg/l [13]. For the juvenile scallop of Chile *Argopecten purpuratus*, the LC<sub>50</sub> (96 hours) Pb, which is 1.47 mg/l [14]. By considering the two LC<sub>50</sub> values, it can be said that it makes sense that the greatest concentration used in this study (0.032 mg/l) has not resulted in death in the giant clam juveniles.



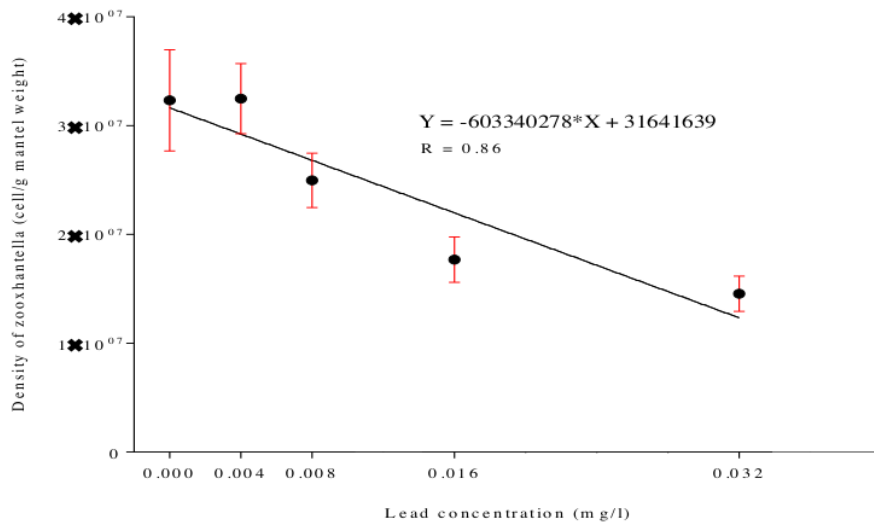
**Figure 1.** Effect of lead on density of zooxanthellae of *Tridacna derasa*. Bars are SEM. Different letters indicated significant differences statistically ( $p < 0.05$ ).

The zooxanthellae densities at treatments of 0, 0.004, 0.008, 0.016 and 0.032 mg/l were 32,322,222.2; 32,488,888.89; 24,977,777.78; 17,676,666.67; and 14,542,222.22 cell/g mantle respectively. In Solomon Island the zooxanthellae density of *Tridacna derasa* is 330,000 cell/g mantle [15]. The density of zooxanthellae of the giant clams used in this study, although in the treatment with the highest concentration (0.032 mg/l) was still more than the density of zooxanthellae of *Tridacna derasa* which is cultivated in Solomon Island [15].

Figure 1 shows a statistically significant reduction of zooxanthellae density at concentrations of 0.016 and 0.032 mg/l. This could be because of the loss of zooxanthellae from the giant clam's mantle due to death or suffered damage. Kuzminov, et al., (2013) exposed Zooxanthellae, *Symbiodinium* spp from coral to 1, 5, 10, 25, 50, 100, 200  $\mu$ M of plumbum (Pb) which is equivalent to 0.21, 1.04, 2.07, 5.175, 10.35, 20.70, 41.40 mg/l, and the results showed that Pb decreases the rate of transport of photosynthetic electrons which have implications for decreasing growth rates [16]. Pb also degrades photochemistry proteins in photosystem II, namely PsbA and PsbD, before it degrades rubisco and ATP synthase enzymes in zooxanthellae [16]. When exposed to Cu 50 mg/l, zooxanthellae of corals die, and at exposure to 20 and 40, the growth rate decreases [17]. Zooxanthellae from giant clam *Tridacna gigas* and *Hippopus hippopus* experienced decreased respiration, which resulted in decreased production when exposed to 5  $\mu$ g Cu l<sup>-1</sup> [18]. Van Dam and co-worker (2011) stated that the inhibition

of photosynthesis in zooxanthellae can cause a decrease in production and will eventually remove zooxanthellae from the host's body or known as bleaching [19].

By comparing with the survival rate, the loss of zooxanthellae from the host mantle is more sensitive in detecting lead toxicity. Bleaching or the loss of zooxanthellae from the host body, according to Jones (1997), provides a tool to assess the response of an organism that is symbiotic with zooxanthellae to natural and un-natural stresses that range from no effect to death. Therefore the release of zooxanthellae has the potential as a biomarker for detecting environmental stresses caused by metal pollutants and other pollutants in coral reef habitats, including the giant clam, *Tridacna derasa* [8].



**Figure 2.** Linear regression of zooxanthellae density and serial dilution of lead.  $R^2 = 0.72$  ( $p < 0.05$ ).

The sensitivity of bleaching as a biomarker is confirmed by the regression curve in Figure 2, which shows the value of a strong correlation coefficient of 0.86 (table 1) and a coefficient of determination of 0.72, which concludes 72% of zooxanthellae density is influenced by the lead concentrations. This means that the higher the lead concentration, the lower the zooxanthellae density in the host mantle (Figure 2).

In this study, the measured water quality parameters were pH, temperature, and salinity, which were 8,  $28.42 \pm 1.40$  °C, and  $32.03 \pm 0.167$  o/oo, respectively. These parameters are still in accordance with the conditions needed by the giant clam to grow optimally. Giant clam, *Tridacna derasa*, which is cultivated on Solomon Island has the following water quality conditions: 24-28 °C, salinity between 34 and 36 o/oo, and pH between 8.0 and 8.4 [15].

**Table 1.** Correlation coefficient [20].

R coefficient value (positive or negative)	Meaning
0.00 – 0.19	Very weak
0.20-0.39	Weak
0.40-0.69	Moderate
0.70-0.89	Strong
0.90-1.00	Very strong

#### Acknowledgment

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