

Original article

## Vitamin B<sub>12</sub> effect to reduce turbidity stress on *Moina macrocopa*

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**Keywords:** Turbidity; Vitamin B<sub>12</sub>; *Moina macrocopa*.

Received: 18 July 2017 / Accepted: 16 September 2017

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### Introduction

In recent years, there has been an increasing concern that climate alteration has impact on the amount, intensity, and frequency of precipitation which leads to extreme weather [1-3]. Consequently, the period and severity of rainfall become inevitably and might lead to the effects, such as runoff and flood. A sudden environmental change, during short period of time, such as flood, usually associated with loading of suspended sediment that effect to the turbidity of water [4,5].

Suspended sediment has correlation with turbidity [6] which might have both an adverse and beneficial consequences to zooplankton as filter-feeder. Several studies had been conducted and proved that continuous exposure of suspended sediments to cladoceran had related to effectivity of food assimilation [7-9] which is affected the survivorship and growth.

Sudden change in turbidity condition during short-period of time is necessary to be assessed considering the occurrence of heavy rain or flood in natural environment and it is assumed to affect the fitness of zooplankton. Water-soluble vitamin, vitamin B<sub>12</sub>, is expected have a role in relieving such stress, considering the vitamin have a vital role in normal development and growth [10-12]. Thus, this study attempt to assess one of water-soluble vitamin, vitamin B<sub>12</sub>, to overcome effect of stress caused by sudden turbidity change in *Moina macrocopa*.

### Materials and methods

The test organism, *Moina macrocopa*, were obtain from cyst and cultured in our laboratory using aerated tap water in 10L polycarbonate tank. For experimental purpose, selection were carried out to avoid dissimilarities result. The neonates hatching from the female then also isolated until become adult and giving the offspring, and finally we used the offspring for experiments. In total, there were 144 offspring needs for experiments (6 turbidity level × 4 vitamin B<sub>12</sub> doses × 6 replicates).

The offspring were fed by *Chlorella vulgaris* (Gene

Bank of National Research Institute, Mie, Japan) free of vitamin B<sub>12</sub> which is cultured continuously using modified f/2 medium (we removed vitamin B<sub>12</sub>). Method of food stock preparation for animal test were adopted from OECD [13]. To prepare vitamin B<sub>12</sub> solution, crystalline cyanocobalamin (Wako Pure Chemical Industry, co. Ltd, Japan) were diluted with distilled water until desired doses are obtained (0 µg/L, 5 µg/L, 15 µg/L, and 25 µg/L).

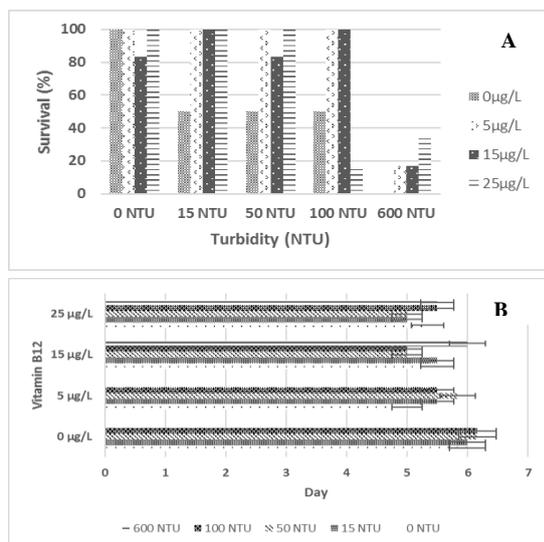
The offspring were placed individually into six-well microplate. The medium in each well contain of 4ml aerated tap water, 1ml of *Chlorella vulgaris* (~10<sup>5</sup> cells/ml) and 1ml of vitamin B<sub>12</sub> based on the dose applied. The neonates gradually exposed to different turbidity levels (0, 15, 50, 100, and 600 NTU) for 3 hours by replacing 90% of the initial medium with kaolin solution using pipette. After 3 hours, the neonates were moved to the new microplates containing the initial fresh medium (mentioned before). The exposure was carried out during the lifespan until all individual are died. During experiment, all animal tests were placed in incubator with 20 ± 1°C (12L:12D).

The observation was carried out daily to record the mortality and start of reproduction. The number of neonates were counted and size of neonates also measured. Prior the measurements, the neonates were separated from the mother and fixed by 5% formalin solution. The measurement of neonate number was easily observed using naked eye, while neonates size measurement was observed under light microscope (×40 magnification) completed by micrometer lens.

### Results and discussion

The presence of vitamin B<sub>12</sub> has significant effect ( $p < 0.05$ ) to enhance the survivorship of *Moina macrocopa* under short-time turbidity change (Fig. 1A). The highest turbidity exposure (600 NTU or ~312.85 mg/L of kaolin) showed the lowest survivorship among other group treatments, however with presence of vitamin B<sub>12</sub> allows animal to survive compare to the group without vitamin B<sub>12</sub> (no data recorded caused by early mortality). Such amount of sediment was rarely

happened in the natural environment. In addition, delay on maturity time also recorded on the group treatment without vitamin B<sub>12</sub> (Fig. 1B). In case of neonate number, there was no significant different among group treatment, however the highest number of neonates were recorded in the highest doses of vitamin B<sub>12</sub> (Table 1).



**Fig. 1.** (A) The survivorship of *Moina macrocopa* until the 6th day of experiment.; (B) Time of maturity of *M. macrocopa*. The absence of vitamin B<sub>12</sub> (0µg/L) showed delay on maturity.

The amount of kaolin particle that consumed by *Moina macrocopa* is believed to inhibit and to decrease food assimilation [9,14]. In this study, the presence of vitamin, resulted animals to uptake other beneficial consequences that may support them to overcome the unfavorable environment. Generally, in higher temperature and food concentration, the growth of cladoceran might be faster [12]. Hence, in this study, delayed at time of maturity might be result from low food supply caused by the presence of kaolin particles. Although the exposure time of this study was very short compare to other studies, late of maturation occurred was consistent with the previous study using *Daphnia magna* exposed for 24 h of 70-312 mg/L of kaolin [14]. The presence of vitamin B<sub>12</sub> might assist in stress tolerance of animals, caused by starvation, related to its function in methionine synthase, as it is proved had been worked for heat tolerance in green algae, *C. reinhardtii* [15].

**Table 1.** Mean value ± SD of *Moina macrocopa*'s neonates number

Vitamin B <sub>12</sub>	Turbidity (NTU)				
	0	15	50	100	600
0 µg/L	10.5±3.1	11.2±5.1	7.3± 3.8	5.0±3.8	N/A
5 µg/L	13.0±5.5	12.2±5.8	7.1±5.5	12.5±4.9	N/A
15 µg/L	5.0±3.6	11.0±5.1	4.5±2.9	13.0±0.0	5.0±2.4
25 µg/L	11.8±6.1	16.7±5.3	13.8±3.9	8.2±5.7	7.5±0.7

N/A means no offspring recorded caused by early mortality before reproduction occurred.

## Conclusions

The short-time change of turbidity had affected the survival and reproduction of *Moina macrocopa*. However, the presence of vitamin B<sub>12</sub> have taken a role to make organism more survive under short-time turbidity change.

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