



Effect of vitamin B₁₂ supplementation on the reproduction of *Daphnia magna* (Straus 1820) under sudden turbidity change

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ABSTRACT

Sediment loading related to heavy rainfall has become an issue to aquatic animals. Here, we reported a study revealing how vitamin B₁₂, as an important nutrient exclusively produced by bacteria, can minimize damage caused by sudden turbidity changed in Cladocera zooplankton, *Daphnia magna*. The animals were exposed individually during its lifespan to various turbidity levels (0, 15, 50, 100, 600, 1200 NTU (Nephelometric Turbidity Unit)) using kaolin clay and fed by *Chlorella vulgaris* with different level of vitamin B₁₂ supplementation (0, 5, 15, 30 µg L⁻¹) in the grown media. Exposing *Daphnia* to the turbidity had no effect to the lifespan and the survivorship, but the fecundity. In addition, supplementation of vitamin B₁₂ showed positive effect to the quality of offspring. Although it is found very subtle, the ratio of total malformation (ranged from 0.002 – 0.022) and male offspring (0.025 for 0 µg L⁻¹ and 0.014 for 5 µg L⁻¹) produced by exposed *Daphnia* were lessen recorded in proportional with the increasing dose of vitamin B₁₂. Collectively our data indicate that pulse turbidity exposure to *D. magna* had affected the offspring condition and presence of vitamin B₁₂ can contribute to normal development offspring of *D. magna* in the changing environment.

Keywords: *Daphnia magna*, Cladocera, kaolin, turbidity, vitamin B₁₂

INTRODUCTION

Turbidity is one of the important water quality factors in the aquatic ecosystem, which can be contributed by both organic and inorganic particles, but sediment loading (clay, silt particles) often tops of the list of material causing turbidity (Gilbert 1990; Kirk 1991). Increasing sediment loading to the aquatic ecosystem was well correlated with rainfall events and intensity (Gelda *et al.* 2013; Lee *et al.* 2016). The problem will be aggravated with more frequent and intense runoff related to ongoing climate change (Pendergrass *et al.* 2017), albeit anthropogenic factors have a major contribution (IPCC 2014; Häder and Gao 2015). The negative impacts of suspended sediment have been well documented for a wide

variety of aquatic organism, start from the producer to the higher consumer in the aquatic food chain. Firstly, it will limit the light attenuation of autotroph taxa to carried out photosynthesis (Bilotta & Brazier 2008; Häder and Gao 2015). Lastly, filter and deposit feeder organisms, such as daphniids, can also be affected indirectly due to particles ingestion possibility that may clog the gut and result in suboptimum food intake, causing stress, or further may even death (Bilotta and Brazier 2008; Chen *et al.* 2012; Hasenbein *et al.* 2013; Maisanaba *et al.* 2015).

There is an increasing concern in the interaction between bacteria, zooplankton and their environment, and how those interactions affect zooplankton performance in the fluctuating environment and stressful condition (Peerakietkhajorn *et al.* 2015; Sison-Mangus *et al.* 2015; Mushegian *et al.* 2016; Manakul *et al.* 2017). The presence of bacteria is reported to play nutritional role for daphniids by producing certain enzymes or nutrients that beneficial for their reproduction and growth (Peerakietkhajorn *et al.* 2015). One of the nutrients that exclusively produced by bacteria and readily available in the aquatic environment is vitamin B₁₂ (Yu *et al.* 1989; Croft *et al.* 2005; Tang *et al.* 2010; Fang *et al.* 2017; Helliwell 2017). It is well-known for decreasing stress (Ghemrawi *et al.* 2013; Ferrer *et al.* 2016) and plays a role as an antioxidant (Bito *et al.* 2017). Vitamin B₁₂ also reported have a protective effect on the cells from nutritional stress (Ghemrawi *et al.* 2013). Research has consistently found that this vitamin plays an important role, especially for phytoplankton dynamics since most of algae are vitamin B₁₂ auxotroph (Yu *et al.* 1989; Croft *et al.* 2005; Helliwell *et al.* 2016). However, there is still little information about its role for zooplankton in the natural environment. Previous studies have reported in the laboratory scale that supplementation of vitamin B₁₂ both in the media and/or food have beneficial for improving growth and fecundity for *Daphnia pulex* (Keating 1985), marine rotifer *Branchionus plicatilis* (Hirayama *et al.* 1989; Yu *et al.* 1989; Hayashi *et al.* 2007), and *Daphnia magna* (Kusari *et al.* 2017). Thus, the aim of present study is to examine vitamin B₁₂, that exclusively produced by bacteria, to obtain a better understanding in respect of bacteria-zooplankton relationship. *Daphnia magna* (Straus 1820), herbivorous freshwater zooplankton and a key species in the aquatic food web, was chosen because of its sensitivity to changing the abiotic environment and widely used as a standard test

organism (OECD 2012). The short-time life cycle and parthenogenetic reproduction allow the animal to be observed for its response in the life-history change. It is also passive filter-feeder that susceptible to turbidity change caused by sediment loading exposure. We assumed that the presence of vitamin B₁₂ have a contribution to minimizing damage caused by turbidity change in *Daphnia*.

MATERIALS AND METHODS

Experimental design

D. magna which is obtained from Gene Bank, National Institute for Environmental Studies, Ibaraki Prefecture, Japan, was grown for many generations in our laboratory at 20±1°C using commercial mineral water. They were cultured in several 1 L jars of commercial mineral water with the density ~10 ind L⁻¹ and fed by commercial Chlorella (Chlorella V-12, Chlorella Industry Inc., Fukuoka, Japan) for maintaining a healthy culture.

For experimental purposes, *Chlorella vulgaris* (Beijerinck 1890) (Gene Bank of National Research Institute of Aquaculture, Mie, Japan) was cultured in 3 L of modified f/2 medium (Andersen *et al.* 2005) and used as the sole diet for experiment. Vitamin B₁₂ content was not included in the algal media culture. Food stock preparation was carried out by centrifuged the 5 days harvested algal culture at 4000 rpm for 30 minutes and remove the supernatant. The algal suspension was diluted using 200 ml distilled water and stored at 4±1°C until use for feeding. The final cells density was measured using hemocytometer Thoma under a light microscope with an estimated amount of 300 × 10³ cells ml⁻¹.

A commercial kaolin clay (Al₂Si₂O₅(OH)₄) (approximately 0.4 µm particle size in diameter) was used as turbidity material. Kaolin has been prevalently utilized as standard solution for turbidity measurement, with 1 NTU = ~1 mg L⁻¹ of kaolin clay (JWRC, 2008). Kaolin were diluted with commercial mineral water used for culture until the desired turbidity applied. Turbidity concentration was measured using handheld colorimeter for water quality measurement (HACH DR890, HACH Co., Ltd., USA) with commercial water as a blank. Kaolin solution was prepared daily, and the turbidity was maintained during the exposure time to prevent suspension.

For the animal test and experimental procedure, the selection was applied to maintain parthenogenetically reproduction before the experimental start. The 5th brood neonates were gained from 30 mothers. The experiment was performed in the six-well microplates, where each well contains one neonate (at age < 24 hours old). The neonates were exposed daily during their lifespan by different turbidity levels (15, 50, 100, 600, 1200 NTU) for 3 hours. After turbidity exposure, the animals were moved to a new medium and fed by *C. vulgaris* with 3 different doses of vitamin B₁₂ (5, 15, 30 µg L⁻¹). Crystalline Cyanocobalamin (Wako Pure Chemical Industry, Co. Ltd, Japan) was used and diluted in the distilled water until the desired doses applied. The control treatment consists of pure medium, fed by only *C. vulgaris* in clear water without the addition of vitamin B₁₂. In total there are 144 neonates (6 turbidity level × 4 vitamin B₁₂ × 6 neonates in microplates) used for experiment and observation was carried out until the animals are dead. All the animals were fed by the similar amount of food, approximately 300 × 10³ cells ml⁻¹ of *C. vulgaris* and kept at incubator with the temperature set up 20 ± 1°C and photoperiod 12:12 L: D cycle.

A daily observation was performed to record the mortality and the day when the onset of reproduction occurred. Once reproduction started, we separated the neonates from the mothers and neonates are fixed by 5% formalin for further body size measurement. The neonates were counted, and the sex of neonates was observed under a dissecting microscope with × 40 magnification. For body size measurement, light microscope equipped by eyepiece micrometer was used with ×40 magnification.

Data analysis

The following reproductive parameters were calculated during the treatment: total number of offspring per female until the 5th reproduction, male appearance/total female offspring per group treatment, offspring malformation/total normal offspring per group treatment, each offspring size per female, time to first brood per female, and frequency of reproduction occurred per female during lifespan.

Total lifespan, offspring number per female, and frequency of reproduction occurred per female were compared between group treatments using two-way ANOVA with significant value $P < 0.05$, followed by Tukey post hoc test to gain differences between treatments. Levene Test was also performed prior data

analysis to meet ANOVA assumption. In the case of offspring size, we use Kruskal Wallis non-parametric test followed by Dunn Test because each individual produces a different number of offspring that result in unequal sample size in group treatments. Time to first brood per female was calculated as a mean difference of the time to first brood per female in treated animals and the mean of the time to first brood per female in control ± standard deviation. The malformation offspring were defined as the ratio of malformation offspring to the total normal offspring observed in each vitamin B₁₂ doses group. The male offspring was defined as the ratio of male offspring to the total normal female offspring observed in each vitamin B₁₂ doses group.

RESULTS

Lifespan

Exposing *D. magna* with kaolin clay for 3 hours with or without vitamin B₁₂ supplementation in the grown media did not affect the lifespan in all vitamin B₁₂ treatment groups as shown in Figure 1. However, 2-way ANOVA followed by Tukey post-hoc test confirmed that the treatment group without supplementation of vitamin B₁₂ showed significantly longer lifespan ($P < 0.05$;

Table 1; Table 2) compare to that receive vitamin B₁₂ supplementation (ranged from 42.33 ± 1.03 to 43.00 ± 0.00 days).

Age at first reproduction

The time to start the first reproduction of *D. magna* were significantly different in the clear water and low turbidity exposure (15 NTU) without vitamin B₁₂ supplementation. The mothers start their reproduction at the day 10th of the experiment. However, the higher turbidity exposure without supplementation of vitamin B₁₂ and other groups with supplementation of vitamin B₁₂ respond similarly, most of them were starting their reproduction earlier, at the day 9th of the experiment (Figure 2).

Total offspring number and frequency of reproduction

In the case of offspring number, we observed the offspring number both in each reproduction and continuously recording during the lifespan. The offspring number was significantly higher ($P < 0.05$) in the treatment with supplementation of 30 µg L⁻¹ of

vitamin B₁₂ until the 5th reproduction (approximately for 20-22 days experiments, when the viability of animal test was 100%). Although the offspring number were not turbidity-dependent in every vitamin B₁₂ treatment groups, it is shown the fewest offspring number was attained in the highest turbidity value (1200 NTU) as depicted in Figure 3 and

Table 2. None of the differences measured were statistically significant for total offspring number during the lifespan.

Two-way ANOVA confirmed that each factor, turbidity exposure and the presence of vitamin B₁₂, were significantly affected the frequency of reproduction of *D. magna*. The animals tend to reproduce more often when exposed at the highest turbidity (1200 NTU), $8.00 \pm 0.63 - 9.33 \pm 1.21$ times during lifespan, compared to the animal grown in the clear water,

ranged from 6.00 ± 1.09 to 7.50 ± 1.76 times during lifespan. Turbidity level ranged from 15 to 600 NTU did not differ significantly in the frequency of reproduction as shown in

Table 2. When compared among the vitamin B₁₂ group treatments, supplementation of 15 and 30 μL^{-1} of vitamin B₁₂ showed lesser frequency of reproduction than the treatment group supplemented by the lower dose of vitamin B₁₂ as summarized in Figure 4.

Offspring size

With respect to the offspring body size of *D. magna* exposed to the combination of turbidity and vitamin B₁₂, all the mothers produced nearly similar offspring in size. Each factor, kaolin clay exposure and supplementation of vitamin B₁₂, did not significantly affected the offspring size produced by *D. magna*. The offspring size ranged from 0.56 ± 0.05 to 0.59 ± 0.04 mm in size (Figure 5).

Table 1. Summary of the subsequent univariate analyses (2-way ANOVA) for lifespan, offspring number, and number of reproductions of *D. magna*

| Dependent variables | Source of variation | df | SS | F | P |
|--|------------------------------|-----|--------|--------|--------------------------|
| Lifespan | VB ₁₂ | 3 | 488 | 5.247 | 0.00195* |
| | Turbidity | 5 | 225 | 1.452 | 0.21083 |
| | VB ₁₂ × Turbidity | 15 | 137 | 0.294 | 0.99521 |
| | Residuals | 120 | 3722 | | |
| Offspring number | VB ₁₂ | 3 | 689.9 | 10.221 | 4.81×10^{-16} * |
| | Turbidity | 5 | 100.3 | 0.892 | 0.4890 |
| | VB ₁₂ × Turbidity | 15 | 800.6 | 2.372 | 0.00501 |
| | Residuals | 120 | 2700 | | |
| Offspring number (1 st -5 th reproduction) | VB ₁₂ | 3 | 172.1 | 8.506 | 3.61×10^{-5} * |
| | Turbidity | 5 | 370.1 | 10.978 | 1.02×10^{-8} * |
| | VB ₁₂ × Turbidity | 15 | 291.5 | 2.882 | 0.000677* |
| | Residuals | 120 | 809.2 | | |
| Number of reproductions | VB ₁₂ | 3 | 76.30 | 17.935 | 1.11×10^{-9} * |
| | Turbidity | 5 | 25.90 | 5.179 | 0.00412* |
| | VB ₁₂ × Turbidity | 15 | 32.08 | 1.508 | 0.11270 |
| | Residuals | 120 | 170.17 | | |

Table 2. Summary of Tukey post hoc test for differentiate among treatment groups relative to parameters observed.

| Response variable | Vitamin B ₁₂ ($\mu\text{g L}^{-1}$) | Turbidity (NTU) |
|--|---|--|
| Lifespan | 30 ^a < 5 ^a < 15 ^a < 0 ^b | 0 ^a < 600 ^a < 100 ^a < 50 ^a < 1200 ^a < 15 ^a |
| Offspring number (all) | 15 ^a < 30 ^b < 0 ^b < 5 ^b | 100 ^a < 50 ^a < 600 ^a < 15 ^a < 0 ^a < 1200 ^a |
| Offspring number (1 st -5 th) | 5 ^a < 0 ^a < 15 ^a < 30 ^b | 1200 ^a < 100 ^{ab} < 600 ^{ab} < 50 ^{ab} < 15 ^b < 0 ^c |
| Number of reproductions | 15 ^a < 30 ^a < 5 ^b < 0 ^b | 0 ^a < 100 ^{ab} < 15 ^{ab} < 600 ^{ab} < 50 ^{ab} < 1200 ^b |

Table 3. Total normal and malformation offspring recorded in *D. magna* supplemented by different vitamin B₁₂ doses under turbidity exposure using kaolin clay.

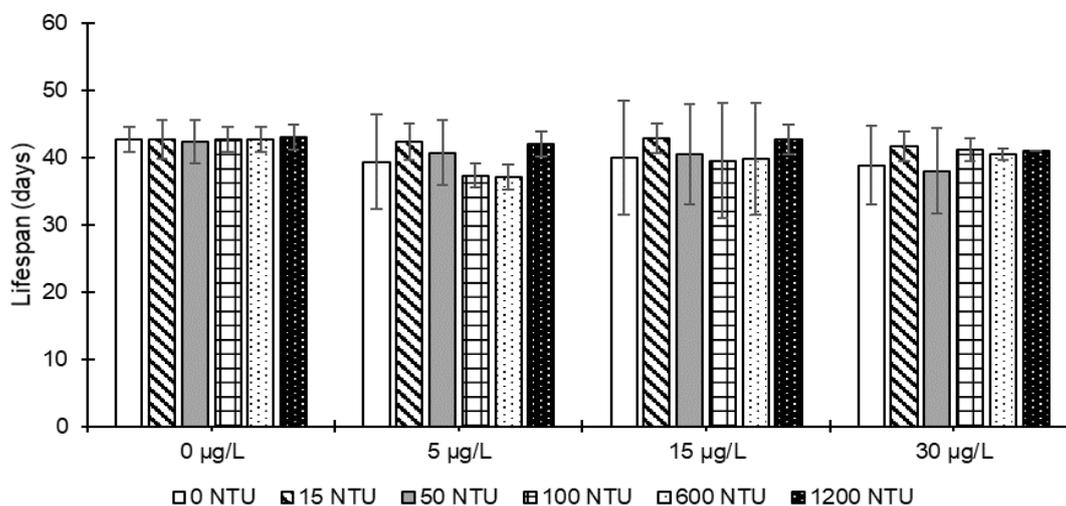
| Turbidity (NTU) | 0 µg L ⁻¹ | | | 5 µg L ⁻¹ | | | 15 µg L ⁻¹ | | | 30 µg L ⁻¹ | | |
|-----------------|----------------------|-----------|--------------|----------------------|-----------|--------------|-----------------------|-----------|--------------|-----------------------|----------|--------------|
| | NO | MO | Ratio | NO | MO | Ratio | NO | MO | Ratio | NO | MO | Ratio |
| 0 | 147 | 0 | 0 | 161 | 0 | 0 | 143 | 0 | 0 | 158 | 0 | 0 |
| 15 | 108 | 3 | 0.027 | 155 | 0 | 0 | 117 | 0 | 0 | 187 | 0 | 0 |
| 50 | 125 | 3 | 0.024 | 154 | 8 | 0.052 | 130 | 6 | 0.046 | 150 | 0 | 0 |
| 100 | 157 | 1 | 0.006 | 136 | 3 | 0.022 | 147 | 1 | 0.006 | 147 | 1 | 0.006 |
| 600 | 136 | 1 | 0.007 | 161 | 1 | 0.006 | 121 | 2 | 0.016 | 151 | 0 | 0 |
| 1200 | 136 | 4 | 0.029 | 155 | 6 | 0.038 | 132 | 2 | 0.015 | 157 | 1 | 0.006 |
| Total | 809 | 18 | 0.022 | 922 | 18 | 0.019 | 790 | 11 | 0.014 | 950 | 2 | 0.002 |

NO = normal offspring, MO = malformation offspring

Table 4. Total female and male offspring recorded in *D. magna* supplemented by different vitamin B₁₂ doses under turbidity exposure using kaolin clay.

| Turbidity (NTU) | 0 µg L ⁻¹ | | | 5 µg L ⁻¹ | | | 15 µg L ⁻¹ | | | 30 µg L ⁻¹ | | |
|-----------------|----------------------|-----------|--------------|----------------------|-----------|--------------|-----------------------|----------|----------|-----------------------|----------|----------|
| | F | M | Ratio | F | M | Ratio | F | M | Ratio | F | M | Ratio |
| 0 | 147 | 0 | 0 | 161 | 0 | 0 | 143 | 0 | 0 | 158 | 0 | 0 |
| 15 | 108 | 0 | 0 | 155 | 0 | 0 | 117 | 0 | 0 | 187 | 0 | 0 |
| 50 | 125 | 12 | 0.096 | 154 | 0 | 0 | 130 | 0 | 0 | 150 | 0 | 0 |
| 100 | 157 | 2 | 0.013 | 136 | 1 | 0.007 | 147 | 0 | 0 | 147 | 0 | 0 |
| 600 | 136 | 6 | 0.044 | 161 | 7 | 0.043 | 121 | 0 | 0 | 151 | 0 | 0 |
| 1200 | 136 | 0 | 0 | 155 | 5 | 0.035 | 132 | 0 | 0 | 157 | 0 | 0 |
| Total | 809 | 20 | 0.025 | 922 | 13 | 0.014 | 790 | 0 | 0 | 950 | 0 | 0 |

F = female offspring, M = male offspring

**Figure 1. Mean lifespan of *D. magna* when exposed by various turbidity and supplemented by different level of vitamin B₁₂. Bars denote mean value ± SD.**

Sex and malformation ratio

Male neonates and malformation offspring were recorded in *D. magna* after short-time turbidity exposure using kaolin clay during their lifespan in very small quantities. Malformation offspring were less

recorded in the group treated with a higher dose of vitamin B₁₂ supplementation as depicted in Table 3. Observed malformation included malformed tail (curved) and stunted body (dwarf-like) (Figure 6). A similar pattern also recorded in the male offspring

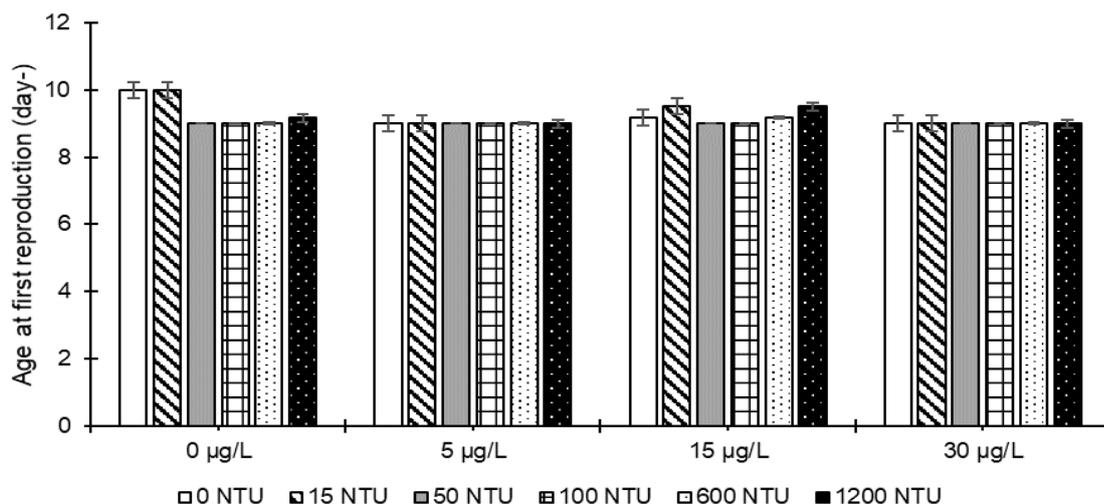


Figure 2. Mean of days of maturity of *D. magna* exposed by various turbidity and supplemented by different level of vitamin B₁₂. Bars denote mean value ± SD.

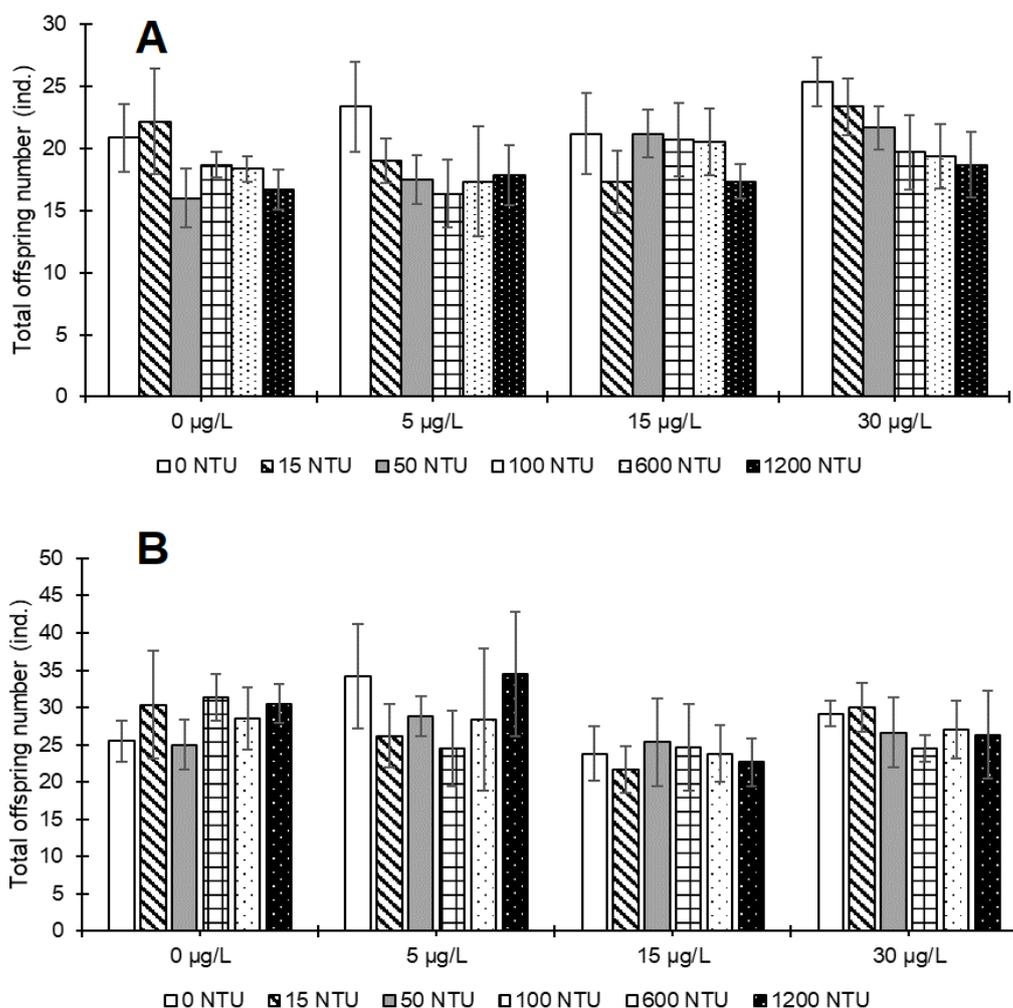


Figure 3 Mean of offspring number of *D. magna* supplemented by different level of vitamin B₁₂ under various turbidity with kaolin clay (A) from the 1st - 5th reproduction (20-22 days of experiments) and (B) during lifespan. Bars denote mean value ± SD. Significance level against the control treatment (0 NTU) in each vitamin B₁₂ group is indicated by **P* < 0.05, ***P* < 0.01.

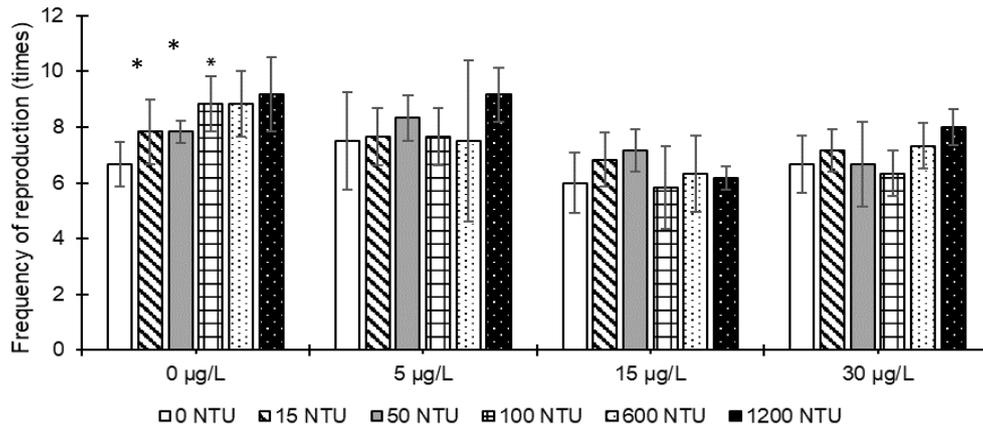


Figure 4. Average frequency of reproduction during lifespan of *D. magna* under various turbidity with kaolin clay and supplemented by different level of vitamin B₁₂. Significance level against the control treatment (0 NTU) in each vitamin B₁₂ group is indicated by * $P < 0.05$, ** $P < 0.01$.

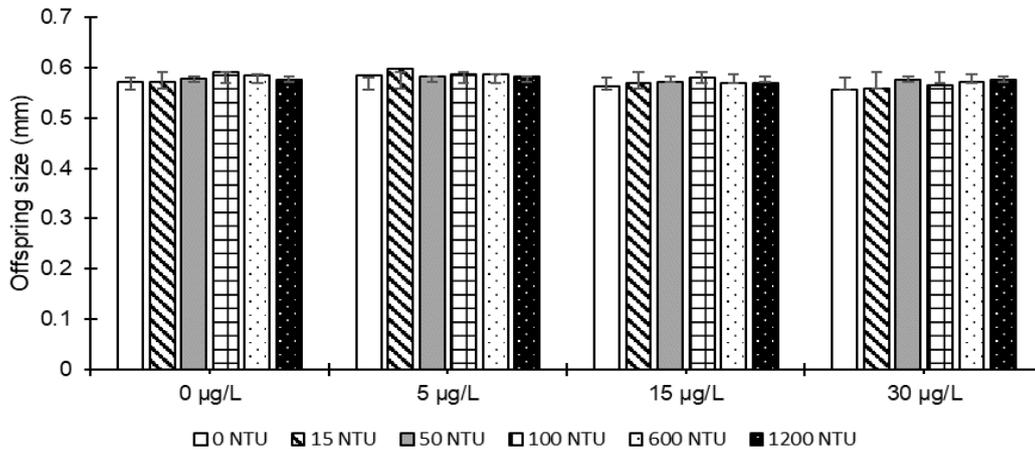


Figure 5. Mean of offspring size of *D. magna* under various turbidity using kaolin clay and supplemented by vitamin B₁₂. Bars denoted mean value \pm SD.

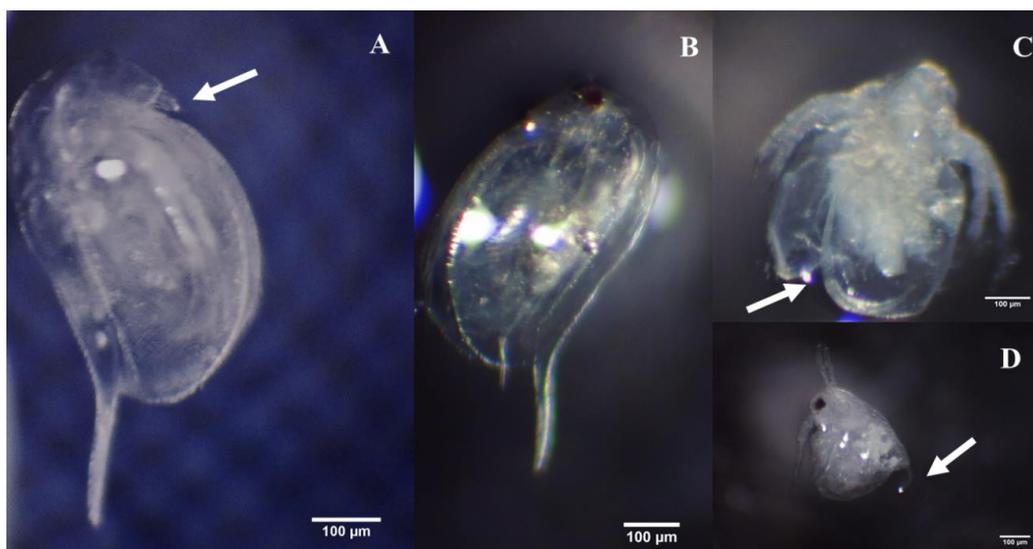


Figure 6. Example of male and malformation offspring found in mother *D. magna* exposed by various turbidity using kaolin clay. (A) normal-male offspring, indicated by the appearance of long antennules (B) normal female offspring (C) curve-tailed offspring (D) stunted-body (dwarf) offspring.

appearance that randomly found in the group treatments without vitamin B₁₂ supplementation and 5 µg L⁻¹ of vitamin B₁₂. The ratio values were not in accordance with the level of turbidity. However, it was found especially in the 50 – 1200 NTU (Table 4). The mother lived in the clear water and very low turbidity (0 and 15 NTU), as well as the mothers that lived in the higher doses of vitamin B₁₂ (15 and 30 µg L⁻¹) in the media produced entirely female offspring. Although the ratios of both the deformities and male offspring were subtle, the total ratio was vitamin B₁₂ dose-dependent.

DISCUSSION

The aim of this study was to observe the role of vitamin B₁₂ in ambient water, which is exclusively produced by bacteria, on freshwater Cladocera *D. magna* at the morphological and life history when exposed to turbidity changed by kaolin clay. The results suggested that in general, *D. magna* was able to tolerate short-time turbidity changed, no significant difference in the lifespan and no mortality was observed until their 5th reproduction (day 20-22), as the standard experimental period by OECD (2012) for reproduction test on *D. magna*. However, the reproduction of *D. magna* as well as quality offspring shown disrupted in the turbid water together with the absence of vitamin B₁₂ in the grown media.

The presence of considerable amount of inorganic clay in the ambient water were suggested can inhibit optimum food uptake for zooplankton, such as Daphniids, as reported in the previous studies (Hart 1992; Rellstab & Spaak 2007; Pietrzak et al. 2010; Robinson et al. 2010). However, the present study showed the survivorship of *D. magna* was not disrupted by turbid water condition. This result was supported by a study carried out by Robinson et al. (2010) that exposing *D. magna* using 800 mg L⁻¹ of kaolin clay, with longer exposure time (24 hours) and at least 48 hours for recovery period did not result in any mortality. Despite of that, exposing *D. magna* in the highest turbidity (1200 NTU), even only short-time periods, led to increasing number of reproduction but fewer offspring (both in each and total reproduction) compare to that exposed by lower turbidity.

Our result demonstrated that supplementation of vitamin B₁₂ efficiently increase the fecundity of *D. magna* and offspring condition (minimize the

malformation and male offspring appearance). The positive effect of vitamin B₁₂ supplementation is in concordance with previous study with higher dose (0.75, 1.5, and 3 mg L⁻¹) of vitamin B₁₂ supplementation in ambient water can increase *D. magna*'s fitness (Kusari et al. 2017). Bioavailability of vitamin B₁₂ was suggested to increase DNA methylation level that is susceptible to external environmental cues, especially it linked to nutritional deficit, and may change the phenotype response. Kleiven et al. (1992) stated that *D. magna* required, at least, simultaneously three stimuli for their sexual reproduction: crowding, photoperiod, and food limitation. Since this study was using an identical amount of food for all group treatments and using optimum photoperiod as well as crowding were not accountable to the reason, we assumed that kaolin clay presences were reduced optimum food uptake for *Daphnia*. In addition, the exposure of this study was conducted in the 1st instar juvenile and continuously during the lifespan, it is assumed that normal development of embryo was failed to be delivered due to kaolin exposure in the critical stage of *Daphnia*, in a long time before maturity. Vitamin B₁₂ in the ambient water might help *Daphnia* to maintain the fitness under such condition. However, the molecular mechanisms in this early investigation were unexplored due to the experiment was carried out in individual basis.

The presence of kaolin clay together with insufficient nutritional uptake (vitamin B₁₂ – deficient) decrease the energy of *Daphnia* for reproduction under environmental change. The limited energy is spent on physiological reaction, including repairing damage and impact to the fecundity failure. The strong effect of food quality suggests that the mothers *Daphnia*'s energy has a role to produce certain offspring with normal development (Sarpe et al. 2014; Choi et al. 2016). This study revealed that vitamin B₁₂ has a role to maintain fitness caused by nutritional stress in *D. magna*, and this function also found when symbiotic bacteria present in the *D. magna* culture (Sison-Mangus et al. 2015). Since bacteria is vitamin B₁₂ producer, it is become clearly proved that *D. magna* requires bacteria to maintain its performance during fluctuating environmental change. The cellular availability of vitamin B₁₂ was reported as a key vitamin for DNA methylation process, that is susceptible to external environment cues (Ducker and Rabinowitz 2017).

Even though this study was performed in the laboratory scale, the results may be interpreted from an ecological point of view. It is assumed that despite the experimental design is somewhat artificial, the present study may explain the role of bacteria for *D. magna* resilience under environmental change, as discussed below. Since vitamin B₁₂ presence in the aquatic ecosystem is exclusively produced by bacteria and archaea (Fang *et al.* 2017; Obeid 2017), and consumed largely by algae (Croft *et al.* 2005; Kazamia *et al.* 2012; Sañudo-Wilhelmy *et al.* 2014; Wells *et al.* 2017), the content of dissolved vitamin B₁₂ in nature will depend on the production-consumption between algae and bacteria. Seasonal dynamics, however, have a contribution to establishing bacterial production in the aquatic ecosystem. During heavy rainfall and flood, primary production of phytoplankton is limited due to insufficient light penetration in turbid water.

Consequently, the organic carbon content in the water as major countershaft for bacterial production (Cole *et al.* 1988; Farjalla *et al.* 2006) is also limited. This condition will indirectly effect the amount of vitamin B₁₂ in the water, and shifting in high-demand of vitamin B₁₂ phytoplankton auxotroph (Gobler *et al.* 2007; Hou 2018). *Daphnia*, that negatively affected by turbid water and/or flood will be helped by the nutritional role of bacteria present. It is assumed, somehow, has a contribution for the resilience of filter-feeding zooplankton due to changing an environment (Häder & Gao 2015).

CONCLUSION

Overall, the observations outlined in the present study, showing a beneficial effect of vitamin B₁₂ in the ambient water to maintain *D. magna*'s reproduction under turbid condition. This study provide novel information about the role of vitamin related to the environmental change in the aquatic ecosystem. Further research might explore the molecular metabolism of this vitamin with larger group of individual and other environmental cues.

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