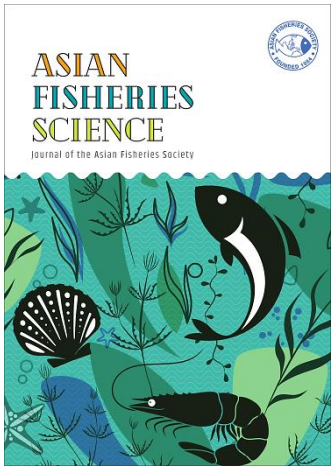

Reduced Reproductive Capacity in *Moina micrura* Kurz, 1875 Exposed to Toxic *Microcystis* spp.

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Abstract

Toxic cyanobacterial blooms are considered harmful to all consuming organisms along the aquatic food chain and top consumers, including humans. Hence this study was conducted to assess the impacts of two toxic *Microcystis* spp. on a tropical cladoceran, *Moina micrura* Kurz, 1875. Population growth studies and chronic bioassays were conducted using *Microcystis aeruginosa* (Kützing) Kützing, 1846, *Microcystis viridis* f. *viridis* (A. Braun) Elenkin, 1938, and a green alga, *Chlorella vulgaris* f. *tertia* Fott et Novakova, 1972 (as the control). Both *Microcystis* spp. negatively affected *M. micrura*. The population growth rate of *M. micrura* fed with *C. vulgaris* was 0.51 day^{-1}, while growth rates were negative when fed with *M. aeruginosa* (- 0.33 day^{-1}) and *M. viridis* (- 0.13 day^{-1}). In the chronic bioassay, the exposure of *M. micrura* to *M. aeruginosa* resulted in delayed production of *M. micrura*'s first batch of offsprings, which only occurred on day 6 compared to *M. micrura* fed with *C. vulgaris* which produced their first batch of offsprings earlier on day 3. This study showed that exposure of *M. micrura* to both toxic *Microcystis* spp. reduced the population density, fecundity, total offspring production and longevity of *M. micrura* compared to those fed with *C. vulgaris*.

Keywords: reproductive capacity, population growth, *Moina micrura*, *Microcystis* spp.

Introduction

Increase in cyanobacterial blooms is a threat to freshwater ecosystems because their presence is associated with deteriorating water quality and eutrophication, mainly resulting from anthropogenic activities related to nutrient loadings (Paerl and Otten, 2013). In addition, the global warming stimulates cyanobacterial growth at a relatively higher temperature compared to other eukaryotic algae (Paerl and Huisman, 2009). Over the years, cyanobacterial blooms have sparked the intention of many researchers because of the side effect they bring not only to aquatic animals (Landsberg, 2002) but also human health (Carmichael et al., 2001). High-density algae blooms can cause fish kills due to hypoxia/anoxia in the surrounding environment (Lim et al., 2012). Moreover, cyanobacterial blooms involving species such as *Anabaena, Aphinozomenon* and *Microcystis* commonly found in freshwater bodies are able to produce secondary metabolites that are harmful such as anatoxin, endotoxin and microcystin (Wiegand and Pflugmacher, 2005). Microcystin, for example, caused broken filaments and lamella in the gill tissue of a carp fish (Jiang et al., 2011). Mass fish mortality in fish ponds, in random cases, such as in Bangladesh and Siberia (Jewel et al., 2003; Drobac et al., 2016) was related to *Microcystis* and *Aphinozomenon* blooms leading to economic losses to fish farmers.

Toxic cyanobacterial species and the cyanotoxins variants can be determined using a few methods such as biochemical, analytical, molecular and biological assay. Enzyme-linked immunosorbent assay (ELISA) and high performance liquid chromatography (HPLC) are widely used to detect the presence and variants of cyanotoxins but the presence of a wide range of toxins limits these detection methods which are in turn dependent on standard solutions (McElhiney and Lawton, 2005, Sivonen, 2009). The discovery of microcystin genes cluster by Tillet et al. (2000), led to extensive research in molecular biological methods. From the conventional polymerase chain reaction

(PCR) to DNA microarray, these approaches have been proven to be rapid and cost- effective toxic species identification (Pearson and Neilan, 2008). Several cyanobacteria genera of *Microcystis, Oscillatoria, Anabaena and Nostoc* produce microcystin as their secondary metabolites. Microcystin synthethase (*mcy*) gene cluster consists of gene coding of non-ribosomal peptide synthetases (NRPSs), polyketide synthases (PKSs) and a series of enzymes. The *mcy* gene cluster is around 55 kb and consists of 10 open reading frames (*mcy*A-*mcy*J) (Sivonen, 2009). There is a direct relationship between the existences of *mcy* genes with microcystin production. Thus, the microcystin producer could be determined with the presence of the *mcy* genes cluster in the cells (Kurmayer et al., 2002; Hisbergus et al., 2003; Kumar et al., 2011).

Zooplankton such as cladocerans, copepods and rotifers are primary consumers that feed on suspended particles and phytoplankton (Dao et al., 2010). Cladocerans have rapid population growth but are sensitive to toxic microalgae and hence they are suitable as ecotoxicological test subject. Acute toxicity study often shows the median lethal concentration while chronic bioassay involves demographic responses such as life span, age at first reproduction and reproductive rates. These responses exhibited physiological and behavioural reactions of zooplankton to toxicant (Sarma and Nandini, 2006). Meanwhile, population dynamics reflect small changes in zooplankton population under stressful condition (Jonathan et al., 2010). Zooplankton-cyanobacteria interaction may respond differently to demographic variables; for example, microcystin might reduce the longevity and generation time but increased the offpring production (Nandini et al., 2017). Some interactions might have consistent effect between toxicity level on population growth and reproduction (Herrera et al., 2015), while some might have developed some adaptation toward toxicant through history of exposure (Gustafsson et al., 2005). Among cladocerans, *Daphnia* and *Ceriodaphnia* have been routinely used in toxicity testing but in Malaysian waters, *Moina micrura* Kurz, 1875, is one of the most commonly found cladocerans (Idris and Fernando, 1981). Moreover, they can be easily cultured in laboratory and can be used as valuable live feed to fish and freshwater prawns (Alam et al., 1991). Therefore, this study aimed to determine the toxic *Microcystis* spp. and to evaluate their effects on the population growth rates and reproductive capacity of a tropical cladoceran, *M. micrura*.

Materials and Methods

Culture of Moina micrura

Moina micrura was isolated from a pond, in Universiti Putra Malaysia (02°59.26 N, 101°42.52 E) in February 2017 using 60 µm zooplankton net. The single parthenogenetic female of *M. micrura* was cultivated in autoclaved filtered pond water (using 1.2 µm filter paper) until at least three generations were produced to offset the maternal effect. *Moina micrura* was fed *ad libitum* with *Chlorella vulgaris* f. *tertia* Fott et Novakova, 1972 at the concentration of 4×10^6 cells.mL^{-1}. During the culture period, the medium was renewed regularly to avoid accumulation of dead cells. *Moina micrura* culture was maintained in 10 L aquarium containing 7 L autoclaved filtered pond water (pH 7.8 ± 2.0) at room temperature of 27.0 ± 2.0 °C and under a light intensity of 60 ± 2.0 µmoles photon m^{-2}.sec^{-1}. The population density of *M. micrura* was maintained below 1000 individuals.L^{-1} to avoid overcrowding.

Culture of microalgae

Water samples were collected from Putrajaya Lake, Malaysia (02°56.174 N, 101°41.147 E) from July to September 2015. The samples were left overnight to allow cells to float on the water surface. Microalgae were isolated using three types of isolation techniques; micromanipulation, streak plating and serial dilution (96-well up to 6-well cell culture plates). All three techniques were combined to isolate *Microcystis aeruginosa (Kützing)* Kützing, 1846, because of its colonial characteristic while only serial dilution was used for *Microcystis viridis* f. *viridis (A. Braun)* Elenkin, 1938, because of its sticky properties. Micromanipulation was done using a dissecting microscope because the colony of *M. aeruginosa* was visible under naked eyes. The cells were washed a few times with milli-Q water to rinse away all the possible microorganisms associated with the colony. After that, a few distinct colonies were streaked onto agar plate containing solidified BG-11 media (https://www.ccap.ac.uk) with agar following manufacturer's instruction (Merck, Darmstadt, Germany). This step was repeated several times until a single, pure species was obtained. The unialgal culture of both *M. aeruginosa* (UPMCA0038) and *M. viridis* (UPMCA0039) was then subjected to both morphological and molecular identification, and the sequences were later submitted to NCBI database under the accession number of KX447651 for *M. aeruginosa* and KY009735 for *M. viridis*. Agar plates were incubated at 25 °C with light intensity of 80–100 µmol photon.m^{-2}.sec^{-1}, and a photoperiod of 12 h light:12 h dark for two weeks. Algae cells were harvested at the late exponential growth phase for feeding regimes. The number of cells for feeding was determined by cell counts using a haemocytometer (GmBH & Co. KG, Wertheim, Germany).

Toxin analysis

Approximately 2 mL microalgae culture was harvested during the exponential growth phase. The DNA extraction was done using Dneasy Plant Mini Kit according to the manufacturer's protocol (Qiagen, Germany). Two specific oligonucleotide primer; Tox 2F 5′-GGAACAAG TTGCACAGAATCCGC-3′ and Tox 2R 5′ - CCAATCCCTATCTTAACACAGT AACTCGG-3′

(Dittmann et al., 1999) were used to screen *mcyB* genes. Primer pairs were synthesised by Integrated DNA Technologies (USA). Then, the gene amplification was conducted in DNA thermal cycler (Biometra T Professional, Germany). 25 µL PCR master mix (Promega) for *mcyB* gene amplification consisted of 14.35 µL autoclaved Milli-Q water, 5.0 µL 5X GoTaq® Flexi buffer, 2.0 µL 25 mM magnesium chloride ($MgCl_2$), 1.0 µL 10 mM dNTPs, 0.25 µL 5u GoTaq® DNA polymerase, 0.2 µL 20 pmol Tox 2F, 0.2 µL 20 pmol Tox 2R, and 2 µL DNA of *Microcystis* were used. The PCR amplification was optimized according to the manufacturer's instruction to suit the annealing temperature of primer pairs. The protocol was run with an initial denaturation at 94 °C for 2 min, 35 cycles of denaturation at 94 °C for 10 s, annealing at 60 °C for 30 s, extension at 72 °C for 1 min and the final elongation at 72 °C for 5 min. After the amplification was completed, 5 µL PCR products were resolved on 1.0 % agarose gel (Promega, USA), in 1 × TAE buffer at 80 volt (V) for 50 min. The gel was spiked with gel red for staining before electrophoresis. 100 base pair (bp) DNA ladder (Fermentas, USA) was used as the expected product was around 300 bp–400 bp. The gel was visualised and photographed using gel documentation system UV Doc (Protein Simple, CA). Then, the agarose gel containing the PCR product was excised for purification using a QIAquick PCR purification kit (Qiagen, Germany) according to the manufacturer's protocol. The purified PCR product was sent for sequencing at the 1st Base Sdn. Bhd. (Seri Kembangan, Malaysia).

Population growth study

Two different species of cyanobacteria; *M. aeruginosa* and *M. viridis*, and a green alga *C. vulgaris* (as the control) were used in this experiment. Each treatment was conducted in triplicate. Two hundred females of *M. micrura* were transferred into an Erlenmeyer flask containing 1L filtered-sterilized pond water and fed with the algae *ad libitum* (stock of 1.0×10^6 cells.mL^{-1}) once daily. Culture flasks were manually shaken every day to ensure homogenisation of algal cells. The culture conditions were maintained at room temperature (27.0 ± 2.0 °C) under a photoperiod of 12 h light:12 h dark (60 ± 2 µmoles photon.m^{-2}.sec^{-1} of light intensity) with pH 7.4 ± 0.3. All particles (broken exoskeleton and waste) at the bottom were carefully removed with pipette tip without disturbing the *M. micrura* population. The experiments were terminated when the population of the control (*M. micrura* fed with *C. vulgaris*) began to decline. The population density was estimated every day by subsampling 25 mL of the well-mixed culture and examined it in a petri dish under a dissecting microscope.

Chronic bioassays (>10 days)

Based on a preliminary study, algae density of 1.0×10^5 cells.mL^{-1} was the optimum concentration to initiate biological responses of *M. micrura* towards all three microalgae species. Thus, this density was used in the following experiments. In addition, the colony size of both *Microcystis* spp. was ensured to be below 35 µm to avoid crowding in experimental containers. Neonates (<24 h) were individually reared in 20 different glass vials containing 15 mL filtered-sterilised pond water and kept under 30 ± 4.0 µmoles photon.m^{-2}.sec^{-1} of light intensity. All the glass vials were checked daily (at 12 h intervals) to determine the age at first reproduction. Once the female has completed hatching, it was transferred to a new vial with fresh culture media and maintained until it died to determine longevity. The frequencies of offspring produced by the female were determined by batches. Each batch of offspring production was transferred to a new vial to avoid miscalculation. Apart from that, the total number of offsprings, and maturity were also determined. Daily routine such as counting the number of individuals under a dissecting microscope, removing faecal materials, broken exoskeleton and replenished culture media were accomplished.

Statistical analysis

The growth of zooplankton was determined using the population growth rate equation: Population growth rate, $\mu = (ln\ X_2 - ln\ X_1)/T$ Where, X_1 = number of *M. micrura* at the start of selected time interval; X_2 = number of *M. micrura* at the final of selected time interval and T = time in days. All data were subjected to normality test and followed by one-way analysis of variance (ANOVA) with Tukey post hoc test to analyse the significant difference ($P < 0.05$) among all treatments. Statistical analyses were performed using SPSS v.23.

Results

Toxin analysis of Microcystis aeruginosa *and* Microcystis viridis

PCR products showed expected base pairs were between 400–500 bp for *M. aeruginosa* and 300–400 bp for *M. viridis* (Fig. 1). The non-microcystin producer strain, *C. vulgaris* yielded negative result. Both sequences revealed 98 % similarity with microcystin synthetase (*mcyB*) gene. This suggested that both isolates were potential toxigenic microcystin producer.

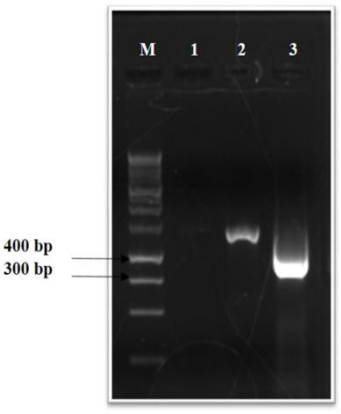

Fig 1. PCR products of microcystin synthetase (*mcyB*) gene. M: DNA ladder of 100bp. Lane 1: PCR products of *Chlorella vulgaris* as the control. Lane 2: PCR products of *Microcystis aeruginosa* (400–500 base pair) Lane 3: PCR products of *Microcystis viridis* (300–400 base pair).

Population growth

The population growth rates of *M. micrura* fed with *M. aeruginosa* and *M. viridis* were significantly reduced ($P < 0.05$) compared to the control, *C. vulgaris* (Figs. 2 and 3). The *M. micrura* population fed with *M. viridis* showed a slight increase on day 3 but subsequently declined. On the other hand, *M. micrura* fed with *M. aeruginosa* could not grow, and all individuals were dead by day 4. *Moina micrura* fed with *C. vulgaris* showed good growth, reaching a peak density on day 6 with the growth rate of 0.51 day^{-1} (Fig. 3). The *M. micrura* population growth rates were negative when fed with *M. aeruginosa* (-0.33 day^{-1}) and *M. viridis* (-0.13 day^{-1}). The mean body length of *M. micrura* was significantly lower ($P < 0.05$) when fed with *M. aeruginosa* compared to the control (Fig. 4). In fact, *M. micrura* exposed to *M. aeruginosa* did not reach maturity and remained much smaller as their mean body size only reached 627.80 µm. In contrast, *M. micrura* exhibited a significantly bigger ($P < 0.05$) body size when fed with *M. viridis* (914.21 µm) compared to those fed with *C. vulgaris* (814.94 µm) (Fig. 4)

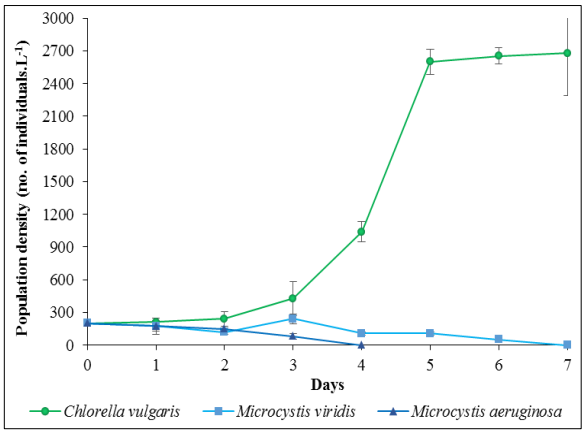

Fig. 2. Population growth curves of *Moina micrura* fed with different diets. Values represent mean ± standard error based on three replicates.

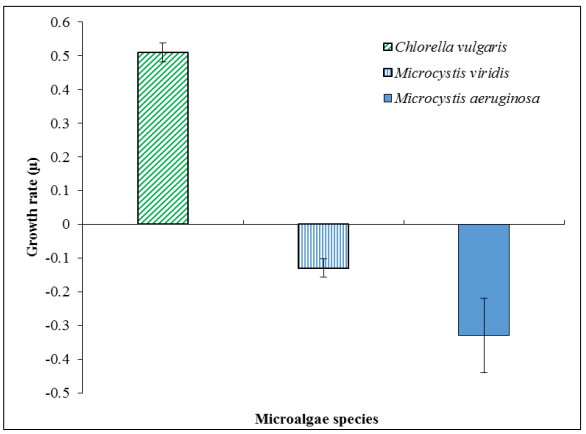

Fig. 3. Population growth rate of *Moina micrura* fed with different microalgae species. Values represent mean ± standard error based on three replicates.

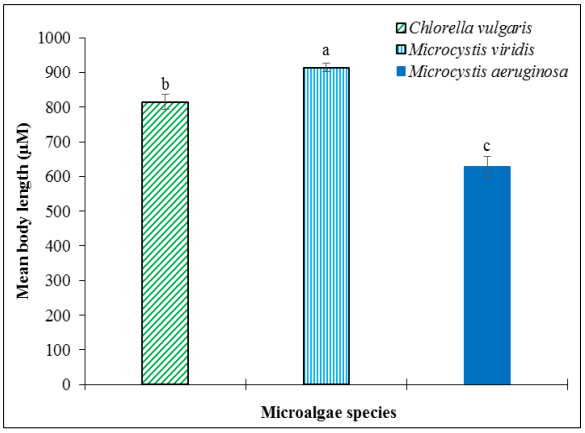

Fig. 4. Mean body length of *Moina micrura* fed with different microalgae species. Values represent mean ± standard error based on three replicates. Different letters indicate significant difference ($P < 0.05$).

Chronic bioassay (>10 days)

The longevity of *M. micrura* was significantly higher ($P < 0.05$) when fed with *C. vulgaris* (9 days) compared to those fed with *M. aeruginosa* and *M. viridis* (Fig. 5). However, no significant difference in longevity was found between populations fed with *M. viridis* (6 days) and *M. aeruginosa* ($P > 0.05$). The exposure of *M. micrura* to toxic *M. aeruginosa* significantly ($P < 0.05$) affected the production of *M. micrura*'s first offspring. Thus, there was a delay in the production of *M. micrura* fed with *C. vulgaris* which only occurred on day 6 compared to *M. viridis* which produced their first offspring as early as on day 3 (Fig. 6). The age of first reproduction in *M. micrura* fed with *M. viridis* and *C. vulgaris* were not significantly different ($P > 0.05$) (Fig. 6). The frequencies of offspring production by *M. micrura* were significantly higher ($P < 0.05$) when fed with *C. vulgaris* compared to both *Microcystis* diets. There was no significant difference ($P > 0.05$) in the production frequency between the two *Microcystis* species. In fact, *M. micrura* fed with *C. vulgaris* produced significantly higher ($P < 0.05$) neonates in the first three batches compared to those fed with both *M. aeruginosa* and *M. viridis* that could only produce two batches of offsprings in a lifetime (Fig. 7). *Microcystis* diets had a tremendous negative effect on the total number of offsprings produced by *M. micrura*. Significantly fewer total offsprings were produced when fed with *M. aeruginosa* and *M. viridis* compared to *M. micrura* fed with *C. vulgaris* (Fig. 8).

Different microalgae species had a significant influence on the percentage of aborted eggs in *M. micrura*. There were only 1.4 % aborted eggs in the control treatment with *C. vulgaris* but in the presence of *Microcystis* spp., the proportion of aborted eggs increased from 1.4 % to 50.0 % (Fig. 9). Even though both cyanobacteria species affect the proportion of aborted eggs in *M. micrura*, the effect was much more severe in *M. micrura* fed with *M. viridis*. *Moina micrura* fed with *M. viridis* had many eggs in their brood

chambers, but only a few were released, and most of them were decomposed (Fig. 10). Out of 20 replicates of *M. micrura*, only seven were able to release one neonate at least, but the neonates were dead not long after they were released.

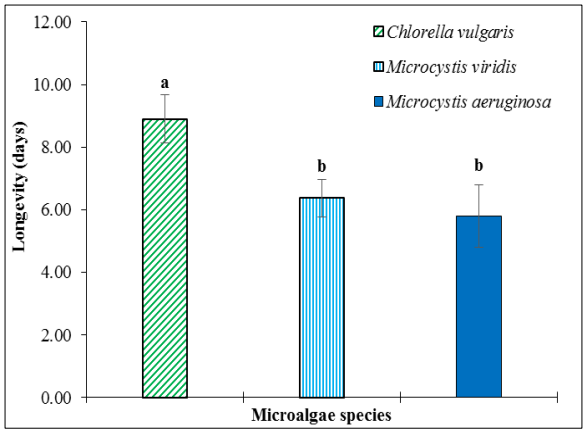

Fig. 5. Longevity of *Moina micrura* fed with different microalgae species. Values represent mean ± standard error (n = 20). Different letters indicate significant difference ($P < 0.05$).

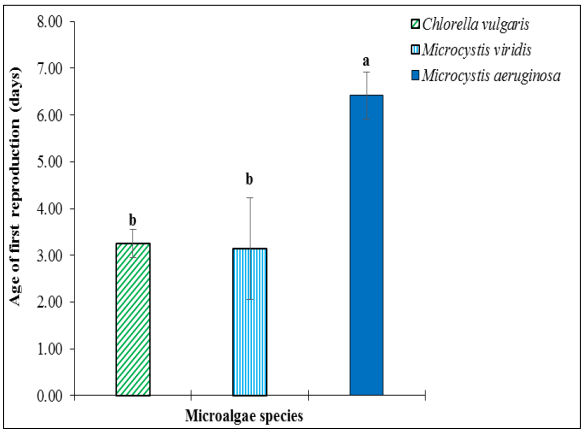

Fig. 6. Age of first reproduction of *Moina micrura* with different microalgae species. Values represent mean ± standard error (n = 20). Different letters indicate significant difference ($P < 0.05$).

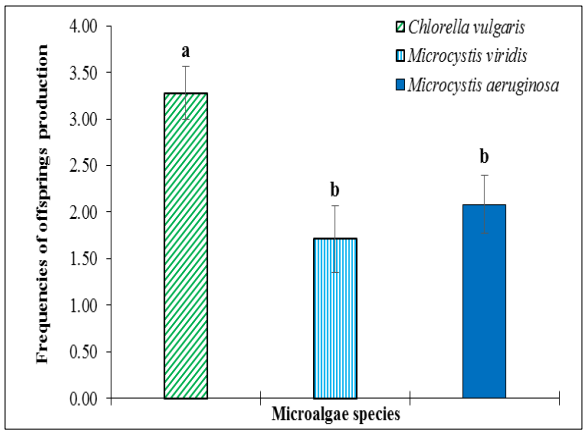

Fig. 7. Frequencies of offspring production of *Moina micrura* fed with different microalgae species. Values represent mean ± standard error (n = 20). Different letters indicate significant difference ($P < 0.05$).

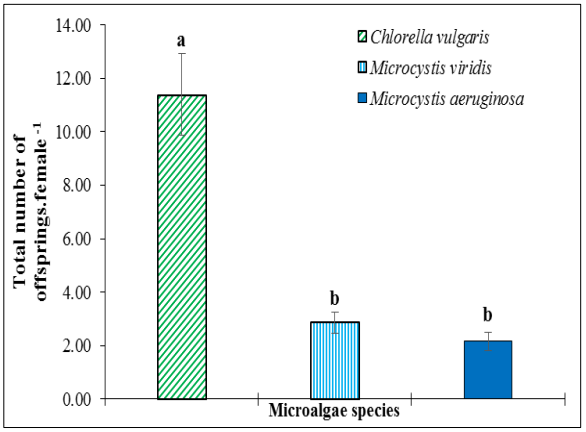

Fig. 8. Total offsprings.female $^{-1}$ of *Moina micrura* fed with different microalgae species. Values represent mean ± standard error (n = 20). Different letters indicate significant difference ($P < 0.05$).

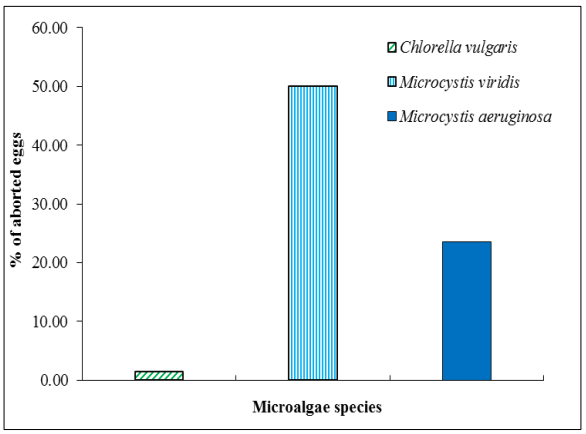

Fig. 9. Percentage of aborted eggs of *Moina micrura* fed with different microalgae species during chronic bioassay (n = 20).

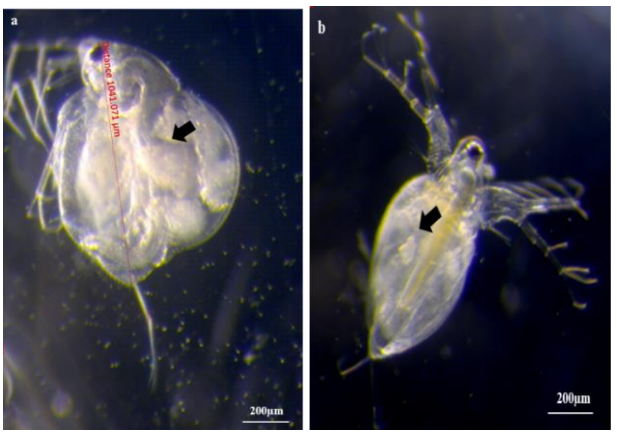

Fig. 10. *Moina micrura* fed with *Microcystis viridis* showed an a) early developmental of egg sac and body cavity of neonates b) aborted egg.

Discussion

The population density and population growth rate of *M. micrura* varied according to the type of algae they fed on. *Moina micrura* population fed on *C. vulgaris* showed significantly higher growth rate ($P < 0.05$) compared to *M. micrura* fed on both of *Microcystis* species. Nandini et al. (2017) also reported that population growth rate of *M. micrura* was negative and suffered total mortality on day 6 when fed with *M. aeruginosa* cells. Cyanobacteria affect zooplankton in three possible ways; morphologically (colonial form), toxicity and nutritionally. Secondary metabolites produced by *Microcystis,* especially microcystin is one of the main causes of mortality in zooplankton. The higher the microcystin content, the higher the mortality of zooplankton populations (Herrera et al., 2015). However, in some cases, there are other possible toxic compounds present in cyanobacteria instead of microcystin that could also affect zooplankton (Dao et al., 2016). Colonial properties of *Microcystis* can also disturb zooplankton growth. In this current study, the effect of bigger colony size (>60 µm) was severe, resulting in total mortality in less than a week compared to the chronic bioassay that had smaller size colonies of *Microcystis* species. The increased colonial size in *Microcystis* when in contact with zooplankton could be associated with the defence mechanism in cyanobacteria to reduce predation risk. Yang et al. (2008) also suggested the role of the grazer (flagellates) in inducing *Microcystis* colony formation. Jang et al. (2003) and Yang et al. (2008) observed the relationship between grazer inducing colonial formation with microcystin concentration in their laboratory.

In addition, the length of the *M. micrura* showed 23 % reduction when fed with *M. aeruginosa.* According to Lürling (2003), there is a relationship between zooplankton maturation and the body length. In this study, *M. micrura* population fed with *M. aeruginosa* had a small body size suggesting they could not reach maturation. Maturation and age of the first offspring production are parameters used in order to measure the fitness level of a cladoceran (Gustafsson et al., 2005). In the present study, the fitness of *M. micrura* was significantly reduced when fed with both *Microcystis* species. The age of the first offspring was significantly prolonged ($P < 0.05$) in *M. aeruginosa* diet and it took almost six days to produce its first neonates. Our findings are in agreement with the data published by Dao et al. (2016). Whereas, the age of first reproduction in *C. vulgaris* and *M. viridis* were not significantly different, and both produced their first offsprings earlier on day 3. In addition, Gustafsson et al. (2005) also described how a maternal effect could enhance cladoceran fitness in the following generation population. Interestingly, this might explain the high tolerance exhibit by certain cladoceran species toward toxic cyanobacteria (Barros et al., 2001; Guo and Xie, 2006). Cladoceran isolated from an environment with toxic *Microcystis* blooms might have high tolerance toward toxic *Microcystis*.

The average life span of *M. micrura* is approximately 13 days (Murugan, 1975). In the present study, the longevity of *M. micrura* fed with *Microcystis* species was significantly reduced compared to those fed with *C. vulgaris*. Nandini et al. (2017) also stated that *Microcystis* diet reduced the average life span of zooplankton. In this study, the reproductive capacity of *M. micrura* was determined by the fecundity, the frequency of egg production and the total number of offsprings produced by each *M. micrura* female. The *M. micrura* female was at its optimum reproductive ability when fed with *C. vulgaris* because it had significantly higher fecundity, reproductive frequency and the total number of offsprings ($P < 0.05$) compared to those fed with *M. aeruginosa* and *M. viridis*. The reproductive capacity of *M. micrura* was significantly reduced when fed with *Microcystis* species. Between the two *Microcystis* species, *M. micrura* reproductive performance was severely affected by *M. viridis* compared to *M. aeruginosa*. Out of 20 tested cladocerans fed with *M. viridis*, only seven were able to produce neonates.

Toxic *Microcystis* affect the zooplankton reproduction not only by suppressing its fecundity but also increasing the risk of potential egg abortion, decomposition and embryo malformation. Egg abortion event is where an organism cannot produce viable eggs while teratogenic offspring is a neonate with unsymmetrical body segments, undeveloped appendages and usually died right after birth because it is incapable of feeding and swimming well (Ianora and Miralto, 2010). In the present study, *M. micrura* fed with different cyanobacterial species showed high percentages of aborted eggs. Only 1.4 % of aborted eggs were observed in the treatment with *C. vulgaris,* whereas the percentage significantly increased to 50 % when fed with *Microcystis* spp. Gustafsson et al. (2005) also discovered that egg abortion occurred in a female *Daphnia* when treated with microcystin. It might be difficult to understand the mechanism of *Microcystis* toxin in a cladoceran as it has a simple reproductive system. A study by Ding et al. (2006) and Zhao et al. (2015) in higher animals with complex reproductive systems, revealed that the toxic *Microcystis* affect the oogenesis in female zebrafish and spermatogenesis in male mice. A comprehensive study should be conducted to better understand the mechanism of microcystin in the impairment of a cladoceran reproductive system. Ger et al. (2010) and Trubetskova and Haney (2006) also suggested the possibility of secondary metabolite other than microcystin that could be harmful to zooplankton.

In the present study, the reproductive capacity in *M. micrura* was affected by both *Microcystis* species probably because both are microcystin producers. In addition, the occurrences of egg abortion and the inability to produce viable eggs in *M. micrura* fed with

M. viridis revealed that harmful effects of *M. viridis* on zooplankton reproductive capacity. Further research is needed to verify the microcystin effects. At the moment, *M. aeruginosa* is recognized by its ability to produce microcystin-LR, whereas, in *M. viridis*, microcystin-RR is more prominent (Yasuno and Sugaya, 1991). Furthermore, *M. viridis* produces significantly higher lipopolysaccharide (LPS) (Forni et al., 1997) compared to other known *Microcystis* species. Thus, it could be possible that these two secondary metabolites, Microcystis-RR and LPS are somehow accountable for reproductive dysfunctional in zooplankton.

Conclusion

This study showed that the exposure of a cladoceran, *M. micrura* to both toxic *M. aeruginosa* and *M. viridis* reduced the zooplankton population growth and impaired its reproductive performance compared to those fed with *C. vulgaris*. *Moina micrura* fed with *C. vulgaris* exhibited a positive growth rate (0.51 day^{-1}) but became negative when fed with both *M. aeruginosa* (-0.33 day^{-1}) and *M. viridis* (-0.13 day^{-1}). Besides that, *M. micrura* fed with *M. aeruginosa* did not reach maturation as their mean body lengths were only 627.80 µm compared to the control, *C. vulgaris* (814. 94 µm). Exposure of *M. micrura* to *Microcystis* species reduced the reproductive capability of *M. micrura* by not only reducing the production frequency and the total offsprings but also increased the number of aborted eggs compared to those fed with *C. vulgaris*.

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