Potential of the water flea *Daphnia magna* to control phytoplankton population in eutrophic waters

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**Abstract.** Water enrichment has become a national problem that needs to be resolved. This study examined the ability of water fleas *Daphnia magna* to grow and to control phytoplankton populations in the waters from eutrophic ponds Situ Cikaret and Situ Gunung Putri in Bogor Regency, West Java. Water samples from these water bodies were used to grow the daphnid populations in the styrofoam aquariums without artificial feeding with an initial density of 3.3 individuals/L. The experiment was carried out with three replications. Daphnid population was counted every 4–5 days to observe its growth and development. The chlorophyll content that reflects the phytoplankton biomass at the beginning of the study in the water of Situ Cikaret and Situ Gunung Putri were 52 and 22 µg/L, respectively. The water from fertile ponds allowed the development of phytoplankton populations and be utilized by daphnids to support their growth and reproduction. The results indicated that daphnids showed a preference for some phytoplankton genera more than other genera because not all phytoplankton populations were consumed. Following an increase in chlorophyll content, the daphnid density also increased to reach a maximum of 197 individuals/L in the water of Situ Cikaret and 169 individuals/L in the water of Situ Gunung Putri on day 15. The chlorophyll content decreased significantly towards the end of the experiment indicating the phytoplankton population is almost depleted. Hence, this study showed the potential of water flea *Daphnia magna* as bio-resources to utilize eutrophic pond waters and to control the phytoplankton population to improve water quality.

1. Introduction

Eutrophication has been currently becoming a major problem in inland water, including lakes, in Indonesia. Lake Limboto in Sulawesi, for example, has been reported to be under eutrophic condition, with N, P, and chlorophyll contents recorded 0.89–1.66 mg/L, 0.12–0.64 mg/L, and 18.43–42.18 µg/L, respectively [1]. Likewise, Lake Maninjau has also been under eutrophic category due to high nutrient contents of 0.37–7.43 mg N/L, 0.02–0.65 mg P/L, and chlorophyll 20.7–77.5 µg/L [2, 3]. There has been a call for national urgency to resolve eutrophication problems and some abatement programs have been launched, but the problem still exists and awaits a more effective resolution.

Eutrophic water is mostly associated with a green scene due to phytoplankton bloom [1, 4], which is the logical consequence of phytoplankton ecological role as autotrophic organisms that opportunistically utilize abundant nutrient sources to grow [5, 6, 7].

Phytoplankton growth gives an impact on the nutrient reduction in the water. However, uncontrolled phytoplankton blooms could give negative impacts, such as oxygen depletion due to dark respiration as well as dead cells organic degradation [8, 9]. Accordingly, there has been some
suggestion to combat water eutrophication using phytoplankton while controlling the population by any herbivorous animals.

Several studies have reported the effectiveness of Cladocera, especially of genus Daphnia due to the habit of filter feeder, to control phytoplankton populations in inland waters [5, 10, 11, 12]. The Daphnia magna has also shown capable in reduce chlorophyll content in the water by more than 80% and pointed out the potential for controlling phytoplankton population in inland waters [13]. This study examines the growth and capability of the water flea Daphnia magna to control phytoplankton bloom in different eutrophic water samples from two small lakes in Bogor Regency, West Java.

2. Materials and Methods

2.1. Tested biota and media

The water fleas used as tested biota was Daphnia magna Straus, 1820, taken from the collection of the Laboratory of Planktonology, the Research Center for Limnology, Indonesian Institute of Sciences (LIPI). The stock of the daphnid population was maintained using water media enriched with catfish feed Hi-Pro-Vite 781 (CP Prima). The water used as the growth medium for Daphnia magna was taken from two small lakes (ponds), Situ Cikaret and Situ Gunung Putri, located in Bogor Regency, West Java. Before the research started, the water was first analyzed to determine the initial conditions of the chemical parameters such as TN, TP, TSS, and chlorophyll in the water.

2.2. The experiment

The study was carried out for 14 days using eight styrofoam boxes as aquariums, each measuring $47 \times 32 \times 30$ cm$^3$ (Figure 1). The boxes were equipped with an aeration system and filled with 30 L of water from each pond. The styrofoam boxes were chosen so as the water temperature during the study was relatively constant. The aquariums were placed in a greenhouse with a room temperature of 25–34°C.

Adult daphnids were selected to be inoculated on the first day of the study with a density of 3.3 individuals/L or 100 individuals per aquarium. During the study, the daphnids were allowed to grow using the available resources according to the different levels of eutrophication of the water without the addition of artificial feeds. The experiment was performed with three replications for both types of water media.

![Figure 1. Daphnia rearing in styrofoam aquariums filled with water samples of Situ Cikaret (center) and Situ Gunung Putri (right) at the beginning of the experiment.](image-url)
2.3. Observations
Observations were made on the main parameters, such as daphnid density, chlorophyll, and Total Suspended Solids (TSS) contents. Chlorophyll and TSS are considered to represent the condition of phytoplankton density in the water. During the experiment, daphnid samples were counted four times (day 1, 5, 10, and 15) to determine their density. The counting was carried out by filtering 1 L of a water sample from each aquarium using a fine nylon filter with a mesh size of 0.5 mm, and then directly counting the number of daphnids in the water sample, both the adults and juveniles.

The chlorophyll and TSS contents were analyzed every 4–5 days by taking 20 to 50 mL of water samples, then filtered using Whatman GF/C filter paper, and immediately stored in the freezer until ready for analysis. Chlorophyll content determination was conducted spectroscopically after extraction in acetone 90% using UV-VIS spectrophotometer, while TSS was analyzed gravimetrically following the APHA standard method [14].

The measurements of the supporting parameters of water quality such as temperature, pH, Dissolved Oxygen (DO), conductivity, Total Dissolved Solids (TDS), and turbidity were carried out every day, while chemical parameters such as Total Nitrogen (TN) and Total Phosphorous (TP) in the water media were analyzed every 4–5 days. The temperature was measured using Thermometer Lutron YK-2001PH, pH using pH meter Lutron pH-201, Dissolved Oxygen using DO meter Lutron DO-201, conductivity and TDS using Lutron YK-2001PH, and turbidity using Turbidimeter Lutron TU-2016.

Media aliquot samplings (100 mL) for TN and TP analysis were conducted on days 1, 5, 10, and 15. The water samples were stored in a refrigerator before analysis, with a storage time of fewer than 14 days. Methods of TN and TP analysis followed APHA [14].

2.4. Data analysis
Data analysis was particularly carried out to evaluate the water trophic status based on the observed chlorophyll and TP contents, according to the formula developed as follow [15]:

\[
\text{TSI}_{\text{Chl}} = 10 \left( 6 - \frac{2.04 - 0.68 \ln \text{Chl}}{\ln 2} \right) \tag{1}
\]

\[
\text{TSI}_{\text{TP}} = 10 \left( 6 - \frac{\ln \text{TP}}{\ln 2} \right) \tag{2}
\]

The Trophic Status Index of the ponds is therefore determined from the average values of TSI(Chl) and TSI(TP).

3. Results and Discussions

3.1. Daphnid growth
During the first five days in the water of Situ Cikaret, the average daphnid density has grown to 29.8 individuals/L (Figure 2), consisting of 17.1 juveniles and 13.9 adults (Figure 3), while in the water of Situ Gunung Putri the population reached 20 individuals/L (Figure 2), consisting of 11.3 juveniles and 8.8 adults (Figure 4). The growth of the daphnid population on day 5 in the water of Situ Cikaret showed a nine-fold increase in daphnid density, while in the water of Situ Gunung Putri the increase was six-fold. In both waters, there were slightly more juveniles observed than adults.

The growth of daphnids until day 15 in the water of Situ Cikaret still shows an increase in density for both juvenile and adult, with a ratio of juvenile to an adult density of 5.6 on day 10 and 3.6 on day 15 (Figure 3). However, in the water of Situ Gunung Putri, the increase of daphnid density reached its maximum on day 10 with the ratio of juvenile to adult density reaching 8.6, while on day 15 the ratio decreased to 6.3 (Figure 4). Daphnid density on day 15 did not show a significant increase compared to its density on day 10. On day 15, the maximum density of daphnids in the water of Situ Cikaret was
197 individuals/L, while in the water of Situ Gunung Putri was 169 individuals/L. Thus, the water of Situ Cikaret gave better support for the growth of the daphnid population.

![Figure 2](image2.png)

**Figure 2.** Daphnid density in the water of Situ Cikaret and Situ Gunung Putri.

The highest increase in daphnid density for both types of water occurred from day 5 to day 10 wherein the water of Situ Cikaret the density of adults has increased by 4.4 individuals/L, while in the water of Situ Gunung Putri was 8.2 individuals/L. On the contrary, there were 17.1 and 11.3 potential juveniles, respectively, in the water of Situ Cikaret and Situ Gunung Putri on day 5 to becoming adults on day 10. If all potential juveniles grew into adults, by day 10 the density of adults should have reached 31.0 and 20.1 individuals/L, respectively, in the water of Situ Cikaret and Situ Gunung Putri instead of 18.3 and 17.0 individuals/L. Similarly, the increase of adult density from day 10 to 15 in the water of Situ Cikaret and Situ Gunung Putri was 24.5 and 6.0 individuals/L. However, there were 101.8 and 146.8 potential juveniles, respectively, in the water of Situ Cikaret and Situ Gunung Putri on day 10 to becoming adults on day 15. Should all of them become adults, the density of adults on day 15 would be 120.1 and 163.8 individuals/L, instead of 42.8 and 23.0 individuals/L, respectively, in the water of Situ Cikaret and Situ Gunung Putri (Figures 3 and 4).

Presumably, there was either death that occurred in adult daphnids or juvenile populations were prevented from growing or developing properly to become adults. The latter could be seen from the decline in the proportions of juveniles to adult individuals that were observed from day 10 to 15 for both water samples (Figures 3 and 4).

![Figure 3](image3.png)

**Figure 3.** The density of juvenile and adult daphnids (left) and the ratio of juvenile to adult (right) in the water of Situ Cikaret.
Figure 4. Density of juvenile and adult daphnids (left) and the ratio of juvenile to adult (right) in the water of Situ Gunung Putri.

3.2. Chlorophyll and TSS dynamics

The results of the chlorophyll and TSS content analysis in the water of both ponds are shown in Figure 5. At the beginning of the study, the chlorophyll contents in the water of the Situ Cikaret and Situ Gunung Putri were 52 and 22 µg/L, respectively, while the TSS contents in the water of the Situ Cikaret and Situ Gunung Putri were 16 and 9 mg/L. Both chlorophyll and TSS contents in the water of Situ Cikaret were much higher than in the water of Situ Gunung Putri on day 1, but the proportion of chlorophyll to the mass of TSS in the water of Situ Cikaret was 0.31%, which is not significantly different from 0.25% of the proportion of chlorophyll to the mass of TSS in the water of Situ Gunung Putri (Figure 6). In the water of Situ Cikaret, the decrease in chlorophyll and TSS contents occurred throughout the experiment to reach minimum values of 4.3 µg/L and 0.5 mg/L on day 15 (Figure 5).

On the other hand, an increase in chlorophyll content occurred on day 10 in the water of Situ Gunung Putri, which was primarily caused by some phytoplankton growth, especially Synedra sp. in which its content in chlorophyll might be higher than other phytoplankton species (Table 1). TSS content in the water of Situ Gunung Putri increased slightly during the first five days (Figure 5), which could be related to the sharp decrease in phytoplankton density from 22,540 individuals/L on day 1 to 2,140 individuals/L on day 5, which produced debris from the excretion of daphnids.

Towards the end of the experiment, the proportion of chlorophyll to TSS tended to be higher in the water of Situ Cikaret, which was 0.74% compared to 0.50% in the water of Situ Cibuntu (Figure 6). This is possibly related to the growth of Synedra sp., Melosira sp., and Mougeotia sp. which did not appear to be consumed by the daphnids population (Table 1). Even though the two values of proportion were not significantly different, but the density of phytoplankton on day 15 in the water of Situ Cikaret was 2.3 times higher than in the water of Situ Gunung Putri.
Table 1 shows that the density of the green algae (Chlorophyta) decreased significantly during the study, except for the genus Mougeotia sp., compared to Bacillariophyta (diatoms). Equipped with an efficient feeding apparatus, the daphnids as filter feeders can even collect the bacteria. However, their food is usually made up of planktonic algae; and the green algae such as *Scenedesmus* sp. are among the best food and the most used food in laboratory experiments [16].

Although there is no information in the literature regarding the ecological functional relationship between daphnids and diatoms, several previous studies have demonstrated the ability of daphnids to select types of food according to their needs. The two identified Bacillariophyta genera, *Synedra* sp. and *Melosira* sp., were not consumed by the daphnids. This could be related to their size or shape. The size of *Synedra* sp. was quite large with its valve length reaching 750 µm [17], while *Melosira* sp. was in the form of long filaments [17, 18]. For the same reason, *Mougeotia* sp. that was also filamentous, cannot be filtered in by the daphnids. In addition, diatoms have cell walls or shells composed of silica that are difficult to digest, so they may be rejected by the daphnids during the food filtering process.

The daphnids were formerly known as filter feeders without feed preference [19]. However, some evidence has been reported to emphasize the dependence of these animals on phytoplankton to meet their nutritional needs, especially essential lipids [20, 21]. The ability of *Daphnia magna* to select more nutritious algae cells for feed has also demonstrated [22], while the ability of these animals to repel toxic blue-green algae during feed filtering was reported [23, 24]. The results of this study emphasize the daphnid's preference in consuming the green algae.
Table 1. Phytoplankton genera are found in the water of both ponds.

| Phytoplankton          | Situ Cikaret |          |          |          |          |          |          |          | Situ Gunung Putri |          |          |          |
|------------------------|--------------|----------|----------|----------|----------|----------|----------|----------|-------------------|----------|----------|----------|
|                        | Day 1        | Day 5    | Day 10   | Day 15   | Day 1    | Day 5    | Day 10   | Day 15   |                   |          |          |          |
| BACILLARIOPHYTA         |              |          |          |          |          |          |          |          |                  |          |          |          |
| Synedra sp.            | 1,800        | 280      | 2,500    | 3,980    | 1,040    | 1,700    | 3,620    | 2,340    |                  |          |          |          |
| Melosira sp.           | 120          | 400      | 660      | 420      | 420      | 40       | 400      | 380      |                  |          |          |          |
| CHLOROPHYTA            |              |          |          |          |          |          |          |          |                  |          |          |          |
| Actinastrum sp.        | 680          | 420      | 220      | -        | 760      | 160      | 60       | -        |                  |          |          |          |
| Dictyosphaerium sp.    | 22,120       | 20,320   | 1,000    | 520      | 19,200   | 220      | 120      | 80       |                  |          |          |          |
| Micractinium sp.       | 200          | -        | -        | -        | 80       | -        | -        | -        |                  |          |          |          |
| Pediastrum sp.         | 720          | 180      | 280      | 80       | 180      | -        | 20       | 80       |                  |          |          |          |
| Mougeotia sp.          | -            | -        | 420      | 3,280    | 60       | -        | 160      | 720      |                  |          |          |          |
| Scenedesmus sp.        | 1,460        | 400      | 240      | 20       | 800      | 20       | 120      | 20       |                  |          |          |          |
| Total density          |             |          |          |          |          |          |          |          | 27,100            |          |          |          |
| (individuals/L)        |              |          |          |          |          |          |          |          | 22,000            |          |          |          |

3.3. TN and TP dynamics

TN concentrations decreased during the study from the initial concentrations of 2.94 and 4.55 mg/L to 0.37 and 0.59 mg/L on day 15 in the water of Situ Gunung Putri and Situ Cikaret, respectively (Figure 7). On the other hand, TP concentrations also decreased during the study from the initial concentrations of 0.16 and 0.20 mg/L to 0.03 and 0.04 mg/L on day 15 in the water of Situ Gunung Putri and Situ Cikaret.

Figure 7 and Table 1 show the phenomenon of a decrease in TN and TP which is in line with the decrease in the abundance of phytoplankton. However, the decrease in nutrients occurred especially in the initial phase of the experiment where the abundance of phytoplankton was still relatively high, while during the experimental period the phytoplankton was not completely depleted so that absorption of nutrients still occurred throughout the experimental time.

3.4. Trophic State Index (TSI)

The chlorophyll content is a description of the algal biomass density contained in the water. High or low chlorophyll concentration is considered as the most relevant factor to be used in assessing the trophic status of a water body. If the chlorophyll concentration ranges from 20–56 µg/L, the lake in question is eutrophic [25]. In this study, the chlorophyll content in the water of both ponds that matched the eutrophic criteria was only found on day 1 (Figure 5). Also, water samples with TSI values ranging from 40 to 50 are categorized as mesotrophic. from 50 to 70 are eutrophic. and above 70 are hypereutrophic [25]. The calculation of TSI based on chlorophyll and TP contents resulted in
the values of TSI (Table 2) which shows that at the end of the study, the fertility levels of both ponds have decreased, thus becoming the low degree of eutrophy for the water of Situ Cikaret and mesotrophy for the water of Situ Gunung Putri.

| Day | TSI\(_{\text{Chl}}\) | TSI\(_{\text{TP}}\) | TSI value | Trophic status | TSI\(_{\text{Chl}}\) | TSI\(_{\text{TP}}\) | TSI value | Trophic status |
|-----|----------------|----------------|-----------|----------------|----------------|----------------|-----------|----------------|
| 1   | 69.3           | 80.6           | 75.0      | Hypereutrophy  | 61.0           | 77.3           | 69.1      | Eutrophy       |
| 5   | 59.4           | 62.8           | 61.1      | Eutrophy       | 51.5           | 58.8           | 55.2      | Eutrophy       |
| 10  | 52.5           | 44.1           | 48.3      | Mesotrophy     | 60.1           | 53.2           | 56.6      | Eutrophy       |
| 15  | 44.9           | 56.8           | 50.9      | Eutrophy       | 47.3           | 52.8           | 50.0      | Mesotrophy     |

3.5. Water quality

The physical and chemical water parameters of this experiment are shown in Table 3. Water temperature tended to increase during the experiment within the range of 22.4–29.4°C. Daphnids have a wide tolerance for temperature, but the optimal temperature for Daphnia sp. is 24–28°C [26]. At the beginning of the experiment, the pH of the water of Situ Cikaret was 9.0, while in the water of Situ Gunung Putri was 9.2. Then, it decreased on day 2 to 8.6 and stabilized starting on day 3 in the range of 8.4–8.9. Most daphnid species can live in a pH range of 6.5–9.5 with an optimal pH between 7.2 and 8.5 [16]. The optimal pH range to support the life of aquatic biota is 6.5–9.0 [27]. Dissolved oxygen concentration (DO) at the beginning of the experiment was relatively high in the range of 9.3–9.6 mg/L. It slightly decreased during the experiment and became quite stable towards the end of the experiment in the range of 7.4–9.8 mg/L. DO concentration in healthy waters ranges 5–9 mg/L or a minimum of 4 mg/L to be able to support a variety of aquatic biota populations [28]. Water temperature and DO in this study are also following [29] that the optimal temperature is around 25°C and the dissolved oxygen level is above 3.5 mg/L.

TDS in the water experienced a slight increase during the experiment with a range of 121–138 mg/L in the water of Situ Cikaret and 180–193 mg/L in the water of Situ Gunung Putri. Conductivity also increased slightly in the range of 0.179–0.200 mS/cm in the water of Situ Cikaret, while in the water of Situ Gunung Putri it experienced a slight increase in the range of 0.272–0.289 mS/cm. The increase in TDS and conductivity values, although slight, indicates the release of ionic minerals that occur together with the degradation of organic matter in the water. Optimum conductivity values to support the life of fish and macroinvertebrates in lakes or rivers range from 0.15 to 0.50 mS/cm (http://water.epa.gov). In general, the conductivity values will increase if there is an increase in the concentration of pollutants in the water.

Turbidity values at the beginning of the experiment in the water of Situ Cikaret and Situ Gunung Putri were 4.2 and 3.2 NTU, respectively. It then increased to reach a maximum of 5.0 NTU in the water of Situ Cikaret and 3.6 NTU in the water of Situ Gunung Putri on day 3. Subsequently, the turbidity values decreased until the end of the study, and on day 15 the values in the water of Situ Cikaret and Situ Gunung Putri were 0.9 and 0.3 NTU, respectively. Low turbidity values indicate high water clarity. During the experiment, turbidity values were below the threshold recommended by the US EPA for fisheries, which is 25 NTU. Altogether, physical and chemical water parameters tested were following the Class I water quality standard based on the Government Regulation of the Republic of Indonesia No. 82 of 2001.
Table 3. The range values of some physical and chemical water parameters during the experiment.

| No | Parameter               | Situ Cikaret       | Situ Gunung Putri   |
|----|-------------------------|--------------------|---------------------|
|    | Physical parameter      |                    |                     |
| 1  | Temperature (°C)        | 22.5–29.4          | 22.4–25.6           |
| 2  | TDS (mg/L)              | 121.0–138.0        | 185.5–193.0         |
| 3  | Conductivity (mS/cm)    | 0.179–0.207        | 0.277–0.289         |
| 4  | Turbidity (NTU)         | 0.6–5.0            | 0.3–3.6             |
|    | Chemical Parameter      |                    |                     |
| 1  | pH                      | 8.4–9.0            | 8.6–9.2             |
| 2  | DO (mg/L)               | 7.6–9.8            | 7.8–9.8             |

4. Conclusion

This study shows that fertile waters promote the abundance of phytoplankton, which thrives and absorbs nutrients from the water. Furthermore, the abundant phytoplankton becomes a source of feed that supports the growth of the daphnid population. Thus, the daphnids can contribute to the process of improving water quality by cleaning up previously grown phytoplankton populations and removing nutrients from the water.

Acknowledgment

This research was conducted under the funding scheme of the National Priority Project for Maninjau Lake Rehabilitation (DIPA 2019) of Research Center for Limnology - Indonesian Institute of Sciences. We would like to thank Ayu Sriwahyuni and Rani Dwi Safitri for their excellent technical assistance during the study.

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